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Research article

EQUINE WHOLE SALIVA: DAILY REPRODUCIBILITY OF SELECTED BIOCHEMICAL PARAMETERS AND PROTEIN ELECTROPHORETIC PATTERNS (PILOT STUDY)

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Salivary analytes are known to exhibit variability associated with time of day and seasonal influences, reflecting circadian rhythmicity. Whole saliva provides a more comprehensive representation of salivary composition compared to commonly studied parotid saliva. Objective of the study was to investigate reproducibility of selected equine whole-saliva constituents when measured at different times of day across four consecutive days, and to qualitatively access stability of salivary protein fractions using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Six clinically healthy Warmblood horses were enrolled in the study. Saliva samples were taken twice a day, in the morning and in the evening. Data were assessed descriptively and expressed as median (range), with $P<0.05$ considered significant. Linear mixed-effects models revealed that time of day had no significant effect on most salivary parameters in horses, with the exception of adenosine deaminase ($P=0.020$). The sampling day had a significant effect on uric acid ($P=0.003$), total proteins ($P=0.007$), adenosine deaminase ($P=0.011$), alkaline phosphatase ($P<0.001$), γ-glutamyl transferase ($P=0.013$) and calcium ($P=0.005$). Interclass correlation coefficients (ICC) were low for most parameters (ICC≈0.00–0.16), indicating that within-horse variability exceeded between-horse variability. The relative proportions of salivary electrophoretic fractions were highly stable and not significantly affected by sampling day, time of day, or their interaction. Despite the identified variability, the overall stability of the salivary electrophoretic profile supports the potential use of salivary protein fractions as qualitative biomarkers.

Keywords: horse, salivary analytes, SDS-PAGE, variability

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INTRODUCTION

Saliva has emerged as valuable non-invasive sample for studying animal health and physiology since it can be collected without restraining the animal and without causing significant stress. It contains hormones, enzymes, antibodies, and other biomarkers that reflect the animal's metabolic, immune, and stress status. In horses, saliva sampling requires particular care to ensure analytical validity, as contamination with feed and different collecting materials can markedly alter salivary biochemical composition [1]. Although oral cavity lavage is traditionally performed using 400 mL syringe, this approach may require substantial restraint and is frequently facilitated by sedation in order to ensure adequate control [2]. Consequently, alternative approaches such as the potential usage of silicone nasogastric tubes in oral lavage of non-sedated horses, may represent a useful option in equine salivary sampling protocols.

Most equine studies focus exclusively on parotid saliva to minimize the variability in salivary composition attributable to differences among secreting glands [3], with relatively few addressing whole saliva and its natural biological variability. Studying whole saliva provides a more comprehensive representation of salivary composition, while offering a simpler and less technically demanding sampling approach compared to parotid saliva, which may improve feasibility in both clinical and research settings.

Salivary analytes are known to exhibit variability associated with the time of day and seasonal influences, reflecting circadian rhythmicity [4]. In humans, salivary inflammatory biomarkers and total protein concentrations have been shown to vary considerably within the same individual across different days, with this variability influenced by multiple physiological and external factors, including autonomic nervous system activity, medication use, circadian rhythmicity, and physical exercise [5]. Whole saliva is widely used as a biological sample for diagnostics and research, in both humans and animals [6,7]. However, limited information is available regarding biological variability and reproducibility of salivary constituents in horses, particularly when whole saliva is analysed. Understanding this is necessary to ensure that differences observed reflect real biological state and as such can be used in facilitating diagnostics of oral and systemic diseases in horses.

The salivary analytes evaluated in this study included urea, uric acid (UA), total proteins, total adenosine deaminase (ADA), alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), and calcium. Urea and uric acid are products of nitrogen metabolism [8], with urea participating in oral buffering through bacterial urease [9], and uric acid being associated with metabolic and inflammatory processes [10]. Combined with total proteins and adenosine deaminase as markers of inflammation and immune status, and ALP, GGT, and calcium as indicators of abdominal disease and mineral balance, these parameters can offer a comprehensive assessment of overall equine health [11,12].

