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ABSTRACT

This prospective study aims to analyze the serum metabolome of female goat kids just before the first breeding in order to characterize the changes in metabolites during sexual maturation. A better knowledge of these changes could help optimizing the time for first breeding and improving the fertilization rates at first insemination. Weekly blood sampling was performed on twenty 6- to 7-month-old female goats from Alpine breed born in February during the 5 weeks preceding their first contact with bucks in September. Progesterone assays and metabolome analysis using ^{1}H Nuclear Magnetic Resonance Spectroscopy were performed on the serum samples. No spontaneous ovulatory cycle was observed before breeding based on progesterone assays. All female goats had reached the pubertal stage of maturity at breeding

since all got pregnant. Metabolome analysis allowed the identification of 109 spectral bins in sera. When the metabolic profiles were compared between the 5 weeks preceding the first contact with bucks, 6 metabolites were highlighted that could differentiate between week 5 and week 1, 2 and 3: glucose, citrate, creatinine, 3-hydroxy-isobutyrate and two unidentified bins. Citrate and 3-hydroxy-isobutyrate are of particular interest because their level significantly varied during sexual maturation, with a significant decrease of 3-hydroxy-isobutyrate at week 5 and a significant increase of citrate at week 4 and 5. However, the lack of variability in the fertility results did not allow us to conclude on a potential link between these metabolomics profile and reproduction performances. Further studies are necessary to ascertain the reliability of these metabolites as biomarkers of sexual precocity.

Keywords:

Caprine; Metabolites; Sexual maturation; Puberty; Nuclear Magnetic Resonance Spectroscopy.

Introduction

Replacement female goat kids determine the future breeding and milk production performances of the herd. Their rearing costs during this unproductive period (from birth until first kidding) are high, thus management of their first breeding is an essential issue. Success of first breeding is a major concern for goat breeders, since failure to fertilize female goats increases unproductive time and breeding costs. Postponing reproduction beyond 7 months of age implies additional rearing costs. In addition, if the kidding period spreads over a long period, it can make it difficult to manage a fallowing period (necessary for disease control and hygiene), create problems in the labor organization (managing heterogeneous groups of young

female goats, organizing milking groups) and shorten the interval between kidding and reproduction, hence potentially impairing their fertility potential.

There is a clear impact of the age at first kidding on growth performances, milk production (quantity, quality and duration of first lactation) and on reproduction performances at the second breeding. Optimizing the management of young female goats and their integration to adult groups requires a better control of their reproduction. After hormonal induction of estrus and cervical insemination, nulliparous goats tend to be less fertile than primiparous or multiparous goats (Leboeuf et al., 2000). Fertility rates after artificial insemination of young goats are highly variable and rather low (Fatet et al., 2014a). Different assumptions have been made on the causes: heterogeneity of age and weight at first breeding (most female goats are bred at a given date while they were born over a few weeks' period and some of them may not have reached sexual maturity), quality of the first ovulation, variability in the ovulatory response to hormonal synchronization (Fatet et al., 2014b).

In France, Alpine and Saanen breeders generally use two criteria to choose when to breed female goat kids: female goats should be 7 months old and weight more than 30kg or 50% of adult weight (Morand-Fehr et al., 1996; Piedhaut et al., 2014). However, sexual precocity is highly variable between female goats. Goats reproduce seasonally and their sexual development is influenced by environmental changes (food availability, photoperiod and temperature), breed, season of birth (Delgadillo et al., 2007), feed practices and female goats size. A better characterization of the pubertal stage of maturity could help defining practical strategies for successful first breeding and better management of milk production. Anti-Müllerian hormone (AMH), a well-known endocrine marker of the ovarian reserve of follicles in mammals, has been proposed as a predictor of fertility in cattle (Mossa et al., 2017), but previous work conducted on AMH plasmatic levels in goats failed to clearly associate AMH level with the onset of puberty or the precocity of fertility (Fabre et al., 2018). Biomarkers for

sexual precocity have been recently proposed in pigs (Goudet et al., 2019), cattle (Abeni et al., 2019) and humans (Hirschel et al., 2020). Up to now, there is no known biomarker for sexual precocity in caprine.

Omics approaches are powerful tools to identify large numbers of biomolecules in body fluids. Nuclear Magnetic Resonance (NMR) spectroscopy is a repeatable, reliable and stable technique that allows a non-targeted identification of the metabolites present in a biological sample. The metabolome includes low-molecular-weight compounds such as peptides, lipids, carbohydrates, amino acids, vitamins and minerals. It is the signature of a physiological state.

The objective of this prospective study was to analyze the serum metabolome of female goat kids just before the first breeding in order to characterize the changes in metabolites during sexual maturation.

Materials and methods

Animals, housing and blood samples collection

This experiment was conducted from February to December in the experimental farm PATUCHEV from INRAE Experimental Unit FERLUS (Lusignan, France). All procedures on animals were conducted in accordance with the guidelines for the care and use of laboratory animals issued by the French Ministry of Agriculture. The project was approved by the ethical review committee (Comité d'Ethique en Expérimentation Animale du Poitou-Charentes, COMETHEA n°84) and authorized by the French Ministry of Agriculture with authorization number: APAFIS # 15201.

Twenty female goats from Alpine breed born between February 1st and March 1st were penned together indoor under natural daylight with free access to water. Female goats were weighed weekly from 6 weeks until 4 months of age, then monthly. Female goats were fed *ad libitum* with a high quality milk replacer without animal fat (CHEVRODOR ALTO, Univor,

France) until they weighed 15 kg, which occurred usually between 50 to 60 days of age. They were then weaned and fed *ad libitum* with a complete feed with 20% protein content (NURSY PRIMOR, Alicoop, France) and straw until 20-24 kg (around 4 months of age). At this stage, they received hay produced on-farm from multi-species pastures *ad libitum* and 500 g of concentrate containing 200 g commercial mix of maize and wheat (DUO CEREAL, Alicoop, France), 200 g of grains produced on the farm (triticale and peas) and 100 g of the complete feed previously used. From July 1st, they grazed multi-species pastures (approximate composition fescue 70%, white clover 25% and alfalfa 5%) and were fed the same ration as previously (hay *ad libitum* + 500 to 600 g of concentrate).

Two bucks were introduced with the 20 female goats on September 9th. After 26 days, these bucks were removed and replaced by a single male. Another replacement occurred 28 days later (54 days after initial buck introduction); the latter was removed after 46 more days (100 days after first introduction). All 4 bucks used for breeding were aged eleven to twelve month old (born in October the previous year) when introduced with the female goats.

Weekly blood samples were collected on the twenty female goats from 4 month of age until breeding. Blood was collected in dry tubes, coagulated at room temperature for 15-30 minutes and centrifuged for 15 minutes at 300g and 20°C. The supernatant was transferred in hemolytic tubes, frozen and stored at -20°C until analysis. ELISA progesterone assays were performed on all samples to monitor spontaneous ovulatory activity. Metabolome analysis using ^{1}H Nuclear Magnetic Resonance Spectroscopy was performed on samples collected during the last 5 weeks before the first contact with bucks (August 12th, 19th, 26th, September 2nd, 9th).

Transrectal ultrasonography was performed using a 7.5 MHz linear transducer (ALOKA-SSD 900, Japan Care Co. Ltd, Kobe-Shi, Japan) with the goats in standing position one week before buck introduction. The number of follicles with a diameter ≥ 3mm and presence or

absence of corpora lutea were recorded and measured on both ovaries to check the ovarian status of the goats. Fertility was evaluated by transabdominal ultrasonography 44, 54 and 100 days after buck introduction.

