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1 **Characterization of serum metabolome changes during the 5 weeks prior to breeding in**  
2 **female goat kids.**

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15

16 **ABSTRACT**

17 This prospective study aims to analyze the serum metabolome of female goat kids just before  
18 the first breeding in order to characterize the changes in metabolites during sexual maturation.

19 A better knowledge of these changes could help optimizing the time for first breeding and  
20 improving the fertilization rates at first insemination. Weekly blood sampling was performed  
21 on twenty 6- to 7-month-old female goats from Alpine breed born in February during the 5  
22 weeks preceding their first contact with bucks in September. Progesterone assays and  
23 metabolome analysis using <sup>1</sup>H Nuclear Magnetic Resonance Spectroscopy were performed on  
24 the serum samples. No spontaneous ovulatory cycle was observed before breeding based on  
25 progesterone assays. All female goats had reached the pubertal stage of maturity at breeding

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

36

37 **Keywords:**

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

40

41 **Introduction**

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

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

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

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

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

78 Omics approaches are powerful tools to identify large numbers of biomolecules in body  
79 fluids. Nuclear Magnetic Resonance (NMR) spectroscopy is a repeatable, reliable and stable  
80 technique that allows a non-targeted identification of the metabolites present in a biological  
81 sample. The metabolome includes low-molecular-weight compounds such as peptides, lipids,  
82 carbohydrates, amino acids, vitamins and minerals. It is the signature of a physiological state.  
83 The objective of this prospective study was to analyze the serum metabolome of female goat  
84 kids just before the first breeding in order to characterize the changes in metabolites during  
85 sexual maturation.

86

## 87 **Materials and methods**

### 88 *Animals, housing and blood samples collection*

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

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

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

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

114 Weekly blood samples were collected on the twenty female goats from 4 month of age until  
115 breeding. Blood was collected in dry tubes, coagulated at room temperature for 15-30 minutes  
116 and centrifuged for 15 minutes at 300g and 20°C. The supernatant was transferred in  
117 hemolytic tubes, frozen and stored at -20°C until analysis. ELISA progesterone assays were  
118 performed on all samples to monitor spontaneous ovulatory activity. Metabolome analysis  
119 using <sup>1</sup>H Nuclear Magnetic Resonance Spectroscopy was performed on samples collected  
120 during the last 5 weeks before the first contact with bucks (August 12<sup>th</sup>, 19<sup>th</sup>, 26<sup>th</sup>, September  
121 2<sup>nd</sup>, 9<sup>th</sup>).

122 Transrectal ultrasonography was performed using a 7.5 MHz linear transducer (ALOKA-SSD  
123 900, Japan Care Co. Ltd, Kobe-Shi, Japan) with the goats in standing position one week  
124 before buck introduction. The number of follicles with a diameter  $\geq$  3mm and presence or

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

#### 128 *Progesterone assay*

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

#### 134 *<sup>1</sup>H-nuclear magnetic resonance analysis of serum samples*

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

141 <sup>1</sup>H-NMR spectra were obtained with a Bruker DRX-600 AVANCE-III HD spectrometer  
142 (Bruker SADIS, Wissembourg, France). A “noesypr-1d” sequence was used to acquire 64  
143 scans on a time domain of 64K data points with a spectral width of 7500 Hz. After Fourier  
144 transformation, baseline and phase correction were applied.

145 NMRProcFlow (version 1.1, INRA UMR 1332 BFP, Bordeaux Metabolomics Facility,  
146 France) (Jacob et al., 2017) was used to reduce <sup>1</sup>H-NMR spectra into a data table containing  
147 variable integrated regions. Spectral intensities, named by compound name if known or by B-  
148 chemical-shift if unidentified, were scaled to the reference region (TSP). Assignments were

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

### 151 *Multivariate analysis*

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

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

167

## 168 **Results**

### 169 *Spontaneous ovulatory activity and fertility*

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

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

211 In order to seek the VIP (metabolites) shared between the side-by-side comparison (week 5  
212 *versus* week 1, week 5 *versus* week 2, week 5 *versus* week 3), a Venn diagram (Bardou et al.,  
213 2014) (<http://jvenn.toulouse.inra.fr>) was established and shared metabolites could be assessed  
214 as potential biomarkers of sexual precocity (Figure 3). The Venn diagram allows highlighting  
215 shared metabolites when comparing different conditions. The shared metabolites between the  
216 three weeks comparison (meaning week 5 and week 1, week 5 and week 2, week 5 and week  
217 3) are 3-hydroxy-isobutyrate (3HO-isobutyrate), citrate, creatinine, glucose and two  
218 unidentified bins (B2-8505 and B3-5801). Among these six metabolites, 3HO-isobutyrate and  
219 creatinine levels progressively decreased whereas the others increased from week 1 to week 5.