Therefore, the aim of the present study was to investigate the reproducibility of selected equine whole-saliva constituents when measured at different times of the

day across four consecutive days, and to qualitatively assess the stability of salivary protein fractions using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

MATERIALS AND METHODS

Animals

The study was approved by Ethical Committee at the Faculty of Veterinary Medicine, University of Belgrade, Serbia (permission number: 01-07/2024).

Six clinically healthy and privately-owned horses analysed in the research were randomly selected from the Equestrian Club at the Hippodrome in Belgrade, Serbia, covering a four-day period in September 2025. The population was composed of three mares, two geldings and one stallion with 8.5 [7-21] (median [min-max]) years of age, all of which were Warmbloods. The horses were bright, alert and responsive, showed no signs of pain and had heart and respiratory rate within normal limits. They were kept individually in conventional horse stalls, and used in show-jumping or for recreational purposes. All horses were fed hay and oats three times a day from 7 AM to 7 PM, at consistent intervals. They had *ad libitum* access to water.

Saliva sampling

Throughout the study, the same experienced veterinarians handled all the animals. Saliva samples were collected twice a day, before feeding. Due to the fact that conventional 400 mL syringes are less practical in non-sedated horses, often requiring greater force and increased head fixation, mouth wash was performed using a flexible silicone nasogastric tube which enabled controlled lavage pressure and efficient removal of food debris, while the horses were minimally restrained. Five minutes after flushing [1], saliva samples were collected using a 5 × 5 cm cotton gauze pad clipped on a metal forceps, which was introduced into the horses' mouth through the bars. Horses were let to chew on the pads for one minute. Samples were taken on four consecutive days. After saliva collection, cotton pads were placed in tubes and centrifuged immediately at 4000 rpm (1790 RCF) for 5 min to obtain saliva specimens, which were stored at -20°C until analyses. Out of 48 samples obtained, only one sample was excluded from the analyses due to the insignificant amount obtained.

Saliva composition analysis

Biochemistry profile of the specimens was determined using Mindray BS-240 analyser (Mindray, Shenzhen, China) and manufacturer's corresponding chemistry for urea, UA, total proteins, ADA, ALP, GGT and calcium.

Salivary protein bands were assessed by SDS-PAGE [13]. Samples were prepared using sampling buffer with addition of 5% β-mercaptoethanol and run on 12% polyacrylamide gel. Electrophoresis was performed at a constant voltage of 80 V for 15 minutes, with subsequential run at 120 V for 1h and 30 minutes. Gels were stained in 0.25% Coomassie Brilliant Blue (CBB) solution and destained in 30% methanol/10% acetic acid solution, until blue bands appeared. The gels were conserved in 7% acetic acid. Gel images were acquired using ChemiDoc 2.0 (Bio-Rad, Hercules, CA, US), and scans were analysed using Total Lab TL 120 software (GE HealthCare LifeScience, NJ, USA) to determine the relative distribution of clusters of protein bands. The former were analysed in clusters due to the even distribution of bands into three groups. First cluster consisted of proteins with a molecular weight over 55 kDa, second cluster from 25 to 55 kDa, and the third cluster consisted of fractions less than 25 kDa. The molecular weight was determined in accordance with molecular weight standards run with protein samples (PageRuler™ Prestained Protein Ladder, Thermo Scientific).

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics (version 20). Data were assessed descriptively and expressed as median (range). Effects of time of day (morning/evening), sampling day, and their interactions were evaluated using a linear mixed-effects model (LMM) with restricted maximum likelihood (REML) estimation. To evaluate within-horse stability, the interclass correlation coefficient (ICC) was calculated from variance components of the mixed model. Statistical significance was set at $P<0.05$.

RESULTS

Daily salivary biochemical parameters are presented in Table 1, with morning and evening samples presented in Table 2. Values obtained show similar median values for morning and evening samples, with wide but overlapping ranges.

Comparisons of morning and evening values across sampling days and their reliability are presented in Table 3. Linear mixed-effects models revealed that time of day had no significant effect on most salivary parameters in horses, with the exception of ADA. In contrast, the sampling day had a significant effect on the majority of analytes, including UA, total proteins, ADA, ALP, GGT and calcium. Significant interactions between time of day and sampling day were observed for ADA and ALP. Interclass correlation coefficients were low for most parameters (ICC≈0.00–0.16), indicating that within-horse variability exceeded between-horse variability; ICC for total proteins could not be reliably estimated due to model constraints.

