Running Title: Biochar Treatment for Fat Androstenone

1 **Effect of Genetic Line and Steroidogenic Capacity on Response to Dietary**
2 **Supplementation of Biochar to Control Fat Androstenone Levels in Boars[1]**

3

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11 **LAY SUMMARY**

12 Boar taint describes an undesirable odor or taste present in pork from some entire male
13 pigs (boars). To prevent it, male pigs are typically castrated, which is a painful and stressful
14 procedure. We recently demonstrated that dietary supplementation with biochar (BC), a
15 charcoal-based binding agent, successfully prevented boar taint in up to 89% of affected
16 animals. While this suggests that dietary BC treatment could be a promising alternative to
17 castration, its effectiveness relies on identifying animals that respond positively. To evaluate
18 the broader applicability of BC as a dietary treatment for boar taint, boars from two different
19 genetic lines were fed a BC-supplemented diet for four weeks. Additionally, boars were
20 classified as having either high or low capacities for steroid production to investigate whether
21 this trait could influence treatment response. Dietary BC effectively controlled boar taint in a
22 subset of affected boars from both genetic lines and steroid production groups, including 100%
23 of those with low steroid production capacity. These findings may help identify boars most
24 likely to benefit from BC treatment, so that it can be provided in a targeted manner to
25 effectively control boar taint.

26 **TEASER TEXT**

27 A 5% biochar (BC) diet successfully controlled boar taint in a subset of affected boars
28 from two different genetic lines. Within this subset, BC was 100% effective in animals with
29 low steroidogenic capacity, suggesting that steroidogenic capacity may help to predict dietary
30 treatment success.

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32 **ABSTRACT**

33 Boar taint, an unpleasant odor in heated pork, is caused primarily by androstenone
34 accumulation in fat. Previous research demonstrated that biochar (BC), a charcoal-based
35 adsorbent, can reduce androstenone accumulation in the fat when used as a dietary additive.
36 The objective of this study was to compare the effectiveness of BC as a dietary treatment for
37 boar taint in two genetic lines of boars from Alliance Genetics Canada (AGC) and Pig
38 Improvement Company (PIC), and examine the influence of steroidogenic capacity on
39 treatment response. All boars were fed a 5% BC diet for four weeks starting at a bodyweight
40 (BW) of 75.25 ± 1.42 kg and 111.10 ± 0.86 days of age (DOA), followed by a two-week
41 recovery period where BC was removed from the diet. Plasma androstenone, estrone sulfate
42 (E_1S), and growth performance parameters were measured weekly, and fat androstenone was
43 measured biweekly. We found that 71% of PIC boars and 47% of AGC boars never
44 developed boar taint, as the fat androstenone concentrations remained below the threshold of
45 1 μg/g throughout the trial. Of those with the capacity to develop boar taint, 89% of AGC
46 boars responded to BC treatment, compared to 67% for PIC. However, no differences in
47 plasma or fat androstenone levels were observed between the two genetic lines. Additionally,
48 all growth performance parameters were not different between AGC and PIC boars except
49 gain:feed (G:F) during the treatment period and average daily gain (ADG) during recovery,
50 where PIC boars performed better (P = 0.001). Plasma E_1S concentrations measured at
51 approximately 130 kg live weight were used to classify boars as either high (plasma E_1S > 16
52 ng/mL) or low steroidogenic capacity (plasma $E_1S \leq 16$ ng/mL). High steroidogenic capacity
53 boars had greater plasma E_1S concentrations (P < 0.05) across all time points compared those
54 with low steroidogenic capacity, although growth rates remained not different between

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55 groups. In both genetic lines, boars that never developed boar taint as well as those responded
56 to BC treatment consisted of a mix of high and low steroidogenic capacity animals, while
57 boars that did not respond to BC treatment were exclusively high steroidogenic capacity.
58 These results suggest that steroidogenic capacity may help to predict BC treatment response,
59 although additional research is needed to identify genetic and biological markers associated
60 with treatment success in order to effectively tailor BC treatment to animals most likely to
61 benefit.

62

63 **Keywords:** androstenone, biochar, boar taint, enterohepatic circulation, genetic lines,
64 steroidogenic capacity

65

66 **List of Abbreviations**

67 AC: activated charcoal

68 ADG: average daily gain

69 AGC: Alliance Genetics Canada

70 BC: biochar

71 BW: body weight

72 DOA: days of age

73 EHC: enterohepatic circulation

74 ELISA: enzyme-linked immunosorbent assay

75 E_1S: estrone-1-sulfate

76 G:F: gain:feed

77 HPLC: high-performance liquid chromatography

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78 PIC: Pig Improvement Company

79 RIA: radioimmunoassay

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80 **INTRODUCTION**

81 Boar taint is an off-odor or flavor that develops in heated pork products from some
82 entire male pigs. A major cause of boar taint is androstenone (5α-androst-16-en-3-one) in the
83 fat, which is a testicular steroid pheromone produced by sexually mature boars (Patterson,
84 1968; Gower, 1972). Consequently, surgical castration remains the most effective method for
85 preventing boar taint, although it raises animal welfare concerns and negatively impacts growth
86 performance (EFSA, 2004).

87 The development of effective alternatives to castration has been challenging, as boar
88 taint is a complex issue influenced by several physiological and metabolic processes that
89 regulate circulating androstenone levels (Squires et al., 2020). Of particular interest is the
90 suspected recycling of androstenone via enterohepatic circulation (EHC), a process in which
91 compounds are first metabolized by the liver and released into the intestinal tract through
92 biliary excretion (Hofmann, 2009). In the small intestine, gut microbes can convert the
93 metabolites back into their active forms, which are reabsorbed into the hepatic portal vein and
94 transported back to the liver (Roberts et al., 2002; Okour and Brundage, 2017). Compounds
95 that are not recycled to the liver may instead enter the systemic circulation or pass through the
96 digestive tract for excretion (Roberts et al., 2002; Hofmann, 2009).

