# Selection for feed efficiency elicits different postprandial plasma metabolite profiles in response to poor hygiene of housing conditions in growing pigs

Alícia Zem Fraga[1¶], Isabelle Louveau[2¶], Paulo Henrique Reis Furtado Campos[3&], Luciano Hauschild[1&], Nathalie Le Floc'h[2*]

[1] Department of Animal Science, São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Jaboticabal, São Paulo, Brazil

[2] PEGASE, INRAE, Institut Agro, Saint Gilles, France

[3] Department of Animal Science, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil

*Corresponding author:

Email: nathalie.lefloch@inrae.fr

¶These authors contributed equally to this work.

&These authors also contributed equally to this work.

# Abstract

Selection for residual feed intake (RFI), a measure of feed efficiency, may affect the ability of pigs to adapt their metabolism in response to poor environmental conditions. This study was conducted to compare postprandial plasma concentrations of insulin, energy related metabolites, and amino acids measured after a 6-week challenge consisting of exposure to good or poor hygiene of housing conditions of 24 growing pigs divergently selected for low-RFI (LRFI) and high-RFI (HRFI). Blood indicators of immune responses were assessed from samples collected before (week 0 or W0), and 3 (W3) and 6 weeks (W6) after pigs transfer to their respective housing hygiene conditions. Plasma haptoglobin concentrations and blood neutrophil granulocyte numbers were greater in poor than in good conditions at W3. Plasma concentrations of total immunoglobulin G were greater ($p$ = 0.04) in poor than in good hygiene conditions at W6. At W6, pigs were fitted with an intravenous catheter for serial blood samplings. Low-RFI pigs had greater insulin ($p$ < 0.001) and lower triglyceride ($p$ = 0.04) average plasma concentrations than HRFI pigs in both conditions. In poor hygiene conditions, the peaks of insulin and glucose occured earlier and that of insulin was greater in LRFI than in HRFI pigs. Irrespective of genetic line, average plasma concentrations of histidine, isoleucine, leucine, methionine, threonine, valine, and alanine were greater in poor compared with good conditions. Only HRFI pigs had greater lysine, asparagine, proline, and tyrosine plasma concentrations in poor than in good hygiene conditions. Conversely, arginine, tryptophan, proline, and tyrosine plasma concentrations were lower only for LRFI pigs housed in poor hygiene conditions. The impact of poor hygiene of housing conditions on insulin, triglycerides, and AA profiles differed between RFI lines. More specifically, our results suggest

41 that, contrary to HRFI, LRFI pigs increased or maintained their utilization of Trp, Arg,
42 and Lys when housed in poor hygiene conditions. This difference may contribute to
43 the better capacity of LRFI to cope with the poor hygiene of housing conditions.

# 44 Introduction

45 Selection of pigs for residual feed intake (RFI) has been used to improve feed
46 efficiency. Briefly, for a similar production level, high-RFI (HRFI) pigs eat more than
47 predicted based on average requirements for growth and maintenance and therefore
48 are less efficient than low-RFI (LRFI) pigs [1]. Difference in feed efficiency between
49 genetic lines is explained by physical activity [2], heat production [3], and metabolism
50 [4]. Genetic selection for RFI results in changes in the partition of nutrients between
51 maintenance and growth [5]. Differences in nutrient partitioning alter pig ability to
52 allocate nutrients for stress and immune responses when facing environmental
53 challenges [6]. In commercial farms, pigs are often exposed to stressful situations like
54 weaning, mixing, high stocking density, transport, and poor hygiene conditions
55 resulting in immune system hyper-activation. In turn, immune hyper-activation,
56 including inflammation, result in changes in nutrient metabolism to support the
57 immune responses, which therefore reduce nutrient availability for growth [7].

58 The better ability of LRFI compared with HRFI growing pigs to cope with an immune
59 challenge caused by poor hygiene of housing conditions was previously reported [8].
60 We hypothesized that this difference in coping ability between RFI pigs may involve
61 changes in their metabolism after a 6-week exposure to contrasted hygiene of
62 housing conditions. To evaluate this metabolic response, we analysed the patterns of
63 plasma postprandial concentrations of insulin, nitrogen- and energy-related
64 metabolites as it has been shown previously that they reflect changes in the use of

nutrients for anabolic and catabolic processes. The same methodology was recently reported to describe the metabolic status of growing pigs in response to an inflammatory challenge and high ambient temperature [9] or to compare castrated and entire male pigs [10]. Therefore, the present study was carried out to compare, in LRFI and HRFI pigs, the effects of poor hygiene of housing conditions on pre- and postprandial plasma concentrations of insulin, energy-related metabolites, and free amino acids (AA).

# Material and methods

The experiment was conducted at INRAE UE3P (Saint-Gilles, France) in accordance with the ethical standards of the European Community (Directive 2010/63/EU), and was approved by the Regional ethical committee (CREEA number 07).

## Animals, Diets, and Experimental Design

The experiment was conducted on a subset of 24 Large-White pigs from a larger study (n = 160 pigs) previously described [8]. Animals with representative body weight of each experimental group were selected at 12 weeks of age. Selected pigs were fed, housed, and submitted to the same experimental procedures as the whole set of pigs, before being involved in the serial blood sampling 6 weeks later.

Pigs originated from the 8th generation of a selection program for divergent RFI conducted at INRAE. Briefly, the lines were established using the RFI selection criterion between 35 and 95 kg body weight (BW), calculated as: RFI = ADFI − (1.24 × ADG) − (31.9 × BFT), where ADFI was the average daily feed intake (g/day), ADG the average daily gain (g/day) and BFT was the backfat thickness (mm) at 95 kg [11].