Table 1. Salivary biochemical parameters in horses across four consecutive sampling days (N=6). Values are presented as median [min–max].

Parameter (unit)	Day 1	Day 2	Day 3	Day 4
Urea (mmol/L)	2.44 [1.67–7.62]	2.83 [0.96–5.28]	1.59 [0.69–4.42]	2.96 [1.52–5.82]
UA (µmol/L)	45.05 [20.5–70.8]	34.8 [25.5–56.5]	17.25 [8.6–58.2]	45.7 [11.5–89.8]
TP (g/L)	1.25 [0.7–2.52]	1.08 [0.57–1.91]	0.65 [0.19–1.84]	1.18 [0.39–2.6]
ADA (U/L)	28.89 [13.02–69.43]	26.67 [7.01–54.18]	14.3 [4.21–32.43]	24.85 [10.14–68.62]
ALP (U/L)	51.40 [14.3–103.3]	46.9 [20.6–69.2]	15.45 [2.9–34.9]	33.75 [8.8–77.6]
GGT (U/L)	37.8 [16.6–75.2]	31.6 [14.6–48]	16.75 [6.4–45.9]	28.95 [9.7–84.8]
Ca (mmol/L)	2.53 [1.65–4.86]	3.13 [1.36–4.51]	2.12 [1.35–3.4]	3.39 [2.44–5.21]

Abbreviations: **UA** – uric acid, **TP** – total proteins, **ADA** – total adenosine deaminase, **ALP** – alkaline phosphatase, **GGT** – γ glutamyl transferase, **Ca** – calcium.

Table 2. Salivary biochemical parameters in horses (N=6). Values are presented as median [min–max] for morning and evening samples pooled across four consecutive sampling days.

Parameter (unit)	Morning	Evening
Urea (mmol/L)	2.49 [0.86–5.46]	2.19 [0.69–7.62]
UA (µmol/L)	34.30 [12.60–79.40]	43.10 [8.60–89.80]
TP (g/L)	1.09 [0.30–2.26]	1.10 [0.19–2.60]
ADA (U/L)	27.26 [7.25–68.62]	17.68 [4.21–69.43]
ALP (U/L)	34.95 [8.40–74.40]	37.80 [2.90–103.30]
GGT (U/L)	29.85 [7.80–84.80]	32 [6.40–75.20]
Calcium (mmol/L)	2.84 [1.55–4.95]	2.85 [1.35–5.21]

Abbreviations: **UA** – uric acid, **TP** – total proteins, **ADA** – total adenosine deaminase, **ALP** – alkaline phosphatase, **GGT** – γ glutamyl transferase.

Table 3. Effects of time of day (morning and evening), sampling day (Day 1–4), and their interaction on salivary biochemical parameters in horses (N=6), assessed using linear mixed-effects models with horse as a random effect.

Parameter (unit)	Diurnal effect	Daily effect	Diurnal vs. Daily effect	ICC(≈)
Urea (mmol/L)	P=0.991	P=0.093	P=0.297	0.16
UA (µmol/L)	P=0.220	P=0.003, F=5.42	P=0.675	0.00
TP (g/L)	P=0.993	P=0.007, F=4.71	P=0.178	*
ADA (U/I)	P=0.020, F=5.91	P=0.011, F=4.22	P=0.038, F=3.10	0.10
ALP (U/I)	P=0.718	P<0.001, F=9.36	P=0.044, F=2.97	0.12
GGT (U/I)	P=0.874	P=0.013, F=4.11	P=0.206	0.15
Calcium (mmol/L)	P=0.657	P=0.005, F=5.02	P=0.881	0.12

Abbreviations: **UA** – uric acid, **TP** – total proteins, **ADA** – total adenosine deaminase, **ALP** – alkaline phosphatase, **GGT** – γ glutamyl transferase. Note: F-values (**F**) represent the test statistic for fixed effects; only significant effects are reported (P<0.05). Interclass correlation coefficients (**ICC**) are provided to estimate within-horse reproducibility. *modelling issues.