97 Although the EHC of androstenone has not been directly demonstrated, binding agents
98 intended to disrupt this process and promote excretion have shown potential as dietary
99 treatments for boar taint. Previous studies have evaluated activated charcoal (Jen and Squires,
100 2011), mineral-based adsorbents (Park and Squires, 2025), and biochar (BC) (Parent et al.,
101 2025), with BC showing the most promise as an effective, cost-efficient feed additive. However,
102 in one study, less than 50% of the boars fed a BC-supplemented diet had the capacity to develop

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103 boar taint, and of these animals, approximately 10% did not respond to treatment (Parent et al.,
104 2025). Therefore, the success of dietary BC as a treatment for boar taint depends on identifying
105 animals that both require intervention and are likely to respond positively.

106 The incidence of boar taint differs between animals with high and low capacities for
107 steroidogenesis (Zamaratskaia and Squires, 2009; Squires and Bone, 2025). Steroidogenic
108 capacity may also influence BC treatment response, as testicular steroids have been shown to
109 alter key components of EHC in pigs, including the hepatic expression of metabolic enzymes
110 and gut microbiome composition (He et al., 2019; Kojima, 2020; Wang et al., 2020; Bone and
111 Squires, 2024). In sexually mature boars, differences in steroidogenic capacity are reflected in
112 plasma concentrations of estrone-1-sulfate (E_1S), which is one of the most abundant steroids
113 produced by boars and a proxy for overall steroid hormone levels (Schwarzenberger et al.,
114 1993; Zamaratskaia et al., 2005b).

115 Therefore, this study examined the efficacy of BC treatment across two different
116 genetic lines of boars to evaluate its broad applicability as a dietary intervention for boar taint.
117 Additionally, treatment responses were compared between animals with high and low plasma
118 E_1S levels to determine whether steroidogenic capacity influences responsiveness to BC
119 treatment. We hypothesized that dietary BC treatment would control boar taint similarly across
120 both genetic lines but differ in efficacy between boars with high or low steroidogenic capacity.

121 **MATERIALS AND METHODS**

122 ***Animals and Housing***

123 The experimental procedures were approved by the University of Guelph Animal Care
124 Committee and followed Canadian Council on Animal Care guidelines (CCAC, 2009; Animal

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125 utilization protocol #4600). A total of 69 commercial cross [(Yorkshire × Landrace) × Duroc]
126 boars were used from two genetic lines: 52 from Pig Improvement Company (PIC) and 17 from
127 Alliance Genetics Canada (AGC). Boars from PIC were housed four per pen, and those from
128 AGC in groups of two to three (13.75 ft^2 per pig in both cases), with ad libitum access to feed
129 and water.

130 ***Feeding Trial Design and Sampling***

131 The feeding trial was conducted in two blocks per genetic line (PIC: n = 27 and n = 25;
132 AGC: n = 9 and n = 8), spaced two months apart. The experimental timeline consisted of a 7-
133 day acclimation period (day −7 to 0), a 4-week treatment period (day 0 to 28), and a 2-week
134 recovery period (day 28 to 42), as previously described by Jen and Squires (2011) and Parent
135 et al. (2025). At the start of the acclimation period, boars averaged 67.27 ± 1.02 kg body weight
136 (BW) and 103.45 ± 0.63 days of age (DOA) and were fed a standard finisher diet (Floradale
137 Feed Mill Limited, Floradale, ON, Canada). Boars were transitioned to a 5% BC diet
138 (purchased from Blue Rock Animal Nutrition, Innisfail, AB, Canada, and formulated as
139 described by Parent et al., 2025) during the treatment period, then returned to a standard finisher
140 diet in the recovery period, allowing each animal to serve as its own control by assessing its
141 ability to develop boar taint in the absence of treatment. Properties of the BC treatment were
142 as follows: 78.5% carbon, 44.5% moisture (wet weight), surface area 443 m^2/g. One block of
143 AGC boars (n = 8) remained on the acclimation and treatment diets for an additional four days
144 each.

145 Blood sampling began on the first day of the acclimation period (day –7) and continued
146 weekly until the end of the trial (day 42). The blood was collected into EDTA-coated tubes

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147 (Fisher Scientific, Toronto, ON, Canada) and centrifuged at 1789× *g* for 15 minutes at 4℃ to
148 separate plasma. Backfat biopsies were performed every two weeks using a biopsy device
149 (Baes et al., 2013), starting on the first day of the treatment period (day 0) and ending on the
150 final day of the recovery period (day 42). All plasma and backfat samples were stored at −20℃
151 until analysis. Additionally, individual pig BW and per pen feed intake were recorded weekly
152 throughout the trial (day –7 to 42) and used to calculate average daily gain (ADG) and gain:feed
153 (G:F) during the acclimation period, treatment period, recovery period, and over the entire trial.

154 ***Hormone Assays and Animal Classifications***

155 Concentrations of androstenone in plasma and backfat samples were quantified using
156 an androstenone-specific enzyme-linked immunosorbent assay (ELISA; cross-reactivity of
157 0.3% for 5α-androsten-17β-ol-3-one, 0.2% for 5α-androstan-3-ol-17-one, and 2.6% for 4-
158 androsten-3,17-dione), originally described by Claus et al. (1988), modified by Squires and
159 Lundström (1997). Briefly, backfat biopsies were melted, and androstenone was extracted from
160 the liquid fat using methanol. The extract was then diluted in PBS for analysis, while plasma
161 samples were analyzed directly by ELISA. Plasma E_1S concentrations were quantified by
162 radioimmunoassay (RIA), as previously described by Raeside et al. (1988). For both ELISA
163 and RIA, intra-assay variation was less than 15%.

164 Fat androstenone concentrations were used to assess the boar taint status of each animal
165 and the response to dietary BC treatment, as described by Parent et al. (2025). Animals were
166 determined to have boar taint when fat androstenone concentrations exceeded the established
167 threshold of 1 μg/g (Bonneau et al., 1992). Those that developed boar taint only during the
168 recovery period (day 28 to 42), after BC treatment was removed, were classified as responding

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169 to treatment. In contrast, animals that developed boar taint while still receiving dietary BC (day
170 0 to 28) were classified as not responding to treatment. Animals whose fat androstenone
171 concentrations remained below the 1 μg/g threshold throughout both the treatment and
172 recovery periods (day 0 to 42) were classified as not developing boar taint.

