**OBESITY, UREA, URIC ACID: DONKEYING AROUND WITH SUBCLINICAL METABOLIC IMBALANCES (PILOT STUDY)**

A Mandić1, B Matorkić1, K Spariosu1, M Radaković1, A Mitrović2, K Nenadović3, M Kovačević Filipović1

1 Department of Pathophysiology, Faculty of Veterinary Medicine, University of Belgrade, Serbia

2 Department of Ruminants and Swine Diseases, Faculty of Veterinary Medicine, University of Belgrade, Serbia

3 Department of Animal Hygiene, Faculty of Veterinary Medicine, University of Belgrade, Serbia

**Short running title**: Obesity, urea and uric acid in donkeys

**Corresponding author**:

Ana Mandić, DVM, PhD Candidate, Junior research assistant

Department of Pathophysiology, Faculty of Veterinary Medicine, University of Belgrade, Bulevar Oslobođenja 18, Serbia

e-mail: [ana.mandic@vet.bg.ac.rs](mailto:ana.mandic@vet.bg.ac.rs)

ORCID: <https://orcid.org/0009-0002-6268-4412>

**Summary**

* **Background** – Obesity is a key precipitating factor in laminitis development and frequent problem in many donkey farms across Europe. Stoic nature of donkeys makes laminitic changes often going unnoticed or mistakenly attributed to hoof neglect.
* **Objectives** – To investigate link between obesity, metabolic profile, inflammation, and laminitis, focusing on physical examination and serum biomarkers.
* **Study design** – Cross-sectional observational study.
* **Methods** – Ten randomly chosen lactating jennies over four years of age, from one farm, being fed with hay and bran, were enrolled in the study. Body condition score (BCS) was assessed using Pearson’s system. Grading scales for fat accumulations and laminitic hoof deformities were developed. Welfare was assessed using AWIN protocol. Blood samples for biochemistry analysis were collected after fasting. Data were presented as median [minimum, maximum] and analysed with MedCalc® software, with *p*<0.05 considered significant.
* **Results** – Laminitic hoof deformities (0.5 [0, 1]), were in strong positive correlation with BCS (7 [4, 8], *p*=0.008) and fat accumulations (1.5 [0, 2.5], *p*=0.017). The category of jennies with BCS≥7 had higher insulin than group with BCS<7 (*p*=0.044). Insulin (34.86 pmol/L [8.33, 75]) showed positive correlation with glucose (4.12 mmol/L [3.8, 5.4], *p*<0.001), cholesterol (1.92 mmol/L [1.53, 2.38], *p*=0.008), and AST (461.6 U/L [379.1, 1037.6], *p*=0.023). Uric acid (0.20 mmol/L [0.09, 0.62]) showed positive correlation with BCS (*p*=0.033) and urea (6.46 mmol/L [3.84, 8.68], *p*=0.048). Urea levels exceeded reference range in eight, and globulins in all the jennies.
* **Main limitations** – Small sample size, unknown gestational state, unawareness of individual food intake.
* **Conclusions** – In lactating, normoinsulinaemic and normoglycaemic jennies, over-conditioning is linked to subclinical laminitis. However, clear metabolic link is missing. Increased globulins suggest subclinical chronic inflammation, while the increase in urea and variable levels of UA suggest the need for thorough assessment of proper feeding management.

**Keywords**: jennies; laminitis; bran; globulins; insulin

# Clinical relevance

* In order to avoid metabolic derangements in lactating jennies, it is of outmost importance to provide balanced nutrition in energy demanding period, such as lactation.
* Uric acid may provide an early warning of the upcoming metabolic syndrome in donkeys and reference range formation should be of interest in future studies.
* The importance of understanding subclinical metabolic imbalances and the need for a thorough clinical assessment of hoof deformities, in order to improve the welfare of farm donkeys.

# 1. Introduction

The growing market demand for jennies’ milk in Serbia has led to the development of semi-extensive farms managing indigenous Balkan donkey populations for dairy production (Ivanov 2007). Dairy farming with this autochthonous breed presents unique challenges, requiring a balance between their evolutionary adaptations and the specialized needs of dairy farming. Donkeys have evolved towards relatively low energy requirements, approximately 60% of those needed by ponies of a similar size (Mendoza et al., 2018a). As donkey dairy farming is a young agricultural practice, there are no standardized guidelines for feeding management, particularly during periods of increased nutritional demand, such as lactation (Raspa et al., 2019). At present, good feeding practices are assessed using body condition scoring (BCS) and ensuring the availability of adequate water (AWIN 2015).

The Balkan donkey weighs approximately 125 kg (Stanišić et al., 2015) and, based on practical experience, milk yield is estimated to be 70-80 litres per lactation, excluding the amount consumed by the nursing foal. The usual practice for lactating Balkan dairy jennies is supplementation with high-energy feed, mostly corn (Lazarević et al., 2017) or bran, but without constant monitoring of oscillations in BCS for better feeding adjustments. Indeed, obesity and hoof neglect are the main welfare concerns in donkey farms across Europe (Dai et al., 2016). In addition, obesity predisposes donkeys to insulin dysregulation and laminitis, which are all the main features of donkey metabolic syndrome (DMS). These features could also appear separately and independently of DMS (Mendoza et al., 2018b). Several scoring systems have been developed to assess obesity or over-conditioning in donkeys, while dynamic testing is important to prove insulin dysregulation. However, diagnosing laminitis in donkeys is challenging, as acute episodes are often alternating with subclinical ones during which no clinical signs of pain are evident, despite pathological changes occurring within the foot (Thiemann et al., 2021).