Salivary SDS-PAGE enabled continuous visualization of 15 protein bands with molecular weight between 15 to 180 kDa (Figure 1).

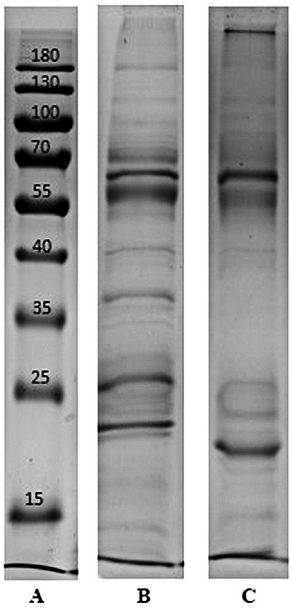

Figure 1. Representative salivary SDS-PAGE protein profile of the same horse: (**A**) Protein Ruler, (**B**) Morning saliva, (**C**) Evening saliva.

The relative distribution of salivary protein band clusters is shown in Table 4. The results show similar distribution between morning and evening samples, with comparable median values and substantial overlap of ranges across all clusters.

Table 4. Relative distribution (%) of salivary protein band clusters determined by SDS-PAGE in morning and evening samples. Values are presented as median [min–max] across all sampling days.

Parameter (kDa)	Morning	Evening
First cluster (>55)	38.16 [34.78–42.79]	39.65 [36.17–43.25]
Second cluster (25–55)	26.69 [18.86–29.10]	26.20 [18.41–33.31]
Third cluster (<25)	36.26 [30.33–39.43]	35.64 [26.48–41.13]

The relative proportions of salivary electrophoretic fractions were highly stable and were not significantly affected by sampling day, time of day, or their interaction (Table 5).

Table 5. Comparison of morning and evening salivary band clusters across sampling days in horses (N=6) assessed using linear mixed-effects models with horse as a random effect.

Parameter (kDa)	Diurnal effect	Daily effect	Diurnal vs. Daily effect
First cluster (>55)	P=0.302	P=0.996	P=0.601
Second cluster (25–55)	P=0.757	P=0.772	P=0.372
Third cluster (<25)	P=0.686	P=0.660	P=0.615

Note: $P<0.05$ is considered significant.

DISCUSSION

Since whole saliva represents a mixture of secretions from multiple salivary glands with distinct composition, variability in its biochemical profile is expected [14]. Salivary urea and UA are primarily blood-derived analytes that enter the saliva by passive diffusion or filtration across the salivary gland epithelium [15], whilst ADA, ALP, and GGT are mainly released from salivary gland cells, oral epithelium, inflammatory and immune cells, and periodontal tissues [16,17]. On the other hand, salivary calcium is mostly actively secreted by salivary acinar cells [18].

The results obtained in the present study indicate the absence of the diurnal effects for the majority of the salivary analytes. In contrast, ADA activity showed a clear diurnal pattern, suggesting a potential circadian influence on this enzyme. A circadian pattern of salivary ADA has previously been reported in horses [4], supporting the biological

plausibility of time-dependent regulation. Such variation may be related to the role of adenosine metabolism in the regulation of the sleep-wake cycle, as suggested by earlier studies on adenosine-metabolizing enzymes [19,20].

In contrast to the limited influence of time of day, the sampling day exerted a significant effect on most analytes, including UA, total proteins, ADA, ALP, GGT and calcium. This finding suggests that repeated sampling over consecutive days may reflect cumulative physiological effects, potentially related to repeated handling, adaptation to the sampling procedure, or short-term metabolic adjustments. These results imply that several salivary biomarkers such as UA, total proteins, ADA, ALP, GGT and calcium, may be more sensitive to transient metabolic or stress-related processes than to diurnal variation alone.

Significant interactions between time of day and sampling day observed for ALP and ADA, further support the dynamic nature of salivary enzyme regulation. Such variability may reflect dynamic regulation of enzyme secretion and activity in response to fluctuating metabolic demands, stress load, or inflammatory status.