220

## 221 **Discussion**

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

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

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

247 2017; Zhang et al., 2019). This could be due to differences in the analytical techniques.  
248 Alternatively, this suggests that pubertal development could be related to variations of the  
249 metabolome, and that further potential biomarkers of sexual maturation could be identified by  
250 serum metabolome analysis.

251 When the metabolic profiles were compared between the five weeks before the first contact  
252 with bucks, six metabolites were identified that could differentiate between week 5 and week  
253 1, week 2 and week 3: 3-hydroxy-isobutyrate, citrate, creatinine, glucose and two unidentified  
254 bins. These metabolites could be assessed as potential biomarkers of sexual development  
255 before puberty. Several studies supporting this assertion have been published. In a study  
256 focusing on the relationships between age at puberty and blood metabolic profiles in dairy  
257 heifers from 6 to 15 months of age, Abeni and collaborators showed modifications of the  
258 glucose and creatinine levels with age before puberty (Abeni et al., 2019). In heifers,  
259 creatinine levels increased with age whereas glucose level decreased. When analyzing the  
260 alterations in blood serum chemistry values in the puberty period in goats, Devrim and  
261 collaborators showed that creatinine levels significantly increased with age (Devrim et al.,  
262 2015). Urinary excretion of creatinine was showed to peak around puberty in healthy  
263 schoolchildren; boys excreting significantly more creatinine compared with girls before and  
264 during puberty, reflecting greater muscle mass (Skinner et al., 1996). Citrate was detected in  
265 urine from children and similar rates were observed in both male and female subjects until  
266 puberty during which time citrate excretion became significantly higher in girls (Kirejczyk et  
267 al., 2014). Moreover, 3-hydroxy-isobutyrate has been identified in urinary metabolome from  
268 8–9 years old children (Maitre et al., 2017) and serum metabolome of lambs (Pereira et al.,  
269 2020). Among 78 metabolites for discrimination between central precocious puberty (CPP)  
270 and healthy individuals, 6 urinary compounds including creatinine provided a good prediction  
271 (Qi et al., 2012). The plasma and urine metabolome of 12-15-year-old adolescents showed

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

285 In addition to these six identified metabolites, the side-by-side comparison (week 5 *versus*  
286 week 3, week 5 *versus* week 2, week 5 *versus* week 1) highlighted several metabolites such as  
287 amino acids (alanine, glycine, isoleucine, leucine, tryptophan, valine, 3-methyl-histidine) and  
288 overlapping of amino acids (leucine + isoleucine and glutamate + methionine + glutamine,  
289 due to the inability to discern these metabolites). In human, puberty has been shown to be  
290 related to significant modifications of some amino acids levels in serum such as alanine,  
291 glycine, tryptophan, valine, methyl-histidine and leucine + isoleucine (Cominetti et al., 2020;  
292 Hirschel et al., 2020). They may be biomarkers of sexual maturation. However, since diet may  
293 influence their concentration, amino acids may not be robust biomarkers. The side-by-side  
294 comparison also highlighted lactate, TMAO and histamine. Metabolomics analysis in human  
295 and equine showed that follicular fluid contain lactate, TMAO and histamine, which could be  
296 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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318