The study was performed as a 2 × 2 factorial design including four experimental groups: HRFI and LRFI pigs housed in good hygiene conditions (good-HRFI, good-LRFI); and HRFI and LRFI pigs housed in poor hygiene conditions (poor-HRFI, poor-LRFI). Briefly, poor hygiene conditions consisted of no cleaning nor sanitation of the room after the previous occupation by non-experimental pigs [8]. In contrast, good housing conditions included room cleaning, disinfection, and adoption of strict biosecurity precautions.

According to their allocation, pigs were placed in one of the two experimental rooms (good or poor hygiene conditions) for 6 weeks. In each room, pigs were housed in individual concrete floor pens (85 × 265 cm) equipped with a feed dispenser and a nipple drinker. Pigs had free access to water and were fed *ad libitum* a standard diet composed of wheat 32.2%, barley 30%, corn 15%, soya bean 7%, and bran 5%, and formulated to meet or exceed the nutritional requirement of growing pigs (9.47 MJ of net energy/kg, starch 44.2%; fat 3.1%; crude protein 15.3% and 8.3 g of standardized digestible lysine (Lys), Lys/kg). Lysine, threonine (Thr), methionine (Met), and tryptophan (Trp) were added as free synthetic AA. After 6 weeks, the 24 selected pigs (n = 6 per experimental group) were fitted with an intravenous ear catheter following a minimally invasive procedure. Briefly, after an overnight fast, pigs were premedicated with 15 mg/kg of ketamine injected intramuscularly (Imalgène 1000, Merial, Lyon, France) and were then anesthetized by inhalation of sevoflurane (Sevoflurane, Baxter, Maurepas, France) using a facemask. An intravenous catheter was inserted through a small incision on the flap of the ear. The external part of the catheter was fixed on the ear skin and a connector was added for blood samplings the day after catheter insertion. No drug was used to avoid interference with the health status of pigs.

## Blood Sample Collection

For measuring blood indicators of immune responses (immunoglobulin G (IgG), haptoglobin, and number of blood neutrophil granulocytes), blood samples were collected at fasted state by jugular vein puncture at week zero (W0; before pig transfer to the respective hygiene conditions) and week three (W3), and from the intravenous ear catheter at week six (W6). Serial blood samplings were performed at W6 the day after catheter insertion. After being fasted overnight, each pig was offered 300 g of the standard diet at 08-h. This meal size was determined to ensure that all pigs eat their meal in less than 10 min [10] and corresponded to 15% of the average daily feed intake. Blood samples (6 ml) were collected from the catheter before the meal delivery (fasted state; t0), and then at 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, and 240 min after the meal delivery. Blood samples were collected on ethylenediaminetetraacetic acid (EDTA) tubes for insulin, glucose, free fatty acid (FFA), triglyceride, and urea measurements; and on heparinized tubes for AA analyses. Samples were immediately placed on ice, except for EDTA tubes used for measuring the number of blood neutrophil granulocytes, and then centrifuged (1800 × g) for 10 min at 4°C. Plasma was collected and stored at -80°C for AA, and at -20°C for other plasma parameters.

## Blood Cell and Plasma Variable Analyses

The number of blood neutrophil granulocytes was measured with a haematology automated cell counter calibrated for pigs (MS9; Melet Schloesing Laboratories, Osny, France). Quantitative sandwich ELISA tests were used to quantify total IgG plasma concentrations [12]. Plasma concentrations of haptoglobin (phase

haptoglobin assay T801; Tridelta Development Ltd, Maynooth, Ireland), glucose (Kit Glucose RTU, ref. 61269; Biomérieux, Marcy-l'Etoile, France), and free fatty acids (Kit WAKO NEFA; Sobioda, Montbonnot-Saint-Martin, France), were analysed by an automated enzymatic method using commercial kits and a multianalyzer apparatus (Konelab 20i, ThermoFisher Scientific, Courtaboeuf, France). For plasma triglyceride and urea concentration measurements, kits were obtained from Thermo Fisher Diagnostics SAS (Asnieres-Sur-Seine, France). Plasma insulin concentrations were determined using a commercial immunoassay kit (ST AIA-PACK IRI) and the AIA-1800 device (Automated Immunoassay Analyzer; TOSOH Bioscience, Tokyo, Japan). Assay sensitivity was 0.5 µUI/ml and the intra-assay CV was below 5%. Plasma free AA concentrations were determined by an ultra-performance liquid chromatography (UPLC) apparatus (Waters Acquity Ultra Performance LC, Waters, Milford, MA, USA) after derivatization of samples using the AccQ Tag Ultra method (MassTrak AAA; Waters, Milford, MA, USA) as previously described [4].

## Calculations and Statistical Analysis

The pig was the experimental unit. Average plasma concentrations of insulin, energy metabolites, and AA were calculated from pre and postprandial concentrations. Average concentrations and concentrations at each sampling time were analysed using the linear MIXED procedure (SAS Inst. Inc., Cary, NC). The model included the genetic lines (LRFI or HRFI), housing hygiene conditions (poor or good), sampling time (time), and their interactions as fixed effects. The repeated measurements option was used with a compound symmetry covariance structure to account for animal effect over sampling time. Adjusted means were compared using the Bonferroni test. Plasma concentrations of total IgG and haptoglobin, and number of

neutrophil granulocytes were compared between the two hygiene conditions with a non-parametric test (Median test) using the NPAR1WAY procedure of SAS. Probabilities less than 0.05 were considered significant.