173 Steroidogenic capacity was determined from plasma E_1S concentrations measured at
174 approximately 130 kg live weight, which is considered a typical slaughter weight in Canadian
175 commercial swine production. This measurement occurred either on the final day of the
176 recovery period (day 42) at an average BW of 129.60 ± 1.25 kg (standard trial timeline), or on
177 the final day of the treatment period (day 36) at an average BW of 139.34 ± 2.05 kg (only AGC
178 pigs from one block with the extended trial timeline). Boars were classified as having high or
179 low steroidogenic capacity based on a plasma E_1S threshold of 16 ng/mL, which was identified
180 from the distribution of E_1S concentrations across PIC and AGC boars and is consistent with
181 previously reported threshold values (Zamaratskaia et al., 2005a; Bone and Squires, 2021).
182 Animals with plasma E_1S concentrations greater than 16 ng/mL were classified as high
183 steroidogenic capacity boars, while those with E_1S concentrations less than or equal to 16
184 ng/mL were classified as low steroidogenic capacity boars.

185 ***Statistical Analysis***

186 All statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, USA),
187 with a significance level of $P < 0.05$. Data were first assessed for normality using the Shapiro-
188 Wilk test. Differences in hormone concentrations (fat androstenone, plasma androstenone, and
189 plasma E_1S) between boars from PIC and AGC genetic lines were evaluated using a two-way
190 repeated measures ANOVA with a Tukey-Kramer post hoc adjustment, according to the
191 statistical model shown in Equation [1]:

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192 $$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + C_k + e_{ijk} \qquad [1]$$

193 Where Y_{ijk} was the hormone concentration; μ was the overall mean; A_i was the fixed
194 effect of genetic line; B_j was the fixed effect of time; AB_{ij} was the interaction between genetic
195 line and time; C_k was the random effect of block; and e_{ijk} was the experimental error. Similar
196 models were used to assess differences in hormone concentrations between boars that
197 responded to BC treatment, did not respond, or did not develop boar taint, as well as differences
198 in hormone concentrations and BW between animals with high and low steroidogenic
199 capacities. In these models, Y_{ijk} represented either hormone concentration or BW and A_i
200 represented the fixed effect of treatment response or steroidogenic capacity classification.

201 A two-way ANOVA with a Tukey-Kramer post hoc adjustment was also used to
202 compare growth performance parameters (ADG, G:F, BW, and DOA) between PIC and AGC
203 boars. These comparisons were made during the acclimation period, treatment period, recovery
204 period, and over the entire trial, using the statistical model shown in Equation [2]:

205 $$Y_{ij} = \mu + A_i + B_j + e_{ij} \qquad [2]$$

206 Where Y_{ij} was the growth performance parameter; μ was the overall mean; A_i was the
207 fixed effect of genetic line; B_j was the random effect of block; and e_{ij} was the experimental
208 error.

209 **RESULTS**

210 ***Effect of BC Treatment on Fat Androstenone Concentrations***

211 To evaluate the broad applicability of BC as a dietary treatment for boar taint, fat
212 androstenone concentrations were assessed in PIC and AGC boars across both treatment (day

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213 0 to 28) and recovery (day 28 to 42) periods. Fat androstenone concentrations in backfat biopsy
214 samples collected on the final day of the recovery period (day 42) were used to first determine
215 whether individual boars had the capacity to develop boar taint in the absence of dietary BC
216 treatment. Only 35% (15 PIC and 9 AGC) of animals had developed boar taint at this time,
217 while 71% of PIC and 47% of AGC boars did not develop boar taint. Androstenone levels in
218 fat were similar between PIC and AGC boars at all sampling time points. Therefore, boars from
219 both genetic lines were grouped by BC treatment response to more accurately assess the effect
220 of dietary BC on fat androstenone concentrations.

221 Figure 1 shows fat androstenone concentrations over time (days 0, 14, 28, and 42) in
222 boars that either responded to BC treatment, did not respond, or never developed boar taint,
223 relative to the established boar taint threshold of 1 μg/g. Among the 24 animals that developed
224 boar taint, 75% (10 PIC and 8 AGC) responded to BC treatment, leaving 5 PIC boars and 1
225 AGC boar that did not respond. During the treatment period (day 0 to 28), fat androstenone
226 concentrations remained similar between boars that responded to dietary BC treatment and
227 those that never developed boar taint. In contrast, fat androstenone concentrations in boars that
228 did not respond to treatment increased from day 14 to 28 ($P = 0.0040$) and were greater on day
229 28 than those in boars that responded to treatment ($P < 0.0001$) and those that never developed
230 boar taint ($P < 0.0001$). However, by the end of the recovery period (day 42), fat androstenone
231 concentrations were similar between boars that responded to BC treatment and those that did
232 not respond, and greater ($P < 0.0001$) than levels in boars that never developed boar taint.

233 ***Effect of BC Treatment on Plasma Androstenone Concentrations***

234 We next assessed the effect of dietary BC treatment on circulating androstenone levels
235 by comparing plasma androstenone concentrations between PIC and AGC boars at all blood

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236 sampling time points (days –7, 0, 7, 14, 21, 28, 35, and 42). Consistent with fat androstenone
237 concentrations, plasma levels did not differ between PIC and AGC boars at any time point, and
238 boars from both genetic lines were again combined and evaluated by treatment response
239 classification (Figure 2). There were no differences in plasma androstenone concentrations
240 over the acclimation and treatment periods in boars that responded to BC treatment; however,
241 levels increased between day 28 and day 42 (P = 0.0001). Additionally, plasma androstenone
242 concentrations were similar between boars that responded to BC treatment, those that did not
243 respond, and those that never developed boar taint from day –7 to day 35. By day 42,
244 concentrations were higher in boars that responded to BC treatment compared to those that
245 never developed boar taint (P = 0.003).

