Animal 17 (2023) 101029

Contents lists available at ScienceDirect

Animal
The international journal of animal biosciences

Comparative targeted metabolomic profiles of porcine plasma and serum

Samuele Bovo [a], Giuseppina Schiavo [a], Giuliano Galimberti [b], Flaminia Fanelli [c], Francesca Bertolini [a], Stefania Dall'Olio [a], Uberto Pagotto [c], Luca Fontanesi [a,*]

[a] Department of Agricultural and Food Sciences, Division of Animal Sciences, University of Bologna, 40127 Bologna, Italy
[b] Department of Statistical Sciences "Paolo Fortunati", University of Bologna, 40126 Bologna, Italy
[c] Department of Surgical and Medical Sciences, Endocrinology Unit, University of Bologna, 40138 Bologna, Italy

ARTICLE INFO

Article history:
Received 7 July 2023
Revised 1 November 2023
Accepted 2 November 2023
Available online 10 November 2023

Keywords:
Animal model
Biofluid
Metabolite
Pig
Sus scrofa

ABSTRACT

Metabolomics has been used to characterise many biological matrices and obtain detailed pictures of biological systems based on many metabolites. Plasma and serum are two blood-derived biofluids commonly used to assess and monitor the organismal metabolism and obtain information on the physiological and health conditions of an animal. Plasma is the supernatant that is separated from the cellular components after centrifugation of the blood that is first added with an anticoagulant. Serum is obtained after centrifugation of the blood that has been coagulated. The choice of one or the other biofluid for metabolomic analyses is related to specific analytical needs and technical issues, to problems derived by the collection and preparation steps, in particular when specimens are sampled from animals involved in field studies. Thus far, most of the metabolomic studies that compared plasma and serum have been carried out in humans and very little is known on the pigs. In this study, we used a targeted metabolomic platform that can detect about 180 metabolites of five biochemical classes to compare plasma and serum profiles of samples collected from 24 pigs. To also obtain a cross-species comparative metabolomic analysis, information for human plasma and serum derived from the same platform was retrieved from previous studies. Statistical analyses included univariate and multivariate approaches aimed at identifying stable and/or differentially abundant metabolites between the two porcine biofluids. A total of 154 (~83%) metabolites passed the initial quality control, indicating a good repeatability of the analytical platform in pigs. Discarded metabolites included aspartate and biogenic amines that were already reported to be unstable in human studies. More than 80% of the metabolites had similar profiles in both porcine biofluids (average correlation was 0.75). Concentrations were usually higher in serum than in plasma, in agreement with what was already reported in humans. The univariate analysis identified 44 metabolites that had statistically different concentrations between porcine plasma and serum, of which 28 metabolites were also confirmed by the multivariate analysis. The obtained picture described similarities and differences between these two biofluids in pigs and the related human-pig comparisons. The obtained information can be useful for the choice of one or the other matrix for the implementation of metabolomic studies in this livestock species. The results can also provide useful hints to valuing the pig as animal model, in particular when metabolite-derived physiological states are relevant.

Implications

This study showed that porcine plasma and serum metabolomic profiles are similar, even if differences for a few metabolites are present. Some metabolites were also quite unstable or difficult to quantify. What was observed in the two biofluids in pigs matched the profiles already reported in humans but, again, several metabolites had contrasting profiles in the two species. The results will serve as a reference point for about 180 biomolecules of plasma and serum in pigs. The obtained information will be useful to value the pig as animal model when metabolomic profiles are useful to link physiological and health conditions.

Introduction

A variety of biofluids (e.g., blood, urine, saliva, milk, rumen fluid) can be sampled in livestock and then used for metabolomic

* Corresponding author.
E-mail address: luca.fontanesi@unibo.it (L. Fontanesi).

https://doi.org/10.1016/j.animal.2023.101029
1751-7311/

analyses. The obtained profiles can disclose valuable information to understanding the physiological states of the animals and assessing the effect of different nutrients, feeding strategies, treatments, pathological conditions, and genetic potentials, among many other factors that may vary the metabolic profiles of the animals (Ametaj et al., 2010; Sun et al., 2015; Fontanesi, 2016; Goldansaz et al., 2017).

Blood is a commonly used biofluid as, according to its circulating nature, its metabolic profile and cellular components provide a global snapshot of clinical relevance that reflects an overall condition of the whole organism at the sampling time point. This profile can be useful to investigate the effect of applied direct or indirect perturbations. Blood is composed of two main components: (i) the cellular fraction, constituted by red blood cells, white blood cells and platelets and (ii) the liquid fraction, which is a straw-coloured carrier that accounts for about 50–55% of blood volume (Luque-Garcia and Neubert, 2007). Plasma and serum are two blood-derived biofluids mostly used in blood metabolomic studies. Plasma is the supernatant that is separated from the cellular components after centrifugation of the blood that is first added with an anticoagulant (e.g., ethylenediaminetetraacetic acid known as **EDTA**, sodium citrate and heparin), which inhibits the blood from clotting. Serum is obtained after centrifugation of the blood that has been coagulated (Luque-Garcia and Neubert, 2007).

Blood metabolomics can be applied both to whole blood and to its derived biofluids. However, the choice of the biological matrix may be due to peculiar analytical needs or by logistic and technical issues related to the sampling, handling of the specimens and preparation steps, particularly when samples are collected in animals involved in field studies. Metabolomics applied to whole blood would allow to capture additional molecules, such as cofactors and antioxidants, both highly present and characterizing red blood cells but that would be lost when applying plasma or serum metabolomics (Nagana Gowda and Raftery, 2023). However, as they are considered unstable, handling these cell-derived metabolites is currently one of the major bottlenecks of whole blood metabolomics, leaving the space to the characterisation of more stable molecules from the two blood-derived biofluids (Gil et al., 2015). Compared to serum, plasma preparation is usually considered more reproducible and faster since there is no need to wait for the blood to clot, it has a lower risk of haemolysis and thrombocytosis, and it is not usually affected by any postcentrifugal coagulation interference which can occur in serum (Hsieh et al., 2006; Luque-Garcia and Neubert, 2007). On the other hand, plasma could still contain some platelets originally present in the blood, as centrifugation might not always be able to completely remove these cells, which, in turn, can alter the metabolic content of this biofluid (Lesche et al., 2016). Both plasma and serum contain about 95% water and several other components, but the process of coagulation makes serum qualitatively different from plasma because, in addition to all cells that are removed, clotting removes fibrin clots and related coagulation factors, which are left in the plasma (Luque-Garcia and Neubert, 2007).

Although the clotting process makes these two biofluids different for several clinical-relevant components (such as metal ions, proteins and enzymes), plasma and serum can be used interchangeably in many laboratory assays (Luque-Garcia and Neubert, 2007). In terms of small molecules (i.e., metabolites), these two matrices have very similar compositions, which do not substantially alter their gross metabolome profile, as shown in human blood-derived biofluids (Liu et al., 2010; Psychogios et al., 2011; Wedge et al., 2011; Yu et al., 2011; Breier et al., 2014; Suarez-Diez et al., 2017). However, more detailed investigations, that compared these two biofluids in humans, indicated that serum contains higher metabolite concentrations than plasma for a few metabolite classes and that additional minor differences or absence of some metabolites in one or the other biofluid occur (Yu et al., 2011; Liu et al., 2018b; Kiseleva et al., 2021; Sotelo-Orozco et al., 2021; Vignoli et al., 2022). It is however not clear if similar differences are also present between plasma and serum prepared from pigs.

Few studies carried out in pigs analysed both blood matrices in the same experimental design. Bovo et al. (2016) investigated plasma and serum-targeted metabolomic profiles to identify differences between two pig breeds. Other studies carried out in pigs for several different aims analysed only one of the two biofluids, and comparisons of their metabolomic profiles could not be possible (Solberg et al., 2010; Bovo et al., 2015; Metzler-Zebeli et al., 2015; Liu et al., 2018a; Luise et al., 2020; Wu et al., 2020).

