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1 Feeding behaviour and pre-prandial status affect post-prandial plasma energy metabolites and
2 insulin kinetics in growing pigs fed diets differing in fibre concentration

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14

15 **Running head:** Regulation of voluntary feed intake in pigs

16

17 **Keywords:** feeding behaviour: pig: dietary fibre: wheat aleurone: metabolism

18

19 **Abbreviations:** ADFI: average daily feed intake; BW: body weight; CF: crude fibre; CP:
20 crude protein DE: digestible energy; DF: dietary fibre; DM: dry matter; EE: ether extract; HF:
21 high fibre; LF: low fibre; NDF: neutral detergent fibre; NE: net energy; ST: starch.

22

23 Preliminary results of this work have been published in Quemeneur K, Labussiere E, Le Gall
24 M *et al.* (2018) Joint dynamics of voluntary feed intake, glycaemia and insulinemia in *ad*
25 *libitum* fed pigs. Book of Abstracts of the 69th Annual Meeting of the European Federation of
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35 Abstract

36 Variations in feeding behaviour between animals result from individual variations in their
37 metabolism as affected by diet composition. The study aimed to link the within-day dynamics
38 of voluntary feed intake and those of blood metabolites and insulin in growing pigs having *ad*
39 *libitum* access to feed and receiving diets differing in dietary fibre levels and aleurone
40 supplementation. Forty pigs (body weight: 35 kg) had access to diets provided ad libitum and
41 differing by fibre content (13 or 18% NDF) and aleurone supplementation (0, 2 or 4 g/kg).
42 Feeding behaviour was individually recorded during one week. The kinetic of plasma
43 metabolites and insulin was followed during 1 hour after a voluntary test meal. Dietary fibre
44 level did not affect daily feed intake but increased meal size and meal duration. Aleurone
45 supplementation (4 g/kg) decreased daily feed intake and number of meals. Dietary fibre level
46 only decreased insulin concentration measured 15 minutes after meal beginning. Aleurone
47 supplementation (4 g/kg) decreased glycaemia during the first hour after the meal and
48 insulinemia 15 minutes after the meal. Free access to feed led to high variability in pre-
49 prandial metabolites and insulin concentrations resulting in different test meal size
50 irrespective of diet composition. Animals were then spread over different profiles combining
51 feeding behaviour and fasted status to explain different profiles of regulation of feed intake.
52 Plasma metabolites and insulin kinetics were affected by diet composition but also by animal
53 characteristics. Individual variability should be considered when diet composition is used to
54 modulate feeding behaviour.

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56 Introduction

57 Dietary fibre (DF) modulates daily voluntary feed intake in association with changes in
58 feeding behaviour: number of meals, meal volume, meal duration and within day spreading of
59 meals ⁽¹⁾. In monogastric animals as pigs and humans, these effects may differ according to
60 the origin and composition of DF and body weight (BW) of the subject. As an example,
61 dietary inclusion of sugar beet pulp (Neutral Detergent Fibre (NDF) content =27%) reduced
62 average daily feed intake (ADFI), meal volume and feeding rate whereas inclusion of wheat
63 bran (NDF=23%) did not affect ADFI in pigs of 14 kg BW ⁽²⁾. The impact of DF on satiety
64 can be explained either by 1.) dietary energy dilution, 2.) a direct effect due to the increase of
65 digestive content volume with activation of mechanoreceptors, 3.) an indirect effect via
66 metabolic regulation pathways, or 4.) fermentations with a production of short chain fatty
67 acids, as reviewed by Bach Knudsen ⁽³⁾ and Li and Patience ⁽⁴⁾. The variety of animal response
68 to the dietary inclusion of DF suggests that various hormonal, metabolic, physical and
69 microbial pathways are involved in the regulation of feed intake. Testing DF fractions is
70 needed to better distinguish the overall effect of DF on feed intake from the specific and
71 functional properties of some DF fractions ⁽³⁾. Wheat aleurone layer, a fraction of wheat bran
72 containing fibres (arabinoxylans and β -glucans) and phenolic compounds, ferulic acids and
73 lignans ⁽⁵⁾, is a suitable candidate as DF-source that can affect glucose and insulin
74 postprandial peaks as demonstrated in horses ⁽⁶⁾.

75 To our knowledge, few studies co-investigated the effect of DF level and the
76 supplementation of a particular fraction of DF on ADFI, feeding behaviour, plasma
77 metabolite and hormone kinetics. Therefore, this current study aimed to link the within-day
78 dynamics of voluntary feed intake, plasma metabolites, and insulin in growing pigs that had
79 *ad libitum* access to feed and received diets differing in DF level and wheat aleurone
80 inclusion. We hypothesized that DF level in the diet and dietary aleurone supplementation
81 modifies feeding behaviour in relation with changes in metabolites and insulin kinetics after a
82 meal, considering that feeding behaviour is closely linked with metabolite and insulin
83 variations ⁽⁷⁾.

84 The implication of this study is the modulation of feeding behaviour in pigs. Limiting
85 voluntary feed intake by dietary composition rather than quantitative feed restriction may
86 benefit animal welfare and facilitate increased feed efficiency ⁽⁸⁾. Due to similitudes in
87 digestion and metabolism between pigs and humans, results obtained in this study also
88 furnished hypotheses regarding human food intake regulation.

89 **Methods**

90 The experiment was performed at the INRA experimental facilities in Saint-Gilles
91 (France) (agreement number: C35-275-32) in compliance with the French directive on animal
92 experimentation and care (2013-118) after evaluation by a competent ethical committee in
93 animal experimentation under the responsibility of the French ministry of research
94 (authorization number 2016072907418869).

95 **Animals, housing and experimental design**

96 The experiment was conducted with forty castrated males ((Pietrain × (Large White ×
97 Landrace)) with an average initial BW of 25.6 ± 3.0 kg (70 days of age). To avoid pain and
98 perturbations during time-series blood sampling, a catheter was inserted in the external
99 jugular vein through a collateral vein according to a protocol previously described by
100 Melchior et al. ⁽⁹⁾ under general anaesthesia (ketamine and isoflurane). After the surgery, pigs
101 had two weeks of recovery and adaptation to experimental diets and to individual housing.
102 Then, pigs were weighed and individually housed in a metabolic cage (60 × 120 cm) with
103 metal floor placed in an enclosure (1.7 m³) and equipped with a weighing scale in order to
104 continuously record feed consumption during seven days. The study was conducted during
105 thirteen batches of three or four littermates each. For each batch, the three or four littermates
106 with a similar BW were selected and successively housed during one week in the enclosure as
107 only one was available. The temperature in the enclosure was maintained constant at 24°C
108 and a 12h lighting time span (0730 to 1930 hours) was used. Each pig had free access to water
109 and one of the six experimental diets. During all the experiment, animals were carefully
110 observed to detect potential injuries of welfare and disease.

111 **Experimental diets**

112 Two concentrations of DF (low fibre: LF (NDF=10%) and high fibre: HF (NDF=15%))
113 and three levels of aleurone supplementation (GrainWise Wheat Aleurone; Horizon Milling;
114 Minneapolis, Minnesota, United States of America; 0 g/kg: A0, 2 g/kg: A2 and 4 g/kg: A4)
115 were tested in six experimental diets (table 1). The HF diet contained a mixture composed of
116 50% of wheat bran, 33% of soya hulls and 17% of sugar beet pulp. This mixture of three DF-
117 rich feedstuffs having different physicochemical properties permitted to study an overall
118 effect of DF level rather than the effect of DF from a specific source ⁽³⁾. Experimental diets
119 were balanced on the ratio of digestible lysine to net energy (NE). Energy content was lower
120 in the HF diets due to the introduction of DF (-6% of NE per kg of dry matter (DM) for HF
121 diet ⁽¹⁰⁾).