246 ***Growth Performance of PIC and AGC Boars***

247 To assess the effect of dietary BC treatment on growth performance, we evaluated
248 performance parameters in PIC and AGC boars over the entire trial and across several key time
249 points (Table 1). No differences in ADG were observed between genetic lines during the
250 treatment period or across the entire trial. However, ADG during the recovery period was
251 greater in PIC than in AGC boars (P = 0.001). Additionally, G:F over the entire trial and during
252 the recovery period was similar between both genetic lines, but lower in PIC compared to AGC
253 boars (P = 0.001) during the treatment period. Finally, there were no differences in BW or
254 DOA between the two genetic lines at any of the time points assessed.

255 ***Plasma E_1S Concentrations and Steroidogenic Capacity Classifications***

256 Plasma E_1S concentrations were next evaluated as an indicator of steroidogenic
257 capacity. There were no differences in plasma E_1S levels between PIC and AGC boars at any

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258 sampling time point. Overall, 51% of animals were classified as high steroidogenic capacity
259 boars (26 PIC and 9 AGC) and 49% as low steroidogenic capacity (26 PIC and 8 AGC). Plasma
260 E_1S concentrations diverged between the steroidogenic capacity groups (Figure 3a), with
261 greater levels observed in boars with high steroidogenic capacity compared to those with low
262 steroidogenic capacity at all time points. In high steroidogenic capacity boars, E_1S
263 concentrations increased between day 28 and day 35 (P = 0.028), and again between day 35
264 and day 42 (P = 0.0002), but remained relatively consistent in low steroidogenic capacity boars
265 throughout the trial. Despite these differences in plasma E_1S concentrations between the
266 steroidogenic capacity groups, BW increased consistently over time in both high and low
267 steroidogenic capacity boars and remained comparable between the groups at all sampling time
268 points (Figure 3b).

269 ***Steroidogenic Capacity and BC Treatment Response***

270 To determine whether steroidogenic capacity influences responsiveness to dietary BC,
271 boars from PIC and AGC genetic lines were grouped based on both steroidogenic capacity and
272 response to dietary BC treatment. The percentage of boars with high or low steroidogenic
273 capacity that were classified as responding to BC treatment, not responding, or not developing
274 boar taint (Figure 4) was numerically similar between the two genetic lines. The majority of
275 animals that did not develop boar taint were low steroidogenic capacity boars (59% PIC and
276 62.5% AGC). Additionally, 60% of PIC and 62.5% of AGC animals that responded to BC
277 treatment were high steroidogenic capacity boars, while the remaining 40% of PIC and 37.5%
278 of AGC boars were low steroidogenic capacity. For both genetic lines, only high steroidogenic
279 capacity boars did not respond to dietary BC treatment. Therefore, all low steroidogenic
280 capacity animals either did not develop boar taint or responded to treatment.

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281 **DISCUSSION**

282 Boar taint is a multifactorial issue influenced by numerous genetic, nutritional,
283 environmental, and physiological factors (Squires et al., 2020). As a result, not all boars will
284 develop boar taint or respond to treatments in the same way, and accounting for these
285 differences is key to accurately evaluating strategies for controlling boar taint without
286 castration. Recently, we assessed BC as a possible dietary treatment for boar taint (Parent et
287 al., 2025), as it has a high pore-to-surface area ratio that allows binding to non-polar compounds
288 like androstenone with high affinity (Weber and Quicker, 2018; Schmidt et al., 2019; Gęca et
289 al., 2022). When individual differences in boar taint status and treatment response were
290 considered, BC treatment reduced fat androstenone accumulation in 89% of boars with the
291 capacity to develop boar taint (Parent et al., 2025). In the present study, we applied the same
292 approach to compare the effects of dietary BC treatment between two genetic lines of boars to
293 assess broader applicability of BC as an intervention for boar taint.

294 Although fat androstenone concentrations were similar between PIC and AGC boars at
295 all sampling time points, the incidence of boar taint and the percentage of boars that responded
296 or did not respond to BC treatment differed between the genetic lines. Specifically, 71% of PIC
297 and 47% of AGC boars never developed boar taint, and among those that did, 67% of PIC and
298 89% of AGC boars responded to BC treatment. These differences reflect the well-established
299 variability in boar taint incidence that exists both between and within breeds and genetic lines
300 (Hortos et al., 2000; Zamaratskaia and Squires, 2009). However, due to the limited sample size
301 for each genetic line in this study, these percentages may not accurately represent the true boar
302 taint incidence or BC treatment response in each population.

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303 Despite differences in boar taint incidence and treatment response between genetic lines,
304 dietary BC effectively maintained fat androstenone concentrations below the 1 µg/g boar taint
305 threshold in 75% of all boars with the capacity to develop boar taint. During the treatment
306 period (day 0 to 28), fat androstenone concentrations were similar between boars that
307 responded to BC treatment and those that never developed boar taint. However, fat
308 androstenone concentrations in boars that responded to treatment increased during the recovery
309 period (day 28 to 42) once BC treatment was removed, and were comparable to levels in boars
310 that did not respond to treatment by day 42. This pattern was consistent across both genetic
311 lines. Additionally, growth performance parameters, including ADG, G:F, BW, and DOA were
312 generally similar between PIC and AGC boars; however, PIC boars had greater ADG during
313 the recovery period and lower G:F during the treatment period compared to AGC boars. These
314 differences likely stem from genetic variation between lines rather than from the effects of BC
315 treatment, as we previously demonstrated that boars fed a 5% BC diet had similar growth
316 performance parameters to those receiving a control diet (Parent et al., 2025). Collectively, our
317 findings support the broad applicability of dietary BC treatment for boar taint.

