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

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Running head: Regulation of voluntary feed intake in pigs

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

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Abstract

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

Introduction

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

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

The implication of this study is the modulation of feeding behaviour in pigs. Limiting voluntary feed intake by dietary composition rather than quantitative feed restriction may benefit animal welfare and facilitate increased feed efficiency ⁽⁸⁾. Due to similitudes in digestion and metabolism between pigs and humans, results obtained in this study also 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 \times (Large White \times
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 \times 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⁾).

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

Feeding behaviour recording

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

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

Blood collection

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

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

Chemical analyses

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

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

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

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

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

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

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

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

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

Results

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

Influence of diets on feeding behaviour.

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

Influence of diets on plasmatic metabolites and insulin concentrations.

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

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

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

Characterization of clusters.

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

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

Discussion

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

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

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

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

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

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

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

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

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

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Conflict of interest

E. L. and L. M. had no conflict of interest. M. L. G., Y. L. are permanent salaries and K.Q. contractual salaries (3 years) from Provimi France that is a part of the Cargill Group. The wheat aleurone tested in the study is commercialized by Horizon Mills that is also a part of the Cargill group (Minneapolis, Minnesota, United States). M. L. G., Y. L. participated to the funding of this work, to the discussion of the results and to the reviewing of the manuscript but did not have any additional role in the study design, data collection and analysis, and decision to publish, or preparation of the manuscript.

Authorship

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

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- 562

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

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

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

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⁾

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

	Fibre		Aleurone			<i>P</i> †		
	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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Table 3. Effect of dietary fibre and aleurone supplementation on pre-prandial concentrations 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

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

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

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

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

‡ RSD= residual standard deviation.

§ Logarithmic transformation was previously performed to validate normality test according to Shapiro-Wilk's test of residual. Values are presented in table as means of the estimated 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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Table 4. Contributions¹ of each variable to components 1 and 2 in the Multiple Factorial Analysis performed on pre-prandial plasmatic metabolite and insulin concentrations and 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

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

Table 5. Characterization of groups after clustering according feeding behaviour, voluntary 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

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

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

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

‡ RSD= residual standard deviation.

§ Logarithmic transformation was previously performed to validate normality test according 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

Figure legend

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

* Significant difference at that time between levels of fibre (C) or aleurone (D), $P<0.05$.

Fig. 2. Effect of dietary fibre and aleurone supplementation on plasma triglyceride concentration in growing pigs fed experimental diets. Results are expressed as means, $n=26$. The experiment was conducted with 14 growing pigs fed LF diet, 12 growing pigs fed HF diet and with 9 growing pigs fed A0 diet, 9 growing pigs fed A2 diet and 8 growing pigs fed A4 diet. Analyse of variance was performed according a General linear mixed model using MIXED procedure of SAS including the fixed effect of dietary fibre level, aleurone supplementation, interaction between dietary fibre and aleurone, time, interaction between time and dietary effect, size of the test meal, pre-prandial concentration of the metabolite or insulin and the random effect of batch. P-values are specified with their residual standard deviation (RSD) for the following effects: F: fibre, A: aleurone, F \times A: interaction between dietary fibre and aleurone. Effect of the time is significant ($P<0.01$) but interactions between time and dietary fibre level or aleurone supplementation are not significant. ^{a,b,c} significant dietary effect on mean triglycerides concentration during the hour after the test meal ($P<0.05$).

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.