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122 Pigs received 500 g of a commercial growing feed (NDF=14%) on the first day after the
123 surgery, and then 1000 g per day during four days with a transition from the commercial diet
124 they fed before surgery to one of the six experimental diets (80%/20%, 60%/40%, 40%/60%,
125 20%/80%). One level of DF with its three levels of aleurone supplementation was tested
126 within each batch. Pigs had free access to experimental diets between 0900 and 1630 and
127 between 1700 and 0600 that permitted mimicking an *ad libitum* feed intake at the scale of the
128 day. Each day, feed allowance was determined to be greater than voluntary daily feed intake.
129 To avoid feed spillage, feed was provided twice a day at 0845 and at 1645, before trough
130 emptying. The daily feed allowance was weighed and a sample of offered feed was cumulated
131 each day of measurements for each pig for immediate DM measurement. For each
132 experimental diet, samples were pooled over the whole experiment for chemical analyses.
133 Feed refusals and spillages were collected at the end of each week of measurements and were
134 oven-dried to determine the refused DM.

135 Feeding behaviour recording

136 After one day of adaptation in the enclosure, feeding and drinking behaviour were
137 analysed during the 6 following days. For feed intake calculations, a day of measurement
138 began at 0900 and finished at 0600. For water intake calculations, a day of measurement
139 began at 0900 and finished at 0800 to take into account water meals when the feed trough was
140 locked. The feed trough and the water container were on a weighing scale for recording
141 feeding and drinking behaviour. The water container was filled each morning with tap water.
142 The weight of the feed trough and the water pit was recorded every 10 seconds. A
143 consumption of feed was defined as a negative variation of the weight of the feed trough. The
144 feed intake size was calculated as the difference between the stabilized weight of the trough
145 before and after the variation. The feed intake duration was defined as the duration between
146 the beginning and the end of the weight variation. In order to compare our results with other
147 studies, successive feed bouts were grouped into the same meal using a meal criterion defined
148 as the maximum length between two feed bouts owing to the same meal ⁽¹¹⁾. Feed bouts
149 separated by intervals shorter than the meal criterion were considered to be part of the same
150 meal and were clustered accordingly. The size of a meal was the sum of the size of the feed
151 bouts and the duration of the meal was the duration between the beginning of the first feed
152 bout and the end of the last feed bout of the meal. In our study, the meal criterion was
153 estimated using the log curves technique ⁽¹²⁾ and was established at four minutes. The DM
154 feed intake per meal was calculated as the feed intake per meal adjusted for the percentage of

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155 DM of the feed measured weekly for each pig. Meals with a size below 10 g were not kept for
156 statistical analysis but spread over other meals of the day. The ADFI, expressed in g of DM
157 per day, was calculated for each pig as the average of dried feed intake from the 6 days
158 considered in the analysis. From the generated database, daily number of meals, average feed
159 intake per meal (g of DM), average duration per meal (min), daily feed intake (g of DM per
160 day) and total ingestion duration per day (min) were calculated as indicators of feeding
161 behaviour. Daily total water intake was calculated as the difference of the water container
162 weight during 23-h periods.

163 **Blood collection**

164 On day 7, blood was sampled to analyse metabolites and insulin plasmatic concentrations.
165 The catheter was externalized outside of the enclosure and permitted to collect blood samples
166 without disturbing the animal. The first meal of day 7 was considered as a test meal. Blood
167 (10 mL) was sampled at 0830 and 0845 (referred as pre-prandial), at the beginning of the
168 meal and 15, 30, 45, 60 minutes after the beginning of the meal. Blood samples (10 mL per
169 sample; 5 mL collected on EDTA and 5 mL collected on heparin) were stored on ice before
170 centrifugation during 10 min at 3000 g (4°C). Plasma was separated into aliquots and stored at
171 -20°C until metabolites and hormone concentration analyses. For each blood sampling, the
172 haematocrit (H) was measured and a basal haematocrit (BH) was calculated as the mean of
173 pre-prandial haematocrits of this pig. Plasma metabolite and insulin concentrations were
174 adjusted as follows to take into account possible dilution because of water intake and saline
175 solution perfusion to flush the catheter line between successive samplings:

$$\text{Adjusted concentration} = \text{Real concentration} \times \frac{(100 - BH)}{(100 - H)}$$

176 **Chemical analyses**

177 The experimental diets were analyzed for DM, ash, gross energy, nitrogen, ether extract,
178 crude fibre, different fibre fractions (NDF, ADF, ADL) and starch according to standard
179 procedures ⁽¹³⁾. Commercial kits were used to determine plasma concentrations of glucose
180 (Glucose RTU; bioMérieux, Craponne, France), lactate (Lactate PAP; bioMérieux, Craponne,
181 France), free fatty acids (NEFA-HR(2); Wako Chemicals GmbH, Neuss, Germany), urea
182 (Urea UV 250; bioMérieux, Craponne, France), α -amino nitrogen (Protéines-kit; bioMérieux,
183 Craponne, France) and triglycerides (Triglycérides Enzymatique PAP 150; bioMérieux,
184 Craponne, France). These parameters were measured with a Konelab 20i device (Thermo

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185 Fisher Scientific, Courtaboeuf, France) according to manufacturers' recommendations.
186 Plasma concentrations of insulin were measured using an RIA kit (INSULIN-CT; CisBio
187 Bioassays, Codolet, France). Intra-assay CV was less than 5%.

188 **Data analyses**

189 The NE concentration of the diets was calculated with the equation
190 $NE=0.703 \times DE+1.58 \times EE+0.47 \times ST-0.97 \times CP-0.98 \times CF$ with DE for Digestible Energy, EE for
191 Ether Extract, ST for Starch, CP for Crude Protein and CF for Crude Fibre ⁽¹⁰⁾

192 Based on feeding behavior, between four to eight animals per treatment are necessary to
193 achieve the statistical power of 90% with an alpha level at 5% to see a difference of 100 g on
194 ADFI and a difference of 2 meals per day.

195 Pigs were individually housed in an enclosure. A batch was constituted by 3 or 4
196 littermates that were successively measured during one week each. One level of DF and three
197 levels of aleurone supplementation were tested during one batch and the other level of DF
198 (with its three levels of aleurone supplementation) was tested during the following batch. For
199 the last replicate, 4 littermates were used, fed with LF-A0, LF-A2, LF-A4 and HF-A0 to
200 adjust number of pigs per treatment.

201 The analysis thereafter was conducted using linear mixed model with the MIXED
202 procedure of SAS (SAS Inst. Inc., Cary, NC) and the assessment of significance of the factors
203 tested in each model was based on "Type 3 tests of fixed effects" as experimental design is
204 not strictly balanced. For variables considered as repeated effect, the REPEATED statement
205 was used in the procedure. An unstructured covariance matrix (type = UN) was used for these
206 models, because it was the covariance structure that displayed the lowest AIC and BIC in
207 comparison with other covariance structure that were tested (compound symmetry, Toeplitz
208 or autoregressive). Data were tested for normality according to Shapiro-Wilk's test of
209 residual.

210 Variables describing feeding behaviour (ADFI, NE intake, number of meals, meal size,
211 meal duration and average interval between two meals) were analysed in a linear mixed
212 model with levels of DF, levels of aleurone and their interaction considered as main factors,
213 batch as a random factor and day of the week as a repeated effect with the pig as the
214 experimental unit.

215 Water intake per day was analysed in a linear mixed model with levels of DF, levels of
216 aleurone and their interaction considered as main factors, feed intake level and number of

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217 meals per day as covariates, batch as a random factor and day of the week as a repeated effect
218 with the pig as the experimental unit.

219 Only data from pigs (n=26) that were fed two voluntary meals between 0900 and 1700 on
220 the day of blood kinetic were considered in the statistical analysis of plasma metabolites and
221 insulin concentrations.