318 While the mechanism by which dietary BC treatment reduces fat androstenone
319 concentrations has not been demonstrated, it is suspected to involve disruption of the EHC, a
320 pathway known to regulate estrogen concentrations and proposed to similarly affect circulating
321 androstenone levels (Gorbach and Goldin, 1987; Ruoff and Dziuk, 1994; Jen and Squires,
322 2011). Specifically, BC may bind androstenone in the gut and prevent it from being recycled
323 to the liver or reabsorbed into systemic circulation, instead promoting its excretion in feces.
324 However, we found that dietary BC treatment reduced fat androstenone concentrations without
325 altering plasma androstenone levels, which remained similar across boars that responded to BC,

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326 did not respond, and never developed boar taint, as well as between boars from PIC and AGC
327 genetic lines. These results are consistent with our previous findings (Parent et al., 2025),
328 although reductions in plasma androstenone levels have also been reported following dietary
329 activated charcoal treatment (Jen and Squires, 2011).

330 Similar inconsistencies have been observed in humans and rodents, where disruption of
331 the EHC by activated charcoal or antibiotics was reported to either reduce biliary excretion and
332 lower plasma levels of synthetic estrogen and progestin steroid analogues (Back et al., 1980;
333 Heimer and Englund, 1986; Heikinheimo et al., 1989) or have no significant effect on their
334 circulating concentrations (Orme and Back, 1990; Elomaa et al., 2001). Although the reasons
335 for these inconsistencies are unclear, it has been proposed that individuals with higher rates of
336 hepatic metabolism and more easily disrupted gut microbiota may be more susceptible to
337 reductions in circulating synthetic steroid levels following treatment with activated charcoal or
338 antibiotics (Orme and Back, 1990). The development of boar taint is similarly influenced by
339 multiple physiological systems that differ between individual animals, including the balance
340 between the rate of androstenone synthesis in the testis and metabolism in the liver, as well as
341 gut microbiome composition, although the latter remains poorly characterized (Claus et al.,
342 1988; Robic et al., 2014). For these reasons, plasma androstenone concentrations do not always
343 correlate with levels in fat (Lundström et al., 1978), which may explain why dietary BC
344 treatment reduced fat androstenone concentrations in a subset of boars, despite no
345 corresponding changes in plasma androstenone levels.

346 Steroidogenic capacity, reflected by plasma E_1S levels, is a well-established contributor
347 to individual differences in boar taint incidence, as boars with high steroidogenic capacities
348 have an increased potential for androstenone synthesis and a greater risk of boar taint

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349 development (Bonneau, 2006). However, recent studies have identified associations between
350 plasma E_1S levels and both the hepatic metabolism of boar taint compounds (He et al., 2019;
351 Kojima, 2020; Wang et al., 2020; Bone and Squires, 2024), and the accumulation of
352 androstenone in fat via regeneration from its sulfated metabolite (Bone and Squires, 2021),
353 suggesting that the relationship between steroidogenic capacity and boar taint is more complex
354 than previously understood. Therefore, in the present study, we classified boars as having either
355 high or low steroidogenic capacity based on plasma E_1S levels, to evaluate the influence of
356 steroidogenic capacity on BC treatment response.

357 Although plasma E_1S concentrations were similar between PIC and AGC boars at all
358 time points, boars with high steroidogenic capacity consistently had higher E_1S levels than
359 those with low steroidogenic capacity. Despite these differences, BW remained comparable
360 between high and low steroidogenic capacity boars throughout the trial, suggesting that
361 differences in steroidogenic capacity do not directly influence growth. The minimum steroid
362 hormone levels required to support growth in boars is unknown; however, the lower levels in
363 boars with low steroidogenic capacity appear sufficient. This likely reflects the unique nature
364 of testicular steroidogenesis in boars, which occurs in a low-frequency, pulsatile manner
365 (Baulieu et al., 1967; Tan and Raeside, 1980; Schuler et al., 2018) and produces inactive steroid
366 sulfates like E_1S in higher quantities than the corresponding free, biologically active forms
367 (Claus and Hoffmann, 1980; Schwarzenberger et al., 1993; Zamaratskaia et al., 2004;
368 Hoffmann et al., 2010). While the physiological significance of these steroid sulfates is unclear,
369 enzymatic reactivation has been demonstrated and is believed to maintain circulating bioactive
370 steroid levels between pulses of steroidogenesis (Schuler et al., 2018). Therefore, future

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371 research should characterize the plasma profiles of additional steroid hormones between high
372 and low steroidogenic capacity boars to better define these phenotypes.

373 Interestingly, we demonstrated that steroidogenic capacity influences both boar taint
374 incidence and response to BC treatment. In both genetic lines, approximately 60% of the boars
375 that never developed boar taint were classified as low steroidogenic capacity animals, and
376 approximately 60% of those that developed boar taint and responded to BC treatment were
377 high steroidogenic capacity boars. The most notable finding was that, in both genetic lines, low
378 steroidogenic capacity boars that developed boar taint consistently responded to dietary BC
379 treatment, suggesting that steroidogenic capacity may help to predict BC treatment
380 responsiveness.

381 It is possible that high and low steroidogenic capacity boars exhibit distinct
382 physiological and metabolic profiles, as testicular steroids have been shown to influence both
383 the expression (Doran et al., 2002; Nicolau-Solano and Doran, 2008) and activity of enzymes
384 responsible for the metabolism of boar taint compounds (Rasmussen et al., 2011; Zamaratskaia
385 et al., 2012; Zamaratskaia and Berger, 2014). Additionally, increased steroid hormone
386 production during puberty was found to induce significant, sex-specific changes in gut
387 microbiome composition (Harada et al., 2016; Korpela et al., 2021). These interactions are
388 bidirectional as gut microbial-derived β-glucuronidases and/or β-galactosidases (Flores et al.,
389 2012; Ervin et al., 2019; Hu et al., 2023), encoded by 60 bacterial genera in humans, can
390 reactivate estrogens from inactive metabolites, influencing estrogen recycling through the EHC
391 and levels in the systemic circulation (Kwa et al., 2016; Bucurica et al., 2023; Simpson et al.,
392 2024). Therefore, additional research comparing the hepatic metabolism and gut microbiome
393 composition of boars with high or low steroidogenic capacity, both with and without boar taint,

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394 may help explain the differences in both boar taint incidence and BC treatment response
395 observed in this study. This may also facilitate the identification of genetic and/or biological
396 markers that can predict both steroidogenic capacity and boar taint status, which are needed to
397 support the targeted delivery of dietary BC treatment to animals most likely to respond.