In this study, we used a targeted metabolomic analytical approach, which measured about 180 metabolites belonging to several biochemical classes, coupled with a specifically applied statistical methodology, to investigate differences in metabolomic profiles between plasma and serum prepared from pigs. The results that we obtained in the two porcine biofluids were then used for a comparative analysis against the human plasma and serum metabolomic profiles reported by previous studies with the same targeted metabolomic platform (Yu et al., 2011; Breier et al., 2014). The results obtained by comparing the metabolomic profiles of the two porcine biofluids and the pig-human comparative analysis provided interesting information (i) that will serve as reference point in pigs, (ii) that will be useful to choose one or the other biofluid in studies aimed to defining biomarkers of physiological perturbations in pigs, and (iii) that will be relevant to value the pig as animal model when links with the human physiological conditions can be described at the metabolome level.

Material and methods

All pigs used in this study were kept according to the Italian and European legislations for pig production. All described procedures followed the Italian and European Union regulations for animal care and slaughter. Pigs were not raised or treated in any way for the purpose of this study. All animals were slaughtered in a commercial and authorised abattoir following standard procedures. Therefore, no other ethical statement is needed.

Pigs and blood samples, plasma and serum

A total of 24 healthy pigs were included in this study: 12 Italian Large White and 12 Italian Duroc pigs. Six castrated males and six entire gilts were considered in each breed. Animals were part of the same batch, fed and handled in the same ways. When animals were about 155 ± 5 kg live weight, they were subject to a fasting period of ~12 h, transported to a commercial abattoir and slaughtered in the morning at about 0800 h, after electrical stunning. Animals entered the slaughtering plan within 5 min, and blood was collected just after jugulation directly from the draining carotid artery into two different tubes (the serum tube included a gel separator and clot activator). For all animals, samples were processed within two hours, including the centrifugation step at 4 °C. We obtained 12 tubes of plasma and 12 tubes of serum that were divided into aliquots and frozen at −80 °C till metabolomic analysis. Additional details on the pigs, their feeding, the slaughtering and blood sampling procedures and the processing of the collected blood are given by Bovo et al. (2016).

Metabolomic analyses of pig samples and quality control

A targeted metabolomic approach was adopted to explore the plasma and serum metabolomes of the pigs. Analyses were based on the Biocrates AbsoluteIDQ^{TM} p180 kit (Biocrates Life Science AG, Innsbruck, Austria) that allows to quantify a panel of a total of 186

metabolites, covering seven analyte subclasses: acylcarnitines (n. 40), amino acids (n. 21), biogenic amines (n. 19), monosaccharides (hexoses including glucose; n. 1), lyso-phosphatidylcholines (n. 14), phosphatidylcholines (n. 76) and sphingomyelins (n. 15). Samples were included in one single Biocrates plate, and metabolomic analyses were run on an analytical platform composed by a Series 200 HPLC system (Perkin Elmer, Inc., Waltham, MA, USA) coupled with an API 4000 QTrap mass spectrometer (AB-Sciex, Framingham, MA, USA). The different analyte classes were subjected to either liquid chromatography-tandem mass spectrometry or flow injection analysis-tandem mass spectrometry. The Biocrates plate also included three replicated samples that were used as quality control standards to evaluate the quantification reliability. Based on quality controls, a CV was derived for each small molecule; metabolites presenting a CV > 20% were excluded from the bioinformatic analyses. More details about the metabolomic analyses, the list of targeted metabolites and data quality check and filtering procedures are reported in Bovo et al. (2015 and 2016) and in Supplementary Table S1.

Statistical analyses for pig plasma and serum metabolites

For each metabolite, Pearson's correlation coefficient was used to evaluate the agreement between plasma and serum measurements. A nominal $P < 0.05$ was used to consider correlation coefficients statistically valid.

Differences between the two porcine blood-derived biofluids and the identification of differentially abundant metabolites were tested by applying univariate and multivariate statistics as follows. Principal Component Analysis (**PCA**) was run on the quality-checked dataset. Before PCA, variables (metabolite concentrations) were scaled to have unit variance. Considering the paired structure of the data, Wilcoxon signed-rank test was used to assess, in a univariate way, differences in metabolite abundances. Differences were evaluated at a nominal $P < 0.05$ and considering the Bonferroni correction ($P < 0.05/154$). We then applied Sparse Multi-Level Partial Least Squares Discriminant Analysis (**sML-PLS-DA**) as supervised multivariate approach to detect differentially abundant metabolites. Briefly, two matrices describing the within-subject variation and between-subject variation were obtained (Westerhuis et al., 2010), the former subjected to sparse PLS-DA for variable selection as described by Luise et al. (2020).

We also evaluated the relative concentration difference between serum and plasma (**Δ%**; Bovo et al., 2016) for each metabolite (i), expressed as $\Delta\%_i = \frac{\bar{x}_i^S - \bar{x}_i^P}{\bar{x}_i^S} \times 100$, where $\bar{x}_i^S$ and $\bar{x}_i^P$ denote the average metabolite abundance of the i^{th} metabolite in serum and plasma, respectively.

To evaluate the relationship between unique pairs of metabolites we used Spearman's Rank correlation coefficient ($\boldsymbol{\rho}$). Serum and plasma datasets were analysed separately. Two correlation matrices were obtained and then used to evaluate the difference between the two pig biofluids by subtraction of matrices and obtaining a third correlation matrix (serum minus plasma).

Correlations, Wilcoxon signed-rank test P and Δ% measures were calculated within each breed and for the combined overall population (i.e., all 24 pigs considered together, analyses at the pig population level). PCA and sML-PLS-DA were run at the population level only. All analyses were carried out in R v.3.4.2 (R Development Core Team, 2022).

Human plasma and serum metabolomic profiles used in the comparative analyses

Results of two studies carried out in humans, obtained by using the same Biocrates targeted metabolomic platform, applied to compare the concentration of plasma and serum metabolites, were retrieved from the related literature (Yu et al., 2011; Breier et al., 2014). The study of Yu et al. (2011) reported the concentration of 122 metabolites measured in plasma and serum samples collected from 83 healthy adult humans (about 50% males and 50% females, with age that ranged from 51 to 84 years) and the study of Breier et al. (2014) reported the concentration of 159 metabolites measured in plasma and serum samples collected from 20 healthy humans with a mean age of 30 years (1/4 males and 3/4 females). These studies can be considered to have a compatible experimental design with that of our study in pigs, where one sampling time point of adult animals was considered and plasma and serum were sampled with the same anticoagulant and clotting system used in the human studies. Supplementary Table S2 reports information on the analysed metabolites in these two human studies, the list of filtered metabolites, their average plasma and serum concentration used to compute Δ% statistics, and the Wilcoxon signed-rank test P-values used in the comparative analyses with the results obtained in pigs.

Results

Pig metabolomic profiles: Filtered metabolites

A total of 154 out of 186 analysed metabolites (~83%) passed the quality control step as they presented a CV% < 20. Of the 154 retained pig metabolites, a total of 62 metabolites (~40%) had a CV% ≤ 5 and a total of 109 metabolites (~70%) had a CV% ≤ 10. The complete list of the retained and discarded metabolites and their CV%s is reported in Supplementary Table S1.

Six metabolites (aspartate, histamine, 3-nitro-tyrosine, cis-4-Hydroxyproline, phenylethylamine, symmetric dimethylarginine) had measured concentrations lower than the limit of detection in the QCs replicates. Four of these metabolites (histamine, 3-nitro-tyrosine, cis-4-Hydroxyproline and phenylethylamine) had the same problems also in the study of Breier et al. (2014) who analysed human plasma and serum samples [(no problems related to the limit of detection were reported by Yu et al. (2011)].

Of the 32 metabolites that were discarded either because of CV% or limit of detection issues (26 and six metabolites, respectively), 13 were also excluded from subsequent analyses by Breier et al. (2014) and 17 (out of 25 possible matches; the study lacks the quantification of biogenic amines) were also excluded from further analyses by Yu et al. (2011) for problems of potential instability or heterogeneity of results in humans (Supplementary Tables S1 and S2). Seven of these metabolites (C16:1-OH, C18:1-OH, lysoPC a C24:0, lysoPC a C26:0, PC aa C26:0, PC aa C30:2, SM C22:3), that showed high CV% and that might represent highly unstable metabolites, were discarded by our study in pigs and by both studies in humans. The Venn diagrams reported in Supplementary Fig. S1 show the overlapping set of metabolites that were retained and discarded in our study with those that were considered by the two studies in humans (Yu et al., 2011; Breier et al., 2014).