Although urea concentrations did not exhibit significant diurnal or day-to-day variations, low ICC (0.16) indicates a limited reproducibility at the individual level. Comparing urea levels in the serum and saliva would give as a clear view in regard to the dynamics and nature of this limitation.

The present study demonstrated that within-horse variability exceeds between-horse variability, which is consistent with previous findings by Eckersall *et al.* (1985). This observation highlights the substantial influence of individual physiological fluctuations on salivary composition and underscores the importance of repeated measurements and precise determination of specific pathological conditions in which saliva samples can be considered valuable tools.

Equine saliva contains a complex mixture of glandular secretions, plasma-derived proteins, enzymes, and mucins, whose expression may vary between individuals and across physiological states. Electrophoretic profiling enables visualization of dominant protein fractions, detection of low-molecular-weight polypeptides, and identification of inter-individual or temporal differences in band patterns [21]. This approach is particularly relevant in horses, whose saliva production is closely linked to feeding behaviour, stress and overall health [4].

In the present study, approximately 15 protein bands ranging from 15 to 180 kDa were consistently observed, which is in accordance with Lopez-Martinez *et al.* (2024). While the overall electrophoretic profile remained largely conserved, variability was noted in a protein band at approximately 35 kDa. This band was not consistently present across all horses or sampling points, showing differences in appearance and intensity across days and time of sampling. Although such variability may reflect differences in physiological state or management conditions at the time of sampling, these observations remain exploratory, as the study design did not allow for controlled assessment of specific influencing factors. Previous studies have identified a protein

band of similar molecular weight as carbonic anhydrase in equine saliva [22]. The molecular weight of the variable band observed in the present study is consistent with these findings; however, definitive protein identification was beyond the scope of this study. Carbonic anhydrase activity in human saliva has been shown to decrease following exercise, potentially due to sympathetic nervous system activation, reduced parasympathetic stimulation of salivary glands, dehydration and transient metabolic alterations [23]. Such mechanisms may contribute to the variability in salivary protein profiles, although this interpretation should be considered preliminary since equine salivary flow rate is higher than in humans [9]. Despite the variability in individual protein bands, the general distribution of electrophoretic fractions was stable across morning and evening and did not show significant daily effects. This suggests that while specific salivary proteins may be dynamically regulated, the overall electrophoretic profile is maintained under relatively tight physiological control, suggesting that analysis of specific salivary proteins may be an interesting research direction toward the identification of specific and sensitive biomarkers of pathological states or dysregulation of homeostasis.

The limitations of this study, including small sample size, heterogeneous population of horses and absence of experimental grouping, restrict the ability to draw definitive conclusions regarding the cause factors influencing saliva's variability. Nevertheless, these findings provide a basis for future investigations and validation of the qualitative stability of the protein fractions, incorporating larger, homogeneous populations and targeted protein identification approaches.

CONCLUSION

The present study demonstrated substantial variability in biochemical parameters of equine whole saliva, underscoring that most of the analytes are not reproducible in whole saliva under the conditions provided in this study. Despite this variability, the overall stability of the salivary electrophoretic profile supports the potential use of salivary protein fractions as qualitative biomarkers, but still requires validation.

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Authors' contributions

AMandić and MKF conceived the study. AMandić, AMitrović and SĐ performed the study. AMandić, MR and SR performed the analyses. AIB did the statistical analysis. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

Declaration of conflicting interests

The authors declared no conflicting interests.

Statement of Informed Consent

The owner understood the procedure and agrees that results related to investigation or treatment of their companion animals could be published in Scientific Journal Acta Veterinaria-Beograd.