398 In conclusion, dietary treatment with 5% BC effectively prevented the development of
399 boar taint in animals from two different genetic lines, without any apparent negative impact on
400 growth performance. These results support the broad applicability of dietary BC treatment;
401 however, its success was limited to a subset of boars from each genetic line, since not all
402 animals will develop boar taint or respond to treatment the same way. Steroidogenic capacity
403 appears to influence both boar taint incidence and BC treatment response. In both genetic lines,
404 high steroidogenic capacity boars were more likely to develop boar taint, and boars with low
405 steroidogenic capacity that developed boar taint always responded to BC treatment. Future
406 studies with larger sample sizes, additional genetic lines, and diverse management systems are
407 needed to investigate the mechanism of action for BC and to determine the minimum effective
408 dose and feeding duration required to reliably reduce androstenone levels in the fat.
409 Additionally, research characterizing the hepatic metabolism and gut microbiome of boars with
410 divergent steroidogenic capacities and boar taint status may help clarify the physiological basis
411 for individual differences in boar taint incidence and BC treatment response, and support the
412 identification of predictive markers for treatment success. This is needed to effectively tailor
413 dietary BC to animals capable of responding favorably, which could support its use as a
414 targeted treatment strategy for boar taint.

415 **DISCLOSURES**

416 The authors declare no conflicts of interest.

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421

422 **LITERATURE CITED**

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Running Title: Biochar Treatment for Fat Androstenone

602 **Figure 1.** Fat androstenone concentrations (µg/g) in boars that responded to BC treatment, did
603 not respond, and did not develop boar taint, shown relative to the boar taint threshold of 1 µg/g.
604 Data are presented as means ± standard error and represent PIC and AGC boars combined.
605 Different letters denote significant differences ($P < 0.05$) between sample time points within a
606 treatment response group. Different numbers denote significant differences ($P < 0.0001$)
607 between treatment response groups at a given time point.

608

609 **Figure 2.** Plasma androstenone concentrations (ng/mL) in boars that responded to BC
610 treatment, did not respond, and did not develop boar taint. Data are presented as means ±
611 standard error and represent PIC and AGC boars combined. Different letters denote significant
612 differences ($P < 0.05$) between sample time points within a treatment response group. Different
613 numbers denote significant differences ($P < 0.01$) between treatment response groups at a
614 given time point.

615

616 **Figure 3.** (A) Plasma E_1S concentrations (ng/mL) and (B) body weight (kg) in high and low
617 steroidogenic capacity boars. Data are presented as means ± standard error and represent PIC
618 and AGC boars combined. Differences between high and low steroidogenic capacity boars at
619 a given time point are indicated by * ($P < 0.05$). Different letters denote significant
620 differences ($P < 0.05$) between time points within a steroidogenic capacity group.

621

622 **Figure 4.** The percentage of high and low steroidogenic capacity boars from PIC and AGC
623 genetic lines that responded to BC treatment, did not respond to treatment, or did not develop
624 boar taint.

625

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626 **Table 1**. Comparison of average daily gain (ADG), gain:feed (G:F), body weight (BW), and
627 days of age (DOA) between boars from Alliance Genetics Canada (*n* = 17) and Pig
628 Improvement Company (*n* = 52) genetic lines that received dietary biochar treatment.

Performance Parameters	**Alliance Genetics Canada**	**Pig Improvement Company**	***P*-value**
ADG over trial, kg/day	1.39 ± 0.035	1.34 ± 0.017	0.50
ADG during treatment, kg/day	1.31 ± 0.050	1.24 ± 0.020	0.57
ADG during recovery, kg/day	1.40 ± 0.044	1.57 ± 0.035	0.001
G:F over trial, kg feed/kg gain	2.65 ± 0.088	2.75 ± 0.018	0.26
G:F during treatment, kg feed/kg gain	2.74 ± 0.11	2.39 ± 0.028	0.001
G:F during recovery, kg feed/kg gain	2.92 ± 0.086	2.82 ± 0.050	0.70
BW at start of acclimation, kg	71.71 ± 2.09	65.82 ± 1.10	0.30
BW at start of treatment, kg	82.71 ± 3.07	73.20 ± 1.19	0.34
BW at end of treatment, kg	125.74 ± 4.14	107.94 ± 1.43	0.34
BW at end of recovery, kg	144.58 ± 4.72	129.91 ± 1.48	0.33
DOA at start of acclimation, day	103.65 ± 1.50	103.38 ± 0.69	0.92
DOA at start of treatment, day	112.53 ± 2.00	110.38 ± 0.69	0.76
DOA at end of treatment, day	143.35 ± 2.75	138.38 ± 0.69	0.63
DOA at end of recovery, day	157.35 ± 2.75	152.38 ± 0.69	0.63

629 Values are reported as mean ± standard error. ADG, BW, and DOA are calculated per animal,
630 and G:F is calculated per pen.