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322

323 **Author contribution statement:**

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

332

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334

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451

## 452 **Figure legends**

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

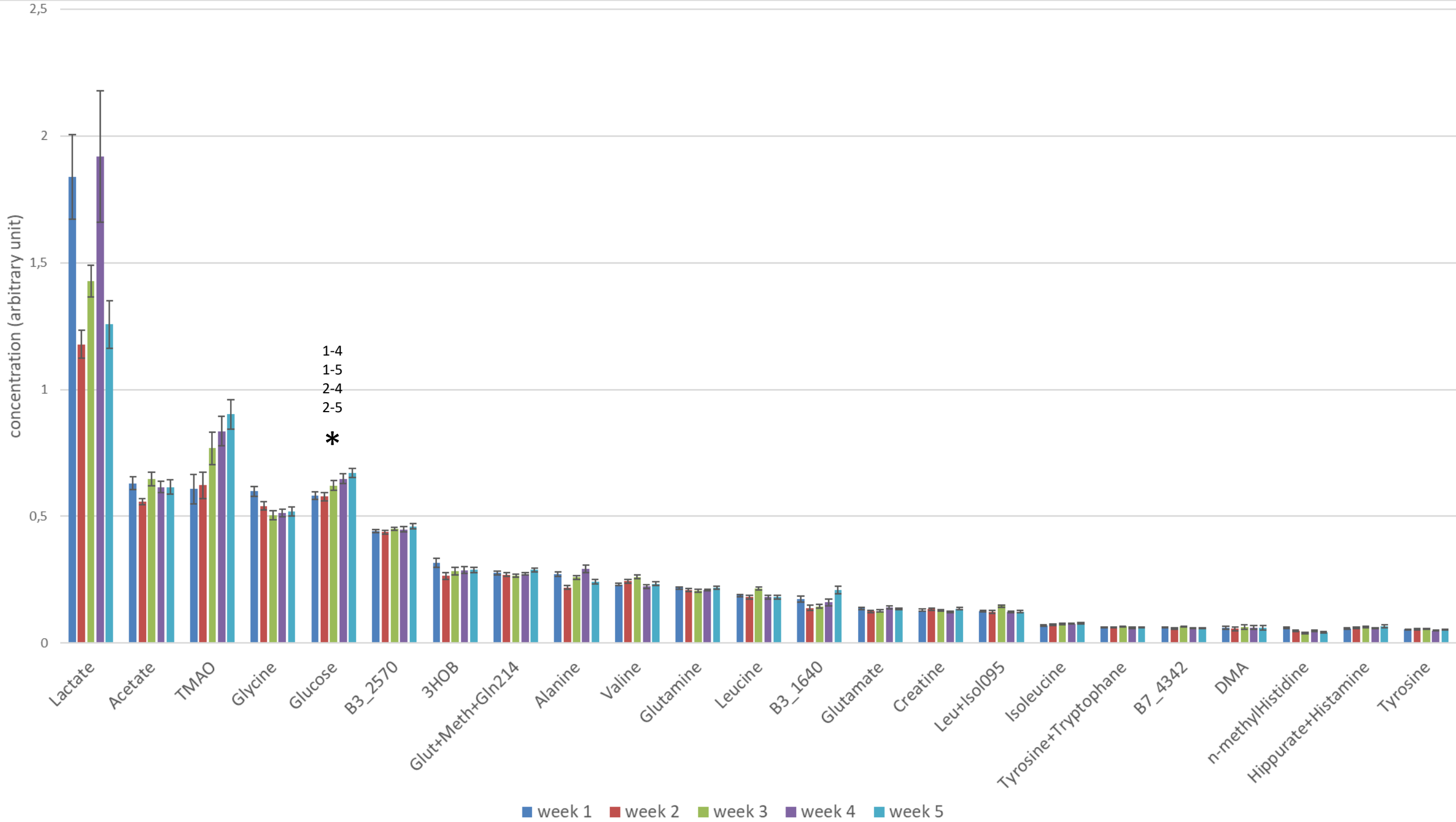
455 (mean + SEM). Spectral intensities, named by compound name if known or by B-chemical-  
456 shift if unidentified, were scaled to the reference region. The y-axis was standardized to the  
457 maximal concentration so that the variations are easy-to-read. \* shared metabolites between  
458 the three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

