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SHORT COMMUNICTION

Obesity, Urea and Uric Acid: Potential Indicators of Subclinical Metabolic Imbalance in Donkeys (Pilot Study)

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A B S T R A C T

Obesity is a known precipitating factor in laminitis development; therefore, objective of the study was to investigate link between obesity, inflammation, and laminitis, through physical examination and serum biomarkers. Ten randomly chosen lactating jennies aged >4 years, fed hay and bran, were enrolled in the study. Laminitis grading scale was developed. Fasted blood samples were used for biochemical analysis. Laminitis was in strong positive correlation with body condition score (BCS) (P=0.008). Jennies with BCS≥7 had higher insulin than group with BCS<7 (P=0.044). Insulin showed positive correlation with glucose (P<0.001), cholesterol (P=0.008), and AST (P=0.023). Uric acid showed positive correlation with BCS (P=0.033) and urea (P=0.048). Urea exceeded reference range in eight, and globulins in all the jennies. In lactating, normoinsulinemic, normoglycemic jennies, over-conditioning is linked to laminitis. Increased globulins suggest subclinical chronic inflammation, while elevated urea and variable uric acid levels indicate the need for thorough evaluation of feeding management.

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INTRODUCTION

Growing market demand for donkey's milk in Serbia has led to development of semi-extensive farms managing indigenous Balkan donkey populations for dairy production. As donkey dairy farming is a young agricultural practice, there are no standardized guidelines for feeding management, particularly during periods of increased nutritional demand (Raspa *et al.*, 2019). At present, good feeding practices are assessed using body condition scoring (BCS) and ensuring availability of adequate water. In Balkan donkey farms, lactating jennies are given high-energy feed as supplement (Lazarević *et al.*, 2017), without constant BCS monitoring for feeding adjustments. This often leads to obesity, the major welfare concern that also predisposes animals to insulin dysregulation and laminitis (Sullivan and Boocock, 2025), key signs of donkey metabolic syndrome. This study aimed to investigate the link between obesity, inflammation and laminitis in lactating Balkan 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) as potential markers of immune and metabolic status.

MATERIALS AND METHODS

The owner of a dairy, semi-extensive donkey farm in Southern Serbia, provided consent and the study was approved by Ethical Committee at the Faculty of Veterinary Medicine, University of Belgrade and permission number: 323-07-07930/2022-05 was acquired from the Ministry of Agriculture, Forestry and Water Management, Republic of Serbia. Farm, visited in June 2024, uses free-range system and no preventative parasitic treatments. Ten, randomly chosen jennies, over 4 years old, were assessed. Jennies were in lactation (3rd - 9th month) and of unknown gestational state. They were housed collectively and maintained on forage and bran. Twice daily, they were led to drink from a shared trough. All were bright, alert, and responsive during physical exam. The Animal Welfare protocol for donkeys was used to assess overall welfare (AWIN, 2015).

Donkey-specific BCS system (Pearson and Ouassat, 2000) classified animals as: thin (1-3), normal (4-6), or over-conditioned (7-9). Donkey-specific Neck Score (NS) provided by Mendoza *et al*. (2015) was used to determine the fatty crest.

Hooves were assessed by two experienced veterinarians whose assessments coordinated; one was blinded to body condition, while the other assessed the hooves on-farm. Healthy hoof criteria were straight wall, no white line separation, even growth, no concavity in the front and minimal struggle with breakover in case of overgrowth. Laminitic hoof criteria were "slipper toe" appearance, stretched white line, concaved front, uneven growth due to circulation disturbances, wall ridges and discomfort on hard surface (Collins *et al.,* 2011). Hoof assessment grades were 0 (healthy hoof), 0.5 (subclinical laminitis) and 1 (chronic laminitis).

The fasted animals received some hay to minimize stress, and sampling occurred in the morning with peripheral blood collected via the jugular vein using 18-gauge needles and plain collection serum tubes (BD Vacutainer® CAT Serum Tubes, Becton-Dickinson, New Jersey, US). Samples remained at room temperature for 20 minutes, then centrifuged at 4000rpm for 5 minutes. The resulting sera were without hemolysis and lipemia. Aliquots were stored at -20°C prior to analysis.

Biochemistry profile was determined using Mindray BS-240 analyzer (Mindray, Shenzhen, China). Ceruloplasmin (CP), and haptoglobin (HPT) were measured as described previously (Karić *et al.,* 2024). Insulin was measured using Tosoh AIA 360 analyzer (Tosoh Corporation, Tokyo, Japan). Serum protein fractions were separated by routine agarose electrophoresis. Specific protein concentrations were calculated using ImageJ software (Windows 64-bit Java 8).

