Research Article

Behavioral and Emotional Recovery in Horses with Equine Metabolic Syndrome: A Longitudinal Ethological Study

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Abstract | Equine Metabolic Syndrome (EMS) is commonly defined by regional adiposity, insulin dysregulation, and a predisposition to laminitis. While its metabolic and locomotor components have been well studied, the behavioral and psychological dimensions of EMS remain poorly understood. This longitudinal study evaluated both behavioral and physiological parameters in EMS-affected horses before and after a 90-day non-pharmacological intervention that included dietary restriction, controlled physical activity, and housing in socially enriched paddocks. Twenty adult geldings (Barb and Arab-Barb crossbreeds) were monitored through standardized ethological protocols, including direct observation, video analysis, and EquiFACS coding. Key behavioral metrics—such as spontaneous locomotion, social interaction, aggression, stereotypies, and indicators of anhedonia—were recorded alongside physiological markers (insulinemia, glycemia, and plasma cortisol). Before the intervention, horses exhibited reduced movement, signs of apathy, social withdrawal, and facial expressions consistent with pain and discomfort. After 90 days, most subjects showed increased voluntary locomotion a higher frequency of affiliative behaviors, improved social behaviors, reduced pain scores, and lower physiological stress markers. Changes in voluntary locomotion, affiliative behaviors, and stress markers significantly correlated with improvements in body condition scores and insulin/glucose profiles, suggesting a concurrent recovery of metabolic and emotional status. These findings highlight the need to integrate behavioral assessments into EMS management. The results support a neuro-metabolic interaction between systemic inflammation, chronic pain, and mood alterations in horses reinforcing the hypothesis that metabolic disturbances can significantly affect emotional states and welfare. This study provides novel evidence for behavioral recovery and emotional rebalancing in EMS horses following a multidisciplinary intervention.

Keywords | EMS, Insulin dysregulation, Obesity, Behavior, Welfare

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INTRODUCTION

Equine Metabolic Syndrome (EMS) is a well-established endocrinopathy characterized by regional adiposity, insulin dysregulation, and an increased risk of laminitis (Stefaniuk-Szmukier *et al*., 2023). While the metabolic and locomotor manifestations of EMS have been extensively investigated, its behavioral and psychological dimensions remain underexplored in both veterinary and ethological literature. However, the chronic low-grade inflammation, adipose accumulation, and locomotor discomfort typical of EMS may substantially impact a horse's emotional state, daily activity, and social behavior (Zak *et al*., 2020).

In horses, obesity is frequently associated not only with reduced physical activity but also with social withdrawal, diminished environmental engagement, and subtle indicators of chronic pain and stress (Geor and Frank, 2009; Toribio, 2011). Comparable behavioral disturbances have been observed across species. In horses, as in humans and rodent models, metabolic syndrome may induce altered behaviors—such as reduced motivation, diminished exploratory activity, and social withdrawal—mediated by systemic inflammation and neuroendocrine dysregulation (Capuron *et al*., 2011; Gregor and Hotamisligil, 2011). The neurobiological mechanisms underlying these behavioral effects involve multiple pathways. Systemic inflammation—mediated by cytokines such as Tumor Necrosis Factor alpha (TNF-α) and Interleukine-6 (IL-6)—can disrupt central nervous system function and affect motivation and emotional regulation (Dantzer *et al*., 2008). Chronic hyperinsulinemia may impair brain glucose metabolism and plasticity, thereby influencing cognition and mood (Gkikas and Tsiknakis, 2023). Dysbiosis of the gut microbiota, a condition commonly associated with both human and equine metabolic syndromes, is hypothesized to alter behavior via the gut-brain axis and neuroimmune mechanisms (Cryan *et al*., 2019). Recent studies have further demonstrated that specific alterations in equine microbiota can modulate brain function through neuroactive metabolites and immune signaling pathways (Mach *et al*., 2020; Boucher *et al*., 2024).

Additionally, in horses, these changes manifest as decreased voluntary movement, reluctance to move, weight-shifting postures to avoid limb pain, and an increase in stereotypic or apathetic behaviors associated with chronic discomfort (Torcivia and McDonnell, 2020). Even in obese horses without active laminitis, altered feeding patterns and reduced locomotion have been documented, suggesting that obesity alone may contribute to secondary behavioral changes (Kleinert *et al*., 2018). As prey animals, horses tend to express pain and distress through subtle behavioral cues, emphasizing the need for ethologically validated assessment tools. Recent advances in equine behavioral science have shown that horses' emotional states can also be assessed using experimental behavioral tests—such as novel object tests, judgment bias tasks, and startle response assays—that are analogous to those used in rodent research (Mach *et al*., 2020). Furthermore, equine body language, facial expression (e.g., the Equine Facial Action Coding System), and social behavior are increasingly recognized as reliable indicators of emotional valence and welfare status (Gkikas and Tsiknakis, 2023).