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MEŠOVITA PLJUVAČKA KONJA: DNEVNA PONOVLJIVOST ODABRANIH BIOHEMIJSKIH PARAMETARA I PROTEINSKIH FRAKCIJA DOBIJENIH ELEKTROFOREZOM (PILOT-STUDIJA)

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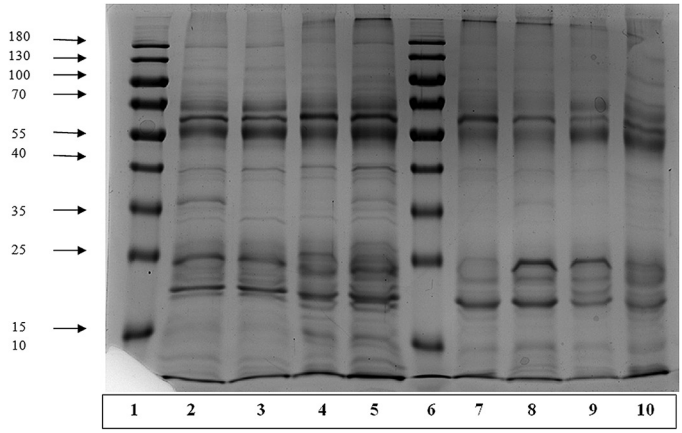
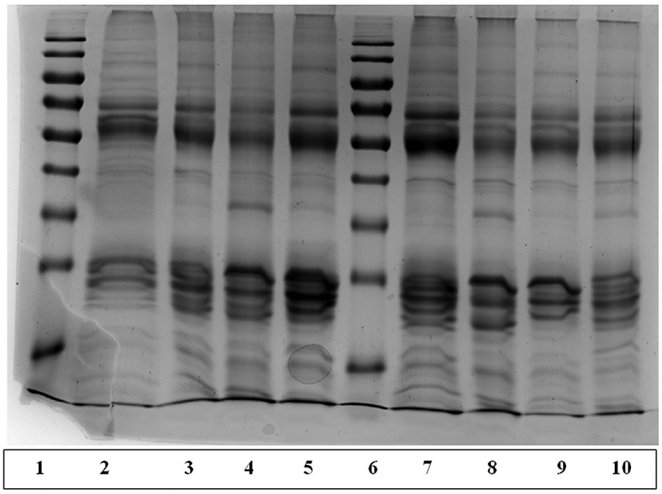
Biohemijski parametri pljuvačke podložni su varijacijama uslovljenim dobom dana i sezonskim faktorima, što odražava njihov cirkadijalni ritam. Mešovita pljuvačka pruža sveobuhvatniji prikaz njenog sastava u poređenju sa često ispitivanom parotidnom pljuvačkom. Cilj ove studije bio je da se ispita ponovljivost odabranih parametara u mešovitoj pljuvački konja merenih u različito doba dana tokom četiri uzastopna dana, kao i da se kvalitativno proceni stabilnost proteinskih frakcija pljuvačke primenom poliakrilamidne gel elektroforeze. U istraživanje je uključeno šest klinički zdravih toplokrvnih konja. Uzorci pljuvačke prikupljani su dva puta dnevno, ujutru i uveče. Podaci su analizirani deskriptivno i prikazani kao medijana (raspon), a vriednost $P<0,05$ smatrana je statistički značajnom. Linearni mešoviti modeli pokazali su da doba dana nije imalo značajan uticaj na većinu pljuvačnih parametara, sa izuzetkom adenozin deaminaze ($P= 0,020$). Dan uzorkovanja imao je značajan uticaj na koncentraciju mokraćne kiseline ($P=0,003$), ukupnih proteina ($P=0,007$), adenozin deaminaze ($P=0,011$), alkalne fosfataze ($P< 0,001$), γ-glutamil transferaze ($P=0,013$) i kalcijuma ($P=0,005$). Koeficijenti interklasne korelacije (*interclass correlation coefficent, ICC*) bili su niski za većinu parametara ($ICC\approx0,00–0,16$), što ukazuje na to da je varijabilnost unutar istog konja bila veća od one između različitih jedinki. Relativni udeli elektroforetskih frakcija pljuvačke pokazali su visoku stabilnost i nisu bili pod uticajem dana uzorkovanja, doba dana niti njihove međusobne interakcije. Uprkos uočenoj varijabilnosti parametara, ukupna stabilnost elektroforetskog profila pljuvačke ukazuje na potencijalnu primjenu proteinskih frakcija kao kvalitativnih biomarkera.