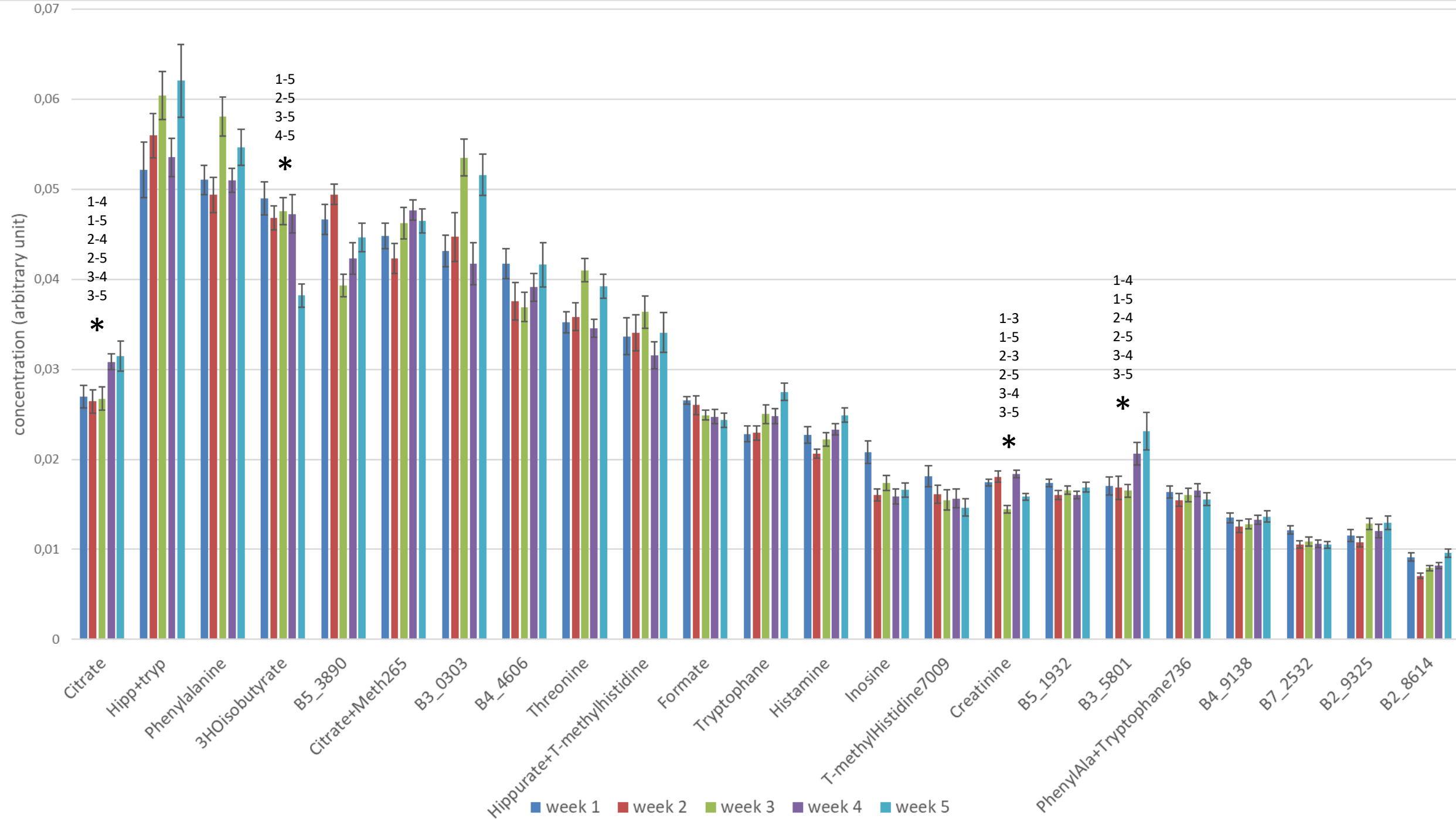
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465 **Figure 2.** Score scatter plot resulting from the OPLS-DA model on  $^1\text{H-NMR}$  spectra of  
466 female goat kid sera. Each dot correspond to one animal. Week 1 in green dot and week 5 in  
467 blue dot.  $R^2Y(\text{cum}) = 0.99$ ,  $Q^2(\text{cum})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