222 Pre-prandial plasmatic concentration of metabolites (glucose, lactate, free fatty acids,
223 triglycerides, urea and α -amino nitrogen) and insulin were analysed in a linear mixed model
224 with levels of DF, levels of aleurone and their interaction considered as main factors, the
225 interval since the last meal and the size of this last meal as covariates, batch as a random
226 factor and time as a repeated effect with the pig as the experimental unit.

227 Plasma metabolites and hormone concentrations were analysed in a linear mixed model
228 with levels of DF, levels of aleurone and their interaction considered as main factors, the size
229 of this test meal (expressed as g/kg BW^{0.60}) and pre-prandial concentration of analysed
230 metabolites as covariates, batch as a random factor and time as a repeated effect with the pig
231 as the experimental unit. When the effect of interaction between time and DF level or
232 aleurone supplementation were significant, post hoc tests were performed through Tukey's
233 pairwise comparison of the means for each time. LS-means were calculated to obtain adjusted
234 means from the statistical model. When data are unbalanced, LS-means give better mean
235 estimates than simple means (LSMEANS statement in SAS).

236 Data were further analysed by multiple factorial analysis and hierarchical classification
237 on principal components to discriminate clusters of pigs using of the R statistical package
238 "FactoMineR" ⁽¹⁴⁾. As blood metabolites and insulin concentrations are affected by feeding
239 behaviour ^(7; 15), size of the test meal and pre-prandial metabolites and insulin concentrations,
240 pigs were discriminated on these different factors of variation to analyse their effects on
241 plasma metabolites and insulin kinetics. Three classes of variables were included in the
242 analysis: pre-meal plasma concentrations of metabolites (glucose, lactate, free fatty acids, α -
243 amino nitrogen, urea and triglycerides) and insulin; variables characterizing feeding behaviour
244 (number of meals, average meal size (g/kg BW^{0.60}), average duration of meal (minutes), total
245 duration of feed intake per day (minutes); and ADFI (g/kg BW^{0.60})) and the size of the test
246 meal (g/kg BW^{0.60}). After cluster identification, data were further tested by linear mixed
247 model as previously described replacing the DF, aleurone supplementation effects and their
248 interaction by a cluster effect.

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249 Data for pre-meal insulin concentration and concentrations of glucose, insulin, urea, free
250 fatty acids and triglycerides during the hour after the test meal were log-transformed before
251 statistical analyses.

252 Differences between treatments were considered significant for $P < 0.05$ whereas
253 $0.05 < P < 0.10$ was discussed as a trend. Data are shown as least-squares means in tables and
254 means in graphics with the residual square deviation (RSD) as an indication of the variability.

255 **Results**

256 Two pigs fed the LF-A0 and HF-A4 diets exhibited fever associated with a low ADFI on
257 the week of measurements. They were excluded from the experiment and subjected to
258 antibiotic treatments (150 mg Amoxicillin per 10 kg for BW). Consequently, 38 pigs were
259 considered in the final analysis. Only the data from 26 pigs that were fed two voluntary meals
260 between 0900 and 1700 on the day of blood kinetic were considered in the statistical analysis
261 of plasma metabolites and insulin concentrations.

262 *Influence of diets on feeding behaviour.*

263 There was no interaction between DF level and aleurone supplementation on any variables
264 characterizing feed intake and feeding behaviour. Pigs had less ($P < 0.01$) but larger (+19%,
265 $P < 0.01$) meals with high level of DF, without effect on ADFI and NE intake (table 2).
266 Whereas average meal duration (+10%, $P < 0.01$) and interval between two meals increased
267 (+14%, $P < 0.01$) with DF level, the total daily feed intake duration was similar. The decrease
268 in ADFI (-20%, when compared with other diets; $P < 0.01$) for aleurone supplementing diet (4
269 g/kg) was associated with a decreased number of meals per day ($P < 0.01$). Pigs spent less time
270 per day to eat and interval between two meals was extended ($P < 0.01$) with diets
271 supplemented with 4 g/kg of aleurone. Neither DF level nor aleurone supplementation
272 modified total water intake, considering feed intake and number of meals as covariates.

273 *Influence of diets on plasmatic metabolites and insulin concentrations.*

274 There was no interaction between DF level and aleurone supplementation on pre-prandial
275 plasmatic concentration of metabolites and insulin. High level of DF decreased pre-prandial
276 insulin concentration ($P = 0.01$) and tended to decrease pre-prandial glucose concentration
277 ($P = 0.06$) when compared with the diets with low level of DF (table 3). The pre-prandial
278 insulin concentration was the greatest with aleurone supplemented diet at 2 g/kg compared to
279 others aleurone supplementations ($P < 0.01$). The pre-prandial α -amino-nitrogen concentration
280 was lower with aleurone supplemented diet at 4 g/kg ($P = 0.04$). Pre-prandial lactate, urea, free
281 fatty acids and triglycerides concentrations were not affected by the dietary factors. An

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282 increase of the interval between the test-meal and the last meal decreased pre-prandial
283 concentration of insulin ($P<0.01$) and increased pre-prandial free fatty acids concentration
284 ($P=0.05$) whereas an increase of the size of the last meal increased pre-prandial concentration
285 of insulin ($P<0.01$) and decreased pre-prandial free fatty acids concentration ($P=0.04$).

286 The size of the voluntary test meal was greater with high level of DF (368 vs 242 g of
287 DM, $P=0.04$) and longer (19.4 min. vs 13.8 min., $P=0.02$). It was not modified with aleurone
288 supplementation (data not shown). Except for urea concentration ($P=0.18$), concentrations of
289 all metabolites and insulin significantly changed during the first hour after the test meal
290 ($P<0.01$, figure 1). During the first hour, there was no interaction between DF level and
291 aleurone supplementation for α -amino-nitrogen, urea, free fatty acids and insulin
292 concentrations and only a tendency for glucose and lactate concentrations ($P=0.08$).
293 Moreover, triglycerides concentration was lower for HF-A4 diet than for any other diets
294 ($P=0.02$; figure 2). Dietary fibre level did not affect any kinetics of metabolites and insulin
295 throughout the first postprandial hour whereas aleurone supplementation decreased glycemia
296 ($P<0.01$; figure 1(b)). Insulin concentration at 15 minutes after the meal was lower for pigs
297 fed high level of DF ($P=0.03$) and was lower for pigs fed aleurone supplemented diet (4 g/kg)
298 than pigs fed diet without aleurone supplementation ($P=0.04$).

299 *Characterization of clusters.*

300 Two principal components accounting for 49% of total variance among pigs were retained
301 from the multiple factorial analyses with 30% for the first component and 19% for the second
302 component. Contributions of each variable to these two major components are presented in
303 table 4. Based on the multiple factorial analyses, the hierarchical classification discriminated
304 pigs according to four clusters. Dietary treatment only partially agreed with cluster
305 classification (table 5). Clusters 1 and 2 grouped the lightest pigs (34.9 and 37.5 kg,
306 respectively) having low ADFI (1209 and 1174 g/d, respectively) and few number of meals
307 per day. Clusters 3 and 4 grouped the heaviest pigs (44.9 and 43.0 kg, respectively) having
308 high ADFI (1580 and 1838 g/d, respectively) and a large number of meals per day.
309 Additionally, pigs grouped in cluster 4 ate a more important voluntary test meal than those
310 grouped in other clusters ($P<0.01$). Pre-prandial plasma concentration of free fatty acids was
311 greater for pigs in clusters 1 and 3 than for pigs in clusters 2 and 4 ($P<0.01$). Pre-prandial α -
312 amino-nitrogen concentration varied from 516 mg/L for pigs from cluster 1 to 762 mg/L for
313 pigs from cluster 4 ($P<0.01$). Mean urea concentration varied from 112 mg/L for cluster 2 to
314 195 mg/L for cluster 1 ($P<0.01$).