For indispensable AA, when an interaction between genetic line and housing hygiene conditions was significant, plasma profiles were analysed by nonlinear regression using a one-compartment model with Erlang retention times [13] in combination with a constant basal concentration. The Erlang distribution was previously described by [14]. The model used to describe the change in AA concentrations over time as an asymmetric bell-shaped curve was:

$$\text{Amino acid concentration } C\,(t) = \frac{k\left(\lambda^{n} \times (t)^{n-1}\right) \times \exp(-\lambda \times t) + C\text{basal}}{(n-1)!}$$

where *C*basal is the basal concentration, *k* is a scale parameter, *λ* and *n* are shape parameters of the Erlang distribution of residence times, and *t* is the time. For each AA plasma concentration curve, the shape parameter *n* was tested using values ranging from one to four, and the result with the lowest residual SD was retained. The model was parameterized to obtain AA concentration at $t = 0$ (*C*0), the maximum AA concentration (*C*max), and the time when AA concentration is maximum (Tmax). To test whether the parameters of the model differed between the experimental groups, a sum of squares reduction test was used [15]. As our main objective was to compare the effects of the hygiene challenge in LRFI and HRFI pigs, this test was run to compare poor-LRFI to good-LRFI, and poor-HRFI to good-HRFI. The principle was to compare a “full model” where all parameters differ between the two experimental groups with a “reduced model”, which has common parameters. This was done for each parameter successively. An F-test was used to test if the models

were statistically different and a probability less than 0.05 was considered as significant.

# Results

## General Observations

One good-LRFI pig was excluded from analyses because it did not fully consume the 300 g of feed during the allocated time. Performance and blood indicators of immune responses are presented in Figure 1. Pig initial and final body weights were 26.5 ± 2.80 kg and 55.4 ± 6.88 kg, respectively (Fig 1A). No hygiene effect was reported for ADG from W0 to W6 (data not shown; $p = 0.16$). No difference between pigs allotted to poor or good hygiene conditions were reported for any measured variables at W0 ($p > 0.05$). At W3, plasma haptoglobin concentrations and blood neutrophil granulocyte counts were greater in pigs housed in poor than in good hygiene conditions ($p < 0.05$; Figs 1B and 1C). At W6, total IgG plasma concentrations were greater in pigs housed in poor hygiene conditions compared with pigs housed in good hygiene conditions ($p = 0.04$; Fig 1D).

**Fig 1. Body weight [(a)] and blood indicators of immune and inflammatory responses [(b) Haptoglobin, (c) Neutrophil granulocytes, and (d) Immunoglobulin G] before 0 (W0), and 3 (W3) and 6 weeks (W6) after pigs transfer to good or poor hygiene conditions.**

# Plasma Concentrations of Insulin, Energy-related Metabolites and Urea

Average concentrations of insulin, energy-related metabolites, and urea are presented in Table 1. Plasma fasted concentrations did not differ for any measured variables (data not shown, $p > 0.05$). There was a sampling time effect ($p < 0.05$) for all metabolites studied. No hygiene effect and interaction between Line × Hygiene were reported ($p > 0.05$). Regardless of hygiene conditions, LRFI pigs had greater insulin ($p < 0.001$) and lower average plasma concentrations of triglycerides ($p = 0.04$) than HRFI pigs.

**Table 1. Average plasma concentrations of insulin, energy-related metabolites, and urea measured in low and high residual feed intake pigs (LRFI and HRFI) housed in good (Good) or poor (Poor) hygiene conditions at week 6.**

| | LRFI | | HRFI | | | *p*-value[4] | | |
|---|---|---|---|---|---|---|---|---|
| | Good | Poor | Good | Poor | SEM | Line | Hyg | Line×Hyg |
| No.[1] | 5 | 6 | 6 | 6 | | | | |
| Average plasma concentrations[2] | | | | | | | | |
| Insulin, mU/l | 27.8 | 29.3 | 17.3 | 15.3 | 1.87 | <0.001 | 0.89 | 0.37 |
| Glucose, mg/l | 1050 | 1044 | 1011 | 1009 | 22 | 0.11 | 0.86 | 0.93 |
| FFA[3], µmol/l | 99.7 | 125.3 | 134.8 | 107.3 | 23.8 | 0.72 | 0.97 | 0.28 |
| Triglycerides, mg/l | 274 | 262 | 343 | 321 | 30 | 0.04 | 0.58 | 0.87 |
| Urea, mg/l | 140 | 150 | 143 | 158 | 13 | 0.70 | 0.36 | 0.86 |

[1]No. = number of animals per group.

[2]Average plasma concentrations include fasted and postprandial concentrations measured for 4-h after ingestion of 300 g of feed. There was no effect of the experimental treatments on fasted concentrations for any blood variables ($p > 0.05$).

[3]FFA = free fatty acids.

[4]Probability values for the effect of genetic lines (Line), hygiene conditions (Hyg), and their interaction. There was an effect of sampling time ($p < 0.05$) for all variables studied. The interactions with time are presented in Fig 2.

The Line × Hygiene × Time interaction was significant for insulin, glucose, and triglycerides (Fig 2). In poor hygiene conditions only, the maximum insulin concentration occurred earlier (45 min) and was greater in LRFI compared with HRFI pigs ($p < 0.001$; Fig 2A). For glucose, the peak value did not differ between lines ($p = 0.98$) but occurred earlier (30 min) in LRFI than in HRFI pigs when housed in poor conditions ($p < 0.001$; Fig 2B). Regarding plasma triglyceride concentrations, the minimum concentration did not differ between the four experimental groups but occurred earlier (45 min) in HRFI pigs housed in poor conditions compared with the three other groups (75 min; Fig 2D).

**Fig 2. Postprandial plasma profiles of [(a)] insulin and metabolites [(b) Glucose, (c) Free fatty acids; FFA, (d) Triglycerides, and (e) Urea] measured in low and high residual feed intake pigs (LRFI and HRFI) housed in good or poor hygiene conditions at week 6**.