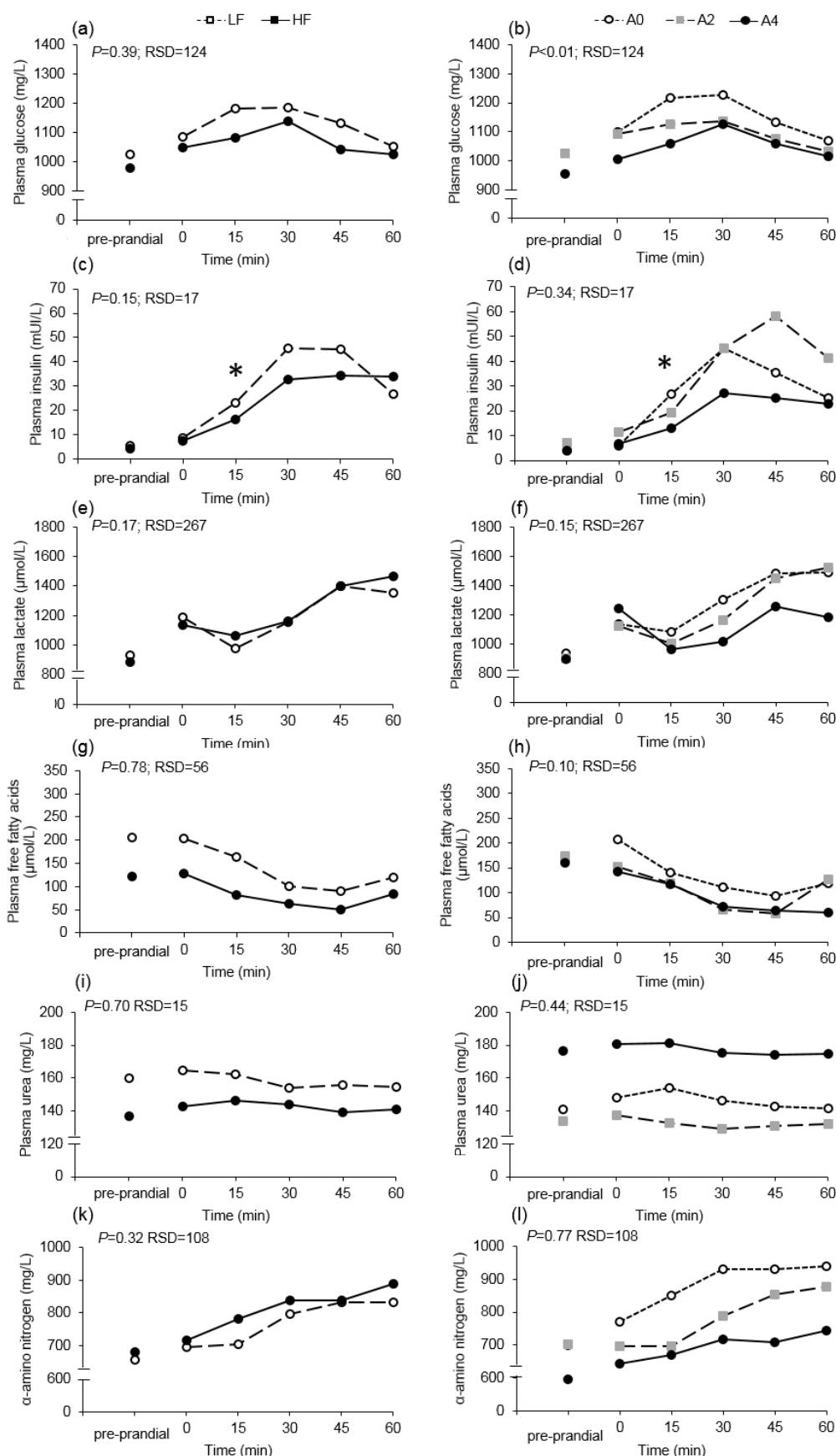


Fig. 1.

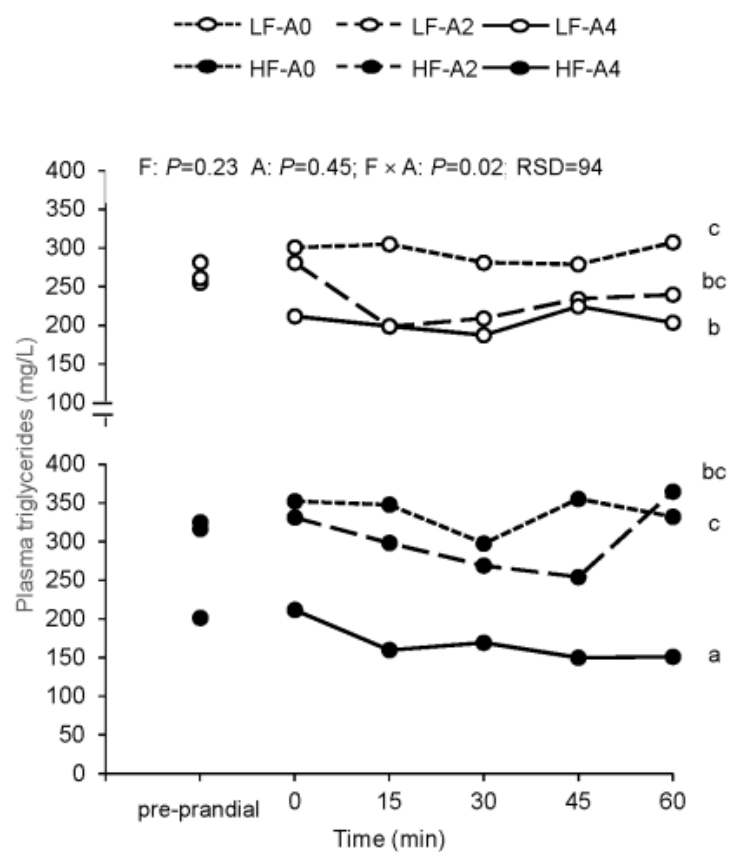


Fig. 2.