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315 During the first hour after the meal, glucose, lactate, triglycerides and insulin kinetics did
316 not differ between clusters whereas free fatty acids, α -amino nitrogen and urea concentrations
317 were affected (figure 3). Free fatty acids concentration was greater for pigs grouped in
318 clusters 1 and 3 than for pigs grouped in clusters 2 and 4 ($P<0.01$; figure 3(d)). Concentration
319 of α -amino nitrogen increased between pigs in cluster 1 and pigs in cluster 4 ($P<0.01$; figure
320 3(e)). Urea concentration was higher for pigs grouped in clusters 1 and 4 than for pigs
321 grouped in clusters 2 and 3 ($P<0.01$; figure 3(f)).

322 Discussion

323 Dietary fibre level or aleurone supplementation modified feeding behaviour and blood and
324 insulin kinetics in pigs having free access to feed during 20.5 hours per day. No interaction
325 between DF level and aleurone supplementation was found. Dietary fibre level did not affect
326 ADFI but increased meal size (+ 28 g) and meal duration (+ 1 min) while reducing the
327 number of meals per day (-1 meal). Aleurone supplementation (4 g/kg) decreased ADFI (-
328 314 g) and the daily number of meals (-1.5 meals per day). These results suggest differences
329 in the importance of each regulation pathways (digestive, metabolic, hormonal and microbial
330 regulations) of voluntary feed intake between the two dietary strategies that were tested to
331 affect feeding behaviour.

332 In pigs fed diets enriched in DF diluting energy concentration (-25% of digestible energy),
333 regulations of voluntary feed intake often lead to increased ADFI (+850 g of DM) to satisfy
334 energy requirements⁽¹⁶⁾. In our study, DF enrichment, diluting the NE concentration by only
335 6% when compared with the control diet, did not reduce ADFI. Similarly, a NE dilution of
336 5% did not modify feed intake in growing pigs between 35 and 50 kg of BW⁽¹⁷⁾.
337 Nevertheless, HF diet modified feeding behaviour, with pigs eating fewer meals per day of
338 greater size than pigs fed the LF diet. The reduction of meal frequency with HF diet was in
339 accordance with previous studies showing that introduction of DF can increase satiety and
340 delay the initiation of the next meal⁽¹⁸⁾. This delay could be due to the production of short
341 chain fatty acids by microbial bacteria, using DF as substrates for fermentation⁽¹⁹⁾. Meal size
342 is partly regulated by activation of mechanoreceptors and the distension of the stomach
343 leading to an early volumetric satiety⁽²⁰⁾. With the HF diet and its bulking properties, meals
344 were expected to end sooner, resulting in smaller meal size due to an earlier stimulation of
345 mechanoreceptors after meal onset. In our study, greater meals suggest that this regulation
346 pathway was not enhanced with HF diet compared with LF diet and a possible adaptation of
347 stomach to counteract the physical volumetric effect of DF⁽²¹⁾. Physical effects of DF on meal

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348 size depend on the introduction rate and nature of fibrous sources, on the BW of the pig, and
349 on the duration of the adaptation period to the DF-rich diet ^(2; 22). In the present study, a
350 mixture of three DF sources (wheat bran, sugar beet pulp and soya bean hulls) with different
351 physical properties was used to test an overall DF effect rather than the consequences of a
352 specific property ⁽²²⁾. In addition, the BW of pigs (37 kg) and the adaptation period (2 weeks)
353 were probably sufficient to satisfactorily adapt the digestive tract of the pigs with an increase
354 of the empty stomach weight (276 g vs 293 g for LF and HF diets respectively, $P<0.01$, data
355 not shown). Moreover, meal size and duration are also regulated by metabolite concentrations
356 as suggested by the glucostatic theory of feed intake regulation ⁽²³⁾. In our study, this
357 metabolic regulation could be more important than bulking effect of high DF level. With DF
358 level, longer (+ 1 min) and greater (+28 g) meals may be explained by a longer duration to
359 reach glucose homeostasis. At the end of the meal (13.8 min vs 19.4 min for pigs fed LF and
360 HF diets respectively), although glycaemia did not significantly differ between HF and LF
361 diets (1112 and 1050 mg/L for pigs fed LF and HF diet respectively, as predicted from the
362 kinetics of plasma metabolites, $P=0.28$), insulinemia was lower for HF diet than LF diet (15.2
363 mUI/L and 8.2 mUI/L, for pigs fed LF and HF diet respectively, $P=0.05$). In the present
364 study, the three fibre sources used in the HF diets are known to delay gastric emptying and to
365 reduce digestibility of starch sources ⁽²⁴⁾. Increased duration of meals may thus permit to pigs
366 to reach glucose homeostasis. The delayed end of the meal may be due to the lower
367 concentration of insulin with DF level, which underlines the importance of some hormones in
368 regulation of feed intake.

369 The decrease in NE intake with aleurone supplementation (4 g/kg) suggested that
370 regulations of ADFI did not result in a constant intake of NE in all cases. The decreased ADFI
371 was explained by a decreased meal number without increase of meal size. This result suggests
372 an extension of satiety which could be partially explained by microbial activity ⁽²⁵⁾. In our
373 study, postprandial free fatty acids concentration tended to be lower with aleurone
374 supplementation during the first 45 min after the meal. Short chain fatty acids decrease free
375 fatty acid concentrations with a possible inhibition of hormone-sensitive lipase ⁽²⁶⁾. As
376 suggested in previous studies in pigs ⁽²⁷⁾ and rats ⁽²⁸⁾, wheat aleurone-rich flour increased short
377 chain fatty acids production in the caecum and colon as the consequence of arabinoxylan
378 fermentation. Short chain fatty acids can be used as energy substrate (up to 14% of pig
379 maintenance requirements ⁽²⁹⁾) and they can modify long term energy homeostasis ⁽³⁰⁾. Short
380 chain fatty acids stimulate satiety hormones like GLP-1 or PYY, thus inhibiting the initiation

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381 of a next meal ^(31; 32). Satiety can also be linked to a period of elimination of metabolic waste
382 resulting from hepatic oxidation and nitrogen metabolism ⁽³³⁾. Regulation of metabolic waste
383 elimination occurs probably more than one hour after the meal. Because urea concentration
384 did not differ between treatments, the present results did not support this hypothesis. The
385 increase of the interval between meals also observed with high DF level might partially result
386 from similar mechanisms due to the presence of aleurone in DF-rich diets that contain wheat
387 and wheat bran.

388 The different pathways permitting the regulation of feed intake (digestive, hormonal,
389 metabolic or microbial) coexist but their relative importance at a specific time might depends
390 on diet composition (DF, aleurone) and animal characteristics. In our trial, as pigs had free
391 access to feed during 22.5 hours of the day, an important variability in feeding behaviour
392 between pigs was observed. The daily number of meals ranged from 2 to 31 and the meal size
393 ranged from 69 g to 379 g DM ingested per meal. In most of the experiments studying
394 metabolite or hormone kinetics, animals were fasted during at least 12 hours before receiving
395 a test-meal of a fixed amount ⁽³⁴⁾ whereas in commercial farms, pigs do not have similar ADFI
396 or feeding behaviour. Consequently, it is needed to let pigs eat with constraints as limited as
397 possible to assess the importance of each regulation mechanism behind this variability to
398 optimize nutritional solution. On the day of blood kinetics measurements, the interval
399 between the meal preceding the test meal and the test meal varied from 115 to 911 minutes.
400 The size of this last meal varied from 21 to 385 g of DM. Pigs did not have the same
401 metabolic status at the beginning of the kinetic measurements. Pre-prandial glycaemia ranged
402 from 689 to 1201 mg/L and pre-prandial free fatty acids concentration ranged from 45 to
403 485 $\mu\text{mol/L}$. Moreover, as the test meal was a voluntary meal, its size was extremely variable
404 between animals, ranging from 98 to 710 g of DM. This variability of feeding behaviour as
405 well as pre-prandial concentrations of metabolites and insulin induced different responses
406 after the voluntary test meal. Apart from a direct analysis of variance according to the
407 experimental design, a discriminant analysis leading to clustering of animals was attempted to
408 aggregate animals in groups with similar pre-prandial metabolic status. Metabolic responses
409 of pigs grouped in cluster 3 exhibited a large increase of glycaemia and insulinemia after the
410 ingestion of the meal test and fitted to metabolic responses of control groups in other studies
411 ^(7; 35). These animals had almost 9 hours of fasting before the beginning of the kinetic
412 measurements and they were fed the LF diet without aleurone supplementation, leading to
413 usual basal conditions before feeding. Cluster 2 grouped pigs that were fed the HF diet or