## Free Plasma AA Average Concentrations

Average concentrations of plasma free AA are presented in Table 2. Except for cysteine (Cys), there was a sampling time effect ($p < 0.05$) for all AA. Regardless of

hygiene conditions, LRFI pigs had greater Met, Thr, asparagine (Asn), Cys, glutamine (Gln), glutamate (Glu), and glycine (Gly) ($p < 0.05$), and lower histidine (His), valine (Val), and alanine (Ala) plasma concentrations ($p < 0.05$) than HRFI pigs. Irrespective of genetic line, average plasma concentrations of His, isoleucine (Ile), leucine (Leu), Met, Thr, Val, and Ala were greater ($p < 0.05$) and Gly were lower ($p = 0.02$) in poor than in good hygiene conditions. For dispensable AA, the interaction between Line and Hygiene was significant for aspartate (Asp), proline (Pro), serine (Ser), and tyrosine (Tyr). Average concentrations of Asp were greater in poor than in good conditions in HRFI pigs only ($p < 0.001$). When housed in poor housing conditions, LRFI pigs had lower and HRFI had greater plasma concentrations of Pro ($p < 0.05$) than in good conditions. Low-RFI pigs had greater average concentrations of Ser in poor than in good conditions ($p < 0.001$) whereas average concentrations did not differ in HRFI pigs ($p = 0.72$). Poor hygiene conditions resulted in lower average Tyr concentrations in LRFI and greater in HRFI pigs compared with good conditions ($p < 0.001$). For indispensable AA, the interaction between Line and Hygiene was significant for arginine (Arg), Lys and Trp. These results are described in details in the following paragraph.

**Table 2. Average concentrations of plasma free AA (nmol/ml) measured in low and high residual feed intake pigs (LRFI and HRFI) housed in good (Good) or poor (Poor) hygiene conditions at week 6.**

| | LRFI | | HRFI | | | *p*-value[3] | | |
|---|---|---|---|---|---|---|---|---|
| | Good | Poor | Good | Poor | SEM | Line | Hyg | Line×Hyg |
| No.[1] | 5 | 6 | 6 | 6 | | | | |
| Average plasma AA concentrations[2] | | | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Arginine | 151.5[a] | 139.2[b] | 100.3[c] | 99.8[c] | 8 | <0.001 | 0.09 | 0.04 |
| Histidine | 70.3 | 69.3 | 75.8 | 82.8 | 10 | <0.001 | 0.02 | 0.05 |
| Isoleucine | 122.2 | 135.2 | 127.6 | 140.0 | 19 | 0.12 | <0.001 | 0.08 |
| Leucine | 174.7 | 186.6 | 177.6 | 191.3 | 12 | 0.13 | <0.001 | 0.10 |
| Lysine | 243.8[a] | 234.5[ab] | 216.9[b] | 246.2[a] | 15 | 0.16 | 0.02 | <0.001 |
| Methionine | 40.0 | 45.9 | 30.6 | 36.1 | 11 | <0.001 | <0.001 | 0.95 |
| Phenylalanine | 83.2 | 82.3 | 84.5 | 85.8 | 8 | 0.84 | 0.14 | 0.58 |
| Threonine | 120.1 | 129.9 | 84.0 | 95.0 | 17 | <0.001 | <0.001 | 0.44 |
| Tryptophan | 62.5[a] | 51.7[b] | 44.2[bc] | 41.4[c] | 37 | <0.001 | <0.001 | <0.001 |
| Valine | 250.4 | 267.8 | 260.7 | 279.9 | 15 | 0.04 | <0.001 | 0.32 |
| Alanine | 448.3 | 496.4 | 527.0 | 564.5 | 3 | <0.001 | <0.001 | 0.39 |
| Asparagine | 60.3 | 59.1 | 52.3 | 54.1 | 17 | <0.001 | 0.92 | 0.99 |
| Aspartate | 18.2[a] | 18.4[a] | 13.8[c] | 15.2[b] | 5 | <0.001 | 0.16 | <0.001 |
| Cystine | 40.4 | 41.0 | 36.9 | 38.2 | 10 | 0.04 | 0.20 | 0.66 |
| Glutamine | 592.2 | 590.1 | 522.4 | 517.8 | 7 | <0.001 | 0.88 | 0.64 |
| Glutamate | 207.5 | 214.5 | 157.8 | 162.4 | 6 | <0.001 | 0.53 | 0.11 |
| Glycine | 767.2 | 733.9 | 732.0 | 713.0 | 12 | 0.01 | 0.02 | 0.55 |
| Proline | 302.1[a] | 288.6[b] | 248.5[d] | 274.7[c] | 14 | <0.001 | 0.06 | 0.02 |
| Serine | 134.1[b] | 141.6[a] | 125.3[b] | 133.1[b] | 9 | <0.001 | <0.001 | <0.001 |
| Tyrosine | 71.0[a] | 56.9[b] | 48.6[c] | 58.9[b] | 16 | <0.001 | 0.46 | <0.001 |

[1]No. = number of animals per group.

[2]Average plasma concentrations include fasted and postprandial concentrations measured for 4-h after ingestion of 300 g of feed.

[3]Probability values for the effect of genetic lines (Line), hygiene conditions (Hyg), and their interaction. Except for cystine ($p = 0.18$), there was an effect of sampling time ($p < 0.05$) for all AA studied. The parameters of the model for indispensable AA whose Line×Hyg

interaction was significant are presented in Table 3. There was Line x Time effect for Arg, only ($p < 0.001$).

[a,b,c] Within a row values with different superscripts differed ($p < 0.05$).