Supplementary files



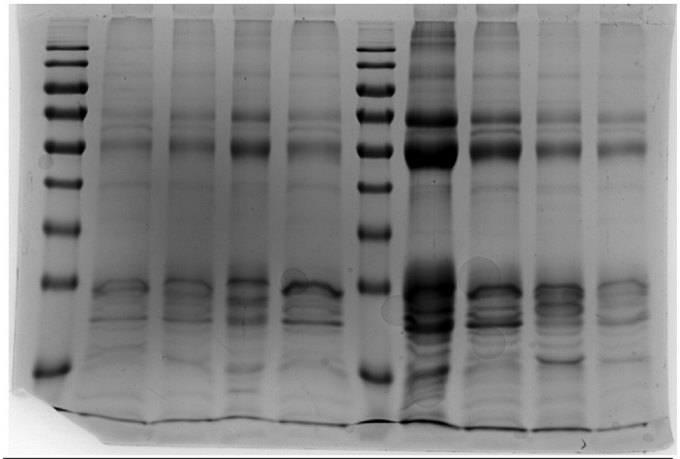
Supplementary Figure 1 – representative SDS-PAGE (Gel 1)

- 1: PageRuler™ Prestained Protein Ladder
- 2: Horse number three, 16th September 2025, morning saliva-Figure 1, B.
- 3: Horse number three, 17th September 2025, morning saliva
- 4: Horse number three, 18th September 2025, morning saliva
- 5: Horse number three, 19th September 2025, morning saliva
- 6: PageRuler™ Prestained Protein Ladder-Figure 1, A.
- 7: Horse number three, 16th September 2025, evening saliva-Figure 1, C.
- 8: Horse number three, 17th September 2025, evening saliva
- 9: Horse number three, 18th September 2025, evening saliva
- 10: Horse number three, 19th September 2025, evening saliva



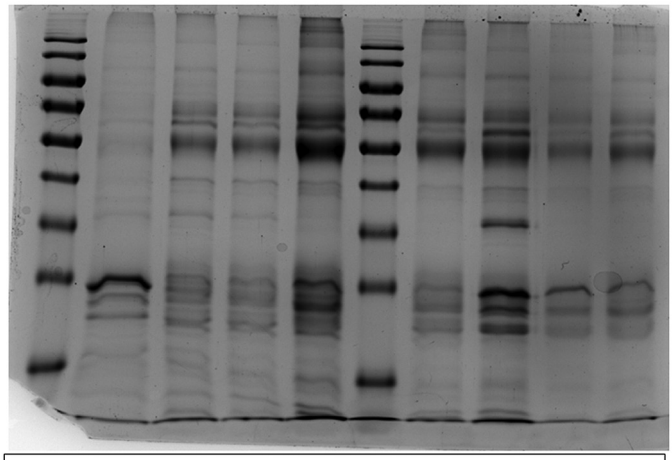
Supplementary Figure 2 – Gel 2

- 1: PageRuler™ Prestained Protein Ladder
- 2: Horse number one, 16th September 2025, morning saliva
- 3: Horse number one, 17th September 2025, morning saliva
- 4: Horse number one, 18th September 2025, morning saliva
- 5: Horse number one, 19th September 2025, morning saliva
- 6: PageRuler™ Prestained Protein Ladder
- 7: Horse number one, 16th September 2025, evening saliva
- 8: Horse number one, 17th September 2025, evening saliva
- 9: Horse number one, 18th September 2025, evening saliva
- 10: Horse number one, 19th September 2025, evening saliva