The aim of this study was to investigate the link between obesity, metabolic profile, inflammation and laminitis in lactating Balkan dairy jennies using physical examination and key serum metabolic and inflammatory biomarkers, including insulin, total globulins, and acute-phase proteins. This study also introduced total adenosine deaminase (ADA) and uric acid (UA), molecules linked to purine metabolism. We hypothesized that the latter may serve as an early marker of DMS in lactating jennies.

**2. Materials and methods**

A dairy, medium-sized, semi-extensive farm in a hilly, forested area of Southern Serbia, was selected for the evaluation due to its convenient accessibility. The owner signed an informed consent, and the Ethical Committee at the Faculty of Veterinary Medicine, University of Belgrade, Serbia, approved the study, and based on the Serbian Law of Animal Welfare, permission was acquired from the Ministry of Agriculture, Forestry and Water Management, Republic of Serbia (permission number: 323-07-07930/2022-05). The farm, operating for 15 years, and numbering 150 donkeys, was visited in June of 2024. The number of lactating dairy jennies on the farm was 20, out of which ten were randomly chosen. All the jennies were older than four years. The farm utilizes a free-range breeding system for its animals, allowing them to roam freely. Lactating dairy jennies were not subjected to preventative parasitic treatments due to organic properties of the farm. At the time of the assessment, the jennies were in lactation (between 3rd and 9th month) and of unknown gestational state. The owner stated that the milking for human consumption starts in the third month after foaling and lasts 6-8 months. The foals were permitted to nurse during that period. The lactating jennies were housed in a collective stable, with small amount of straw used as bedding and maintained on forage diet and bran. Two to three times a day, they were released from the stable in a controlled manner to drink from a shared water trough. Salt blocks were available. The jennies were assessed by two veterinarians whose assessments were in coordination and who had experience in evaluation of laminitic hoof deformities both in horses and donkeys. A physical examination was performed and all the jennies were bright, alert, and responsive.

*2.1. Assessment of donkey’s body condition, fat accumulations (FA) and neck score (NS)*

Each jenny was assessed using a donkey-speciﬁc BCS system ranging from 1 to 9 points (Pearson and Ouassat 2000). This scoring system is based on anatomical features and different sites for fat accumulation, dividing animals into three categories: thin (1-3), normal (4-6), and over-conditioned (7-9).

Jennies were also assessed for regional FA for which the authors of this study developed a new scoring system. The method consisted of visual assessment and palpation of the three body regions: neck, ribs, and tail head. Calcified fat was not graded. Scale ranges from 0 (no fat deposits), 0.5 (not visible, but palpable fat deposits) to 1 (visible and palpable fat deposits). After scoring, the points would be summed to obtain a final score ranging from 0 to 3. The scoring system is presented in Table 1.

Table 1

Donkey-specific Neck Score (NS) provided by Mendoza et al. (2015) was applied to additionally determine the fatty crest. This ranges from a thin neck with the absence of a visible and palpable crest, scoring 0 to a crest grossly enlarged, which cannot be grasped with one hand, scoring 4.

*2.2. Hoof assessment*

The owner denied a history of acute laminitis in lactating dairy jennies. The presence of hoof deformities and subclinical laminitis was assessed according to a grading scale specifically designed for donkeys by the authors of this study. The laminitic hoof deformity was thus scored 0 (no visible hoof deformity), 0.5 (low-grade subclinical laminitis) and 1 (marked subclinical laminitis). Details are presented in Table 2. The inclusion criterion was at least one affected hoof.

Table 2

*2.3. Welfare assessment*

The welfare assessment of the jennies was conducted using the Animal Welfare Indicator (AWIN) assessment protocol for donkeys (AWIN 2015). Only selected parameters, assessing feeding practice, health, and behaviour, were applied in our study. Welfare indicators were scored 0 when welfare was good or 1 when welfare was poor and unacceptable. A higher score represented impaired donkey welfare.

*2.4. Blood sampling*

The animals were fasted overnight, with only a small portion of hay provided to minimize stress. Sampling took place early in the morning. Peripheral blood samples were collected on the same day from all the jennies via the jugular vein, using 18-gauge needles and sterile, plain blood collection tubes for serum (BD Vacutainer® CAT Serum Tubes, Becton-Dickinson, New Jersey, US). The samples were left at room temperature for 20 minutes before being centrifuged at 4000 rpm for 5 minutes. The resulting serum samples were without visible presence of haemolysis and lipaemia. Serum aliquots were stored at -20°C prior to transport. Samples were subsequently transferred to the laboratory in a portable refrigerator on ice and stored at -20°C until analysis.