## Plasma Free Indispensable AA Postprandial Profiles

Average Arg concentrations were lower in poor than in good hygiene conditions in LRFI pigs only ($p = 0.04$; Table 3). This effect was associated with lower *C*0 value in LRFI pigs when housed in poor hygiene conditions ($p = 0.02$; Table 3 and Fig 3A). Lysine average concentrations ($p = 0.02$; Table 3) and *C*max ($p < 0.001$; Table 3 and Fig 3B) value were higher in poor conditions in HRFI pigs only. Lower Trp concentrations were observed in poor hygiene conditions in LRFI pigs only ($p < 0.001$; Table 3). For LRFI pigs, the values of *C*0, and Tmax for Trp were lower in poor than in good conditions ($p < 0.001$; Table 3 and Fig 3C).

**Table 3. Values of parameters describing the postprandial kinetics of plasma free arginine, lysine, and threonine in low and high residual feed intake pigs (LRFI and HRFI) housed in good (Good) or poor (Poor) hygiene conditions at week 6.**

| | LRFI | | | HRFI | | |
|---|---|---|---|---|---|---|
| | Good | Poor | *P*-value[3] | Good | Poor | *P*-value[3] |
| No.[1] | 5 | 6 | | 6 | 6 | |
| Parameter values[2] | | | | | | |
| Arginine | | | | | | |
| C0, μM | 88 ± 6 | 69 ± 5 | 0.02 | 62 ± 6 | 53 ± 6 | 0.28 |
| Cmax, μM | 193 ± 12 | 188 ± 7 | 0.62 | 125 ± 5 | 130 ± 3 | 0.75 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Tmax, min | 86 ± 4 | 81 ± 2 | 0.84 | 87 ± 5 | 96 ± 3 | 0.27 |
| Lysine | | | | | | |
| C0, µM | 126 ± 10 | 127 ± 8 | 0.98 | 145 ± 9 | 132 ± 9 | 0.34 |
| Cmax, µM | 349 ± 2 | 329 ± 7 | 0.12 | 273 ± 5 | 315 ± 3 | <0.001 |
| Tmax, min | 77 ± 3 | 69 ± 2 | 0.42 | 74 ± 7 | 86 ± 3 | 0.61 |
| Tryptophan | | | | | | |
| C0, µM | 39 ± 2 | 27 ± 2 | <0.001 | 25 ± 2 | 22 ± 2 | 0.79 |
| Cmax, µM | 72 ± 0 | 63 ± 4 | 0.17 | 52 ± 2 | 50 ± 7 | 0.88 |
| Tmax, min | 87 ± 3 | 68 ± 5 | <0.001 | 91 ± 6 | 87 ± 4 | 0.29 |

[1]No. = number of animals per group.

[2]Values are means ± SD. C0: the concentration at t = 0, Cmax: the maximum concentration, Tmax: the time in minute at maximum concentration.

[3]Probability values for the effect of hygiene conditions.

**Fig 3. Plasma profiles of free indispensable amino acids [(a) arginine; Arg, (b) lysine; Lys, and (c) tryptophan; Trp] measured in low and high residual feed intake pigs (LRFI and HRFI) housed in good or poor hygiene conditions at week 6. The observed (Ob) and estimated (Es) values are presented.**

# Discussion

This study was conducted to compare the metabolic modifications induced by poor hygiene of housing conditions in LRFI and HRFI growing pigs. Our major and original finding is that the effects of the poor hygiene challenge on the postprandial profiles of insulin and some indispensable AA differ between the two RFI lines. We discuss if

292 such differences may explain the better coping ability of LRFI pigs previously
293 reported [8].

294 To compare metabolic changes induced by the experimental factors, we analysed the
295 average postprandial plasma concentrations of insulin, energy and nitrogen-related
296 metabolites, as well as the pattern of postprandial plasma indispensable AA profiles.
297 Briefly, on the day of serial blood samplings, after being fasted overnight, all pigs
298 received 300 g of the same feed that they consumed in less than 10 min. Thus,
299 differences in plasma profiles were associated to differences in both digestion and
300 postprandial metabolism induced by the experimental factors, namely the genetic
301 line, the housing hygiene condition, and their interaction. In the current study, a
302 model of poor hygiene of housing conditions was used to induce an immune system
303 hyper-activation and systemic inflammation. This challenge alters health and
304 decreases pig growth performance [8]. In the current study, the findings of greater
305 plasma haptoglobin concentrations and blood neutrophil granulocyte counts in poor
306 than in good hygiene conditions at W3 indicate that the model successfully induced
307 an inflammation. Moreover, greater total IgG plasma concentrations in poor
308 conditions at W6 show that the immune system was still overstimulated at the time of
309 serial blood sampling. Contrary to AA, energy-related metabolites and insulin did not
310 differ between poor and good hygiene conditions. It should be noted that in our study,
311 insulin and energy metabolite concentrations were measured 6 weeks after the
312 beginning of the challenge when pigs were probably recovering from inflammation as
313 indicated by lower blood haptoglobin concentrations at W6 compared with W3.
314 Indeed, during an inflammatory challenge, plasma glucose concentrations were
315 restored two days after being temporarily increased in young growing pigs [4]
316 showing the fast return to glucose homeostasis. Pigs housed in poor hygiene

conditions had greater concentrations of Ala, His, Met, Thr, and branched-chain AA (BCAA) than pigs housed in good conditions. Greater AA plasma concentrations may be due to a lower AA retention as muscle protein in immune challenged pigs [16] caused by an increase in protein breakdown and/or decrease in protein synthesis. Indeed, growth rate measured in the whole set of pigs was depressed after 6 weeks of housing in poor hygiene conditions and this effect was caused by the poor health status and hyper-activation of the immune system [8]. Conversely, lower Thr postprandial plasma concentrations were reported in pigs coinfected with *Mycoplasma hyopneumoniae* and influenza virus probably to support a great demand for immunoglobulin synthesis [12]. Such a discrepancy regarding the response of Thr concentrations in our study was unexpected since the hygiene challenge increased immunoglobulin plasma concentrations but this increase was probably too moderate to impact plasma Thr concentrations. From these results, it can be suggested that inflammation and immune system hyper-activation impacted glucose metabolism more rapidly and for a shorter period than protein metabolism, as previously reported after a change in hormonal status in growing pigs [10]. Accordingly, in septic rats, plasma concentrations of tumor necrosis factor α measured 1.5 hours after an experimental infection showed a strong correlation with changes in protein metabolism and body weight two weeks later [17], demonstrating that prolonged effects on nitrogen metabolism may be observed while blood indicators of inflammation are no more detectable or return to normal values.