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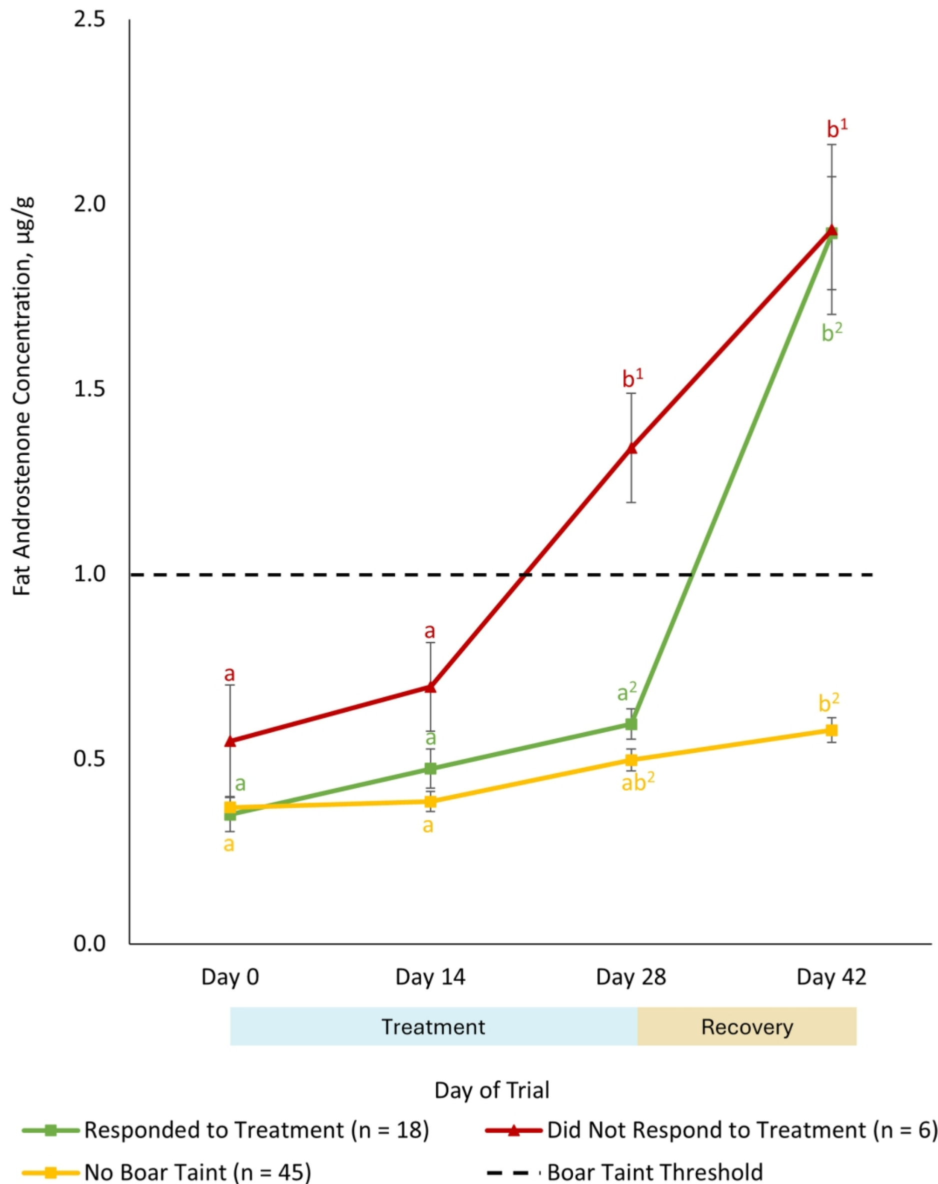

Figure 1. Fat androstenone concentrations (µg/g) in boars that responded to BC treatment, did not respond, and did not develop boar taint, shown relative to the boar taint threshold of 1 µg/g. Data are presented as means ± standard error and represent PIC and AGC boars combined. Different letters denote significant differences ($P < 0.05$) between sample time points within a treatment response group. Different numbers denote significant differences ($P < 0.0001$) between treatment response groups at a given time point.

160x197mm (300 x 300 DPI)

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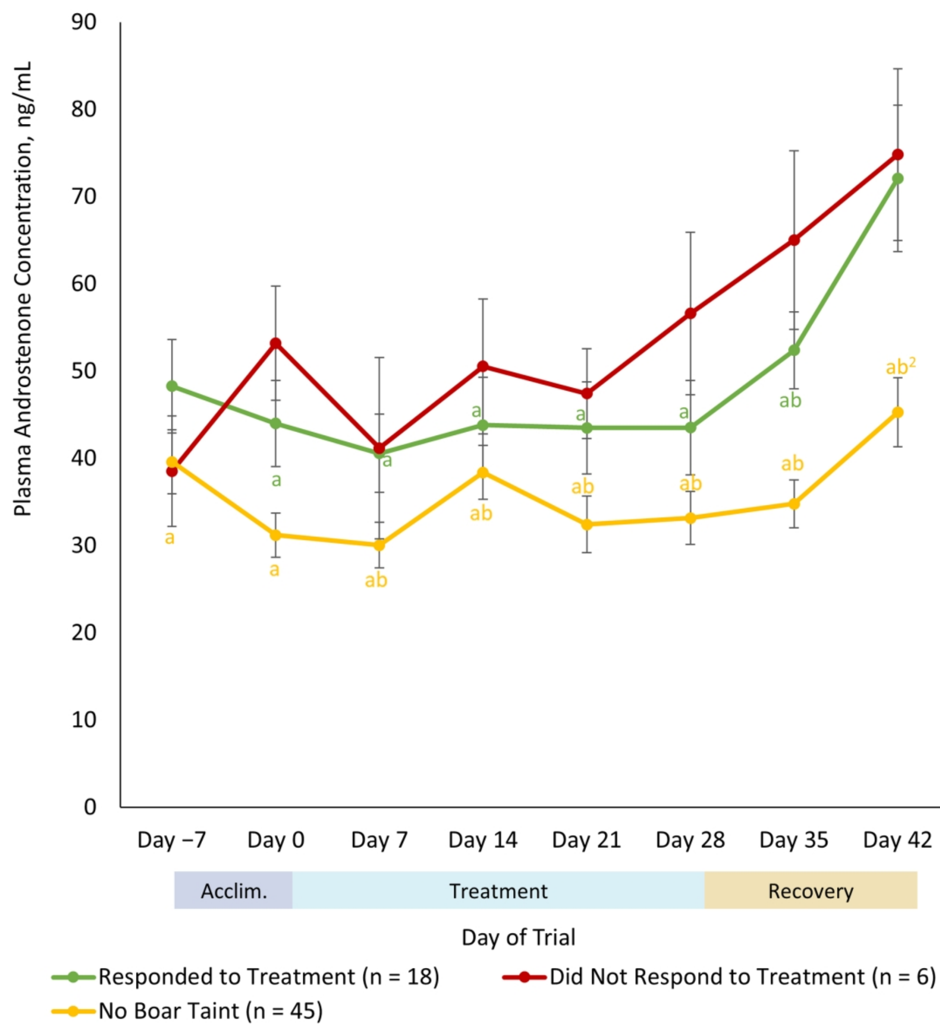

Figure 2. Plasma androstenone concentrations (ng/mL) in boars that responded to BC treatment, did not respond, and did not develop boar taint. Data are presented as means ± standard error and represent PIC and AGC boars combined. Different letters denote significant differences ($P < 0.05$) between sample time points within a treatment response group. Different numbers denote significant differences ($P < 0.01$) between treatment response groups at a given time point.