*2.5. Laboratory methods*

At the time of the analysis, serum samples were thawed at room temperature for 20 minutes. The biochemistry profile was determined using an automated analyser Mindray BS-240 (Mindray, Shenzhen, China). Laboklin GMBH & CO. (Germany) reference ranges for biochemistry parameters were used. Acute phase proteins determination included: paraoxonase 1 (PON1), ceruloplasmin (CP), and haptoglobin (HPT). Measurement of PON1 was carried out by utilizing 4-nitrophenyl acetate as a substrate (Dantoine et al., 1998). The evaluation of CP was performed through its p-phenylenediamine (PPD) oxidase function, previously described by Hussein et al. (2019), and the absorbance of the resulting purple product was recorded at 630 nm. The determination of HPT was performed by the method using peroxidase activity of haptoglobin-haemoglobin complex (Jones and Mould 1984; Owen et al., 1960). Insulin measurements were performed using an automated analyser Tosoh AIA 360 (Tosoh Corporation, Tokyo, Japan) and corresponding chemistry. Serum protein fractions were assessed by agarose gel (1%) electrophoresis, according to a procedure by Milanović et al. (2017). Specific protein concentrations were calculated based on detected signals, using ImageJ software (Windows 64-bit Java 8 version).

*2.6. Statistical analysis*

Results were presented as median [minimum, maximum]. The correlations between different biochemical parameters, BCS, NS, FA, and laminitic hoof deformities were assessed using the Spearman correlation coefficient (ρ) and presented with heatmap. Mann Whitney Test was conducted to examine the differences in insulin levels according to the donkeys’ BCS. The results were considered significant if *p*<0.05. The statistical analysis was carried out using MedCalc® software version 14.8.1 (MedCalc Software Ltd, Belgium). Heatmap was formulated in GraphPad Prism 8. Inc. (GraphPad Software, USA).

# 3. Results

Most of the selected welfare indicators were in line with good management practices at farms. Identified welfare issues in the lactating dairy jennies were predominantly over-conditioning and laminitic hoof deformities. Eight jennies were considered over-conditioned, and two were of a normal BCS (7 [4, 8]). Signs of hoof neglect were noted in nine jennies. Bad hair condition was present in two and ocular discharge was present in one jenny.

Fat accumulations were observed in nine jennies (1.5 [0, 2.5]). Noticeable crest and patchy fat deposits were present in nine jennies (2 [0, 3]). Based on the authors’ scaling system, six jennies had low-grade subclinical laminitis graded as 0.5, while three had marked subclinical laminitis graded as 1 (Figure 1). Only one jenny displayed visually healthy hooves.

Figure 1

Serum biochemical parameters are shown in Table 3. Eight jennies had total protein levels above the reference range and all of them had total globulin levels exceeding the reference range. Albumin levels remained within the reference range in all the jennies. One jenny had a higher glucose (GLU) level, and one had the GLU level below the reference range. Regarding triglycerides (TG) and total cholesterol (TC), the vast majority of jennies had low TG (8/10), whilst TC levels were within the reference range. Urea levels were dominantly elevated in most of the jennies (8/10), with creatinine being lower than the reference range in three of them. Phosphates and calcium levels were mostly within the reference range, with only one jenny having phosphates level below the reference range. Hepatic enzymes (AST and ALP) exceeded reference ranges, with AST being increased in two and ALP in eight jennies. Total bilirubin and creatine kinase (CK) were found to be within the ranges for all the animals. UA, ADA, β Hydroxybutyrate (BHB) and acute phase proteins (PON1, CP, HPT) were not described in Laboklin reference value sheath for this species, so the authors were unable to define if the measured values were typical for steady state or not. Basal insulin levels were within the reference range.

Table 3

The correlation of different morphological and biochemical parameters of the surveyed jennies is presented in Figure 2. Relationship between different morphological parameters of the jennies’ body showed expected results. In particular, laminitic hoof deformities were in strong positive correlation with BCS (ρ=0.781, *p*=0.008) and FA (ρ=0.727, *p*=0.017), while in a moderately positive with NS (ρ= 0.679, *p*=0.031). Fat accumulations showed a strong positive correlation with BCS (ρ=0.844, *p*=0.002) and NS (ρ=0.853, *p*=0.002), while the interaction between BCS and NS was also significant (ρ=0.781, *p*=0.008). Laminitic hoof deformities (ρ=0.763, *p*=0.010; ρ=0.728, *p*=0.017), BCS (ρ=0.937, *p*=0.001; ρ=0.873, *p*=0.001) and FA (ρ=0.828, *p*=0.003; ρ=0.828, *p*=0.003) showed a strong positive correlation with urea and creatinine levels, respectively. There was a pattern of moderate correlation between NS (ρ=0.673, *p*=0.033), FA (ρ=0.676, *p*=0.031), and TC levels. A moderate positive correlation was shown between BCS and UA levels (ρ=0.672, *p*=0.033). A strong positive correlation was present between albumin and CP (ρ=0.714, *p*=0.020). There was a strong positive correlation between AST and TC (ρ=0.758, *p*=0.014), but a moderate one between AST and TG (ρ=0.681, *p*=0.030). Total globulins had a moderately positive correlation with ADA (ρ=0.671, *p*=0.033). α and β globulins were in a strong negative correlation with γ globulins (ρ=-0.830, *p*=0.003). There was a moderate positive correlation between CP and calcium levels (ρ=0.681, *p*=0.030). Creatinine and HPT showed a highly positive correlation (ρ=0.842, *p*=0.002). A strong positive correlation was presented between GLU and TC (ρ=0.720, *p*=0.019). Insulin was in a strong positive correlation with GLU (ρ=0.915, *p*<0.001), TC (ρ=0.781, *p*=0.008), and AST (ρ=0.705, *p*=0.023). Moderately positive correlation was shown between TC and TG (ρ=0.652, *p*=0.041). Urea showed a moderately positive correlation with UA (ρ=0.636, *p*=0.048).