Contrary to the hygiene challenge that affects metabolism and digestibility [18], the selection for RFI did not affect the digestibility of a standard low fiber feed [19]. Thus, differences in plasma profile between RFI lines are mostly explained by a difference in metabolism. Irrespective of hygiene conditions, LRFI pigs had greater average

plasma concentrations of insulin and lower plasma concentrations of triglycerides than HRFI pigs. In agreement with our findings, Montagne et al. [20] observed greater plasma insulin concentrations after the ingestion of a small meal and Le Naou et al. [21] reported lower plasma triglyceride concentrations measured at fed state in LRFI compared with HRFI pigs. In pigs, plasma triglycerides result from the lipolysis of lipids stored in adipose tissue. Insulin is an anabolic hormone with a potent anti-lipolytic action on adipose tissue [22]. Therefore, lower triglyceride concentrations may be partly explained by the greater insulin concentrations in plasma. Alternatively, lower plasma triglycerides levels may also be attributed to the lower body fat content of LRFI compared with HRFI pigs [23]. Moreover, the impact of the overnight fasting associated with feed restriction on the day of serial blood samplings may be greater in HRFI pigs forcing them to mobilize their body fat reserve. However, feed restriction of HRFI pigs did not affect their plasma triglyceride concentrations [21]. Poor hygiene conditions impacted differently insulin response in LRFI and HRFI. Indeed, the postprandial peak of insulin occurred earlier and was greater in LRFI compared with HRFI pigs when housed in poor hygiene conditions. Such an effect contributes to explain why LRFI pigs maintained their growth rate in poor hygiene conditions [8]. Insulin is indeed the main hormone allowing the postprandial AA utilization for protein synthesis in muscle [24].

Plasma urea concentration did not significantly differ between the two lines. Urea is produced from the deamination of AA and, at fed state, reflects the catabolism of dietary AA that are not used for body protein synthesis and deposition. Our result is in line with a previous study showing that genetic selection for RFI did not affect nitrogen metabolism when HRFI and LRFI pigs were fed the same restricted level of feed [5]. However, differences in plasma free AA average concentrations were

observed between the two RFI lines. For instance, His, Val, and Ala plasma concentrations were lower in LRFI than in HRFI pigs. Lower plasma Ala concentrations at fed state were previously reported in LRFI compared with HRFI pigs [4] suggesting a lower muscular release of Ala for hepatic glucose synthesis (Cahill cycle) in LRFI pigs. This is in accordance with lower energy expenditure in LRFI than in HRFI pigs [5]. The BCAA (Ile, Leu and Val) are the major donors of the amino group for the synthesis of Ala from pyruvate in muscle [4]. In the present experiment, despite that only Val concentrations differed between the two lines, Ile and Leu plasma concentrations were numerically lower in LRFI than in HRFI pigs. Alanine synthesis in muscle and its release in the plasma in LRFI pigs may have been reduced by the decreased availability of BCAA. In the current study, Arg and Trp average and basal plasma concentrations were lower in LRFI pigs when housed in poor compared with good housing conditions whereas the challenge did not affect plasma concentrations of these two AA in HRFI pigs. Besides being proteinogenic AA, both Trp and Arg are known to be involved in immune-related metabolic pathways. During immune hyper-activation, Trp is catabolized in kynurenine by the indoleamine 2,3-dioxygenase (IDO) enzyme, a metabolic pathway involved in the regulation of immune responses [25]. Arginine is an AA serving as a precursor for the synthesis of polyamines that is massively used by rapidly dividing cells like proliferating lymphocytes [26]. It is also involved in the synthesis of creatine that has anti-oxidative and anti-inflammatory functions [27]. Lower C0 values for Trp and Arg may reflect an effect of immune activation that is independent of postprandial use of AA for muscle anabolism. Besides, for Trp, the time-related variations differed between hygiene conditions in LRFI pigs with earlier time at maximum plasma concentration (Tmax) in poor than those housed in good conditions. Such results

might be a consequence of faster postprandial clearance of dietary Trp for both immune responses and muscle anabolism. If genetic selection for LRFI reduces the total tract digestive capacity of pigs challenged with bacterial endotoxin [28], a significant contribution of the digestive tract to Trp postprandial utilization seems unlikely since Trp is not extensively used by the gut [29]. To summarize, lower average concentrations of Trp and Arg in LRFI pigs may result from an increased utilization of these two AA for immune purposes and may contribute to support the greater ability of LRFI pigs to cope with poor hygiene conditions and to maintain their growth rate in challenging conditions [8]. When housed in poor hygiene conditions, HRFI pigs had greater average concentrations and maximum plasma concentration (Cmax) of Lys than those housed in good conditions whereas no difference between hygiene was reported in LRFI pigs. Greater plasma Lys concentrations are probably a consequence of lower body protein synthesis and reduced efficiency of nitrogen utilization for protein retention in pigs when the immune system is overstimulated [30]. Accordingly, Chatelet et al. [8] reported that ADG of the HRFI pigs was more affected by poor hygiene conditions with a difference in ADG between poor- and good-HRFI pigs being twice the differences observed between poor- and good-LRFI pigs.