470

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





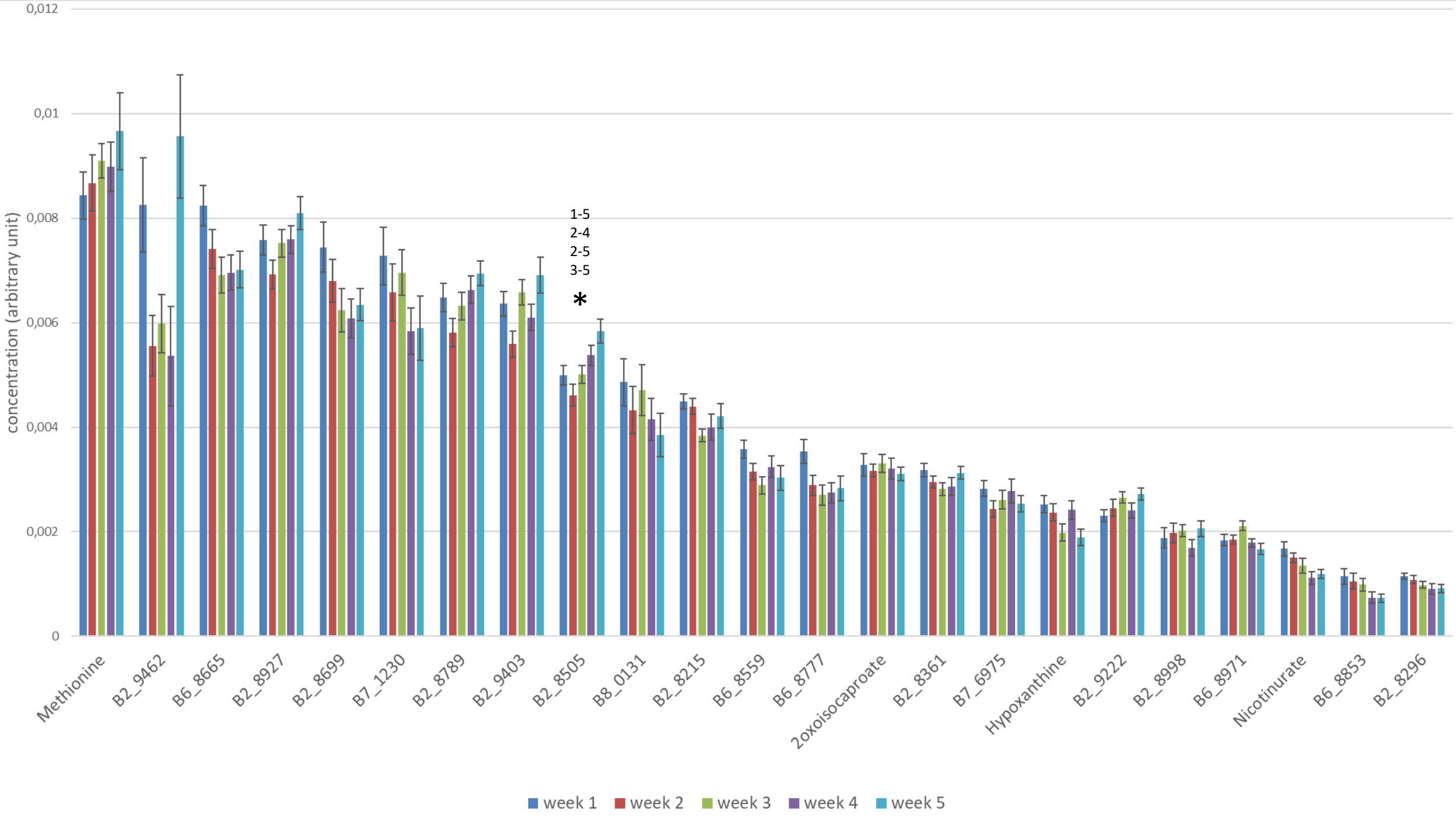