Mann-Whitney U Test showed a significant difference in the insulin levels (*p*=0.044) between two groups of jennies with different BCS. The group with BCS≥7 had median insulin value 41.32 pmol/L (14.58 – 75), while the group with BCS<7 had median insulin value 9.72 pmol/L (8.33 – 11.11).

**4.Discussion**

The present study in lactating jennies identified correlations between morphological parameters indicative of over-conditioning and hoof issues, and biochemical parameters reflecting metabolic changes and subclinical inflammation. However, among biochemical parameters only urea, total globulins and ALP were consistently elevated above the reference interval in majority of jennies.

The farm selected for this study provided suitable living conditions that allowed the donkeys to exhibit their natural behaviours. The main welfare issues observed in the lactating jennies were over-conditioning and laminitic hoof deformities classified as subclinical. Often, owners are not aware that their donkeys are obese. Results obtained by Valle et al. (2017) indicate a substantial disagreement in body condition evaluations between experts and the owners. The FA scoring system, developed by the authors of this study, offers a practical method for estimating a donkey’s body condition without requiring a detailed clinical assessment using the BCS system. Despite being based solely on the palpability and visibility of fat accumulations, it showed a strong correlation with BCS, suggesting its potential for more efficient obesity assessment by the owners. Future studies should focus on validating the suggested FA scoring system.

Obesity is a key precipitating factor in laminitis development and both issues represent a frequent problem in many donkey farms across Europe (Cox et al., 2010; Dai et al., 2018). Although physical examination revealed no signs of active laminitis, the jennies exhibited hoof changes indicative of subclinical laminitis. The reasons are at least two folds: obesity and hoof neglect. Namely, several obesity-related criteria correlated with laminitic hoof deformities, suggesting that metabolic dysregulation contributes to its development. Concerning hoof neglect, in farm-kept donkeys, hoof growth and wear are not naturally balanced, leading to overgrowth that should be constantly managed. Otherwise, hoof overgrowth places excessive strain on the hoof capsule, joints, and tendons. Overgrown hooves have compromised blood supply, increasing the risk of pedal bone remodelling (Reilly 1997), particularly in donkeys with underlying metabolic dysfunction. To make the issues with hooves even worse, the stoic nature of donkeys makes laminitic changes often going unnoticed or mistakenly attributed only to hoof neglect postponing proper care.

Our results showed strong correlations between laminitic hoof deformities and urea and creatinine levels. Donkeys require only about 3% crude protein in their diet to maintain body mass, and exhibit a urea recycling capacity similar to that of ruminants (Engelhardt 1978; Izraely et al., 1989). This makes excess protein intake unnecessary and potentially leading to elevated urea levels. Moreover, high-protein diets trigger a hyperinsulinaemic response and alter amino-acid dynamics in horses with equine metabolic syndrome (EMS) (Loos et al., 2019). This indicates that dietary protein content should be rigorously controlled in the management of insulin dysregulation-prone donkeys. We suggest that the addition of bran, a protein-rich feed, in lactating jennies' diet, could be related to the increased urea levels, over-conditioning, and laminitic hoof deformities. Increased protein metabolism leads to higher metabolic stress on the liver, putting strain on the overall metabolism of the animal. This metabolic derangement can trigger systemic inflammation, and oxidative stress, causing altered blood flow to the hooves, all of which are key factors in the development of laminitis (Bäßler et al., 2021). Creatinine levels were below the reference range in three jennies, even though two of them were over-conditioned. There is a high probability that longer duration of metabolic strain, together with prolonged feet discomfort, leads to muscle wasteness and regional resistance to lipolysis. Namely, the signs of hoof neglect increased the probability of donkeys being lean (Dai et al., 2018).

Bran is not only rich in proteins but also in fructans, polymers of fructose, which is the primary carbohydrate that generates UA during its metabolism (Fox and Kelley 1972; King et al., 2018). Data about UA concentration in donkeys is scarce and do not allow the formation of reference intervals. The median value (minimal and maximal) for UA in our study was 0.20 mmol/L with high variability of values (0.09 – 0.62 mmol/L). In Nigeria, donkeys’ UA values were reported to be in the lower quartile (Bature et al., 2018), while in Brazil the values were slightly higher than the upper quartile of the values measured in our study (Silva et al., 2018), and are probably reflecting nutritional status of individuals. The results of this study show a moderate, positive correlation between BCS and UA, highlighting that over-conditioning is related to higher UA levels. Additionally, a moderate correlation between UA and urea can be explained by the fact that both metabolites are products of nitrogen metabolism, accentuating potential problems with the enrichment of jennies’ feed with bran. Hyperuricaemia is frequently observed in human patients with obesity, metabolic syndrome and type 2 diabetes (Fox and Kelley 1972; King et al., 2018). High UA is a trigger for mitochondrial oxidative stress and insulin resistance (King et al., 2018). Mitochondrial oxidative stress in endothelial cells leads to impairment of insulin-dependent nitric oxide release and glucose delivery to the periphery (Sanchez-Lozada et al., 2012). This impairment also plays an important role in the development of endocrinopathic laminitis in horses (Ertelt et al., 2014). Laminitis-prone ponies had high UA concentrations when lush pastures were rich in fructans (Bailey et al., 2008). This enables us to hypothesize that observed levels of UA, especially those in the upper quartile, are the consequence of the addition of wheat bran in jennies’ diet. Hyperuricaemia may contribute to the pathogenesis of insulin resistance, hepatic lipidosis, and dyslipidaemia (King et al., 2018). Thus, we suggest that further research should investigate whether UA could serve as an early marker of DMS in over-conditioned, normoinsulinaemic, and normoglycaemic donkeys.