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414 aleurone supplementing diets. The low level of free fatty acids during the first hour after the
415 meal might be explained with the low pre-prandial concentration of free fatty acid and with
416 the production of short chain fatty acids which are known to decrease free fatty acids
417 plasmatic concentrations ⁽²⁶⁾. This underlines the importance of microbial activity in changes
418 of feeding behaviour due to DF level. An increase of meal frequency (cluster 4) induced
419 changes of regulation pathways of feed intake. In this cluster, pigs fed different experimental
420 diets suggesting that animal characteristics could be more important than diet composition in
421 some post-prandial responses. This result is in accordance with another study which showed
422 an important variability of feeding behaviour in the same group ⁽³⁶⁾. Despite a low level of
423 plasma free fatty acids, these pigs had a heavier test meal than pigs of the other clusters,
424 which might be due to the heavy BW of animal suggesting a larger stomach and ingestion
425 capacity. The large meal size was associated with a high concentration of α -amino nitrogen
426 whereas glucose and insulin responses were less modified. Although not significant, our
427 results are in accordance with others studies that exhibited less daily fluctuations of glucose
428 and insulin with increased meal frequency ^(7; 37). Other studies reported that kinetics on
429 glucose did not only depend on diet composition but also of feeding behaviour ⁽³⁸⁾. We
430 supposed that frequent meals induced a continuous supply of glucose and other nutrients
431 without important fluctuations of their plasmatic concentration. Glucose metabolism may not
432 be the first regulation pathway which modulates feed intake in these pigs. Finally, whereas
433 others clusters showed a large test meal size (> 220 g) compared with average meal size over
434 the measurement period (< 200 g), pigs grouped in cluster 1, fed predominantly LF diet,
435 showed a test meal more representative of the averaged meal size over other days. As pigs
436 belonging to this cluster were light (34.9 kg), their ingestion capacity may be limited. Due to
437 the small meal size, pigs presented a low α -amino-nitrogen concentration associated with high
438 urea concentrations in pre-prandial situation and one hour after the meal. These results
439 suggest that pigs mobilized protein storages to meet energy requirements.

440 To conclude, there was no interaction between DF level and aleurone supplementation on
441 feeding behaviour in growing pigs. Dietary fibre level in the diet increased meal size and
442 decreased daily meal number without affecting ADFI whereas aleurone supplementation
443 decreased ADFI with a reduction of the meal number per day. The dietary supplementation
444 with wheat aleurone modulated feed intake with effect of satiety regulation pathways, when
445 introduced at 4 g/kg in the diet. Moreover, this trial permitted to show that the variability of
446 feeding behaviour and metabolic responses when pigs have *ad libitum* access to feed might be

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447 due to different regulation pathways with different degrees of importance depending on diet
448 composition and animal characteristics. Inter-individual variability should be considered
449 when diet composition is used to modulate feeding behaviour.

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461 **Conflict of interest**

462 E. L. and L. M. had no conflict of interest. M. L. G., Y. L. are permanent salaries and
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466 the funding of this work, to the discussion of the results and to the reviewing of the
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468 analysis, and decision to publish, or preparation of the manuscript.

469 **Authorship**

470 K. Q., E. L., L. M. designed the study. K. Q., with direction from E. L. and L. M., was
471 responsible for data collection and statistical analysis. E. L. assisted with the study execution.
472 E. L. and L. M. reviewed the manuscript and M. L. G. and Y. L. participated to the reviewing
473 of the manuscript. All authors read and approved the final manuscript. L. M. is the
474 corresponding author.

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	Low Fibre			High Fibre		
	A0	A2	A4	A0	A2	A4
Ingredients (g/kg of DM)						

563 **Table 1.** Ingredients and chemical composition of the experimental diets

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Wheat	339.6	339.6	339.6	219.7	219.7	219.7
Corn	219.7	219.7	21.97	219.7	219.7	219.7
Barley	219.7	219.7	21.97	219.7	219.7	219.7
Soybean meal	185.9	185.9	185.9	157.5	157.5	157.5
Rapeseed oil	5.0	5.0	5.0	5.0	5.0	5.0
Wheat bran	-	-	-	75.0	75.0	75.0
Soya hulls	-	-	-	50.0	50.0	50.0
Sugar beet pulp	-	-	-	25.0	25.0	25.0
Aleurone†	-	2.0	4.0	-	2.0	4.0
Premix ‡	30.0	29.8	29.6	28.3	28.1	27.9

Chemical composition

Analysed (% DM or precised)

Crude protein	18.1	18.3	18.0	16.8	17.7	17.8
Crude fat	3.6	3.3	3.5	3.5	3.5	3.2
Crude fibre	2.6	2.8	2.8	4.8	5.1	5.0
NDF	10.1	10.6	10.4	14.5	14.6	14.9
ADF	3.3	3.5	3.3	5.8	5.8	5.9
ADL	0.5	0.6	0.6	0.9	0.9	0.7
Starch	56.7	53.7	53.4	49.9	47.5	48.2
Gross energy (MJ/kg DM)	18.38	18.24	18.18	18.12	18.20	18.07

Measured (MJ/kg DM)

Digestible energy	16.21	15.80	15.80	15.06	15.02	15.73
Metabolisable energy	15.87	15.37	15.43	14.78	14.66	15.33

Calculated (MJ/kg DM) §

Net energy	11.32	11.14	11.10	10.53	10.57	10.47
------------	-------	-------	-------	-------	-------	-------

564 A0, no aleurone; A2, supplementation of 2 g/kg of aleurone; A4, supplementation of 4 g/kg of
565 aleurone; DM, dry matter

566 † GrainWise Wheat Aleurone, Horizon Mills, Cargill, Minneapolis, Minnesota, United States
567 with 19.3 MJ gross energy/kg DM, 18.5% DM of crude protein, 11.1% DM of crude fibre and
568 52.6% DM of neural detergent fibre.

569 ‡ The premix supplied (per kg diet) for LF diet: -lysine HCL, 3 g; L-threonine, 1 g, L-
570 tryptophan, 0.1 g; DL-methionine, 0.4 g; salt, 4.5 g; calcium carbonate, 5.9 g; dicalcium
571 phosphate, 10.1 g; vitamin A, 5000 UI; vitamin D3, 1000 UI; vitamin E, 20 mg; vitamin B1, 2

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572 mg; vitamin B2, 4 mg; pantothenic acid, 2000 mg; vitamin B6, 1 mg; vitamin B12, 0.022 mg;
573 niacinamide, 15 mg; vitamin K3, 2 mg; folic acid, 1 mg; biotine, 0.2 mg; choline chloride, 50
574 mg; Mg, 0.1 g; Ca, 1.33 g; Fe, 80 g; Cu, 10 mg; Zn, 100 mg; Mn, 40 mg; I, 0.2 mg; Se, 0.15
575 mg and for HF diet and L-lysine HCL, 2.8 g; L-threonine, 0.9 g, L-tryptophan, 0.1 g; DL-
576 methionine, 0.4 g; salt, 4.5 g; calcium carbonate, 4.7 g; dicalcium phosphate, 9.9 g; vitamin
577 A, 5000 UI; vitamin D3, 1000 UI; vitamin E, 20 mg; vitamin B1, 2 mg; vitamin B2, 4 mg;
578 pantothenic acid, 2000 mg; vitamin B6, 1 mg; vitamin B12, 0.022 mg; niacinamide, 15 mg;
579 vitamin K3, 2 mg; folic acid, 1 mg; biotine, 0.2 mg; choline chloride, 50 mg; Mg, 0.1 g; Ca,
580 1.33 g; Fe, 80 g; Cu, 10 mg; Zn, 100 mg; Mn, 40 mg; I, 0.2 mg; Se, 0.15 mg.