Progesterone assay

Progesterone concentrations were determined using an enzyme-linked immunosorbent assay, on 10 µl aliquots of undiluted plasma as previously described (Canepa et al., 2008). The limit of detection of the assay was 0.4 ng/ml and the intra-assay coefficient of variation was 6.6% for quality control plasma samples containing 1.5 ng/ml progesterone. Plasma progesterone levels > 1 ng/ml for two or more consecutive samples indicated ovulatory activity.

^{1}H-nuclear magnetic resonance analysis of serum samples

As previously described by Beauclercq and collaborators (Beauclercq et al., 2016), sera were prepared by cold methanol precipitation of lipids and proteins. After centrifugation and solvent evaporation of the supernatant, dried extracts were stored at -80°C. Prior to ^{1}H-NMR analysis, dried serum samples were dissolved in 220 µl of 0.2 M potassium phosphate buffer in deuterium oxide (pH = 7.4) with 123 µM of tri-methyl-silylpropionic acid (TSP) final concentration.

^{1}H-NMR spectra were obtained with a Bruker DRX-600 AVANCE-III HD spectrometer (Bruker SADIS, Wissembourg, France). A "noesypr-1d" sequence was used to acquire 64 scans on a time domain of 64K data points with a spectral width of 7500 Hz. After Fourier transformation, baseline and phase correction were applied.

NMR*Proc*Flow (version 1.1, INRA UMR 1332 BFP, Bordeaux Metabolomics Facility, France) (Jacob et al., 2017) was used to reduce ^{1}H-NMR spectra into a data table containing variable integrated regions. Spectral intensities, named by compound name if known or by B-chemical-shift if unidentified, were scaled to the reference region (TSP). Assignments were

done using databases as HMDB (http://www.hmdb.ca) and ChenomX NMR suite 8.1 evaluation edition (ChenomX Inc, Edmonton, Canada).

Multivariate analysis

A supervised multivariate statistical data analysis based on PLS-DA (partial least squares discriminant analysis) and OPLS-DA (orthogonal partial least squares discriminant analysis, can rotate the projection so that the model focuses on the effect of interest) were performed on the metabolomics data generated by ^{1}H-NMR using SIMCA 13.0.3 software (Umetrics, Umea, Sweden). Differences between NMR metabolic profiles were sought between week conditions. The model qualities were evaluated after 7-fold cross validation by R^2Y (goodness of fit), Q^2 (goodness of prediction) and CV-ANOVA (Cross Validation-ANalysis Of VAriance). CV-ANOVA is a diagnostic tool for assessing the reliability of models; the returned p-value is indicative of the statistical significance of the fitted model. According to these criteria, metabolites with greater contribution in the separation of the groups were identified. The features with Variable Importance on Projection (VIP) values > 1.0 were responsible for the differences between weeks.

Univariate analysis was performed on VIP using a t-test or a Mann Whitney test according to the normal distribution of the data. The results were considered to be significantly different when $P \leq 0.05$.

Results

Spontaneous ovulatory activity and fertility

Plasma progesterone levels raised > 1 ng/ml punctually in 3 young females during summer. One doe had 2 consecutive samples above the threshold in June (3.7 and 4.9 ng/ml), 2 does had a sample above 1 ng/ml in late July (1.04 and 1.4 ng/ml), probably as a response to the stress of grazing outside daily from July 1st. We considered that animals exhibiting a

174 significant increase in progesterone levels during summer had reached ovarian maturity
175 earlier. However, based on plasma progesterone levels and as confirmed by the absence of
176 corpora lutea when analyzing the ovaries by ultrasound scanning, no spontaneous ovulatory
177 activity was observed during the five weeks before breeding in any of the 20 female goats.
178 Fertility following buck introduction was 100% and most females (n = 18) were fertilized
179 during their first cycle (within 12 days from buck introduction). All female goats had reached
180 the pubertal stage of maturity at breeding since all got pregnant. Thus, potential differences
181 between fertile/non fertile female goats, with short/long latency until gestation could not be
182 studied.
183 *Serum metabolome analysis*
184 Serum metabolome ^{1}H-NMR analysis allowed the identification of 109 spectral bins
185 (supplementary data). Among them, 78 were identified as unique metabolites (citrate,
186 formate, leucine, valine, for example) or an overlapping of metabolites (Glut + Meth + Gln =
187 Glutamate + Methionine + Glutamine, for example). Thirty-one of them were unidentified
188 (B6-8777 = chemical shift at 6.8777 of the spectral bin for example).
189 Serum metabolome from female goat kids contained sugars (glucose), organic acids (acetate,
190 citrate, formate, glutamate, isobutyrate, lactate, nicotinurate), amino acids and derivatives
191 (alanine, creatine, glutamine, glycine, isoleucine, leucine, methionine, methylhistidine,
192 phenylalanine, threonine, tryptophan, tyrosine, valine), organic compounds (creatinine,
193 dimethylamine) and nucleosides (hypoxanthine, inosine).
194 Figure 1 shows the spectral intensities scaled to the reference region of each spectral area
195 from week 1 to week 5 until the first contact with bucks for the 20 studied female goats.
196 Metabolome results were compared between the group of female goats whose progesterone
197 levels raised punctually and the other female goats but no significant differences were
198 observed (data not shown). Metabolome results were compared between female goats with or

without large follicles (diameter $\geq$ 3mm) but no significant differences were observed (data not shown). When considering the 20 female goats, PLS-DA was applied in order to compare the metabolic profiles between the 5 weeks before buck introduction. Unfortunately, no PLS-DA model was found to differentiate the 5 weeks. We then used OPLS-DA to look for biomarker candidates, comparing side by side all weeks. Table 1 summarizes the predictive abilities of the different statistical models obtained by comparing weeks. The best OPLS-DA model was found when comparing week 1 *versus* week 5 (Figure 2) suggesting that regarding the metabolic profiles discrimination between the two weeks is possible. The discriminating spectral bins with VIP values over 1 and their *P*-values are listed in Table 2. According to the quality model criteria, the comparison between week 5 and week 2 (Table 3) or week 5 and week 3 (Table 4) seems interesting because it signs the evolution of some metabolites along weeks.

In order to seek the VIP (metabolites) shared between the side-by-side comparison (week 5 *versus* week 1, week 5 *versus* week 2, week 5 *versus* week 3), a Venn diagram (Bardou et al., 2014) (http://jvenn.toulouse.inra.fr) was established and shared metabolites could be assessed as potential biomarkers of sexual precocity (Figure 3). The Venn diagram allows highlighting shared metabolites when comparing different conditions. The shared metabolites between the three weeks comparison (meaning week 5 and week 1, week 5 and week 2, week 5 and week 3) are 3-hydroxy-isobutyrate (3HO-isobutyrate), citrate, creatinine, glucose and two unidentified bins (B2-8505 and B3-5801). Among these six metabolites, 3HO-isobutyrate and creatinine levels progressively decreased whereas the others increased from week 1 to week 5.

Discussion

The objective of this prospective study was to analyze the serum metabolome of female goat kids before the first breeding in order to characterize pubertal development related variations of the metabolome.

Among livestock metabolomics studies, few were performed on caprine compared to bovine, porcine and ovine (Goldansaz et al., 2017). Metabolomics analyses have been performed on goat milk (Scano et al., 2014; Yang et al., 2016; Caboni et al., 2019), vitreous humor (Locci et al., 2014; Rosa et al., 2015), rumen fluid or epithelium (Mao et al., 2016; Guo et al., 2019; Zhang et al., 2019), hindgut (Tao et al., 2017), urine (Contreras-Jodar et al., 2019), liver (Zhang et al., 2019) and meat (Wang et al., 2019). Two metabolomics studies have been performed on goat serum : the metabolomics alterations induced by a mycotoxin in the serum of dairy goats were analyzed by a NMR-based approach (Cheng et al., 2017) and the metabolome of serum from dairy goat fed with different diets was analyzed by gas chromatography and mass spectrometry (Zhang et al., 2019). However, to our knowledge, NMR characterization of female goat kid serum has never been performed. Our study was successful in performing metabolomics analyses in the serum of 20 female goat kids collected over 5 weeks just before the first breeding.