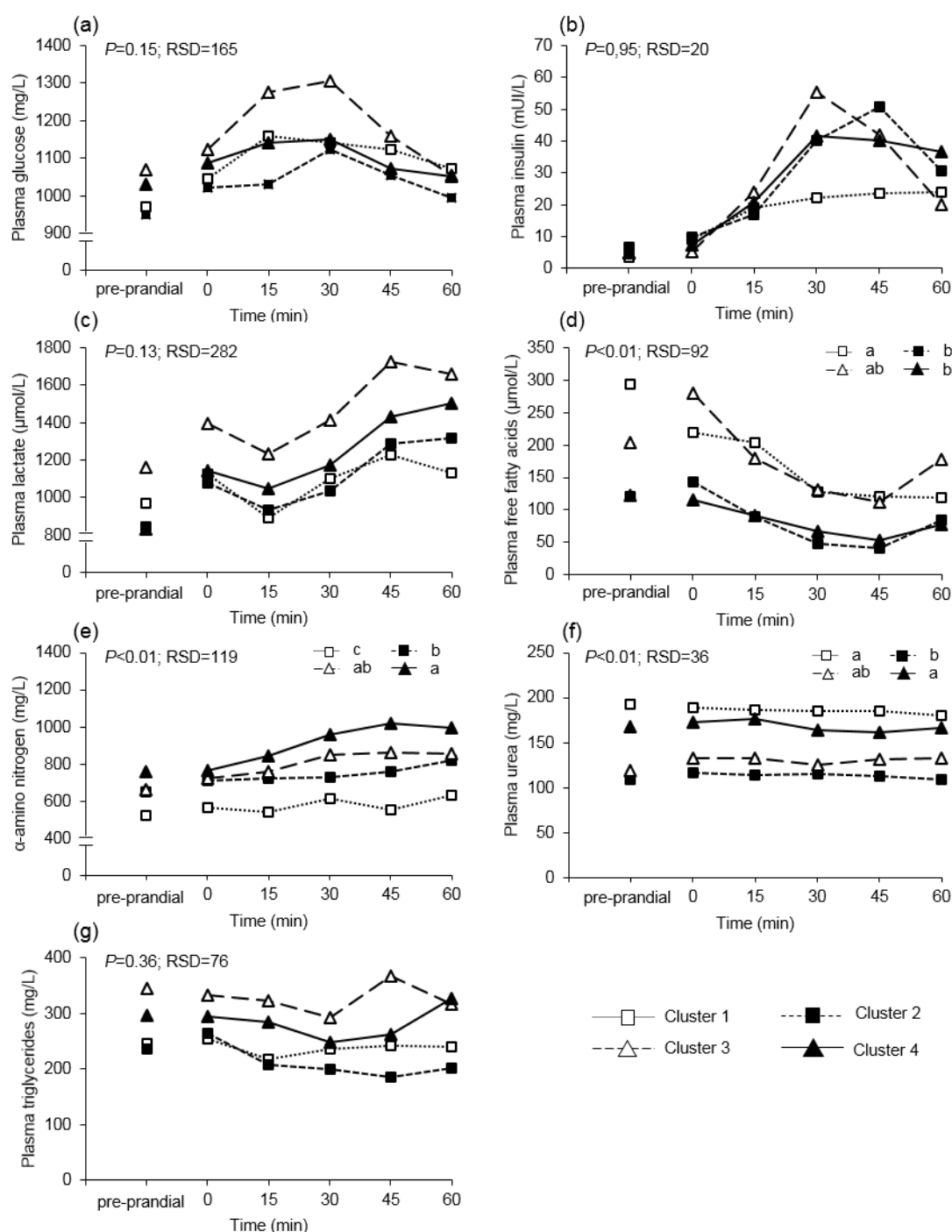


Fig. 3.