Differences between plasma and serum metabolomic profiles in pigs

Pearson's correlation coefficients were calculated for each metabolite over the two biofluids, within each breed and at the population level, i.e. considering the analysed pigs altogether (Supplementary Tables S3–S5). Correlations had a nominal $P < 0.05$ for 94 (61%), 116 (75%) and 129 (84%) out of 154 metabolites, for Italian Duroc, Italian Large White and all pigs together, respectively. For these metabolites, correlations ranged from $r = 0.57$ (glutamine) to $r = 0.98$ (acetylornithine), from $r = 0.55$ (C14:2) to $r = 0.99$ (C2), and from $r = 0.40$ (C16-OH) to $r = 0.98$ (acetylor-

nithine), for the three groups of pigs (i.e., Italian Duroc, Italian Large White and all pigs together), respectively. When correcting the *P*, the number of significant correlations decreased to 16 (min $r = 0.83$), 64 (min $r = 0.82$) and 94 (min r = 0.67) metabolites for Italian Duroc, Italian Large White and all pigs together, respectively. Considering the correlations irrespectively from their significance level, we observed an overall medium–high value between the two matrices, with a mean ± SD of $r = 0.77 \pm 0.10$ (median = 0.78), $r = 0.85 \pm 0.11$ (median = 0.88), $r = 0.75 \pm 0.15$ (median = 0.81) for Italian Duroc, Italian Large White and the whole population, respectively. We observed an overall low correlation for acylcarnitines (Fig. 1a).

Differences between plasma and serum profiles were first investigated using the unsupervised multivariate approach of PCA. The first two principal components accounted for 25 and 15% of the total variance, respectively. The effect of the biological matrix is only slightly evident, as captured by the principal component 2 (Fig. 1b).

According to the Wilcoxon signed-rank test and applying a nominal $P < 0.05$, a total of 65 (Italian Duroc), 86 (Italian Large White) and 98 (all pigs) metabolites had concentration differences between the two biofluids. All results of this analysis are reported in Supplementary Tables S3–S5. Fifty-five metabolites were in common between the two breeds, when the test was run separately in Italian Large White and Italian Duroc groups. Fig. 1a shows both Δ% and *r* values for the whole profile considered in all investigated pigs. In general, considering Δ%, concentrations were higher in serum than in plasma. After applying Bonferroni correction, however, significant results remained for a total of 44 metabolites (28.4%) only when all pigs were analysed together (Table 1 and Supplementary Table S5). These 44 metabolites belong to all the investigated metabolite classes except for monosaccharides. A total of 33 out of 44 metabolites had a concentration higher in serum than in plasma. Amino acids and acylcarnitines were two analyte classes that had only higher concentration in serum. Among the metabolites with significantly higher concentration in serum, five out of 33 had a $\Delta\% > 20$ (arginine, C10:2, putrescine, taurine and serotonin) whereas for metabolites with Bonferroni significantly higher concentration in plasma, eight out of 11 had a $\Delta\% < -20$ (spermine, spermidine, acetylornithine, PC aa C42:0, PC ae C42:0, PC aa C42:1, PC ae C42:1, PC aa C40:1, PC ae C30:0, PC aa C28:1). The metabolites with the largest differences were serotonin ($P = 1.19 \times 10^{-7}$, $\Delta\% = 74.3$) and spermine ($P = 2.20 \times 10^{-5}$; $\Delta\% = -40.5$).

After these evaluations, we applied sML-PLS-DA, a supervised multivariate approach able to select the most discriminant variables (i.e., metabolites) taking into consideration the paired structure of the dataset. Based on the sML-PLS-DA scores t_W1 and t_W2 obtained from the analysis of the within-subject variation matrix, results demonstrated that the metabolomic profiles clearly clustered into two groups representing the plasma and serum biological matrices (Fig. 1c). In particular, separation of the two clusters was due to 28 selected metabolites belonging to five metabolite subclasses (two acylcarnitines, five amino acids, four biogenic amines, 16 phosphatidylcholines and one sphingolipid) having a non-zero regression coefficient. Selected metabolites had a generally higher concentration in plasma (except for six metabolites). It was also interesting to note that all 28 metabolites selected with the multivariate approach were also selected by using the univariate approach. Among these 28 metabolites, only 17 were differentially abundant with a significant correlation between the two biofluids (correlations with nominal $P < 0.05$ in both breeds; correlation at the population level with $P < 0.05$, Bonferroni corrected). Putrescine, asparagine, and lysoPC a C17:0 had the highest (absolute value) regression weights, with absolute delta values that ranged from 8 to 31%.

We then evaluated the relationship between unique metabolite pairs. Fig. 2 shows the correlation matrices (based on Spearman's Rank correlation coefficient) obtained for serum (Fig. 2a) and plasma (Fig. 2b) metabolites. Data are reported in Supplementary Table S6. Both biofluids had medium–high correlation coefficients between metabolites belonging to the same metabolite class whereas correlations between classes were lower. Comparison of correlation coefficients between the two biofluids (Fig. 2c; delta correlation matrix) highlighted stable within-class metabolite correlations. A total of 2 968 and 2 335 correlations (involving 147 and 146 metabolites) had $P < 0.05$ ($|\rho| > 0.405$) in serum and plasma, respectively. By merging these results, a total of 2 029 metabolite pairs had a relationship in at least one biofluid and 1 638 metabolite pairs had a relationship in both. A total of 24 differentially abundant metabolites (out of the 28 identified) had statistically significant correlations. Of those, high difference in delta correlation was observed for the serotonin-taurine metabolite pair [that moved from $\rho = 0.84$ ($P = 3.6 \times 10^{-07}$) in plasma to $\rho = 0.45$ ($P = 0.028$) in serum] and the C14:1-C18 metabolite pair [that moved from $\rho = 0.48$ ($P = 0.015$) in plasma to $\rho = 0.90$ ($P = 2.3 \times 10^{-09}$) in serum].

For the most significant metabolite pairs ($P < 0.001$; $|\rho| > 0.629$), we then evaluated their connectivity (number of

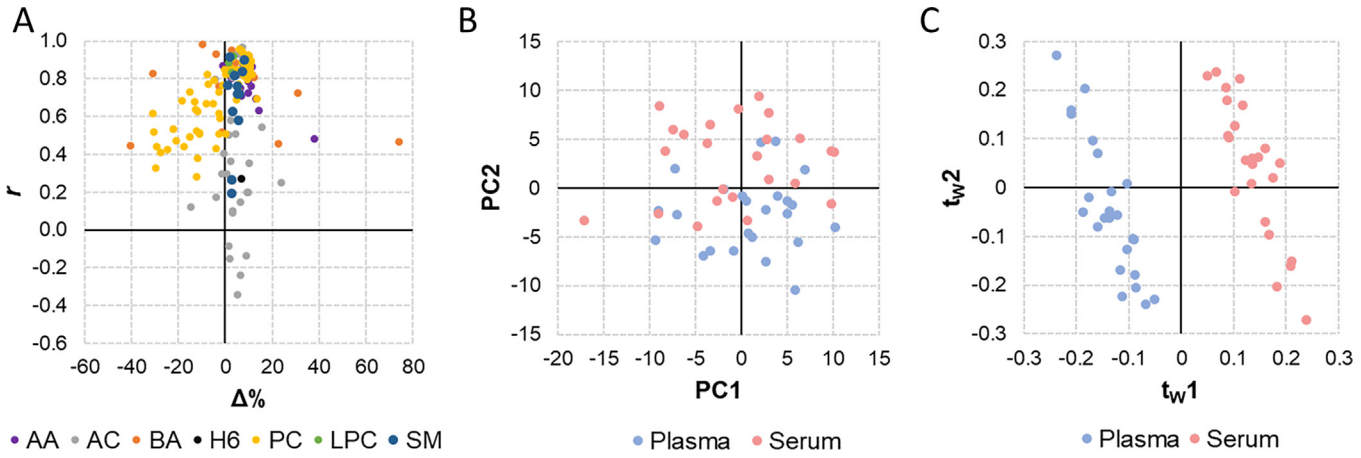

Fig. 1. Representations of metabolomic differences between porcine plasma and serum. (A) Pearson's correlation coefficient (*r*) and Δ% values are presented; each point represents a metabolite; AA: amino acid; AC: acylcarnitine; BA: biogenic amine, H1: hexoses, LPC: lyso-phosphatidylcholine, PC: phosphatidylcholine, SM: sphingomyelin; (B) Principal Component Analysis (PCA); each point represents a pig sample; (C) Sparse Multilevel Partial Least Squares Discriminant Analysis (sML-PLS-DA); each point represents a pig sample.