Among the 109 spectral bins obtained, 78 metabolites were identified. Most of the metabolites identified in the serum of female goat kids were also identified in adult goat serum such as sugars (glucose), organic acids (acetate, citrate, glutamate, lactate), amino acids and derivatives (alanine, creatine, glutamine, glycine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, tyrosine, valine), organic compounds (creatinine), and nucleosides (hypoxanthine, inosine) (Cheng et al., 2017; Zhang et al., 2019). However, according to our study and the literature currently available, some metabolites in the serum of female goat kids were not detected in adult goats, such as trimethylamine-oxyde (TMAO), dimethylamine (DMA), methylhistidine, oxoisocaproate, formate and histamine (Cheng et al.,

2017; Zhang et al., 2019). This could be due to differences in the analytical techniques. Alternatively, this suggests that pubertal development could be related to variations of the metabolome, and that further potential biomarkers of sexual maturation could be identified by serum metabolome analysis.

When the metabolic profiles were compared between the five weeks before the first contact with bucks, six metabolites were identified that could differentiate between week 5 and week 1, week 2 and week 3: 3-hydroxy-isobutyrate, citrate, creatinine, glucose and two unidentified bins. These metabolites could be assessed as potential biomarkers of sexual development before puberty. Several studies supporting this assertion have been published. In a study focusing on the relationships between age at puberty and blood metabolic profiles in dairy heifers from 6 to 15 months of age, Abeni and collaborators showed modifications of the glucose and creatinine levels with age before puberty (Abeni et al., 2019). In heifers, creatinine levels increased with age whereas glucose level decreased. When analyzing the alterations in blood serum chemistry values in the puberty period in goats, Devrim and collaborators showed that creatinine levels significantly increased with age (Devrim et al., 2015). Urinary excretion of creatinine was showed to peak around puberty in healthy schoolchildren; boys excreting significantly more creatinine compared with girls before and during puberty, reflecting greater muscle mass (Skinner et al., 1996). Citrate was detected in urine from children and similar rates were observed in both male and female subjects until puberty during which time citrate excretion became significantly higher in girls (Kirejczyk et al., 2014). Moreover, 3-hydroxy-isobutyrate has been identified in urinary metabolome from 8–9 years old children (Maitre et al., 2017) and serum metabolome of lambs (Pereira et al., 2020). Among 78 metabolites for discrimination between central precocious puberty (CPP) and healthy individuals, 6 urinary compounds including creatinine provided a good prediction (Qi et al., 2012). The plasma and urine metabolome of 12-15-year-old adolescents showed

that pubertal development stage was related to urinary creatinine and citrate content (Zheng et al., 2014). Moreover, it has been reported that the excretion of urinary citrate is regulated by sex hormones such as estrogen (Dey et al., 2002). Therefore, the modifications in the citrate content could be attributed to changes in sex hormones during pubertal development. These data and our results suggest that citrate, 3-hydroxy-isobutyrate and creatinine could be potential biomarkers of sexual maturation in several species, including caprine. Citrate and 3-hydroxy-isobutyrate are of particular interest because their level significantly varied during sexual maturation, with a significant decrease of 3-hydroxy-isobutyrate at week 5 and a significant increase of citrate at week 4 and 5. Creatinine levels were significantly lower at week 3 and week 5. Glucose levels were significantly higher at week 4 and 5 compared to week 1 and 2, but the concentrations of glucose are influenced by diet. Thus, glucose may not be a robust biomarker of sexual maturation. Two unidentified bins are also candidates for potential biomarkers, further studies are in progress to identify them.

In addition to these six identified metabolites, the side-by-side comparison (week 5 *versus* week 3, week 5 *versus* week 2, week 5 *versus* week 1) highlighted several metabolites such as amino acids (alanine, glycine, isoleucine, leucine, tryptophan, valine, 3-methyl-histidine) and overlapping of amino acids (leucine + isoleucine and glutamate + methionine + glutamine, due to the inability to discern these metabolites). In human, puberty has been shown to be related to significant modifications of some amino acids levels in serum such as alanine, glycine, tryptophan, valine, methyl-histidine and leucine + isoleucine (Cominetti et al., 2020; Hirschel et al., 2020). They may be biomarkers of sexual maturation. However, since diet may influence their concentration, amino acids may not be robust biomarkers. The side-by-side comparison also highlighted lactate, TMAO and histamine. Metabolomics analysis in human and equine showed that follicular fluid contain lactate, TMAO and histamine, which could be related to ovary maturation (Dogan et al., 2020; Fernández-Hernández et al., 2020). In human,

297 lactate concentration varies significantly during pubertal development (Cominetti et al.,
298 2020). Such variations in blood biochemical profiles probably reflect changes in energy and
299 carbohydrate metabolism during sexual maturation.
300
301 **Conclusion**
302 This prospective study allowed highlighting six metabolites whose levels significantly
303 changed during sexual maturation five weeks before the first contact with bucks: citrate,
304 creatinine, glucose, 3-hydroxy-isobutyrate and two metabolites that are still being
305 unidentified. However, the lack of variability in the fertility results did not allow us to
306 conclude definitively on a potential link between these metabolomics profile and reproduction
307 performances. Additional work is needed to confirm whether some of these metabolites could
308 be reliable biomarkers of sexual precocity. Moreover, the influence of diet and individual
309 metabolism on their levels should be taken in consideration. Further studies should include
310 reproduction challenges to create more variability in the fertility results, i.e. breeding female
311 goats at an earlier age or following a hormonal induction.
312
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Author contribution statement:

Alice Fatet: Conceptualization, Validation, Investigation, Resources, Writing, Supervision, Funding acquisition. **Lydie Nadal-Desbarats:** Methodology, Validation, Formal analysis, Resources, Writing, Visualization. **Karine Boissard:** Methodology, Validation, Investigation, Resources. **Catherine Antar:** Methodology, Validation, Formal analysis, Resources. **Sandrine Freret:** Conceptualization, Validation. **Maria-Teresa Pellicer-Rubio:** Conceptualization, Validation, Writing. **Danielle Monniaux:** Conceptualization, Validation, Writing. **Ghylène Goudet:** Conceptualization, Validation, Investigation, Writing, Visualization, Supervision.

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References:

Abeni, F., Petrera, F., Le Cozler, Y., 2019. Effects of feeding treatment on growth rates, metabolic profiles and age at puberty, and their relationships in dairy heifers. Animal : an international journal of animal bioscience 13, 1020-1029.

Bardou, P., Mariette, J., Escudié, F., Djemiel, C., Klopp, C., 2014. jvenn: an interactive Venn diagram viewer. BMC Bioinformatics 15, 293-293.

Beauclercq, S., Nadal-Desbarats, L., Hennequet-Antier, C., Collin, A., Tesseraud, S., Bourin, M., Le Bihan-Duval, E., Berri, C., 2016. Serum and Muscle Metabolomics for the Prediction of Ultimate pH, a Key Factor for Chicken-Meat Quality. J. Proteome Res. 15, 1168-1178.

Caboni, P., Murgia, A., Porcu, A., Manis, C., Ibba, I., Contu, M., Scano, P., 2019. A metabolomics comparison between sheep's and goat's milk. Food Res. Int. 119, 869-875.

Canepa, S., Laine, A.-L., Bluteau, A., Fagu, C., Flon, C., Monniaux, D., 2008. Validation d'une méthode immunoenzymatique pour le dosage de la progestérone dans le plasma des ovins et des bovins. Cahier des Techniques de l'INRA 64, 19-30.