In conclusion, our results show that insulin, energy metabolite, and AA postprandial profiles differ between LRFI and HRFI pigs in response to poor hygiene of housing conditions. The findings that only LRFI had lower concentrations of Trp and Arg in poor hygiene of housing conditions may be associated with a greater AA utilization for supporting their coping ability and growth. Conversely, only HRFI pigs had greater concentrations of Lys in poor hygiene conditions, which is in line with the greater impact of the challenge on protein deposition. Although our results clearly showed

that selection for RFI modified the metabolic response to the hygiene challenge, it is not possible to determine whether selection has first modified the immune response or some metabolic pathways.

# Data reporting

The data were deposited in DataINRAE repository: https://doi.org/10.15454/U0RH9F

# Acknowledgements

The authors thank the staff of INRAE PEGASE and UE3P for their collaboration. This study was supported by the European Union's Seventh Framework Programme for Research, Technological Development and Demonstration (grant number 613574, PROHEALTH project). Alícia Zem Fraga was supported by a scholarship from the São Paulo Research Foundation (FAPESP-Brazil; grant number 2018/15559-7 and fellowship number 2018/11807-6).

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1 **Fig 1.**

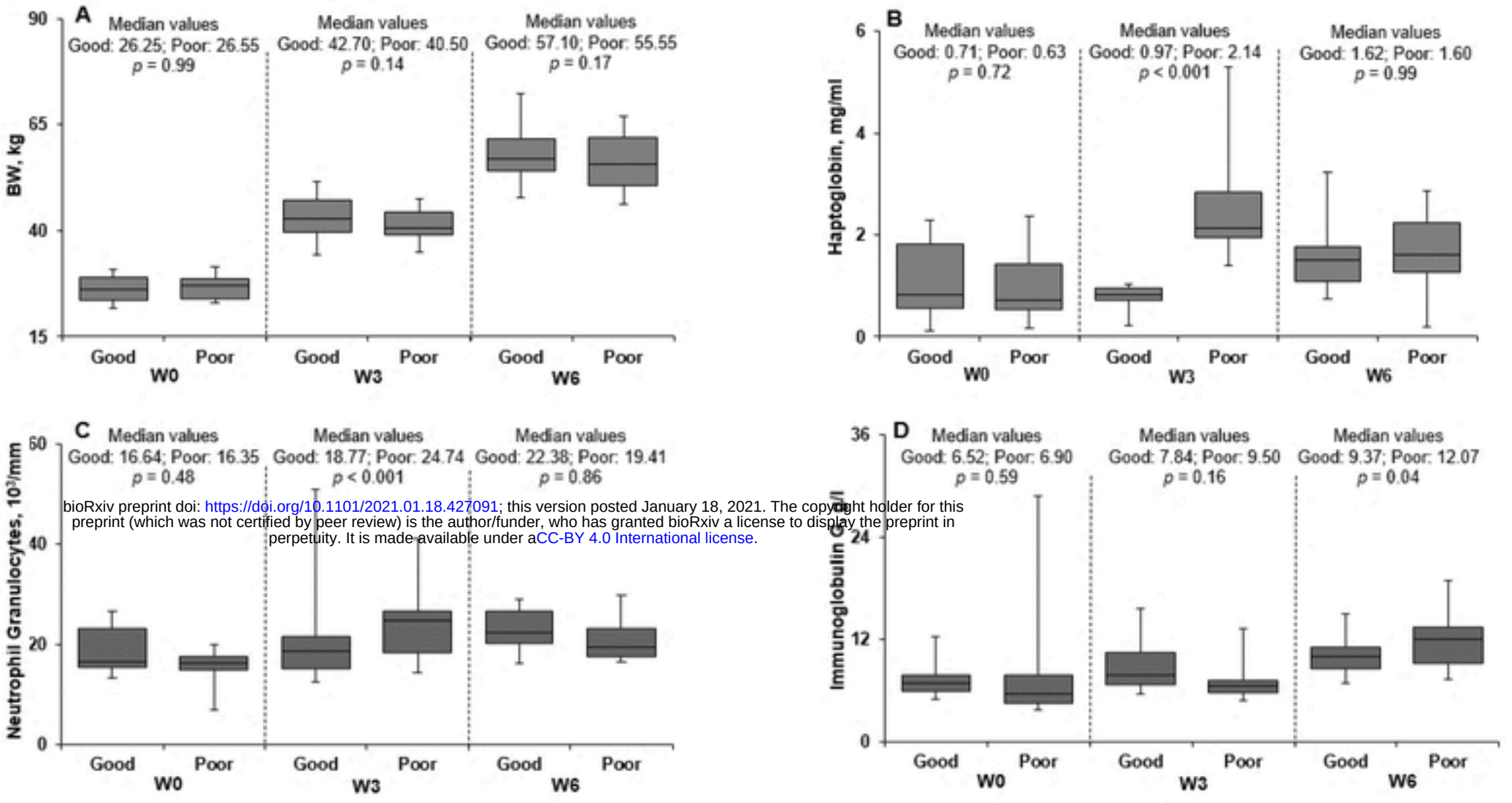




Figure 1

1 **Fig 2.**



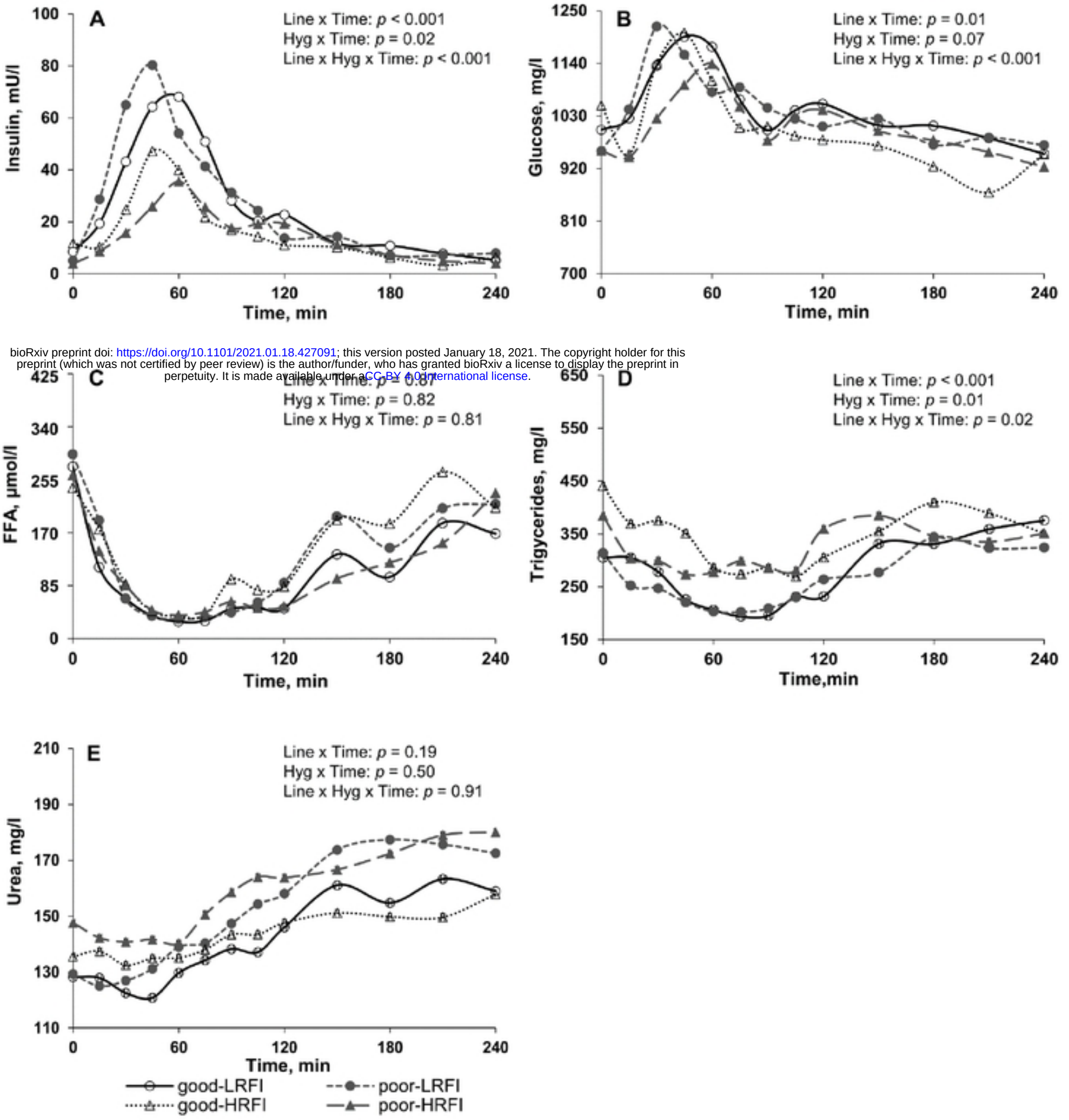


Figure 2

1 **Fig 3.**

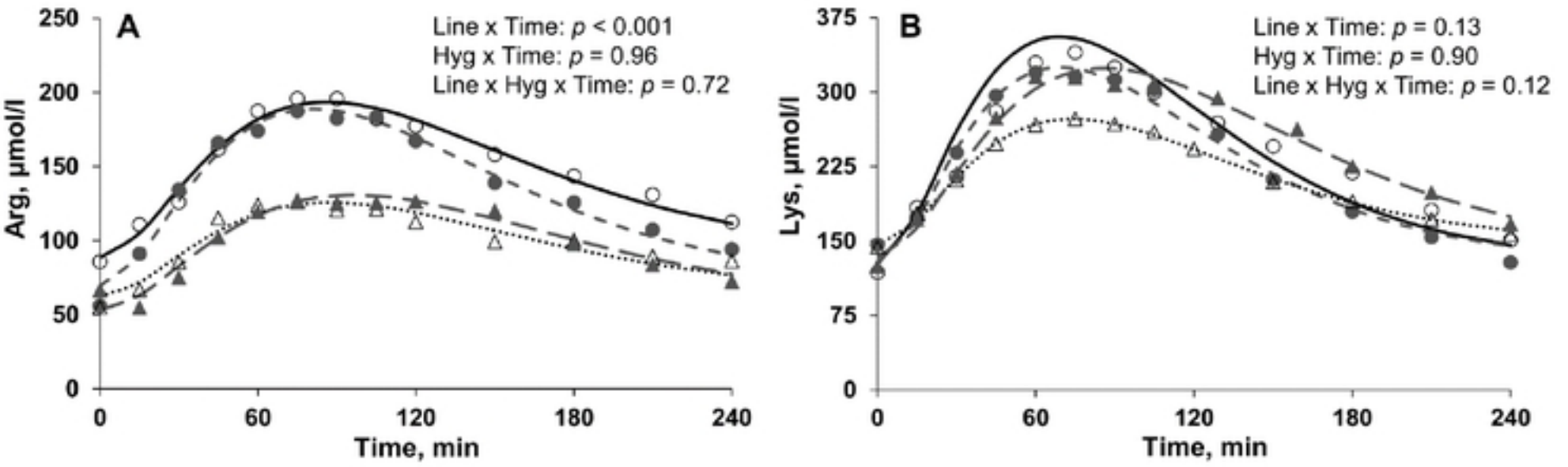




Figure 3