Table 1
Metabolites (n. 44) that showed differences between serum and plasma concentration in the 24 analysed pigs. Metabolites are listed from the lowest to the highest Δ% value.

Class[1]	Metabolite[2]	Plasma (mean ± SD)[3]	Serum (mean ± SD)[3]	*r* (pigs)[4]	Δ% (pigs)[5]	sML-PLS-DA[6]	*r* (humans)[7]	Humans (S/P)[8]	Humans (S/P)[9]
BA	Spermine	0.56 ± 0.13	0.40 ± 0.08	0.448*	−40.51	−0.555	–	–	–
PC	PC aa C28:1	1.17 ± 0.27	0.89 ± 0.29	0.615#	−31.02	−0.443	0.856	S	S
BA	Spermidine	0.82 ± 0.31	0.63 ± 0.25	0.826	−30.57	0.000	–	–	NA^
PC	PC aa C42:0	0.18 ± 0.03	0.14 ± 0.03	0.329	−29.20	−0.645	0.895	S^	NA^
PC	PC ae C42:1	1.61 ± 0.36	1.25 ± 0.33	0.442	−28.80	0.000	0.731	S^	NA^
PC	PC aa C42:1	0.13 ± 0.02	0.10 ± 0.02	0.423	−24.51	−0.696	0.837	S	NA^
PC	PC ae C30:0	0.50 ± 0.10	0.41 ± 0.10	0.532	−22.11	0.000	0.884	S	S
PC	PC aa C40:1	0.36 ± 0.05	0.30 ± 0.06	0.474	−21.07	−0.444	0.790	S	NA^
LPC	lysoPC a C17:0	1.07 ± 0.22	0.92 ± 0.18	0.840*;#	−16.81	−0.667	0.825	S	S
PC	PC ae C42:0	0.58 ± 0.09	0.52 ± 0.09	0.676	−12.82	0.000	0.488	S	NA^
BA	Acetylornithine	10.50 ± 4.92	9.61 ± 4.50	0.984*;#	−9.26	0.000	–	–	NA^
SM	SM C18:1	3.28 ± 0.47	3.57 ± 0.52	0.903*;#	8.12	0.155	0.772	S	S
PC	PC ae C38:5	3.79 ± 0.56	4.13 ± 0.75	0.891*;#	8.23	0.000	0.750	S	S
PC	PC aa C38:5	30.80 ± 4.65	33.70 ± 5.03	0.824*;#	8.61	0.000	0.879	S	S
PC	PC aa C38:4	145.00 ± 24.35	159.00 ± 26.15	0.883*;#	8.81	0.025	0.856	S	S
PC	PC ae C36:4	3.57 ± 0.56	3.93 ± 0.75	0.884*;#	9.16	0.000	0.794	S	S
PC	PC aa C36:1	37.80 ± 5.93	41.70 ± 6.03	0.796*;#	9.35	0.000	0.867	S	S
AA	Asparagine	51.30 ± 10.72	56.60 ± 10.80	0.917*;#	9.36	0.447	–	–	S
AA	Leucine	214.00 ± 29.39	237.00 ± 34.57	0.840*;#	9.70	0.139	0.677	S^	S
PC	PC ae C36:3	3.43 ± 0.63	3.80 ± 0.64	0.863*;#	9.74	0.041	0.823	S	S
AA	Methionine	47.60 ± 9.45	52.80 ± 9.66	0.871*;#	9.85	0.000	0.670	S	NA^
PC	PC aa C34:1	67.60 ± 11.31	75.00 ± 13.21	0.897*;#	9.87	0.057	0.896	S	S
PC	PC ae C34:2	5.10 ± 0.92	5.66 ± 0.98	0.848*;#	9.89	0.000	0.837	S	S
PC	PC aa C40:5	19.70 ± 3.55	21.90 ± 3.94	0.928*;#	10.05	0.152	0.892	S	S
PC	PC aa C36:3	23.60 ± 4.71	26.30 ± 5.14	0.919*;#	10.27	0.074	0.889	S	S
PC	PC ae C36:2	5.06 ± 1.00	5.65 ± 1.15	0.903*;#	10.44	0.017	0.861	S	S
PC	PC aa C36:4	56.30 ± 8.92	63.00 ± 9.97	0.836*;#	10.63	0.031	0.851	S	S
PC	PC aa C38:3	18.30 ± 3.14	20.50 ± 3.79	0.864*;#	10.73	0.029	0.884	S	S
PC	PC ae C38:4	6.88 ± 1.09	7.71 ± 1.53	0.885*;#	10.77	0.000	0.745	S	S
PC	PC aa C34:2	91.80 ± 17.94	103.00 ± 19.53	0.892*;#	10.87	0.047	0.850	S	S
PC	PC aa C36:2	112.00 ± 16.19	126.00 ± 18.56	0.825*;#	11.11	0.093	0.847	S	S
PC	PC aa C34:3	3.03 ± 0.58	3.41 ± 0.63	0.893*;#	11.14	0.076	0.886	S	S
AA	Proline	198.00 ± 35.80	223.00 ± 39.92	0.822*;#	11.21	0.000	0.889	S	S
AA	Histidine	110.00 ± 18.87	124.00 ± 18.53	0.763*;#	11.29	0.000	0.589	S	S
AA	Tyrosine	88.60 ± 18.24	100.00 ± 22.40	0.865*;#	11.40	0.000	0.708	S	S
BA	Sarcosine	11.30 ± 2.31	12.90 ± 2.86	0.807*;#	12.40	0.000	–	–	P
AA	Phenylalanine	83.40 ± 11.04	96.00 ± 13.05	0.696*;#	13.13	0.180	0.575	S	S
AA	Serine	118.00 ± 18.50	138.00 ± 19.58	0.632	14.49	0.142	0.774	S	S
AC	C3-DC (C4-OH)	0.05 ± 0.01	0.06 ± 0.01	0.546	15.66	0.284	0.056	–	–
BA	Taurine	160.00 ± 34.14	206.00 ± 24.03	0.454	22.33	0.440	–	–	S
AC	C10:2	0.02 ± 0.00	0.03 ± 0.00	0.248	23.83	0.411	0.934	–	S
BA	Putrescine	0.91 ± 0.25	1.31 ± 0.32	0.727*;#	30.69	0.469	–	–	S
AA	Arginine	141.00 ± 25.33	229.00 ± 26.45	0.485	38.43	0.455	0.500	S	S
BA	Serotonin	1.37 ± 1.16	5.33 ± 1.98	0.465	74.30	0.515	–	–	S

[1] AA: amino acid; AC: acylcarnitine; BA: biogenic amine; LPC: lyso-phosphatidylcholine; PC: phosphatidylcholine, SM: sphingomyelin.
[2] Metabolites with $P < 0.05$ (Bonferroni corrected) at the Wilcoxon signed-rank test. Full names are given in Supplementary Table S1.
[3] Concentrations are expressed in μM.
[4] Pearson's correlation coefficient (*r*) between serum and plasma concentrations. Metabolites with $P < 0.05$ (Bonferroni corrected) at the pig population level are marked with the star (*) symbol. Metabolites with nominal $P < 0.05$ in both single breeds are marked with the hash (#) symbol.
[5] Positive values indicate higher values in serum than in plasma and *vice versa*.
[6] Regression coefficient. Non-zero values indicate metabolites contributing to differentiate plasma and serum samples. Positive values indicate higher concentrations in serum than in plasma and *vice versa*.
[7] Correlation coefficients as retrieved from the study of Yu et al. (2011). *P*-value was not provided by the authors. Data are reported for the metabolites included in the Biocrates p150 kit (Biocrates Life Science AG, Innsbruck, Austria) and that passed the quality control.
[8] S: higher value in human serum. P: higher value in human plasma.^statistical non-significant difference. Data are reported from the study of Yu et al. (2011) for those metabolites included in the Biocrates p150 kit (Biocrates Life Science AG, Innsbruck, Austria) and that passed the quality control.
[9] S: higher value in human serum. P: higher value in human plasma. NA: info not provided; only the *P* is available.^statistically non-significant difference ($P > 0.01$). Data are reported from the study of Breier et al. (2014) for the metabolites included in the Biocrates p150 kit (Biocrates Life Science AG, Innsbruck, Austria) and that passed the quality control.