Cheng, J., Huang, S., Fan, C., Zheng, N., Zhang, Y., Li, S., Wang, J., 2017. Metabolomic analysis of alterations in lipid oxidation, carbohydrate and amino acid metabolism in dairy goats caused by exposure to Aflotoxin B1. J. Dairy Res. 84, 401-406.

Cominetti, O., Hosking, J., Jeffery, A., Pinkney, J., Martin, F.-P., 2020. Contributions of Fat and Carbohydrate Metabolism to Glucose Homeostasis in Childhood Change With Age and Puberty: A 12-Years Cohort Study (EARLYBIRD 77). Frontiers in Nutrition 7.

Contreras-Jodar, A., Nayan, N.H., Hamzaoui, S., Caja, G., Salama, A.A.K., 2019. Heat stress modifies the lactational performances and the urinary metabolomic profile related to gastrointestinal microbiota of dairy goats. PLoS One 14, e0202457.

Delgadillo, J.A., De Santiago-Miramontes, M.A., Carrillo, E., 2007. Season of birth modifies puberty in female and male goats raised under subtropical conditions. Animal : an international journal of animal bioscience 1, 858-864.
Devrim, A.K., Elmaz, O., Mamak, N., Sudagidan, M., 2015. Alterations in some clinical biochemistry values of Honamli and Native Hair goats during pubertal development. Vet. Arh. 85, 647-656.
Dey, J., Creighton, A., Lindberg, J.S., Fuselier, H.A., Kok, D.J., Cole, F.E., Hamm, L., 2002. Estrogen replacement increased the citrate and calcium excretion rates in postmenopausal women with recurrent urolithiasis. J. Urol. 167, 169-171.
Dogan, B., Karaer, A., Tuncay, G., Tecellioglu, N., Mumcu, A., 2020. High-resolution (1)H-NMR spectroscopy indicates variations in metabolomics profile of follicular fluid from women with advanced maternal age. J. Assist. Reprod. Genet. 37, 321-330.
Fabre, S., Lahoz, B., Chantepie, L., Fatet, A., Alabart, J.L., Monniaux, D., 2018. Relationship between circulating AMH and puberty, fertility and prolificacy of small ruminants. 22. Annual Conference of the European-Society-for-Domestic-Animal-Reproduction (ESDAR). John Wiley & Sons, Cordoba, Spain.
Fatet, A., Boissard, K., Forgerit, Y., Borderes, F., Bruneteau, E., Douteau, E., Pougnard, J.L., 2014a. Insemination in doe kids with cooled vs frozen semen. 21. Rencontres autour des Recherches sur les Ruminants. Institut de l'Elevage - INRA, Paris, France.
Fatet, A., Guérin, H., Boissard, K., Monniaux, D., 2014b. Secretion pattern and fertility predictive value of AMH in the doe kid. 21. Rencontres autour des Recherches sur les Ruminants. Institut de l'Elevage - INRA, Paris, France.
Fernández-Hernández, P., Sánchez-Calabuig, M.J., García-Marín, L.J., Bragado, M.J., Gutiérrez-Adán, A., Millet, Ó., Bruzzone, C., González-Fernández, L., Macías-García, B., 2020. Study of the Metabolomics of Equine Preovulatory Follicular Fluid: A Way to Improve Current In Vitro Maturation Media. Animals : an open access journal from MDPI 10.
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.
Goudet, G., Nadal-Desbarats, L., Douet, C., Savoie, J., Staub, C., Venturi, E., Ferchaud, S., Boulot, S., Prunier, A., 2019. Salivary and urinary metabolome analysis for pre-puberty-related biomarkers identification in porcine. Animal : an international journal of animal bioscience 13, 760-770.
Guo, C., Sun, D., Wang, X., Mao, S., 2019. A Combined Metabolomic and Proteomic Study Revealed the Difference in Metabolite and Protein Expression Profiles in Ruminal Tissue From Goats Fed Hay or High-Grain Diets. Frontiers in physiology 10, 66.
Hirschel, J., Vogel, M., Baber, R., Garten, A., Beuchel, C., Dietz, Y., Dittrich, J., Körner, A., Kiess, W., Ceglarek, U., 2020. Relation of Whole Blood Amino Acid and Acylcarnitine Metabolome to Age, Sex, BMI, Puberty, and Metabolic Markers in Children and Adolescents. Metabolites 10.
Jacob, D., Deborde, C., Lefebvre, M., Maucourt, M., Moing, A., 2017. NMRProcFlow: a graphical and interactive tool dedicated to 1D spectra processing for NMR-based metabolomics. Metabolomics 13, 36.
Kirejczyk, J.K., Porowski, T., Konstantynowicz, J., Kozerska, A., Nazarkiewicz, A., Hoppe, B., Wasilewska, A., 2014. Urinary citrate excretion in healthy children depends on age and gender. Pediatr. Nephrol. 29, 1575-1582.
Leboeuf, B., Restall, B., Salamon, S., 2000. Production and storage of goat semen for artificial insemination. Anim. Reprod. Sci. 62, 113-141.
Locci, E., Scano, P., Rosa, M.F., Nioi, M., Noto, A., Atzori, L., Demontis, R., De-Giorgio, F., d'Aloja, E., 2014. A metabolomic approach to animal vitreous humor topographical composition: a pilot study. PLoS One 9, e97773.
Maitre, L., Lau, C.E., Vizcaino, E., Robinson, O., Casas, M., Siskos, A.P., Want, E.J., Athersuch, T., Slama, R., Vrijheid, M., Keun, H.C., Coen, M., 2017. Assessment of metabolic phenotypic variability in children's urine using (1)H NMR spectroscopy. Scientific reports 7, 46082.

Mao, S.Y., Huo, W.J., Zhu, W.Y., 2016. Microbiome-metabolome analysis reveals unhealthy alterations in the composition and metabolism of ruminal microbiota with increasing dietary grain in a goat model. Environ. Microbiol. 18, 525-541.
Morand-Fehr, P., Broqua, C., Bas, P., Lefrileux, Y., 1996. Dietary recommandations and strategies for dairy young goats. 3. Rencontres autour des Recherches sur les Ruminants. Institut de l'Elevage - INRA, Paris, France.
Mossa, F., Jimenez-Krassel, F., Scheetz, D., Weber-Nielsen, M., Evans, A.C.O., Ireland, J.J., 2017. Anti-Müllerian Hormone (AMH) and fertility management in agricultural species. Reproduction 154, R1-r11.
Pereira, T.L., Fernandes, A.R.M., Oliveira, E.R., Cônsolo, N.R.B., Marques, O.F.C., Maciel, T.P., Pordeus, N.M., Barbosa, L., Buarque, V.L.M., Padilla, A.R.H., Colnago, L.A., Gandra, J.R., 2020. Serum metabolomic fingerprints of lambs fed chitosan and its association with performance and meat quality traits. Animal : an international journal of animal bioscience, 1-12.
Piedhaut, F., Lazard, K., Proust, M., Foisnon, B., Lictevout, V., Lhériau, J.Y., Bossis, N., 2014. Réussir l'élevage des chevrettes, de la naissance à la mise-bas. Institut de l'élevage, Paris.
Qi, Y., Li, P., Zhang, Y., Cui, L., Guo, Z., Xie, G., Su, M., Li, X., Zheng, X., Qiu, Y., Liu, Y., Zhao, A., Jia, W., Jia, W., 2012. Urinary metabolite markers of precocious puberty. Molecular & cellular proteomics : MCP 11, M111.011072-M011111.011072.
Rosa, M.F., Scano, P., Noto, A., Nioi, M., Sanna, R., Paribello, F., De-Giorgio, F., Locci, E., d'Aloja, E., 2015. Monitoring the modifications of the vitreous humor metabolite profile after death: an animal model. BioMed research international 2015, 627201.
Scano, P., Murgia, A., Pirisi, F.M., Caboni, P., 2014. A gas chromatography-mass spectrometry-based metabolomic approach for the characterization of goat milk compared with cow milk. J. Dairy Sci. 97, 6057-6066.
Skinner, A.M., Addison, G.M., Price, D.A., 1996. Changes in the urinary excretion of creatinine, albumin and N-acetyl-beta-D-glucosaminidase with increasing age and maturity in healthy schoolchildren. Eur. J. Pediatr. 155, 596-602.
Tao, S., Tian, P., Luo, Y., Tian, J., Hua, C., Geng, Y., Cong, R., Ni, Y., Zhao, R., 2017. Microbiome-Metabolome Responses to a High-Grain Diet Associated with the Hind-Gut Health of Goats. Frontiers in microbiology 8, 1764.
Wang, W., Sun, B., Hu, P., Zhou, M., Sun, S., Du, P., Ru, Y., Suvorov, A., Li, Y., Liu, Y., Wang, S., 2019. Comparison of Differential Flavor Metabolites in Meat of Lubei White Goat, Jining Gray Goat and Boer Goat. Metabolites 9.
Yang, Y., Zheng, N., Zhao, X., Zhang, Y., Han, R., Yang, J., Zhao, S., Li, S., Guo, T., Zang, C., Wang, J., 2016. Metabolomic biomarkers identify differences in milk produced by Holstein cows and other minor dairy animals. Journal of proteomics 136, 174-182.
Zhang, R.Y., Liu, Y.J., Yin, Y.Y., Jin, W., Mao, S.Y., Liu, J.H., 2019. Response of rumen microbiota, and metabolic profiles of rumen fluid, liver and serum of goats to high-grain diets. Animal : an international journal of animal bioscience 13, 1855-1864.
Zheng, H., Yde, C.C., Arnberg, K., Mølgaard, C., Michaelsen, K.F., Larnkjær, A., Bertram, H.C., 2014. NMR-based metabolomic profiling of overweight adolescents: an elucidation of the effects of inter-/intraindividual differences, gender, and pubertal development. BioMed research international 2014, 537157.