Results were presented as median [min, max]. Correlations between biochemical parameters, BCS, NS, and laminitis were assessed using Spearman correlation coefficient (ρ) and presented with heatmap. Mann Whitney Test was conducted to examine differences in insulin levels according to the BCS. The results were significant at P<0.05. Statistical analysis was conducted using MedCalc® 14.8.1 (MedCalc Software Ltd, Belgium). Heatmap was created in GraphPad Prism 8. Inc. (GraphPad Software, USA).

RESULTS AND DISCUSSION

Eight jennies were considered over-conditioned (7 [4, 8]), while nine had noticeable crest (2 [0, 3]). One jenny displayed visually healthy hooves graded 0, six jennies had subclinical laminitis graded 0.5, while three had chronic laminitis graded 1 (Fig. 1). These were also main identified welfare issues.

Obesity-related criteria (BCS and NS) correlated with laminitis (Fig. 2), suggesting that metabolic dysregulation contributes to its development. Urea levels were dominantly elevated in most of the jennies (8/10), with creatinine being lower than the reference range in three of them (Table 1). Additionally, strong correlations between laminitis, urea and creatinine levels were found (Fig. 2). Bran, protein-rich feed, may contribute to increased urea, over-conditioning, and laminitis in lactating jennies. Elevated protein metabolism strains the liver, triggering inflammation and oxidative stress, which may impair hoof blood flow and cause laminitis (Bäßler *et al*., 2021), increasing the risk of weight loss (Dai *et al.*, 2018). Three jennies had low creatinine; two also had fat deposits, suggesting prolonged metabolic stress and hoof pain may have led to muscle loss and localized lipolysis resistance.

Fig. 1: Different hoof deformities associated with laminitis in lactating jennies. Grade 1 or chronic laminitis: (A) Overgrown hoof with long pointy toe and low heels, big ridges and wall bulges, hairline changed into V shape; (B) Overgrown hooves with toe growing dorso-anteriorly, visible white line and ridges, broken hoof pastern axis; (C) Overgrown hooves with medio-lateral imbalance, coronary band swelling and V shaped hairline; Grade 0.5 or subclinical laminitis: (D) Overgrown hooves with flaring of the hoof wall; (E) Overgrown hooves with the start of change in the base of the coronary band, ridges and hoof wall separation; (F) Overgrown hooves with visible tension in fetlock joints, medio-lateral imbalance and curved hairline.

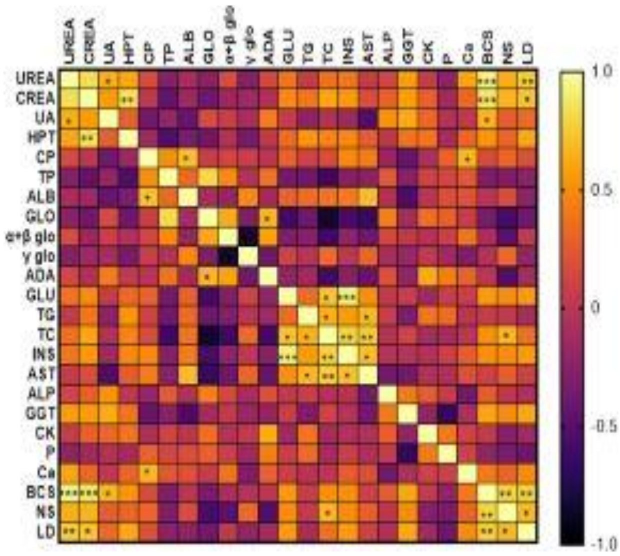

Fig. 2: Heatmap showing statistical significance between the analyzed parameters, both biochemical and morphological. * P<0.05, ** P≤0.01, *** P≤0.001. Abbreviations: CREA – creatinine, UA – uric acid, HPT – haptoglobin, CP – ceruloplasmin, TP – total proteins, ALB – albumin, GLO – total globulins, ADA – total adenosine deaminase, Glu – glucose, TG – triglycerides, TC – total cholesterol, INS – insulin, AST – aspartate aminotranspherase, ALP – alkaline phosphatase, GGT – γ glutamyl transpherase, CK – creatine kinase, P – phosphates, Ca – calcium, BCS – body condition score, NS – neck score, LD – laminitic deformities.

Table 1: Serum biochemistry profile, acute phase proteins and insulin of lactating jennies (N=10).