581 § The net energy (NE) was calculated with the equation $NE=0.703 \times DE+1.58 \times CF+0.47 \times ST-$
582 $0.97 \times CP-0.98 \times CF$ ⁽⁹⁾ with DE for Digestible Energy, EE for Ether Extract, ST for Starch, CP
583 for Crude Protein and CF for Crude Fibre ⁽⁹⁾

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584 **Table 2.** Effect of dietary fibre and aleurone supplementation on feeding and drinking

	Fibre		Aleurone			P †		
	LF	HF	A0	A2	A4	RSD‡	F	A
Number of pigs	18	20	13	14	11			
Body weight (kg)	37.2	37.8	38.0	37.6	36.8	4.0	0.69	0.68
Average daily feed intake (g DM/day)	1420	1445	1562 ^a	1487 ^a	1248 ^b	334	0.73	<0.01
Net energy intake per day (kJ NE/kg BW ^{0.60} /day)§	1684	1777	1900	1667	1581	266	0.35	0.05
Number of meals per day §	9.4	8.2	9.1 ^a	9.4 ^a	7.6 ^b	3.2	<0.01	<0.01
Average feed intake per meal (g DM/meal) §	149	177	173	153	167	48	<0.01	0.40
Average meal duration (min) §	9.9	10.9	10.2	10.2	10.9	3.7	<0.01	0.16
Total meal duration per day (min/day) §	90	87	90 ^a	92 ^a	81 ^b	25	0.17	0.01
Interval between two meals (min) §	145	166	150 ^a	144 ^a	176 ^b	55	<0.01	<0.01
Water intake (g/day) §	2973	3074	2926	3123	3013	1547	0.88	0.89

585 behaviour in growing pigs

586 A0, no aleurone; A2, supplementation of 2 g/kg of aleurone; A4, supplementation of 4 g/kg of
 587 aleurone; DM, dry matter; HF, high fibre; LF, low fibre; NE, net energy.

588 The experiment was conducted with 5 growing pigs fed LF-A0, 7 growing pigs fed LF-A2, 6
 589 growing pigs fed LF-A4, 8 growing pigs fed HF-A0, 7 growing pigs fed HF-A2 and 5
 590 growing pigs fed HF-A4.

591 ^{a,b} Mean values within a column with unlike superscript letters were significantly different
 592 (P<0.05).

593 † Analysis of variance performed using Proc Mixed (SAS) including effects of dietary fibre
 594 (F), aleurone (A), interaction between dietary fibre and aleurone (non-significant) and day
 595 (non-significant). Data are presented in table as least squares means (*lsmeans* statement in
 596 SAS).

597 ‡ RSD= residual standard deviation.

598 § Logarithmic transformation was previously performed to validate normality test according
 599 to Shapiro-Wilk's test of residual. Values are presented in table as means of the estimated
 600 data from MIXED model after exponential transformation of the predicted values. Residual

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601 standard deviation of these variables is calculated as the standard error of the difference
602 between experimental data and estimated data.

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603 **Table 3.** Effect of dietary fibre and aleurone supplementation on pre-prandial concentrations
 604 of plasma metabolites and insulin in growing pigs

	Fibre		Aleurone			RSD¶	P †			
	LF	HF	A0	A2	A4		F	A	I	LMS
Number of pigs	14	12	9	9	8					
Insulin (mUI/L) §	5.8	3.1	3.7 ^a	6.7 ^b	3.0 ^a	2.1	0.01	<0.01	<0.01	<0.01
Glucose (mg/L)	1183	1102	1152	1156	1118	88	0.06	0.70	0.08	0.25
Lactate (µmol/L)	930	882	905	894	919	162	0.50	0.95	0.22	0.46
Free fatty acid (mol/L)	185	143	134	179	179	98	0.34	0.59	0.05	0.04
Triglycerides (mg/L)	257	285	288	286	238	70	0.37	0.35	0.75	0.47
α-amino nitrogen (mg/L)	645	671	697 ^a	701 ^a	576 ^b	95	0.54	0.04	0.90	0.53
Urea (mg/L)	185	161	164	155	199	40	0.20	0.13	0.17	0.32

605 A0, no aleurone; A2, supplementation of 2 g/kg of aleurone; A4, supplementation of 4 g/kg of
 606 aleurone; DM, dry matter; HF, high fibre; LF, low fibre

607 The experiment was conducted with 5 growing pigs fed LF-A0, 4 growing pigs fed LF-A2, 5
 608 growing pigs fed LF-A4, 4 growing pigs fed HF-A0, 5 growing pigs fed HF-A2 and 3
 609 growing pigs fed HF-A4.

610 ^{a,b} Mean values within a column with unlike superscript letters were significantly different
 611 (P<0.05).

612 † Analysis of variance performed using Proc Mixed (SAS) including effects of dietary fiber
 613 (F), aleurone (A), interaction between dietary fiber and aleurone (non significant), interval
 614 since the last meal (I) and the size of the last meal (LMS). Data are presented in table as least
 615 squares means (*lsmeans* statement in SAS).

616 ‡ RSD= residual standard deviation.

617 § Logarithmic transformation was previously performed to validate normality test according
 618 to Shapiro-Wilk's test of residual. Values are presented in table as means of the estimated
 619 data from MIXED model after exponential transformation. Residual standard deviation of

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620 these variables is calculated as the standard error of the difference between experimental data
621 and estimated data.

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622 **Table 4.** Contributions¹ of each variable to components 1 and 2 in the Multiple Factorial
623 Analysis performed on pre-prandial plasmatic metabolite and insulin concentrations and
624 feeding behaviour in growing pigs

	Component 1	Component 2
Voluntary meal size	36.3	11.9
Pre-prandial plasmatic concentration		
α -amino-nitrogen	20.2	0.0
Triglycerides	5.9	11.8
Glucose	5.7	4.7
Free fatty acids	1.9	21.3
Lactate	1.1	21.1
Urea	<1.0	0.1
Insulin	<1.0	2.4
Feeding behaviour during the week of measurements		
Number of meals per day	8.9	8.7
Average daily feed intake	7.9	0.4
Total intake duration	6.2	<1.0
Average meal size	3.5	9.8
Average meal duration	2.2	7.6

625 ¹The contribution of each variable is expressed as % of the variance (inertia) explained by each
626 component.