Figure 2

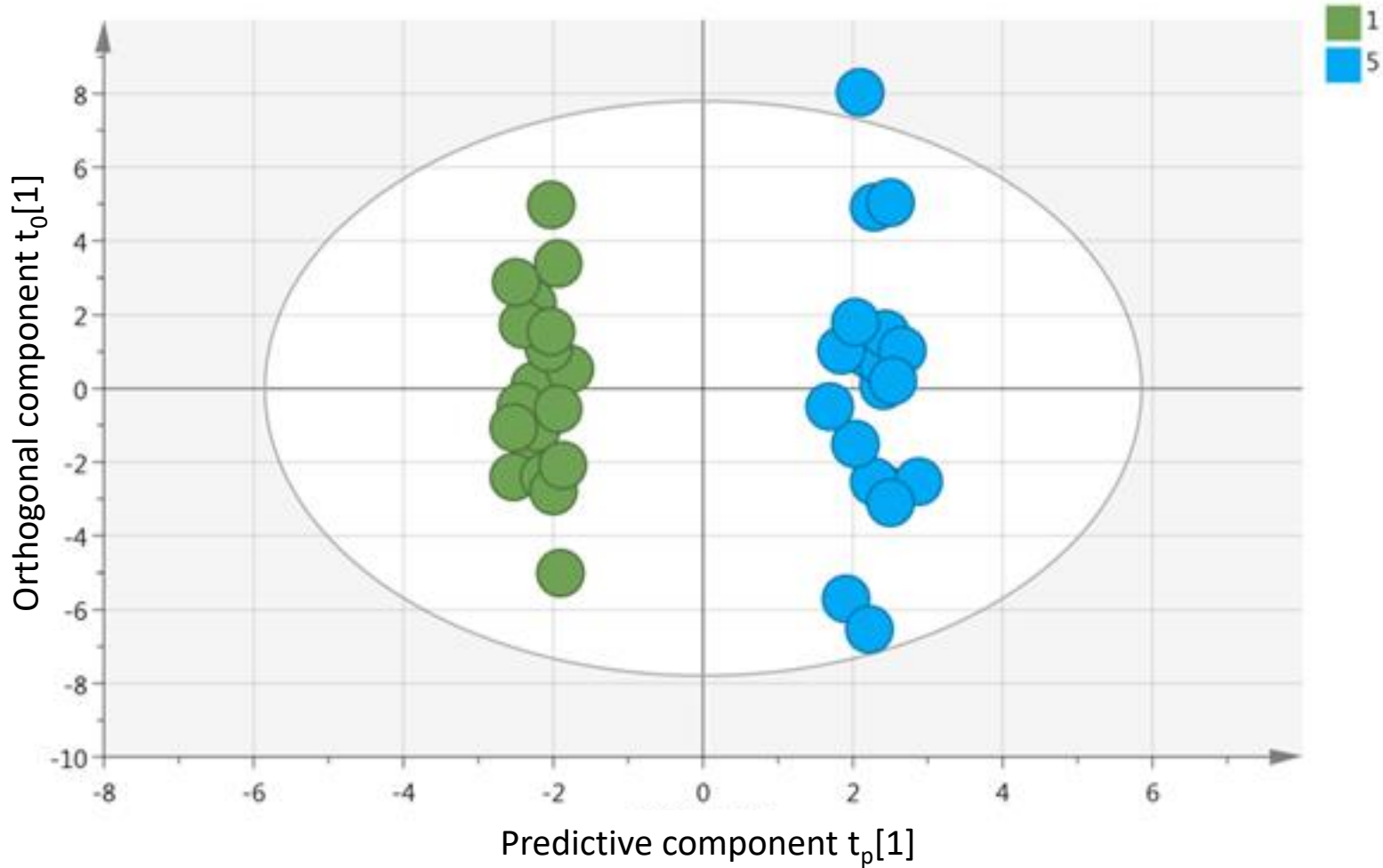
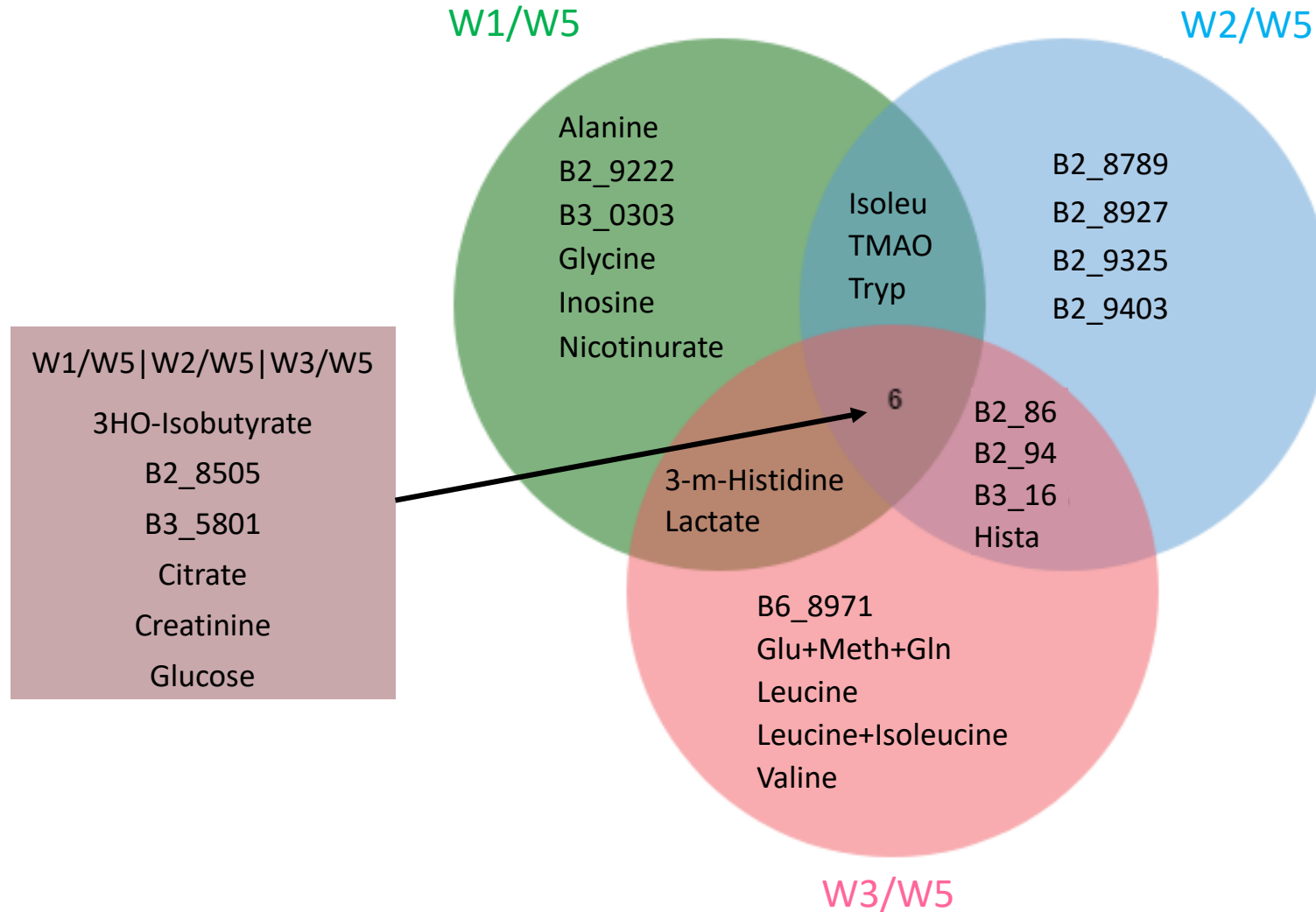