linked metabolites to a given metabolite; Supplementary Table S7). Connectivity was generally higher in serum than in plasma (78 metabolites had more connections in serum than in plasma; 34 metabolites had more connections in plasma than in serum) with a total of 873 involved correlations (metabolite pairs) in serum and plasma, respectively, and involving a similar number of metabolites (110 and 106). Arginine and PC aa C32:2 were the two metabolites that gained and lost more connections, respectively, when the serum and the plasma matrices were considered, respectively.

Comparative metabolomic profiles between pigs and humans

To provide a first comparative picture of plasma and serum metabolomic profiles between humans and pigs, we used the information retrieved from the study of Yu et al. (2011). These authors obtained targeted metabolomic profiles of human plasma and serum using a previous version of the Biocrates platform that detected a total of 163 metabolites. Out of these 163 metabolites, 122 passed the quality control and for 104 their concentration differed between the two biofluids, with a prevalence of higher level

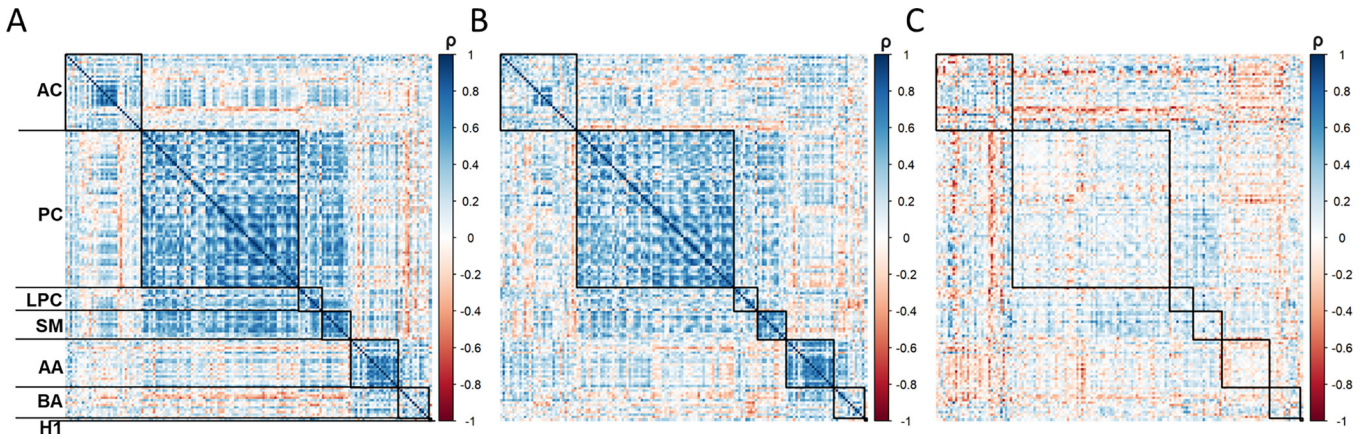

Fig. 2. Correlation matrices that showed the relationships between metabolites in pigs. (A) Serum; (B) Plasma; (C) Difference in correlation between serum and plasma (delta correlation). Metabolites are ordered based on metabolite class. Acronyms of metabolite classes are reported in the legend of Fig. 1 and in Supplementary Table S1.

in serum than in plasma, similar to the results we obtained in pigs. Of these metabolites, 111 (91%: 111/122) also passed the QC in our pig study; therefore, it was possible to compare their level in the two biofluids between the two species. Out of the 44 differentially abundant metabolites in pigs, 32 were also differentially abundant in humans (Yu et al., 2011), 26 of which had a common pattern in humans and pigs (being higher in serum than in plasma in both species), whereas the remaining six had an opposite pattern in the two species (Fig. 3). The Δ% statistics of these 26 metabolites was comparable between the two species except for serine and phenylalanine whose Δ% values were almost twice as higher in humans than in pigs. In both humans and pigs, arginine was the metabolite with the highest Δ% value.

Among the biogenic amines, Breier et al. (2014) reported that only sarcosine had a significantly lower concentration in serum than in plasma. In our pig study, sarcosine concentration between the two biofluids confirmed the difference reported in humans. Spermine could not be compared since in humans, it did not pass QC, whereas serotonin and putrescine had a similar pattern even if in humans, the CV across all plates was above the 25% in reference samples (thus these data in humans should be considered with caution). Taurine had the same profile, whereas the concentration of spermidine and acetylornithine did not differ statistically between the two biofluids.

Discussion

Plasma and serum are blood-derived biofluids commonly used to assess and monitor the organismal metabolism. The information provided by these two blood matrices are important to assess the physiology and health status of an animal. Plasma and serum are, in a few cases and contexts, considered interchangeable matrices for several chemical-biochemical laboratory investigations even if they are inherently different. A first intrinsic source of differences comes from the biological nature of these fluids: during the coagulation process, blood cells are metabolically active, leading to changes in metabolite concentrations. Then, external non-biologically derived factors, such as the addition of an anticoagulant (plasma) or a clot activator (serum), can interfere the metabolite concentration (i) by directly increasing or decreasing the measured values of some metabolites, (ii) by interfering with the detection of some metabolites, and (iii) by contributing to detect additional or artificially added compounds that could be summed to the intrinsically present metabolites (Siskos et al., 2017; Vignoli et al., 2022). These elements are important in clinical metabolomics since the reference range for each metabolite might be related to the type of biofluid. Therefore, these aspects should be considered when metabolomic analyses are aimed to identifying novel blood-derived biomarkers for a variety of animal conditions.

A few studies have already evaluated the metabolite profile differences between human plasma and serum, including the effects of collection and handling procedures, such as the type of collection tube/agent (e.g., EDTA, citrate, clot activator, gel, etc.), the postcollection sample processing time gap (e.g., 2, 4, 8 h postcollection), the storing condition (e.g., 4 °C, 22 °C) and the detection methods and the analytical platforms (e.g., Nuclear Magnetic Resonance, Mass Spectrometry), among several other factors (López-Bascón et al., 2016; Cruickshank-Quinn et al., 2018; Kamlage

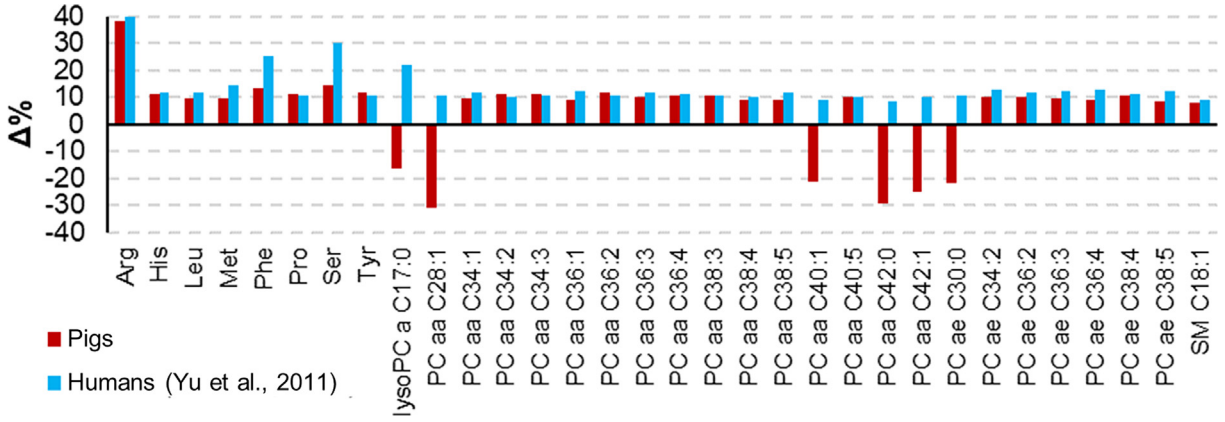

Fig. 3. Comparison between differentially abundant metabolites between plasma and serum (n. 32) identified both in pigs and humans. Acronyms of metabolite classes are reported in Supplementary Table S1.

et al., 2018; Nishiumi et al., 2018; Paglia et al., 2018; Kennedy et al., 2021). All these studies confirmed that the main source of differences is due to the nature of the two biofluids, whereas other factors can alter the within-matrix concentration without any substantial modifications that would confound or reverse the profiles obtained in plasma with that obtained in serum.