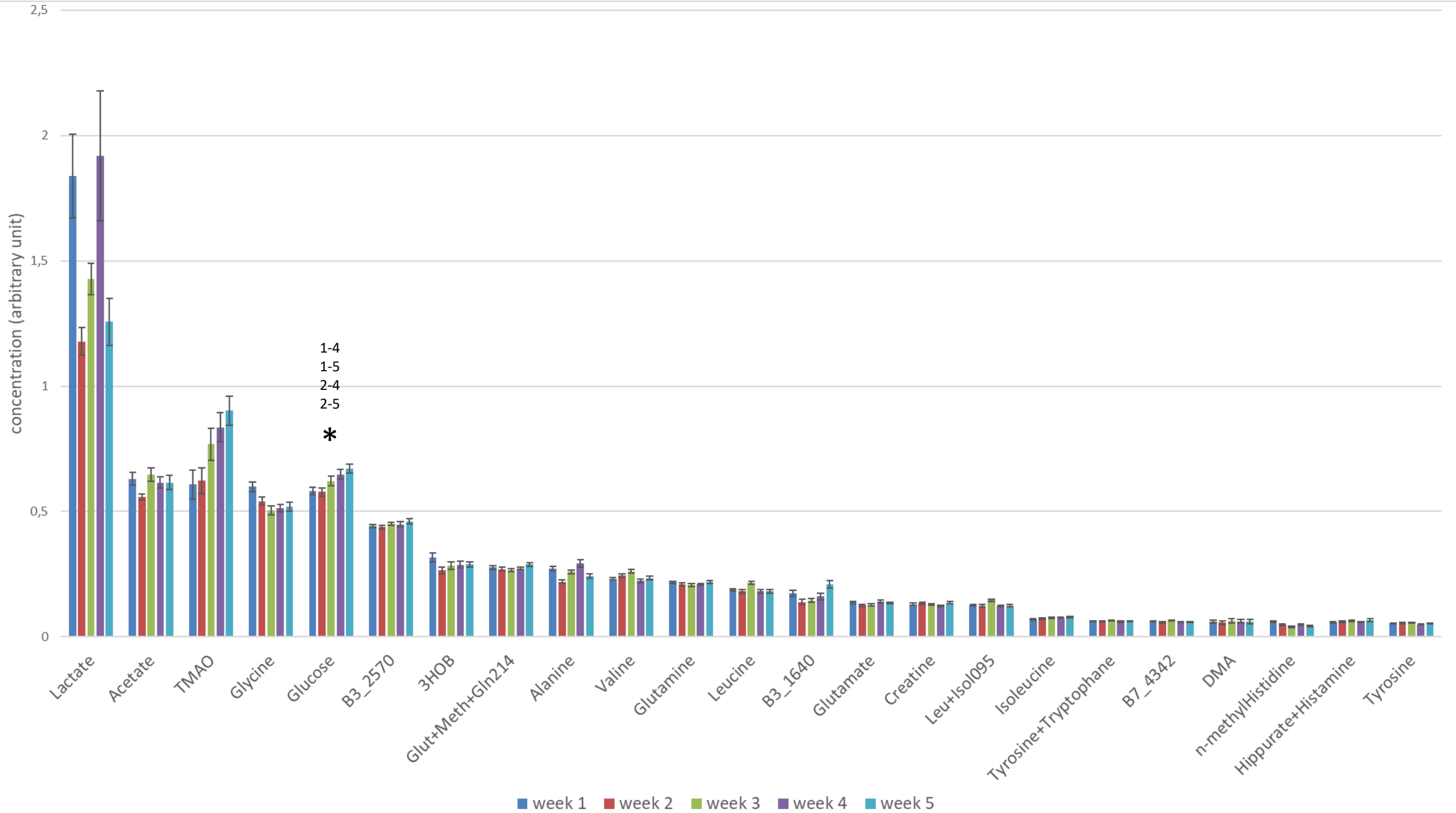
Figure legends

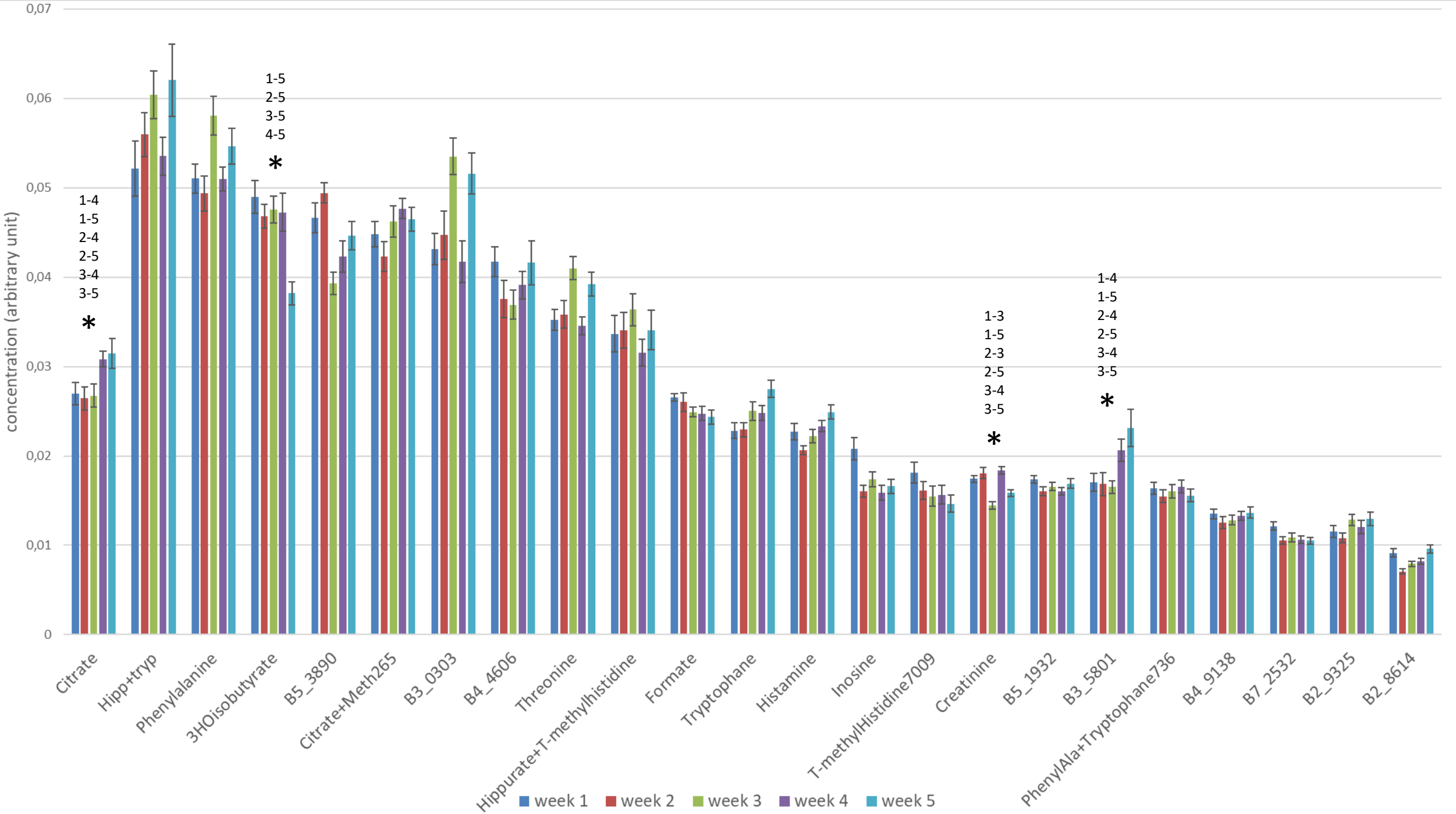
Figure 1. Average serum concentrations of identified and unidentified metabolites (arbitrary unit) for the 20 female goat kids from week 1 to week 5 until the first contact with bucks

(mean + SEM). Spectral intensities, named by compound name if known or by B-chemical-shift if unidentified, were scaled to the reference region. The y-axis was standardized to the maximal concentration so that the variations are easy-to-read. * shared metabolites between the three comparisons week 5 *vs* week 1, week 5 *vs* week 2 and week 5 *vs* week 3 (OPLS-DA). 1-4: levels differ significantly between week 1 and week 4 (t-test; $P \leq 0.05$). TMAO= trimethylamine-oxyde; 3HOB = 3 hydroxybutyrate; Glut + Meth + Gln = glutamate + methionine + glutamine; Leu + isol = leucine + isoleucine; DMA = dimethylamine; Hipp + tryp = hippurate + tryptophane; Phenylala = phenylalanine; 3HOisobutyrate = 3-hydroxy-isobutyrate; Citrate + Meth = citrate + methionine.

Figure 2. Score scatter plot resulting from the OPLS-DA model on ^{1}H-NMR spectra of female goat kid sera. Each dot correspond to one animal. Week 1 in green dot and week 5 in blue dot. $R^2Y(cum) = 0.99$, $Q^2(cum) = 0.9$. The predicted scores t_P [1] (predictive component) versus the orthogonal scores t_O [1] (orthogonal component) of the week 1 (green dots) and the week 5 (blue dots) are shown.