Given these insights, it is essential to incorporate behavioral metrics into EMS management strategies. Ethological assessments not only provide a holistic understanding of EMS-related discomfort but also help refine individualized treatment approaches.

The aim of this study was to assess behavioral and physiological changes in horses diagnosed with Equine Metabolic Syndrome following a structured, non-pharmacological management protocol. Specifically, we sought to:

- Characterize the behavioral progression of EMS horses over a 90-day period;
- Identify patterns of stress, apathy, and aggression during dietary restriction;
- Correlate behavioral outcomes with changes in metabolic and stress-related biomarkers.

MATERIALS AND METHODS

Study Population

This study involved 20 adult geldings diagnosed with Equine Metabolic Syndrome (EMS), including 10 Barb and 10 Arab-Barb horses aged 5 to 19 years (median: 10 years). Before inclusion, horses had been managed under diverse conditions ranging from private stables to semi-extensive systems, often with unrestricted pasture access and/or concentrated feeding. These practices were discontinued upon study entry.

Participants were recruited through regional equine rehabilitation centers and private owners, following informed consent. Inclusion criteria required a body condition score (BCS) ≥ 7/9, basal hyperinsulinemia (> 20 µIU/mL), and either a history or clinical signs of laminitis. Only horses free from other systemic or unrelated pathologies, as confirmed by physical examination and hematological screening, were included.

Upon enrollment, horses underwent a 14-day acclimation period to allow stabilization before baseline assessment. During this time, they were transitioned to standardized management conditions, including housing in dirt paddocks, consistent forage-only diets, regular handling, and daily health monitoring.

At baseline, 12 horses exhibited clinical signs of active laminitis (e.g., hoof sensitivity, divergent growth rings, abnormal stance). The remaining 8 met EMS criteria without acute laminitis signs, enabling subgroup comparisons. Horses were monitored daily by a veterinarian for signs of discomfort or clinical deterioration. To avoid confounding behavioral or physiological markers (e.g., locomotion, cortisol), no systemic analgesics were administered. However, any horse exhibiting signs of severe pain was excluded and provided with appropriate care. Animal welfare was prioritized throughout.

Hoof care was performed every 4–6 weeks by certified farriers using trimming protocols adapted for laminitis horses. Laminitis status was assessed radiographically at baseline and day 90 via lateromedial and dorsopalmar views of the front feet, focusing on rotation and sinking of the distal phalanx (P3). Lameness was scored using the AAEP 5-point scale. On days when a horse scored >2, it was excluded from behavioral assessments to reduce pain-related bias.

Housing, Feeding Conditions and Exercise

During the study, all horses were maintained under standardized and controlled conditions to ensure consistency across management variables.

Table 1: Nutritional composition of oat hay used in the study (dry matter basis).

Parameter	Result	Unit	Comments
Dry Matter (DM)	91.0	% as fed	Normal range for hay
Crude Protein (CP)	8.5	% DM	Moderate; supports maintenance needs
Neutral Detergent Fiber (NDF)	62.3	% DM	Reflects mature plant; slows intake
Acid Detergent Fiber (ADF)	38.7	% DM	Indicates moderate digestibility
Water-Soluble Carbohydrates (WSC)	6.2	% DM	Acceptable for EMS (<10%)
Ethanol-Soluble Carbohydrates (ESC)	4.8	% DM	Low sugar; suitable for insulin-dysregulated horses
Starch	1.2	% DM	Very low; minimizes post-prandial insulin spikes
Non-Structural Carbohydrates (NSC)*	7.4	% DM	NSC = WSC + Starch — EMS-safe threshold is <10%
Digestible Energy (est.)	1.8	Mcal/kg DM	Moderately low; consistent with weight-loss goals
Calcium	0.37	% DM	Within typical range for grass hay
Phosphorus	0.22	% DM	Balanced Ca:P ratio with supplementation if needed

***NSC:** WSC + Starch.

Dietary regimen: Horses were fed oat hay from a single, pre-analyzed batch, offered at 2% of body weight (dry matter/day), without any concentrate supplementation. Laboratory analysis confirmed the hay's suitability for EMS management: dry matter content of 91%, crude protein at 8.5%, and low non-structural carbohydrate (NSC) content of 7.4% (6.2% water-soluble carbohydrates and 1.2% starch), staying below the recommended <10% threshold for insulin-dysregulated horses. This ensured consistent forage quality and minimized intake variability (Table 1).