Therefore, in principle, differences could be also expected between the metabolomic profiles of porcine plasma and serum derived from the same animals. However, this question has not been investigated thus far, as most of the studies in pigs explored one or the other biofluid, but not both biofluids in the same experimental design. It would be also reasonably expected that what was observed in humans could not completely match the picture derived from the plasma and serum of the pigs, despite the physiological similarities between the two species. Therefore, in this context, a more detailed comparison between humans and pigs could give the possibility to dig into their fine differences that would better value the pig as animal model when blood metabolomic information is relevant to establish and interpret the links with the human physiology. It is important to mention here that the comparison of absolute metabolite concentrations between species is always very complicated due to the difficulties in controlling many environmental and technical conditions and match the same physiological status in different species. For these reasons, relative differences between the two biofluids can be more appropriated for an across-species comparison.

The knowledge of difference between plasma and serum metabolomic profiles has also a specific value for the pig when it is needed to understand the mechanisms and physiological changes in response to feeding strategies, treatments, pathological conditions, and many other applied factors and perturbations that might be relevant in pig breeding, husbandry, and health. This is particularly important as some metabolite classes or specific metabolites (that might be considered as markers of different physiological conditions) could be more concentrated or stable in one or the other biofluids.

Most studies carried out thus far in pigs mainly exploited untargeted metabolomics, by disclosing information for a small part of the metabolome (about 10–40 metabolites or metabolomic features). Few studies investigated more complex metabolomic profiles. For example, the Biocrates platform (150–190 metabolites of different analyte classes) was used to analyse the porcine plasma metabolome for a variety of purposes, but without evaluating the repeatability of the results and potential differences between the two blood-derived matrices (Solberg et al., 2010; Bovo et al., 2015; Metzler-Zebeli et al., 2015; Liu et al., 2018a; Luise et al., 2020; Wu et al., 2020).

As a follow-up of our previous study where we investigated metabolomic profile differences between the same two breeds of pigs that we considered here (Bovo et al., 2016), in this study, we aimed to provide, for the first time, differences between plasma and serum-targeted metabolomic profiles of adult pigs. Samples were selected to maximise the power of the metabolomic approach: (i) we included pigs of two different breeds (Italian Large White and Italian Duroc) to capture some within-species variability; (ii) all animals were healthy and raised in the same performance station at the same time, fed and handled in the same ways, sampled at the same age and weight and after the same fasting time, transported on the same vehicle to the same abattoir; (iii) to minimise the effect of other confounding factors, samples were also collected and then handled/processed in the same way and day, by the same operator, and metabolomic analyses were run in a single batch (i.e., one kit plate). In the present study, it was not possible to evaluate if the measured concentrations precisely reflected the physiological basal level of these metabolites in adult pigs as, possibly, the effect of stressing preslaughter conditions (e.g., animal transportation, changes of environment, introduction into the restrainer) could alter the abundance of certain metabolites. However, the analytical process that here considered one time point was optimal to precisely control several potential confounding effects and provide a reliable picture of differences between plasma and serum. This simple experimental design also provided a quite good match with a few similar studies reported in humans (based on the same targeted metabolomic platform) that otherwise, for the intrinsic difficulties to design across-species comparative analyses, would not be possible to consider for the evaluation of the human-pig similarities and differences. One of the major strengths of our pig study compared to any other studies in humans was that we could control many confounding factors that, for obvious reasons, cannot be controlled in humans. On the other hand, it is also worth noting that our study did not provide a longitudinal profile and trend of the metabolomic picture that would have requested the sampling at more time points.

We observed a good repeatability of the pig metabolomic profiles as more than 80% of the metabolites had a CV < 20%. About 5% of the amino acids and 31% of biogenic amines failed the CV quality check, representing the most stable and the most unstable analyte classes, respectively. In particular, only one amino acid (i.e., aspartate) and five biogenic amines (i.e., histamine, 3-nitrotyrosine, cis-4-Hydroxyproline, phenylethylamine, symmetric dimethylarginine) were difficult to quantify or were present with traces below the limit of detection of the applied platform. This was in line with our previous findings, where the same QC procedures were applied for the analysis of porcine plasma of a larger number of samples that also had to be run on more than one Biocrates kit plate, introducing additional variability (Bovo et al., 2015). Instability of aspartate and biogenic amines was also evidenced in other studies that evaluated the cross-laboratory comparability of plasma and/or serum metabolite measurements in humans, mice, and rats with either the Biocrates p180 kit or Biocrates p400HR kit, another similar targeted platform that includes most of the analytes of the kit that we used (Siskos et al., 2017; Thompson et al., 2019).

Considering all pigs together, more than 80% of the metabolites had similar relative profile in plasma and serum, with an average $r = 0.75 \pm 0.15$. These results denoted a quite high stability of the metabolites that we analysed, as also evidenced by a similar result ($r = 0.81 \pm 0.10$) reported in humans, where plasma and serum were analysed with the same targeted metabolomic platform (Yu et al., 2011). When this information was dissected between the two pig breeds, correlations reflected the overall averaged information: in Italian Large White, $r = 0.85 \pm 0.11$; in Italian Duroc, $r = 0.77 \pm 0.10$. Therefore, it seems that differences in the blood metabolomic profiles that we already observed between these two breeds (Bovo et al., 2016) do not impact the respective paired profiles of the two biofluids.

Quantitatively, metabolite concentrations in pigs were usually higher in serum than in plasma (based on Δ% statistics), in line with what was also shown in humans when (i) plasma EDTA and serum with clot activator were used in the preparation of the two biofluids (Yu et al., 2011; Breier et al., 2014), as we did for the pig samples, or when (ii) the same plasma EDTA was used but coupled with a different serum preparation system based on gel tube (Paglia et al., 2018). These studies in humans indicate that slight differences in serum preparation procedures do not substantially modify the serum metabolite profile.

Based on the univariate analysis (Wilcoxon signed-rank test) that we applied to mine the metabolomic pig data, 28.4% of the investigated metabolites had statistically different abundances ($P < 0.05$; Bonferroni corrected) between the two biofluids (when all pigs were considered together). Separation between the plasma and serum profiles was also captured with PCA, even if the reported low explained variance indicated an overall similarity between the pat-

terns of the two biofluids. Comparing the results that we obtained in pigs with the results obtained in humans (Yu et al., 2011; Breier et al., 2014), the human metabolome has more statistically different concentrations between the two biofluids than the porcine profiles. This general picture could give a first level of differences between the two species, even if this information should be considered with caution, as some differences can be ascribed to the methodologies applied to filter the human and pig datasets and to the different statistical powers reached by the human and pig studies.