Figure 3. Venn diagram showing shared metabolites between side-by-side comparison of week 5 *vs* week 1, week 5 *vs* week 2 and week 5 *vs* week 3. 3-HO-Isobutyrate = 3-hydroxy-isobutyrate; Isoleu = isoleucine; TMAO = trimethylamine-oxyde; Tryp = tryptophane; Hista = histamine; 3-m-Histidine = 3-methyl-histidine; Glu + Meth + Gln = Glutamate + Methionine + Glutamine.


2,5
2
1,5
1
0,5
0
concentration (arbitrary unit)
1-4
1-5
2-4
2-5
*
Lactate
Acetate
TMAO
Glycine
Glucose
B3_2570
3HOB
Glut+Meth+Gln214
Alanine
Valine
Glutamine
Leucine
B3_1640
Glutamate
Creatine
Leu+Isol095
Isoleucine
Tyrosine+Tryptophane
B7_4342
DMA
n-methylHistidine
Hippurate+Histamine
Tyrosine
week 1
week 2
week 3
week 4
week 5


0,07
0,06
0,05
0,04
0,03
0,02
0,01
0
concentration (arbitrary unit)
1-4
1-5
2-4
2-5
3-4
3-5
*
1-5
2-5
3-5
4-5
*
1-3
1-5
2-3
2-5
3-4
3-5
*
1-4
1-5
2-4
2-5
3-4
3-5
*
Citrate
Hipp+tryp
Phenylalanine
3HOisobutyrate
B5_3890
Citrate+Meth265
B3_0303
B4_4606
Threonine
Hippurate+T-methylhistidine
Formate
Tryptophane
Histamine
Inosine
T-methylHistidine7009
Creatinine
B5_1932
B3_5801
PhenylAla+Tryptophane736
B4_9138
B7_2532
B2_9325
B2_8614
week 1
week 2
week 3
week 4
week 5