Low-grade chronic inflammation is associated with obesity in horses (Zak et al., 2020). We tested the hypothesis that over-conditioned jennies in our study had systemic inflammation using a combination of acute-phase proteins, total globulins and ADA values. Levels of HPT and CP were within the reference interval provided by Perez-Ecija et al. (2021) for Andalusian donkeys, indicating that there was no acute inflammation present. In horses with EMS, HPT and CP were not increased suggesting that they may not be suitable markers of low-grade inflammation (Zak et al., 2020). On the other hand, the increase in total globulin levels across all the jennies can be linked to the increase in γ globulins and thus the possible underlying subclinical chronic inflammation. ADA is an enzyme involved in purine metabolism and its levels elevate during the activation of the immune system, especially T-lymphocytes and monocytes/macrophages (Reuter et al., 2005). The results of this study indicate that donkeys and horses share a similar pattern of ADA activity, with total serum values being very low (Contreras-Aguilar et al., 2020; Tax and Veerkamp 1978). Nevertheless, a moderately positive correlation between ADA and total globulins, may further indicate its association with increased immune system activity, and confirm low-grade inflammation. Besides over-conditioning, the potential cause of low-grade inflammation could be a high intestinal parasite load, resulting from the lack of deworming practices and the relatively high population density of donkeys on this farm (Mijatović et al., 2022). It can be speculated that obesity and a high parasite burden contribute to low-grade inflammation, which may, in turn, lead to at least partial insulin resistance, further exacerbating conditions that first led to laminitis.

Our study revealed no disturbances in basal GLU, TC, and insulin levels, suggesting the absence of detectable insulin resistance, the hallmark of DMS. However, jennies with a BCS≥7 exhibited higher insulin levels than those with a BCS<7, despite only two jennies falling into the latter category. It is expected that over-conditioned donkeys have higher mean insulin concentrations when compared to moderate and thin donkeys (Pritchard et al., 2019). Dynamic testing, as performed in the aforementioned study, was not conducted in ours. However, it is well-documented that many horses with EMS, which exhibit abnormal hyperinsulinemia during dynamic testing, have normal basal insulin levels (Burns and Toribio 2018). Thus, it is highly possible that tested jennies, classified as normoinsulinaemic, may still have experienced insulin dysregulation without detectable hyperinsulinaemia, reflecting partial insulin resistance. In EMS, insulin resistance may occur selectively in the liver, muscle, or adipose tissue without accompanying hyperinsulinaemia or hyperglycaemia (Frank 2009). Additionally, the observed strong positive correlation between GLU and TC levels suggests that similar underlying factors, like partial insulin resistance, influence changes in their concentrations.

Steady-state concentrations and the positive correlation observed between AST, insulin, and TC may suggest coordinated hepatocyte metabolism in response to low-grade inflammation and partial insulin resistance in some individuals. Our findings neither confirm nor refute the hypothesis that hepatocyte clearance of gut-derived substances linked to laminitis (Frank 2011) might be slightly impaired in surveyed lactating jennies. However, this remains an intriguing possibility that could partially account for the high prevalence of subclinical laminitis observed in this study. Notably, two jennies exhibited a doubling of AST activity. In horses, serum AST activity is highly sensitive (100%) for detecting hepatic lipidosis (Satué et al., 2022), though no specific cut-off value has been established to correlate AST increases with hepatic lipidosis. The reason behind the increase of ALP levels, in most of the surveyed jennies, could not be easily determined since ALP could also be linked to gestational state. It is, therefore, plausible to speculate that these jennies may have experienced hepatic lipidosis or other hepatic insults leading to increased hepatocyte permeability.

The limitations of this study, including small sample size, heterogeneous population of jennies of various ages, unknown gestational state and milk yield, difficulties in determining individual food intake, emphasize the necessity for a more detailed study in the future.

**5. Conclusion**

This study shows that despite the fact that over-conditioned lactating jennies with subclinical laminitis are normoinsulinaemic and normoglycaemic, they exhibit increased urea, indicating the need for nutritional adjustments, and elevated globulins, suggesting subclinical chronic inflammation that requires further investigation and management. The link between urea, UA and BCS indicate the presence of a driving force that connects metabolism derangements with inappropriate food management. The assessment of serum UA level may provide a simple mean of diagnosing early metabolic syndrome in donkeys. However, big variations in its levels, suggest the necessity for a thorough assessment of donkeys’ nutritional needs. Future studies are necessary to confirm these results using larger populations.