Differences in metabolite concentration between the two biofluids in our targeted species, the pig, are also interesting to be pointed out. sML-PLS-DA was applied to obtain a reliable list of differentially abundant metabolites between the porcine plasma and serum profiles, while considering both multiple explanatory variables modelled jointly and the paired structure of data. This approach has also other advantages, especially in the setting of many variables in a small sample size, than the univariate analysis of the data which might suffer from a limited statistical power. The sML-PLS-DA retained 28 metabolites as the most discriminant ones, all of which were also selected with the univariate approach. In this metabolite set, serotonin had the highest positive sML-PLS-DA regression weight (β = 0.51), being also the metabolite with the highest positive delta (Δ% = 74.3; serum concentration higher than the plasma concentration). However, despite the notable difference in abundance, its quantification seems quite challenging as correlation between the two biofluids was almost evident at population level (r = 0.46, P < 0.05) but variable between the two breeds (quite lower for the Italian Duroc pigs r = 0.17, P < 0.05) for the presence of outliers. Possible explanations for this variability could not be only the instability of the metabolite (due to the closeness of their level to the limit of detection) but could also be due to the effect of genetic factors affecting their level and quantification (with different alleles, and then, genotypes, segregating in the analysed pigs). These aspects should be further investigated considering the key role of serotonin as possible biomarker of neurological functions and behaviour of the animals. Similarly, also arginine was in this list of discriminant metabolites for the two biofluids even if it had lower correlations. The same explanations related to this variability could be raised, also suggesting a modified impact depending on the type of biofluid. It is interesting to note that arginine was also the metabolite with the highest difference in concentration between human plasma and serum, as reported by Yu et al. (2011). Metabolites PC.aa.C42.1 and spermine were the porcine analytes with the most negative sML-PLS-DA regression weight (β = -0.69) and negative Δ (−40.5% at population level), respectively. Also in these cases, quantification seems quite unstable: if within breed correlations for PC.aa.C42.1 were in the range r = 0.36–0.52 (P > 0.05), for spermine the situation was quite puzzling, being the sign of correlation in the two breeds opposite (Italian Large White, r = 0.68, P < 0.05; Italian Duroc, r = −0.51, P > 0.05), probably due to the presence of outliers caused by metabolite instability and/or genetic factors segregating in the two breeds (e.g., the analysed pigs could carry different genotypes at major loci affecting their level). Only few metabolites (n.17) had similar profiles between the two porcine matrices while presenting substantial differences in absolute abundances. These metabolites included one biogenic amine (putrescine), three amino acids (asparagine, phenylalanine and leucine), SM:C18.1, lysoPC a C17 and different phosphatidylcholines. The studies of Yu et al. (2011) and Breier et al. (2014) confirmed the same profiles of these metabolites in the human matrices except for lysoPC a C17.0, whose concentration was reversed between humans and pigs (Δ % values had opposite signs). The recent study of Kennedy et al. (2021) confirmed the opposite profile of lysoPC a C17.0 concentration in the human biofluids using a different liquid chromatography-tandem mass spectrometry analytical platform.

Overall, what emerged from these results is the possibility to predict, to some extent, (i) the physiological impacts and (ii) the technical aspects that should be considered when metabolomic analyses are run on different matrices. This has been shown and supported by the double comparative analysis that we carried out, where the metabolomic profiles were evaluated both (i) between matrices in pigs and (ii) across matrices between species. Moreover, this across-species comparison allowed to capture the link between the metabolomic profiles of pigs and humans which can be relevant to interpret and transfer information when the pig is used as animal model.

Conclusions

Thousands of metabolites contribute to describe the profile of animal biofluids. This study focused on porcine plasma and serum, despite it captured one of the largest numbers of targeted metabolites reported thus far in pigs, can give information on just the tip of the "metabolome" iceberg, as many unknown undetected components were not investigated. The obtained picture, however, provided important information that described similarities and differences between these two biofluids in pigs and the related comparisons human-pig, supporting the choice of one or the other matrix for the implementation of extensive metabolomic studies in pigs. The results can also provide useful hints to valuing the pig as animal model, in particular when metabolite-derived physiological states are important to establish the link between humans and pigs.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2023.101029.

Ethics approval

Not applicable.

Data and model availability statement

The data that support the study findings are publicly available at: https://doi.org/10.5281/zenodo.10058406.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not use any artificial intelligence-assisted technologies in the writing process.

Author ORCIDs

Samuele Bovo: https://orcid.org/0000-0002-5712-8211.
Flaminia Fanelli: https://orcid.org/0000-0003-2601-2694.
Giuseppina Schiavo: https://orcid.org/0000-0002-3497-1337.
Giuliano Galimberti: https://orcid.org/0000-0002-9161-9671.
Francesca Bertolini: https://orcid.org/0000-0003-4181-3895.
Stefania Dall'Olio: https://orcid.org/0000-0003-1384-3771.
Uberto Pagotto: https://orcid.org/0000-0003-4182-3110.
Luca Fontanesi: https://orcid.org/0000-0001-7050-3760.

Author contributions

Samuele Bovo: Methodology, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing.
Flaminia Fanelli: Formal analysis, Resources.

Giuseppina Schiavo: Investigation, Data Curation.
Giuliano Galimberti: Methodology.
Francesca Bertolini: Investigation, Data Curation.
Stefania Dall'Olio: Supervision.
Uberto Pagotto: Resources.
Luca Fontanesi: Conceptualisation, Resources, Writing - Original Draft, Writing - Review & Editing, Supervision, Funding acquisition.

Declaration of interest

None.

Acknowledgements

The authors thank the National Pig Breeders Association (ANAS) for their collaboration in designing this study.

Financial support statement

This work was funded by Italian Ministry of University and Research (MUR), PRIN2017, PigPhenomics project.

References

Ametaj, B.N., Zebeli, Q., Saleem, F., Psychogios, N., Lewis, M.J., Dunn, S.M., Wishart, D.S., 2010. Metabolomics reveals unhealthy alterations in rumen metabolism with increased proportion of cereal grain in the diet of dairy cows. Metabolomics 6, 583–594.

Bovo, S., Mazzoni, G., Calò, D.G., Galimberti, G., Fanelli, F., Mezzullo, M., Schiavo, G., Scotti, E., Manisi, A., Samoré, A.B., Bertolini, F., Trevisi, P., Bosi, P., Dall'Olio, S., Pagotto, U., Fontanesi, L., 2015. Deconstructing the pig sex metabolome: targeted metabolomics in heavy pigs revealed sexual dimorphisms in plasma biomarkers and metabolic pathways. Journal of Animal Science 93, 5681–56993.

Bovo, S., Mazzoni, G., Galimberti, G., Calò, D.G., Fanelli, F., Mezzullo, M., Schiavo, G., Manisi, A., Trevisi, P., Bosi, P., Dall'Olio, S., Pagotto, U., Fontanesi, L., 2016. Metabolomics evidences plasma and serum biomarkers differentiating two heavy pig breeds. Animal 10, 1741–1748.

Breier, M., Wahl, S., Prehn, C., Fugmann, M., Ferrari, U., Weise, M., Banning, F., Seissler, J., Grallert, H., Adamski, J., Lechner, A., 2014. Targeted metabolomics identifies reliable and stable metabolites in human serum and plasma samples. PLoS One 9, e89728.

Cruickshank-Quinn, C., Zheng, L.K., Quinn, K., Bowler, R., Reisdorph, R., Reisdorph, N., 2018. Impact of blood collection tubes and sample handling time on serum and plasma metabolome and lipidome. Metabolites 8, 88.

Fontanesi, L., 2016. Metabolomics and livestock genomics: Insights into a phenotyping frontier and its applications in animal breeding. Animal Frontiers 6, 73–79.

Gil, A., Siegel, D., Permentier, H., Reijngoud, D.J., Dekker, F., Bischoff, R., 2015. Stability of energy metabolites – An often overlooked issue in metabolomics studies: A review. Electrophoresis 36, 2156–2169.

Goldansaz, S.A., Guo, A.C., Sajed, T., Steele, M.A., Plastow, G.S., Wishart, D.S., 2017. Livestock metabolomics and the livestock metabolome: A systematic review. PLoS One 12, e0177675.

Hsieh, S.Y., Chen, R.K., Pan, Y.H., Lee, H.L., 2006. Systematical evaluation of the effects of sample collection procedures on low-molecular-weight serum/plasma proteome profiling. Proteomics 6, 3189–3198.

Kamlage, B., Neuber, S., Bethan, B., González Maldonado, S., Wagner-Golbs, A., Peter, E., Schmitz, O., Schatz, P., 2018. Impact of prolonged blood incubation and extended serum storage at room temperature on the human serum metabolome. Metabolites 13, 6.