Parameter (unit)	Median	Min – Max	Reference interval (RI)
Urea (mmol/L)	6.46	3.84 – 8.68	1.5 – 5.2
Creatinine (μmol/L)	64.35	49.3 – 101.9	53 – 118
UA (mmol/L)	0.20	0.09 – 0.62	NA
Haptoglobin (μmol/L)	9.25	7.86 – 12.01	*
Ceruloplasmin (μmol/L)	2.99	2.21 – 4.01	*
Total proteins (g/L)	77.95	72.2 – 82.9	58 – 76
Albumin (g/L)	29.65	26.3 – 31.6	22 – 32
Globulins (g/L)	49.4	42.9 – 52.1	<37
α+β globulins (g/L)	35.93	27.25 – 42	NA
γ globulins (g/L)	19.93	16.51 – 27.47	NA
ADA (U/L)	0.39	0.27 – 0.50	NA
Glucose (mmol/L)	4.12	3.8 – 5.4	3.9 – 4.7
Triglycerides (mmol/L)	0.38	0.22 – 1.0	0.6 – 2.8
Cholesterol (mmol/L)	1.92	1.53 – 2.38	1.4 – 2.9
Insulin (pmol/L)	34.86	8.33 – 75	< 138.89
AST (U/L)	461.6	379.1 – 1037.6	<536
ALP (U/L)	376	208.3 – 578.6	<252
GGT (U/L)	29.85	14.5 – 58.9	<70
CK (U/L)	282.95	218.3 – 353.4	<525
Phosphates (mmol/L)	1.12	0.82 – 1.5	0.87 – 1.97
Calcium (mmol/L)	3.07	2.85 – 3.19	2.2 – 3.4

Abbreviations: UA – uric acid, ADA – total adenosine deaminase, AST – aspartate aminotransferase, ALP – alkaline phosphatase, GGT – γ glutamyl transferase, CK – creatine kinase. NA – not available. *Perez-Ecija *et al.* 2021.

Uric acids showed high variability of values (Table 1). In Nigeria, donkeys' UA values were in the lower quartile (Bature *et al*., 2018), while in Brazil values were slightly higher than the upper quartile (Silva *et al*., 2018) of the values measured in our study. Our results showed moderate, positive correlation between BCS and UA and between UA and urea (Fig. 2) which can be explained by both metabolites being products of nitrogen metabolism. Furthermore, laminitis-prone ponies had high UA concentrations on fructan-rich pastures (Bailey *et al.,* 2008). Measuring serum UA levels might serve in the early detection of metabolic syndrome in people (Nie *et al.,* 2023) and maybe in donkeys. However, high variability highlights the necessity for thorough assessment of donkeys' nutritional needs and timing of UA evaluation.

Obesity in horses is associated with low-grade chronic inflammation (Pratt-Phillips*,* 2024).

In our study, HPT and CP levels were not increased (Table 1), indicating no acute inflammation. Increased total globulins can be linked to increase in γ globulins (Table 1) and the underlying subclinical chronic inflammation. Low ADA activity (Table 1) matched patterns seen in horses (Contreras-Aguilar *et al.,* 2020), and its moderate correlation with globulins (Fig. 2) supports immune activation. Obesity and possibly high parasite burden in these jennies contribute to low-grade inflammation, potentially promoting partial insulin resistance and exacerbating laminitis risk.

No disturbances in basal GLU, TC, and insulin levels (Table 1), suggest the absence of detectable insulin resistance. Group with BCS≥7 (n=8) had median insulin value 41.32pmol/L (14.58–75), while group with BCS<7 (n=2) had median insulin value 9.72pmol/L (8.33–11.11) with P=0.044. It is expected that over-conditioned donkeys have higher mean insulin concentrations when compared to moderate and thin donkeys (Pritchard *et al.,* 2019). Strong positive correlation between GLU and TC levels (Fig. 2) suggests that similar underlying factors, influence changes in their concentrations. Many metabolic-syndrome donkeys with abnormal hyperinsulinemia during dynamic testing, have normal basal insulin levels (Mendoza *et al.,* 2024). Thus, surveyed jennies may still have experienced insulin dysregulation without detectable basal hyperinsulinemia, reflecting partial insulin resistance.

Hepatic enzymes (AST and ALP) exceeded reference ranges in two and eight jennies respectively (Table 1). In horses, serum AST is sensitive marker for hepatic lipidosis (Satué *et al.,* 2022). Correlation between AST, insulin, and TC (Fig. 2) suggests altered liver metabolism linked to low-grade inflammation and partial insulin resistance. The reason for increased ALP levels is unclear but may be related to unknown gestational status. Findings suggest possible hepatic lipidosis or other hepatic insult causing increased hepatocyte permeability in some jennies.

Conclusions: This pilot study found correlations between over-conditioning-related morphology, laminitis, and markers like UA, urea, globulins, and ADA, indicating metabolic changes and chronic subclinical inflammation, emphasizing the need for further research.

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Authors contribution: AM[1] and MKF conceived and performed the study; BM performed blind hoof assessment; KN performed welfare assessment; AM[1], KS, MR and AM[2] performed the analyses; All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

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