**Authors’ contribution**

AM1 and MKF conceived the study; AM1, BM and MKF performed the physical examination; KN performed the welfare assessment; AM1 collected the samples; AM1, KS, MR and AM2 performed the analyses; AM1 and MKF interpreted the data; AM1 and MKF wrote the manuscript; KS, MR and MKF corrected and edited the manuscript. All authors have read and approved the manuscript.

**Acknowledgements**

We acknowledge Sergej Ivanov, DVM, for his willingness to participate in the study and technical support. Special acknowledgment to Radiša Prodanović, DVM, PhD, Associated Professor, Department of Ruminants and Swine Diseases, Faculty of Veterinary Medicine, University of Belgrade, Serbia, for his support with manuscript preparation.

**Funding information**

The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (contract number: 451-03-66/2024-03/200143).

**Conflict of interests’ statement**

The authors have declared no conflicting interests.

**Data integrity**

AM1 had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Ethical animal research**

The Ethical Committee at the Faculty of Veterinary Medicine, University of Belgrade, Serbia, approved the study, and based on the Serbian Law of Animal Welfare, permission was acquired from the Ministry of Agriculture, Forestry and Water Management, Republic of Serbia (permission number: 323-07-07930/2022-05).

**Informed consent**

The owner signed an informed consent.