Kennedy, A.D., Ford, L., Wittmann, B., Conner, J., Wulff, J., Mitchell, M., Evans, A.M., Toal, D.R., 2021. Global biochemical analysis of plasma, serum and whole blood collected using various anticoagulant additives. PLoS ONE 16, e0249797.

Kiseleva, O., Kurbatov, I., Ilgisonis, E., Poverennaya, E., 2021. Defining blood plasma and serum metabolome by GC-MS. Metabolites 12, 15.

Lesche, D., Geyer, R., Lienhard, D., Nakas, C.T., Diserens, G., Vermathen, P., Leichtle, A. B., 2016. Does centrifugation matter? Centrifugal force and spinning time alter the plasma metabolome. Metabolomics 12, 159.

Liu, L., Aa, J., Wang, G., Yan, B., Zhang, Y., Wang, X., Zhao, C., Cao, B., Shi, J., Li, M., Zheng, T., Zheng, Y., Hao, G., Zhou, F., Sun, J., Wu, Z., 2010. Differences in metabolite profile between blood plasma and serum. Analytical Biochemistry 406, 105–112.

Liu, H., Chen, Y., Ming, D., Wang, J., Li, Z., Ma, X., Wang, J., van Milgen, J., Wang, F., 2018a. Integrative analysis of indirect calorimetry and metabolomics profiling reveals alterations in energy metabolism between fed and fasted pigs. Journal of Animal Science and Biotechnology 9, 41.

Liu, X., Hoene, M., Wang, X., Yin, P., Häring, H.U., Xu, G., Lehmann, R., 2018b. Serum or plasma, what is the difference? Investigations to facilitate the sample material selection decision making process for metabolomics studies and beyond. Analytica Chimica Acta 1037, 293–300.

López-Bascón, M.A., Priego-Capote, F., Peralbo-Molina, A., Calderón-Santiago, M., De Castro, M.L., 2016. Influence of the collection tube on metabolomic changes in serum and plasma. Talanta 150, 681–689.

Luise, D., Bovo, S., Bosi, P., Fanelli, F., Pagotto, U., Galimberti, G., Mazzoni, G., Dall'Olio, S., Fontanesi, L., 2020. Targeted metabolomic profiles of piglet plasma reveal physiological changes over the suckling period. Livestock Science 231, 103890.

Luque-Garcia, J.L., Neubert, T.A., 2007. Sample preparation for serum/plasma profiling and biomarker identification by mass spectrometry. Journal of Chromatography A 1153, 259–276.

Metzler-Zebeli, B.U., Eberspächer, E., Grüll, D., Kowalczyk, L., Molnar, T., Zebeli, Q., 2015. Enzymatically modified starch ameliorates postprandial serum triglycerides and lipid metabolome in growing pigs. PLoS ONE 10, e0130553.

Nagana Gowda, G.A., Raftery, D., 2023. NMR Metabolomics methods for investigating disease. Analytical Chemistry 95, 83–99.

Nishiumi, S., Suzuki, M., Kobayashi, T., Yoshida, M., 2018. Differences in metabolite profiles caused by pre-analytical blood processing procedures. Journal of Bioscience and Bioengineering 125, 613–618.

Paglia, G., Del Greco, F.M., Sigurdsson, B.B., Raine, R.J., Volani, C., Hicks, A.A., Pramstaller, P.P., Smarason, S.V., 2018. Influence of collection tubes during quantitative targeted metabolomics studies in human blood samples. Clinica Chimica Acta 486, 320–328.

Psychogios, N., Hau, D.D., Peng, J., Guo, A.C., Mandal, R., Bouatra, S., Sinelnikov, I., Krishnamurthy, R., Eisner, R., Gautam, B., Young, N., Xia, J., Knox, C., Dong, E., Huang, P., Hollander, Z., Pedersen, T.L., Smith, S.R., Bamforth, F., Greiner, R., McManus, B., Newman, J.W., Goodfriend, T., Wishart, D.S., 2011. The human serum metabolome. PLoS ONE 6, e16957.

R Development Core Team, 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Siskos, A.P., Jain, P., Römisch-Margl, W., Bennett, M., Achaintre, D., Asad, Y., Marney, L., Richardson, L., Koulman, A., Griffin, J.L., Raynaud, F., Scalbert, A., Adamski, J., Prehn, C., Keun, H.C., 2017. Interlaboratory reproducibility of a targeted metabolomics platform for analysis of Human serum and plasma. Analytical Chemistry 89, 656–665.

Solberg, R., Enot, D., Deigner, H.P., Koal, T., Scholl-Bürgi, S., Saugstad, O.D., Keller, M., 2010. Metabolomic analyses of plasma reveals new insights into asphyxia and resuscitation in pigs. PLoS ONE 5, e9606.

Sotelo-Orozco, J., Chen, S.Y., Hertz-Picciotto, I., Slupsky, C.M., 2021. A comparison of serum and plasma blood collection tubes for the integration of epidemiological and metabolomics data. Frontiers in Molecular Biosciences 8,. https://doi.org/10.3389/fmolb.2021.682134 682134.

Suarez-Diez, M., Adam, J., Adamski, J., Chasapi, S.A., Luchinat, C., Peters, A., Prehn, C., Santucci, C., Spyridonidis, A., Spyroulias, G.A., Tenori, L., Wang-Sattler, R., Saccenti, E., 2017. Plasma and serum metabolite association networks: comparability within and between studies using NMR and MS profiling. Journal of Proteome Research 16, 2547–2559.

Sun, H.Z., Wang, D.M., Wang, B., Wang, J., Liu, H.Y., Guan, L.L., Liu, J.X., 2015. Metabolomics of four biofluids from dairy cows: potential biomarkers for milk production and quality. Journal of Proteome Research 14, 1287–1298.

Thompson, J.W., Adams, K.J., Adamski, J., Asad, Y., Borts, D., Bowden, J.A., Byram, G., Dang, V., Dunn, W.B., Fernandez, F., Fiehn, O., Gaul, D.A., Hühmer, A.F., Kalli, A., Koal, T., Koeniger, S., Mandal, R., Meier, F., Naser, F.J., O'Neil, D., Pal, A., Patti, G.J., Pham-Tuan, H., Prehn, C., Raynaud, F.I., Shen, T., Southam, A.D., St John-Williams, L., Sulek, K., Vasilopoulou, C.G., Viant, M., Winder, C.L., Wishart, D., Zhang, L., Zheng, J., Moseley, M.A., 2019. International ring trial of a high resolution targeted metabolomics and lipidomics platform for serum and plasma analysis. Analytical Chemistry 91, 14407–14416.

Vignoli, A., Tenori, L., Morsiani, C., Turano, P., Capri, M., Luchinat, C., 2022. Serum or plasma (and which plasma), that is the question. Journal of Proteome Research 21, 1061–1072.

Wedge, D.C., Allwood, J.W., Dunn, W., Vaughan, A.A., Simpson, K., Brown, M., Priest, L., Blackhall, F.H., Whetton, A.D., Dive, C., Goodacre, R., 2011. Is serum or plasma more appropriate for intersubject comparisons in metabolomic studies? An assessment in patients with small-cell lung cancer. Analytical Chemistry 83, 6689–6697.

Westerhuis, J.A., van Velzen, E.J.J., Hoefsloot, H.C.J., Smilde, A.K., 2010. Multivariate paired data analysis: multilevel PLSDA versus OPLSDA. Metabolomics 6, 119–128.

Wu, W., Zhang, L., Xia, B., Tang, S., Liu, L., Xie, J., Zhang, H., 2020. Bioregional alterations in gut microbiome contribute to the plasma metabolomic changes in pigs fed with inulin. Microorganisms 8, 111.

Yu, Z., Kastenmüller, G., He, Y., Belcredi, P., Möller, G., Prehn, C., Mendes, J., Wahl, S., Roemisch-Margl, W., Ceglarek, U., Polonikov, A., Dahmen, N., Prokisch, H., Xie, L., Li, Y., Wichmann, H.-E., Peters, A., Kronenberg, F., Suhre, K., Adamski, J., Illig, T., Wang-Sattler, R., 2011. Differences between Human plasma and serum metabolite profiles. PLoS ONE 6, e21230.