Feeding method: Hay was distributed using double-layered hay nets to prolong eating time and reduce feeding-related stress. Water was available ad libitum. No commercial balancer or pelleted vitamin-mineral supplements were administered; however, horses had continuous access to salt licks enriched with essential trace minerals (sodium, calcium, magnesium, and zinc), supporting baseline micronutrient intake. The nutritional adequacy of the hay was deemed sufficient for this short-term intervention as it met the non-structural carbohydrate (NSC) threshold (<10%) recommended by NRC (2007) guidelines for insulin-dysregulated horses. Forage calcium and phosphorus levels were periodically monitored, and no clinical signs of deficiency were observed. Access to pasture was strictly prohibited throughout the study. All paddocks were grass-free to prevent uncontrolled forage consumption and ensure precise dietary management.

Turnout and housing: Horses were individually housed in 12 × 20 m dirt paddocks with compacted sand-soil footing, free of grass, designed to support movement comfort even in laminitis horses. The daily turnout of 8 hours allowed for natural behaviors and reduced stress from confinement. Paddocks were aligned in parallel rows, offering uniform exposure to environmental conditions and allowing visual and tactile contact among horses.

Weight management: A progressive dietary restriction protocol was implemented, targeting a weekly weight loss of 0.5–1% of body mass. Body weight was recorded weekly using a calibrated livestock scale (±1 kg accuracy), always under standardized conditions (same time of day, before feeding) to enhance reliability.

Exercise protocol: Horses participated in a structured, low-intensity exercise program comprising 20 minutes of in-hand walking per day, five days per week, supervised by the same handler. Turnout in gently sloped paddocks supplemented formal exercise, encouraging voluntary locomotion. Riding and lunging were excluded to avoid mechanical stress on compromised limbs.

Laboratory analysis of the hay batch provided to all horses during the 90-day intervention. Values confirm the forage's suitability for dietary management in horses with EMS.

No statistical tests were conducted to compare the two subgroups (n = 12 with laminitis vs n = 8 without) at baseline. However, all horses met equivalent EMS diagnostic criteria, including insulin levels, body condition, and housing conditions.

Study Design

Behavioral and physiological assessments were conducted at three time points:

- T0: Baseline (prior to dietary restriction)
- T1: After 30 days of dietary restriction
- T2: After 90 days of dietary restriction

No systemic pharmacological treatments were administered to avoid potential confounding effects on behavior. All behavioral and physiological assessments were conducted concurrently across all horses within a standardized time window during the spring season (May–June), ensuring temporal and environmental consistency. To minimize inter-rater variability, all measurements were performed by the same trained research team.

Behavioral Assessment

Observation protocol: Each horse was observed twice daily (morning and afternoon) using a standardized behavioral assessment protocol (Hausberger *et al*., 2008), with each 15-minute session structured into three phases: 5 minutes of undisturbed spontaneous behavior, 5 minutes during forage distribution (including feeding and social interaction) conducted by the same neutral handler to minimize variability in horse-human interaction, and 5 minutes following a human approach, beginning passively and then progressing to active interaction.

Observations combined direct real-time assessments by two observers with synchronized video recordings for retrospective analysis. Fixed HD surveillance cameras were positioned at head height on the front and lateral sides of each paddock to capture both facial and full-body expressions from two angles. This dual-camera setup allowed for the detection of subtle or transient behaviors that might be missed during live observation. Over the course of the study, approximately 60 hours of synchronized video footage were collected across all subjects and time points, providing a robust basis for detailed retrospective EquiFACS coding and behavioral scoring.

To address the limitations inherent in live ethological observation—particularly in prey species such as horses, which may mask signs of discomfort—we followed recommendations by Torcivia and McDonnell (2020), integrating video footage to improve behavioral detection and reduce observer bias. Observers were blinded to the study phase (T0, T1 and T2) during both live and video-based evaluations.

Both observers underwent specific training in the EquiFACS system before the study. Each horse was evaluated independently at each time point. Inter-observer reliability was assessed using Cohen's kappa (κ = 0.84), indicating strong agreement. In cases of discrepancy, final scores were obtained by consensus following a video review. This observation format was adapted from validated ethological protocols (Dalla Costa *et al*., 2014; Minero *et al*., 2016), balancing observational depth with practical feasibility.