**References**

1. AWIN Protocol for Donkeys. Available from: <https://air.unimi.it/retrieve/dfa8b992-3aaa-748b-e053-3a05fe0a3a96/AWINProtocolDonkeys.pdf>.
2. Bailey, S.R., Habershon-Butcher, J.L., Ransom, K.J., Elliott, J. and Menzies-Gow, N.J. (2008) Hypertension and insulin resistance in a mixed-breed population of ponies predisposed to laminitis. *American Journal of Veterinary Research*. 69(1), 122-129. doi: 10.2460/ajvr.69.1.122.
3. Bäßler, S., Kenéz, Á., Scheu, T., Koch, C., Meyer, U., Dänicke, S. and Huber, K. (2021) Association between alterations in plasma metabolome profiles and laminitis in intensively finished Holstein bulls in a randomized controlled study. *Scientific Reports.* 11, 12735. doi: 10.1038/s41598-021-92163-6.
4. Bature, I., Shehu, B. and Barje, P. (2018) Serum biochemical parameters of donkeys (*Equus asinus*) as affected by age, location, and sex in Northwestern Nigeria. *Journal of Animal Production Research.* 1, 123-133.
5. Burns, T.A., Toribio, R.E. (2018) Insulin Dysregulation and Equine Metabolic Syndrome. In: Equine Internal Medicine, 4th edn., Ed: S.M. Reed, W.M. Bayly and D.C. Sellon, Elsevier, Missouri. pp 1085-1100.
6. Contreras-Aguilar, M.D., Tvarijonaviciute, A., Monkeviciene, I., Martín-Cuervo, M., González-Arostegui, L.G., Franco-Martínez, L., Cerón, J.J., Tecles, F. and Escribano, D. (2020) Characterization of total adenosine deaminase activity (ADA) and its isoenzymes in saliva and serum in health and inflammatory conditions in four different species: an analytical and clinical validation pilot study. *BMC Veterinary Research.* 16(1), 384. doi: 10.1186/s12917-020-02574-2.
7. Cox, R., Burden, F., Proudman, C., Trawford, A. and Pinchbeck, G. (2010) Demographics, management and health of donkeys in the UK. *Veterinary Record.* 166, 552–556.
8. Dai, F., Dalla Costa, E., Murray, L., Canali, E. and Minero, M. (2016) Welfare conditions of donkeys in Europe: Initial outcomes from on-farm assessment. *Animals*. 6, 5. doi: 10.3390/ani6010005.
9. Dai, F., Segati, G., Brscic, M., Chincarini, M., Dalla-Costa, E., Ferrari, L., Burden, F., Judge, A. and Minero, M. (2018) Effects of management practices on the welfare of dairy donkeys and risk factors associated with signs of hoof neglect. *Journal of Dairy Research.* 85(1), 30-38. doi: 10.1017/S0022029917000723.
10. Dantoine, T.F., Debord, J., Charmes, J.P., Merle, L., Marquet, P., Lachatre, J. and Leroux-Robert, C. (1998) Decrease of serum paraoxonase activity in chronic renal failure. *Journal of the American Society of Nephrology.* 9, 2082-2088. doi: 10.1681/asn.v9112082.
11. Engelhardt, W.V. (1978) Adaptation to low protein diet in some mammals. In: Proceedings of the Zodiac symposium on adaptation, Wageningen, Netherlands. pp 110-115.
12. Ertelt, A., Barton, A.K., Schmitz, R.R. and Gehlen, H. (2014) Metabolic syndrome: is equine disease comparable to what we know in humans? *Endocrine Connections.* 3(3). doi: 10.1530/EC-14-0038.
13. Fox, I.H. and Kelley, W.N. (1972) Studies on the mechanism of fructose-induced hyperuricemia in man. *Metabolism*. 21, 713-721.
14. Frank, N. (2009) Equine metabolic syndrome. *Journal of Equine Veterinary Science.* 29, 259–267.
15. Frank, N. (2011) Equine metabolic syndrome. *Veterinary Clinics of North America Equine Practice*. 27(1), 73-92. doi: 10.1016/j.cveq.2010.12.004.
16. Hussein, H.A., Bäumer, J. and Staufenbiel, R. (2019) Validation of an automated assay for measurement of bovine plasma ceruloplasmin. *Acta Veterinaria Scandinavica.* 61, 34. doi: 10.1186/s13028-019-0470-4.
17. Ivanov, S. (2007) Indigenous breeds conservation efforts in the Stara Planina mountain area. In: Conference on Native Breeds and Varieties as part of Natural and Cultural Heritage, Book of Abstracts, Šibenik, Croatia. pp 113-114.
18. Izraely, H., Choshniak, I., Stevens, C.E., Demment, M.W. and Shkolnik, A. (1989) Factors determining the digestive efficiency of the domesticated donkey (*Equus asinus asinus*). *Quarterly Journal of Experimental Physiology.* 74(1), 1-6. doi: 10.1113/expphysiol.1989.sp003234.
19. Jones, G.E. and Mould, D.L. (1984) Adaptation of the guaiacol (peroxidase) test for haptoglobins to a microtitration plate system. *Research in Veterinary Science.* 37, 87-92.
20. King, C., Lanaspa, M.A., Jensen, T., Tolan, D.R., Sánchez-Lozada, L.G., Johnson, R.J., Treviño-Becerra, A., Iseki, K., editors. (2018) Uric acid as a cause of the metabolic syndrome. In: Uric Acid in Chronic Kidney Disease, Contrib Nephrol., Basel, Karger, Vol. 192, 88-102. doi: 10.1159/000484283.
21. Lazarević, J., Tasić, T., Popović, S., Banjac, V., Đuragić, O., Kokić, B. and Čabarkapa, I. (2017) Changes in milk composition of domestic Balkan donkeys' breed during lactation periods. *Acta Periodica Technologica.* 48, 187-195. doi: 10.2298/APT1748187L.
22. Loos, C.M.M., Dorsch, S.C., Elzinga, S.E., Brewster-Barnes, T., Vanzant, E.S., Adams, A.A. and Urschel, K.L. (2019) A high protein meal affects plasma insulin concentrations and amino acid metabolism in horses with equine metabolic syndrome. *Veterinary Journal.* 251, 105341. doi: 10.1016/j.tvjl.2019.105341.
23. Mendoza, F.J., Toribio, R.E. and Perez-Ecija, A. (2018) Aspects of clinical relevance in donkeys. In: Equine Internal Medicine, 2nd edn., Ed: S.M. Reed, W.M. Bayly and D.C. Sellon, Elsevier, Missouri. pp 1513-1520.
24. Mendoza, F.J., Toribio, R.E. and Perez-Ecija, A. (2018) Donkey Internal Medicine —Part I: Metabolic, Endocrine, and Alimentary Tract Disturbances. *Journal of Equine Veterinary Science*. 65, 66–74. doi: 10.1016/j.jevs.2018.02.001.
25. Mendoza, F.J., Estepa, J.C., Gonzalez-De Cara, C.A., Aguilera-Aguilera, R., Toribio, R.E. and Perez-Ecija, A. (2015) Energy-related parameters and their association with age, gender, and morphometric measurements in healthy donkeys. *Veterinary Journal*. 204, 201-207. doi: 10.1016/j.tvjl.2015.03.004.
26. Mijatović, B., Pavlović, I., Živkovic, S., Trailović, I., Ćirić, J. and Trailović, D. (2022) Prevalence of endoparasites in the Balkan donkey (*Equus asinus*) from Serbia. *Comparative Parasitology.* 89(2), 115-121. doi: 10.1654/COPA-D-22-00005
27. Milanović, Z., Ilić, A., Francuski-Andrić, J., Radonjić, V., Beletić, A. and Kovačević-Filipović, M. (2017) Acute-phase response in *Babesia canis* and *Dirofilaria immitis* co-infections in dogs. *Ticks and Tick-Borne Diseases.* 8(6), 907-914. doi: 10.1016/j.ttbdis.2017.07.015.
28. Owen, J.A., Better, F.C. and Hoban, J. (1960) A simple method for the determination of serum haptoglobins. *Journal of Clinical Pathology.* 13, 163-164.
29. Pearson, R.A. and Ouassat, M. (2000) Estimation of live weight. In: A Guide to Live Weight Estimation and Body Condition Scoring of Donkeys, 1st edn., Thomson Colour Printers, Glasgow. pp 17-20.
30. Perez-Ecija, A., Buzon-Cuevas, A., Aguilera-Aguilera, R., Gonzalez-De Cara, C. and Mendoza Garcia, F.J. (2021) Reference intervals of acute phase proteins in healthy Andalusian donkeys and response to experimentally induced endotoxemia. *Journal of Veterinary Internal Medicine.* 35(2), 580-589. doi: 10.1111/jvim.15983.
31. Pritchard, A., Nielsen, B., McLean, A., Robison, C., Yokoyama, M., Hengemuehle, S., Bailey, S. and Harris, P. (2019) Insulin resistance as a result of body condition categorized as thin, moderate, and obese in domesticated U.S. donkeys (*Equus asinus*). *Journal of Equine Veterinary Science.* 77, 31-35. doi: 10.1016/j.jevs.2019.02.011.
32. Raspa, F., Cavallarin, L., McLean, A.K., Bergero, D. and Valle, E. (2019) A review of the appropriate nutrition welfare criteria of dairy donkeys: Nutritional requirements, farm management requirements and animal-based indicators. *Animals (Basel)*. 9(6), 315. doi: 10.3390/ani9060315.
33. Reilly, J.D. (1997) The donkey’s foot and its care. In: The professional handbook of the donkey, 3rd edn., Ed: E.D. Svendsen, Whittet Books, London. pp 71-91.
34. Reuter, H., Burgess, L.J., Carstens, M.E. and Doubell, A.F. (2005) Adenosine deaminase activity—more than a diagnostic tool in tuberculous pericarditis. *Cardiovascular Journal of South Africa.* 16(3), 143-147.
35. Sanchez-Lozada, L.G., Lanaspa, M.A., Cristobal-Garcia, M., Garcia-Arroyo, F., Soto, V., Cruz-Robles, D., Nakagawa, T., Yu, M.A., Kang, D.H. and Johnson, R.J. (2012) Uric acid-induced endothelial dysfunction is associated with mitochondrial alterations and decreased intracellular ATP concentrations. *Nephron Experimental Nephrology.* 121. doi: 10.1159/000335389.
36. Satué, K., Miguel-Pastor, L., Chicharro, D. and Gardón, J.C. (2022) Hepatic enzyme profile in horses. *Animals (Basel)*. 12(7), 861. doi: 10.3390/ani12070861.
37. Silva, G., Jones Ferreira Lima da Silva, C., Souza, L., Hunka, M., Ferreira, L., Manso, H.E. and Manso Filho, H. (2018) Hematological and blood chemistry values of donkeys (*Equus africanus asinus*) in different management systems. *Pferdeheilkunde*. 34, 253. doi: 10.21836/PEM20180306.
38. Stanišić, L., Dimitrijević, V., Simeunović, P., Lakić, N., Radović, I., Ivanković, A., Stevanović, J. and Stanimirovic, Z. (2015) Morphological, biochemical and hematological characterization of endangered Balkan donkey breed. *Acta Veterinaria.* 65, doi: 10.1515/acve-2015-0010.
39. Tax, W.J. and Veerkamp, J.H. (1978) Activity of adenosine deaminase and purine nucleoside phosphorylase in erythrocytes and lymphocytes of man, horse and cattle. *Comparative Biochemistry and Physiology Part B.* 61(3), 439-441. doi: 10.1016/0305-0491(78)90151-7.
40. Thiemann, A.K., Buil, J., Rickards, K. and Sullivan, R. (2021) A review of laminitis in the donkey. *Equine Veterinary Education.* 34. doi: 10.1111/eve.13533.
41. Valle, E., Raspa, F., Giribaldi, M., Barbero, R., Bergagna, S., Antoniazzi, S., McLean, A.K., Minero, M. and Cavallarin, L. (2017) A functional approach to the body condition assessment of lactating donkeys as a tool for welfare evaluation. *PeerJ*. 5, e3001. doi: 10.7717/peerj.3001.
42. Zak, A., Siwinska, N., Elzinga, S., Barker, V.D., Stefaniak, T., Schanbacher, B.J., Place, N.J., Niedzwiedz, A. and Adams, A.A. (2020) Effects of equine metabolic syndrome on inflammation and acute-phase markers in horses. *Domestic Animal Endocrinology.* 72, 106448. doi: 10.1016/j.domaniend.2020.106448.