627

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628 **Table 5.** Characterization of groups after clustering according feeding behaviour, voluntary
629 test meal and pre-prandial plasma metabolites and insulin concentrations in growing pigs

	Clusters				RSD‡	P †	
	1	2	3	4		C	BW
Number of pigs fed LF-A0/LF-A2/LF-A4 diets	1/1/2	0/2/0	2/0/1	2/1/2	-	-	-
Number of pigs fed HF-A0/HF-A2/HF-A4 diets	0/0/1	0/3/2	1/0/0	3/2/0	-	-	-
Body weight (kg)	34.9 ^a	37.5 ^a	44.9 ^b	43.0 ^b	3.1	<0.01	-
Feeding behaviour							
Average daily feed intake (g DM/d)	1209 ^{ab}	1174 ^a	1580 ^{bc}	1838 ^c	243	0.03	<0.01
Number of meals per day	7.1 ^a	7.0 ^a	11.0 ^{ab}	12.6 ^b	3.2	0.04	0.71
Day of the blood kinetic							
Test meal size (g DM)	144 ^a	242 ^a	223 ^a	462 ^b	84	<0.01	<0.01
Interval since the last meal (min)	418	511	534	324	171	0.16	0.22
Pre-prandial plasmatic concentrations							
Insulin (mUI/L)§	3.3	5.7	4.6	4.1	5.4	0.14	0.08
Glucose (mg/L)	1057	1034	1221	1174	97	0.14	0.51
Lactate (µmol/L)	989 ^{ab}	855 ^a	1139 ^b	818 ^a	129	<0.01	0.64
Free fatty acid (µmol/L)	290	95	118	91	150	0.17	0.27
Triglycerides (mg/L)	272	249	321	284	65	0.55	0.28
α-amino nitrogen (mg/L)	516 ^a	645 ^b	667 ^b	762 ^c	56	<0.01	0.80
Urea (mg/L)	195 ^b	112 ^a	125 ^a	172 ^b	33	<0.01	0.90

630 A0, no aleurone; A2, supplementation of 2 g/kg of aleurone; A4, supplementation of 4 g/kg of
631 aleurone; BW, body weight; C, cluster; DM, dry matter; HF, high fibre; LF, low fibre,

632 ^{a,b,c} Mean values within a column with unlike superscript letters were significantly different
633 (P<0.05).

634 † Analysis of variance performed using Proc Mixed (SAS) including effects of cluster (C) and
635 body weight (BW). Data are presented in table as least squares means (*lsmeans* statement in
636 SAS).

637 ‡ RSD= residual standard deviation.

638 § Logarithmic transformation was previously performed to validate normality test according
639 to Shapiro-Wilk's test of residual. Values are presented in table as means of the estimated

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640 data from MIXED model after exponential transformation. Residual standard deviation of
641 these variables is calculated as the standard error of the difference between experimental data
642 and estimated data.

643

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644 **Figure legend**

645 **Fig. 1.** Effect of dietary fibre and aleurone supplementation on plasma glucose (a, b), insulin
646 (c, d), lactate (e, f), free fatty acid (g, h), urea (i, j) and α -amino-nitrogen (k, l) concentrations
647 in growing pigs fed diets. Results are expressed as means, n=26. The experiment was
648 conducted with 14 growing pigs fed LF diet, 12 growing pigs fed HF diet and with 9 growing
649 pigs fed A0 diet, 9 growing pigs fed A2 diet and 8 growing pigs fed A4 diet. General linear
650 mixed analysis was performed using MIXED model including the fixed effect of dietary fibre
651 level, aleurone supplementation, interaction between dietary fibre and aleurone, time,
652 interaction between time and dietary effect, size of the test meal, pre-prandial concentration of
653 the metabolite or insulin and the random effect of batch. P-values are specified with their
654 residual standard deviation (RSD) for the effects of dietary fibre level (a, c, e, g, i, k) and
655 aleurone supplementation (b, d, f, h, j, l). Interaction between DF level and aleurone
656 supplementation was never significant. Effect of the time is significant for all metabolites and
657 insulin concentrations except urea concentration. Interactions between time and dietary fibre
658 level or aleurone supplementation are not significant except for insulin and α -amino-nitrogen.
659 * Significant difference at that time between levels of fibre (C) or aleurone (D), $P < 0.05$.

660
661 **Fig. 2.** Effect of dietary fibre and aleurone supplementation on plasma triglyceride
662 concentration in growing pigs fed experimental diets. Results are expressed as means, n=26.
663 The experiment was conducted with 14 growing pigs fed LF diet, 12 growing pigs fed HF diet
664 and with 9 growing pigs fed A0 diet, 9 growing pigs fed A2 diet and 8 growing pigs fed A4
665 diet. Analyse of variance was performed according a General linear mixed model using
666 MIXED procedure of SAS including the fixed effect of dietary fibre level, aleurone
667 supplementation, interaction between dietary fibre and aleurone, time, interaction between
668 time and dietary effect, size of the test meal, pre-prandial concentration of the metabolite or
669 insulin and the random effect of batch. P-values are specified with their residual standard
670 deviation (RSD) for the following effects: F: fibre, A: aleurone, F \times A: interaction between
671 dietary fibre and aleurone. Effect of the time is significant ($P < 0.01$) but interactions between
672 time and dietary fibre level or aleurone supplementation are not significant. ^{a,b,c} significant
673 dietary effect on mean triglycerides concentration during the hour after the test meal ($P < 0.05$).
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675 **Fig. 3.** Effect of clustering according pre-prandial concentrations of plasma metabolites and
676 insulin, feeding behaviour and size of the voluntary test meal on glucose (a), insulin (b),
677 lactate (c), α -amino nitrogen (d), urea (e), free fatty acid (f) and triglyceride (g) concentrations
678 in growing pigs. Results are expressed as means, n=26. The experiment was conducted with 5
679 growing pigs grouped in cluster 1, 7 growing grouped in cluster 2, 4 growing grouped in
680 cluster 3 and 10 growing pigs grouped in cluster 4. General linear mixed analysis was
681 performed using MIXED model including the fixed effects of cluster, time, their interaction
682 and body weight and the random effect of batch. P-values are specified with their residual
683 standard deviation (RSD) for the effects clustering. ^{a,b,c}: significant effect of clustering on
684 average metabolites concentrations during one hour after the test meal ($P<0.05$). Effect of the
685 time is significant for all metabolites and insulin concentrations except urea concentration.
686 Interactions between time and clustering are not significant except for triglycerides.