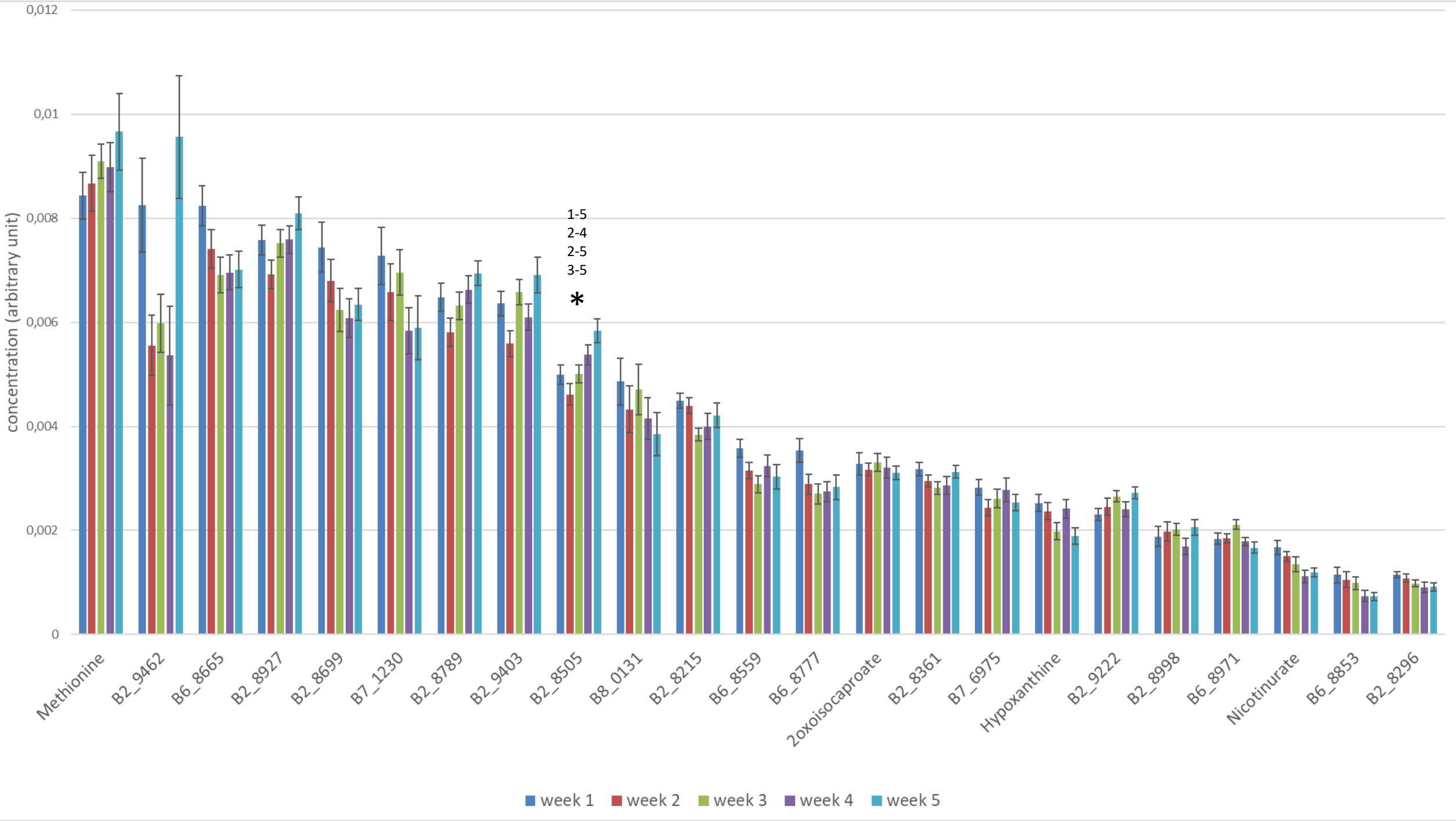

0,012
0,01
0,008
0,006
0,004
0,002
0
concentration (arbitrary unit)
1-5
2-4
2-5
3-5
*
Methionine
B2_9462
B6_8665
B2_8927
B2_8699
B7_1230
B2_8789
B2_9403
B2_8505
B8_0131
B2_8215
B6_8559
B6_8777
2oxoisocaproate
B2_8361
B7_6975
Hypoxanthine
B2_9222
B2_8998
B6_8971
Nicotinurate
B6_8853
B2_8296
week 1
week 2
week 3
week 4
week 5

Figure 2

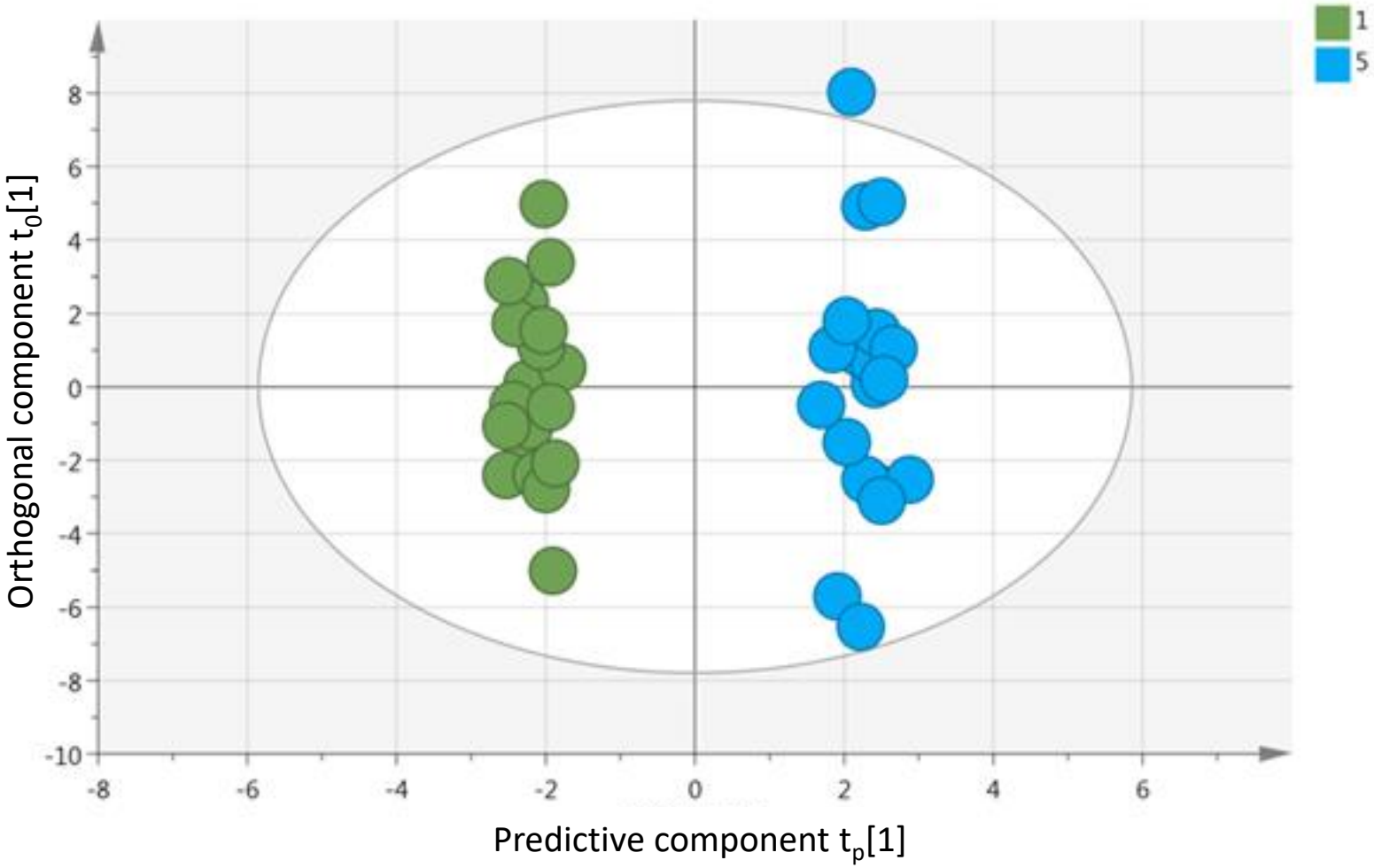

1
5
Orthogonal component t_0[1]
Predictive component t_p[1]
8
6
4
2
0
-2
-4
-6
-8
-10
-8
-6
-4
-2
0
2
4
6