**Figure 1.** Different hoof deformities associated with subclinical laminitis in lactating jennies. Grade 1: (A) Overgrown hoof with big ridges and wall bulges, hairline changed into V shape; (B) Overgrown hooves with visible white line and ridges, broken hoof angle; (C) Overgrown hooves with medio-lateral imbalance and V shaped hairline; Grade 0.5: (D) Overgrown hooves with flaring of the hoof wall; (E) Overgrown hooves with the start of change in the base of the coronary band; (F) Overgrown hooves with visible tension in fetlock joints, medio-lateral imbalance present.

**Figure 2.** Heatmap showing statistical significance between the analysed parameters, both biochemical and morphological. \* p < 0.05, \*\* p ≤ 0.01, \*\*\* p ≤ 0.001.

Abbreviations: TP – total protein, ALB – albumin, GLO – total globulins, Glu – glucose, TG – triglycerides, TC – total cholesterol, CREA – creatinine, P – phosphates, Ca – calcium, T-bil – total bilirubin, AST – aspartate aminotranspherase, ALP – alkaline phosphatase, GGT – γ glutamyl transpherase, CK – creatine kinase, UA – uric acid, ADA – total adenosine deaminase, BHB – β hydroxybutyrate, PON1 – paraoxonase 1, CP – ceruloplasmin, HPT – haptoglobin, INS – insulin, BCS – body condition score, NS – neck score, FA – fat accumulation, HD – laminitic hoof deformities.

**Table 1**. Authors’ scoring system sum for regional fat accumulations (FA): neck, ribs and tail head.

**Table 2.** Authors’ scoring system for the presence of laminitic hoof deformities (HD) associated with subclinical laminitis.

**Table 3**. Serum biochemistry profile, acute phase proteins and insulin of lactating jennies (N=10).