Figure 3



**Table 1:** Summary of the quality criteria of the OPLS-DA models fitted on female goat kid serum <sup>1</sup>H-NMR data. R<sup>2</sup>Y = goodness of fit; Q<sup>2</sup> = 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			
	R <sup>2</sup> Y(cum)	Q <sup>2</sup> (cum)	CV-ANOVA	Number of spectral variables
Week 1 vs week 2	0.84	0.57	4.80E <sup>-5</sup>	64
Week 1 vs week 3	0.78	0.64	1.88E <sup>-7</sup>	58
Week 1 vs week 4	0.95	0.78	5.15E <sup>-8</sup>	62
Week 1 vs week 5	<b>0.99</b>	<b>0.90</b>	<b>2.98E<sup>-8</sup></b>	<b>42</b>
Week 2 vs week 3	0.87	0.68	4.56E <sup>-7</sup>	57
Week 2 vs week 4	0.70	0.51	3.19E <sup>-5</sup>	61
Week 2 vs week 5	<b>0.91</b>	<b>0.80</b>	<b>8.10E<sup>-10</sup></b>	<b>60</b>
Week 3 vs week 4	0.95	0.81	4.58E <sup>-9</sup>	58
Week 3 vs week 5	<b>0.93</b>	<b>0.78</b>	<b>4.41E<sup>-8</sup></b>	<b>59</b>
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	<i>P</i> -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.

<b>Var ID (Primary)</b>	<b>VIP</b>	<b><i>P</i>-value</b>	<b>Week 2 vs Week 5</b>
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.

<b>Var ID (Primary)</b>	<b>VIP</b>	<b><i>P</i>-value</b>	<b>Week 3 vs Week 5</b>
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.