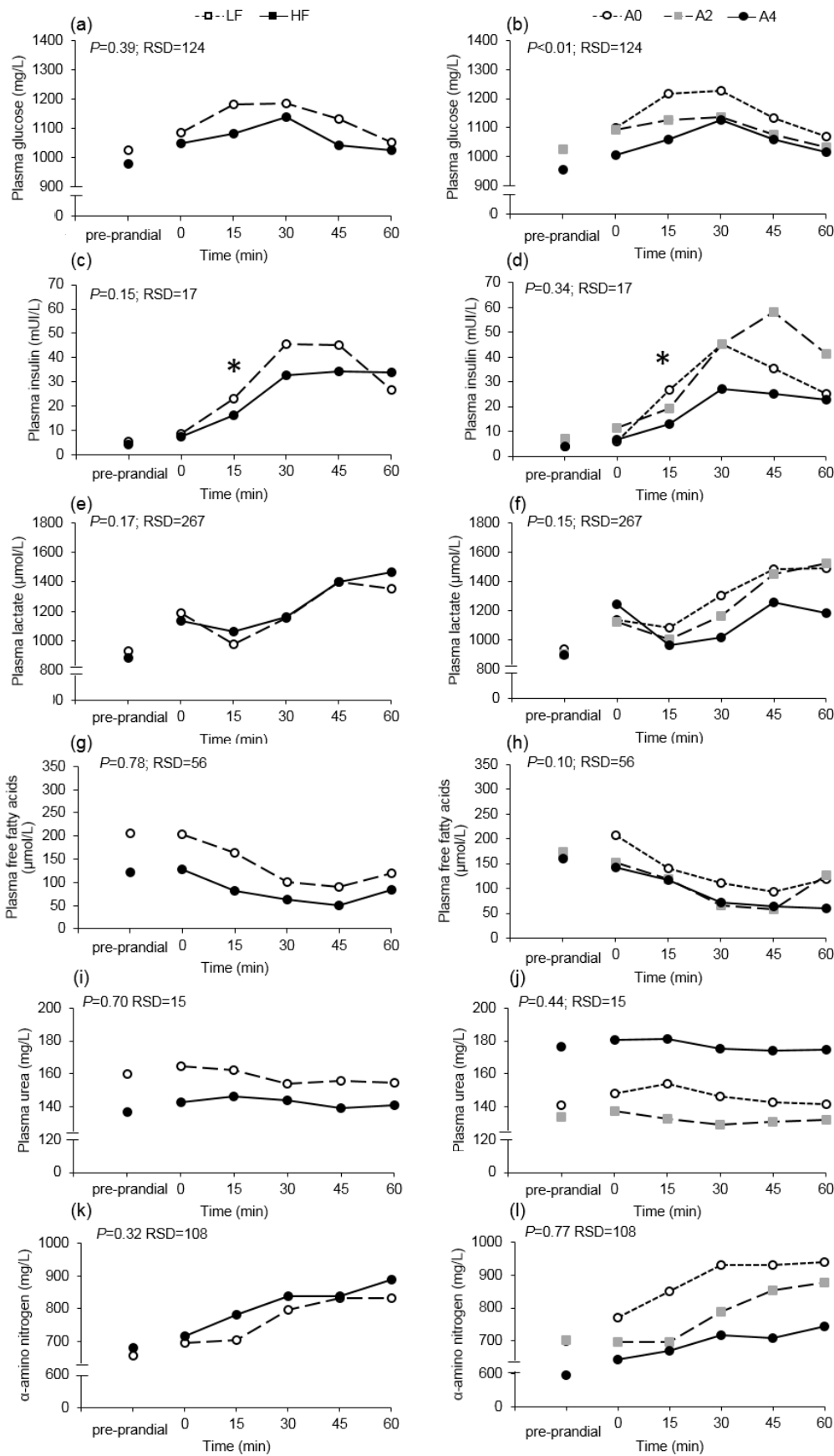


Fig. 1.

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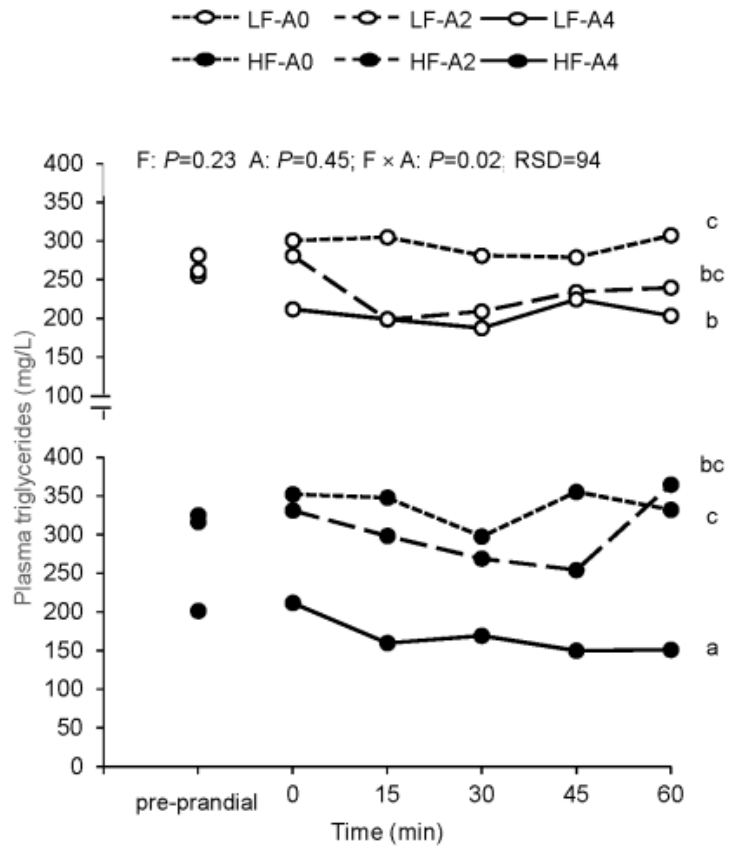


Fig. 2.

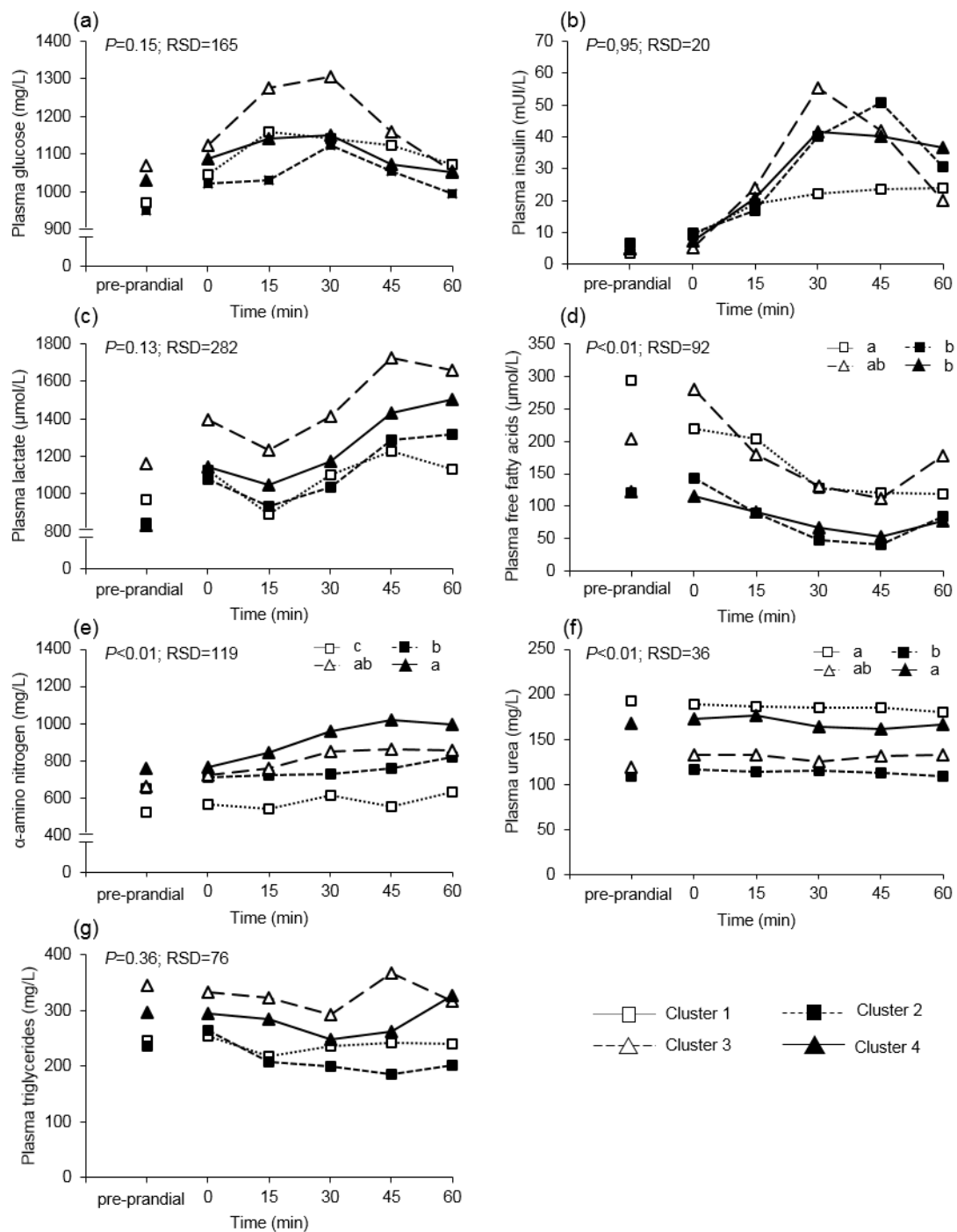


Fig. 3.