Figure 3

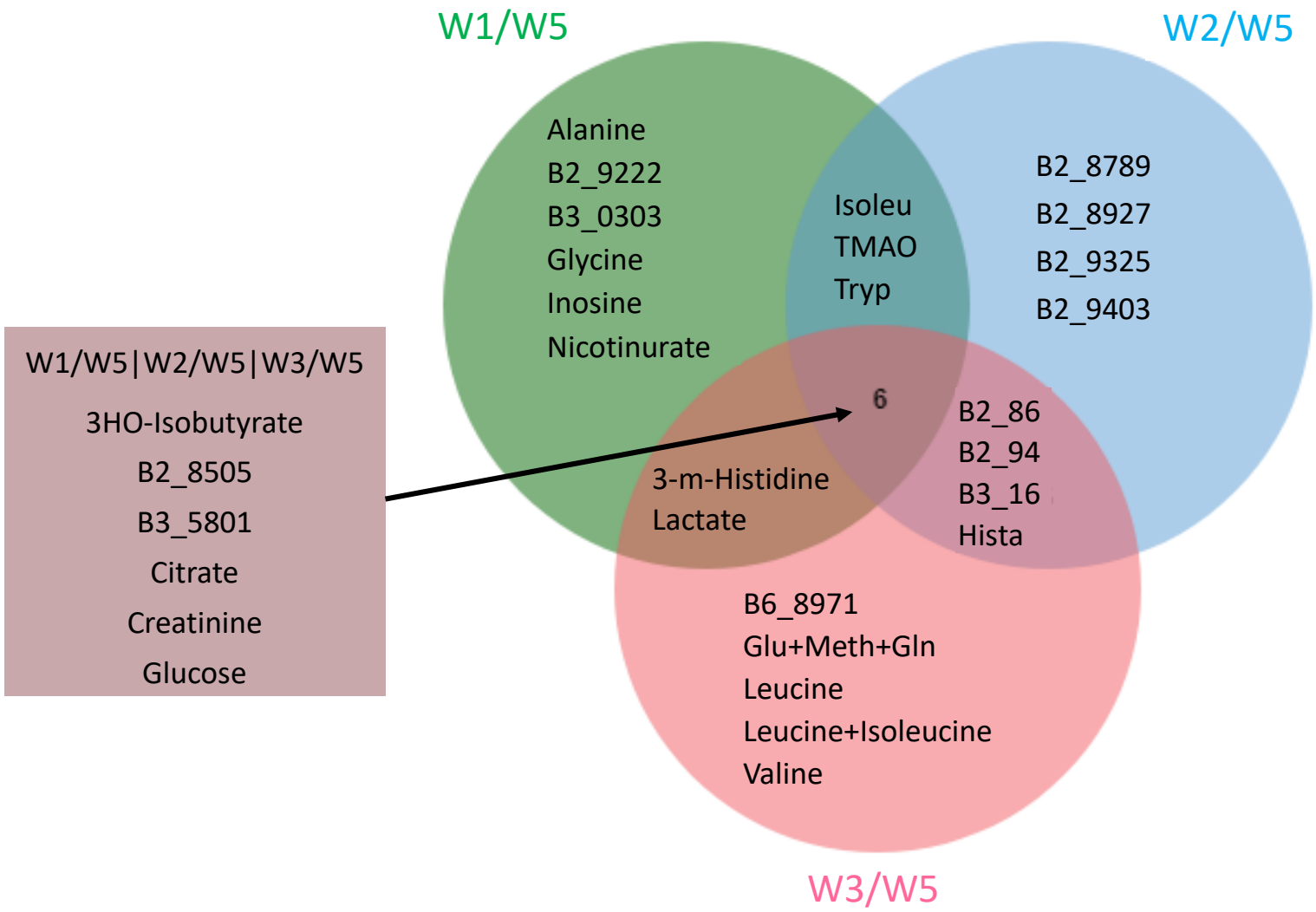

W1/W5
W2/W5
W3/W5
Alanine
B2_9222
B3_0303
Glycine
Inosine
Nicotinurate
Isoleu
TMAO
Tryp
B2_8789
B2_8927
B2_9325
B2_9403
6
B2_86
B2_94
B3_16
Hista
3-m-Histidine
Lactate
B6_8971
Glu+Meth+Gln
Leucine
Leucine+Isoleucine
Valine
W1/W5|W2/W5|W3/W5
3HO-Isobutyrate
B2_8505
B3_5801
Citrate
Creatinine
Glucose

Table 1: Summary of the quality criteria of the OPLS-DA models fitted on female goat kid serum ^{1}H-NMR data. R^2Y = goodness of fit; Q^2 = goodness of prediction; CV-ANOVA = Cross Validation-Analysis Of VAriance. Based on the quality criteria of the OPLS-DA models, the best models are mentioned in bold.

OPLS-DA models	Quality of the models			
	R²Y(cum)	Q²(cum)	CV-ANOVA	Number of spectral variables
Week 1 vs week 2	0.84	0.57	$4.80E^{-5}$	64
Week 1 vs week 3	0.78	0.64	$1.88E^{-7}$	58
Week 1 vs week 4	0.95	0.78	$5.15E^{-8}$	62
Week 1 vs week 5	**0.99**	**0.90**	**$2.98E^{-8}$**	**42**
Week 2 vs week 3	0.87	0.68	$4.56E^{-7}$	57
Week 2 vs week 4	0.70	0.51	$3.19E^{-5}$	61
Week 2 vs week 5	**0.91**	**0.80**	**$8.10E^{-10}$**	**60**
Week 3 vs week 4	0.95	0.81	$4.58E^{-9}$	58
Week 3 vs week 5	**0.93**	**0.78**	**$4.41E^{-8}$**	**59**
Week 4 vs week 5			No model	

Table 2: Serum metabolites identified by OPLS-DA discriminating between week 1 and week 5. VIP = Variable Importance in the Projection, *P*-value obtained by a *t*-test.

Var ID (Primary)	VIP	*P*-value	Week 1 *vs* Week 5
3-HO-Isobutyrate	1,45	0.00004	Up
3-methyl-Histidine	1,32	0.00030	Up
Glucose	1,31	0.00084	Down
TMAO	1,26	0.00107	Down
Tryptophane	1,23	0.00131	Down
Lactate	1,16	0.00005	Up
B2_8505	1,15	0.00681	Down
Creatinine	1,13	0.00717	Up
Glycine	1,12	0.00429	Up
B3_5801	1,11	0.04363	Down
B3_0303	1,11	0.00687	Down
B2_9222	1,09	0.01526	Down
Isoleucine	1,07	0.01921	Down
Citrate	1,04	0.03745	Down
Inosine	1,04	0.00819	Up
Alanine	1,04	0.02593	Up
Nicotinurate	1,01	0.00733	Up

3-HO-Isobutyrate = 3-hydroxy-isobutyrate; TMAO = trimethylamine-oxyde.

Table 3: Serum metabolites identified by OPLS-DA discriminating between week 2 and week 5.
VIP = Variable Importance in the Projection, *P*-value obtained by a *t*-test, *P*-value with (W) is calculated by the Wilcoxon Mann Whitney test.

Var ID (Primary)	VIP	*P*-value	Week 2 *vs* Week 5
Histamine	1.52	< 0.0001	Down
B2_8505	1.52	0.0004	Down
B2_8614	1.49	0.0001	Down
3HO-Isobutyrate	1.47	< 0.0001	Up
B2_8789	1.42	0.0034	Down
B2_9403	1.41	0.0042	Down
Tryptophane	1.38	0.0009	Down
B2_8927	1.35	0.0081	Down
Glucose	1.32	0.0008	Down
B3_1640	1.25	0.0004	Down
TMAO	1.23	0.0011	Down
B2_9462	1.20	0.0039 (W)	Down
Creatinine	1.17	0.0047	Up
B2_9325	1.17	0.0264	Down
B3_5801	1.13	0.0328 (W)	Down
Isoleucine	1.07	0.0379 (W)	Down
Citrate	1.01	0.0236	Down

3-HO-Isobutyrate = 3-hydroxy-isobutyrate; TMAO = trimethylamine-oxyde.

Table 4: Serum metabolites identified by OPLS-DA discriminating between week 3 and week 5.
VIP = Variable Importance in the Projection, *P*-value obtained by a *t*-test, *P*-value with (W) is calculated by the Wilcoxon Mann Whitney test.

Var ID (Primary)	VIP	*P*-value	Week 3 *vs* Week 5
3HO-Isobutyrate	1.52	< 0.0001	Up
Leucine	1.45	0.0017	Up
Leucine+Isoleucine	1.44	0.0022	Up
B3_1640	1.36	0.0007	Down
B2_8505	1.27	0.0070	Down
3-methylHistidine	1.26	0.0020	Down
B6_8971	1.26	0.0038	Up
Valine	1.25	0.0151	Up
B3_5801	1.24	0.0192 (W)	Down
Glucose	1.21	0.0043 (W)	Down
B2_8614	1.20	0.0015 (W)	Down
B2_9462	1.15	0.0075 (W)	Down
Histamine	1.09	0.0223	Down
Glu+Meth+Gln	1.09	0.0292	Down
Creatinine	1.08	0.0217	Down
Citrate	1.00	0.0312	Down

3-HO-Isobutyrate = 3-hydroxy-isobutyrate; Glu+Meth+Gln = Glutamate + Methionine + Glutamine.