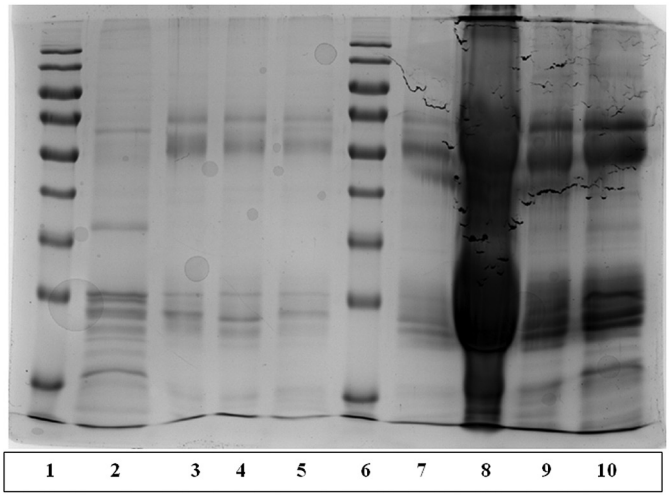
Supplementary Figure 3 – Gel 3

- 1: PageRuler™ Prestained Protein Ladder
- 2: Horse number two, 16th September 2025, morning saliva
- 3: Horse number two, 17th September 2025, morning saliva
- 4: Horse number two, 18th September 2025, morning saliva
- 5: Horse number two, 19th September 2025, morning saliva
- 6: PageRuler™ Prestained Protein Ladder
- 7: Horse number two, 16th September 2025, evening saliva
- 8: Horse number two, 17th September 2025, evening saliva
- 9: Horse number two, 18th September 2025, evening saliva
- 10: Horse number two, 19th September 2025, evening saliva



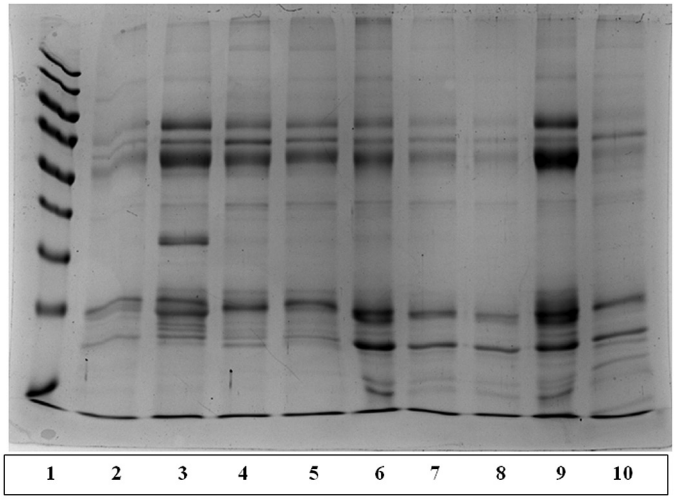
Supplementary Figure 4 – Gel 4

- 1: PageRuler™ Prestained Protein Ladder
- 2: Horse number four, 16th September 2025, morning saliva
- 3: Horse number four, 17th September 2025, morning saliva
- 4: Horse number four, 18th September 2025, morning saliva
- 5: Horse number four, 19th September 2025, morning saliva
- 6: PageRuler™ Prestained Protein Ladder
- 7: Horse number four, 16th September 2025, evening saliva
- 8: Horse number four, 17th September 2025, evening saliva
- 9: Horse number four, 18th September 2025, evening saliva
- 10: Horse number four, 19th September 2025, evening saliva



Supplementary Figure 5 – Gel 5

- 1: PageRuler™ Prestained Protein Ladder
- 2: Horse number five, 16th September 2025, morning saliva
- 3: Horse number five, 17th September 2025, morning saliva
- 4: Horse number five, 18th September 2025, morning saliva
- 5: Horse number five, 19th September 2025, morning saliva
- 6: PageRuler™ Prestained Protein Ladder
- 7: Horse number five, 16th September 2025, evening saliva
- 8: Horse number five, 17th September 2025, evening saliva
- 9: Horse number five, 18th September 2025, evening saliva
- 10: Horse number five, 19th September 2025, evening saliva



Supplementary Figure 6 – Gel 6

- 1: PageRuler™ Prestained Protein Ladder
- 2: Horse number six, 16th September 2025, morning saliva
- 3: Horse number six, 17th September 2025, morning saliva
- 4: Horse number six, 18th September 2025, morning saliva
- 5: Horse number six, 19th September 2025, morning saliva
- 6: Horse number six, 16th September 2025, evening saliva
- 7: Horse number six, 17th September 2025, evening saliva
- 8: Repeated sample.
- 9: Horse number six, 18th September 2025, evening saliva
- 10: Horse number six, 19th September 2025, evening saliva