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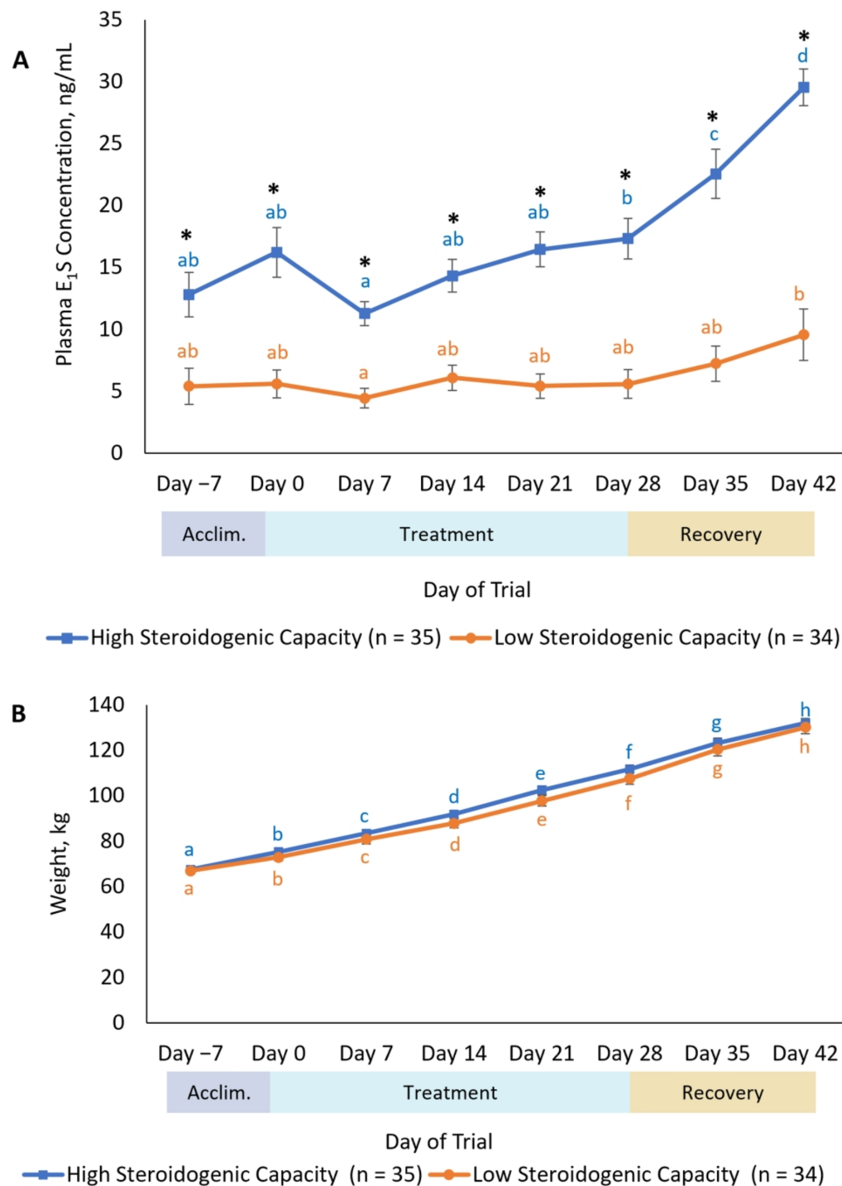

Figure 3. (A) Plasma E_1S concentrations (ng/mL) and (B) body weight (kg) in high and low steroidogenic capacity boars. Data are presented as means ± standard error and represent PIC and AGC boars combined. Differences between high and low steroidogenic capacity boars at a given time point are indicated by * ($P < 0.05$). Different letters denote significant differences ($P < 0.05$) between time points within a steroidogenic capacity group.

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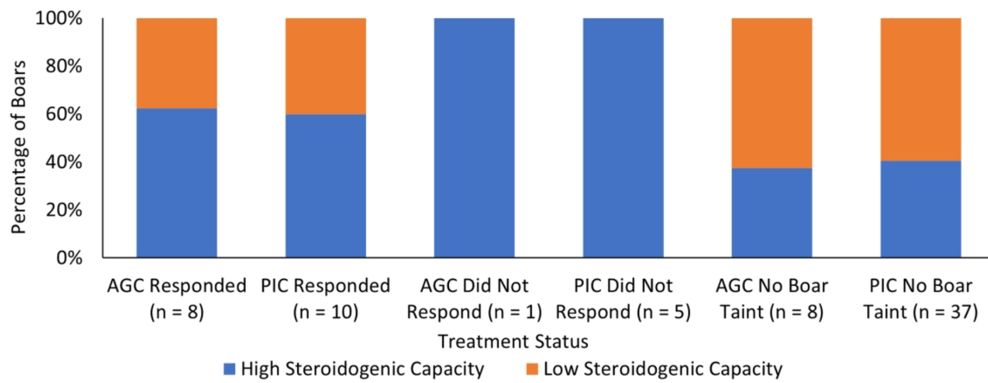

Figure 4. The percentage of high and low steroidogenic capacity boars from PIC and AGC genetic lines that responded to BC treatment, did not respond to treatment, or did not develop boar taint.

180x71mm (300 x 300 DPI)

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