EquiFACS protocol: Facial expressions were analyzed using the Equine Facial Action Coding System (EquiFACS), a validated, objective tool for coding facial muscle movements in horses (Wathan *et al*., 2015). This system allows for the non-invasive assessment of emotional and physiological states by identifying discrete facial movements—referred to as Action Units (AUs)—without inferring internal affective states.

Certified EquiFACS coders independently assessed each horse using synchronized video recordings captured from both frontal and lateral angles, allowing for clear visualization of key facial zones, including the eyes (e.g., narrowing, fixed gaze), ears (e.g., forward, pinned back), mouth and lips (e.g., tension, lip curling), and forehead and cheek muscles (e.g., contraction, furrowing).

Each AU was scored using a standardized 4-point intensity scale, where 0 indicated absence and 3 represented strong activation. Frequently observed AUs included AU101 (inner brow raiser), AU145 (ears pinned back), AU17 (lip tightening), AU25 (lips part), and AU126 (nostril dilator). To enhance interpretive value, EquiFACS coding was supplemented with the EQUUS-FAP (Facial Assessment of Pain) scale, providing clinical context regarding potential pain expression. This dual approach ensured both objective quantification and practical relevance in behavioral assessment.

Behavioral parameters: Table 2 presents the ethological tools and scales used to evaluate core behavioral domains. Although horses were housed in individual paddocks, these were adjacent with shared fencelines, allowing for physical proximity, mutual grooming, and vocal interaction. Affiliative behaviors were defined as approach, ear orientation, synchronized movement, mutual sniffing, and voluntary human approach. These were recorded as indicators of social engagement.

Aggressive behaviors were defined as ear pinning, snapping or biting attempts, striking, tail swishing directed at conspecifics, or vocal threats. An aggressive event was scored from its onset (e.g., ear pinning toward a neighbor) to its resolution (return to neutral posture or behavioral redirection). Repeated acts were considered separate events if separated by at least 10 seconds.

Table 2: Ethological parameters and validated scoring tools used for behavioral assessment.

Domain	Behavior	Tool / Scale
Locomotor activity	Time spent in spontaneous movement (%)	(Minero *et al*., 2016)
Pain expression	Equine facial pain score (0–18)	EQUUS-FAP (Dalla Costa *et al*., 2014)
Horse–human interaction	Voluntary approach / avoidance	Standardized passive human approach test (Hausberger *et al*., 2008)
Aggression	Number of aggressive acts/session	Adapted EquiFACS coding grid (Wathan *et al*., 2015)
Stress indicators	Scratching, vocalization, stereotypies	Direct coded observation (Chen *et al*., 2016)
Depression-like signs	Apathy (> 60 s immobility), isolation	Adapted anhedonia index(Dantzer *et al*.,2008)

Exploratory behavior referred to sustained visual or olfactory inspection of the environment (e.g., novel objects, fence areas, or enrichment items) lasting more than 3 seconds. These were recorded as signs of environmental engagement. While EquiFACS is not an ethogram per se, it provides an anatomically based, objective framework to quantify facial muscle movements. These were interpreted in conjunction with ethologically validated tools such as the EQUUS-FAP scale to assess pain-related expressions in context.

Stereotypic behaviors (e.g., crib-biting, pawing) were recorded both in terms of frequency (events per hour) and duration (seconds). A 60% reduction was observed between T0 and T2, with mean frequency decreasing from 5.8 to 2.1 events per hour.

To ensure methodological consistency across individuals and time points, all facial expressions and behavioral categories were coded using standardized definitions. The coding framework integrated EquiFACS-based facial action units and validated ethological behaviors, and corresponding physiological parameters when relevant. Table 3 summarizes the full list of variables extracted from video recordings, along with their definitions, coding types, and reference sources.

Table 3: Variables used for video coding of EMS horses, based on EquiFACS and ethological tools.

Category	Code / Label	Variable Name / Description	Coding Type	Reference(s)
Facial Action Units (EquiFACS)	AU101	Inner Brow Raiser – emotional tension, pain or alertness	Occurrence	(Wathan *et al*., 2015)
	AU145	Ears Pinned Back – defensive or painful response	Duration	(Wathan *et al*., 2015)
	AU17	Lip Tightening / Chin Raiser – stress, discomfort	Occurrence	(Dalla Costa *et al*., 2014)
	AU25	Lips Part – oral tension, discomfort	Occurrence	(Wathan *et al*., 2015)
	AU126	Nostril Dilator – arousal or respiratory tension	Occurrence	(Wathan *et al*., 2015)
Pain Assessment	EQUUS-FAP	Composite score (0–18) of facial pain indicators	Score (0–18)	(Dalla Costa *et al*., 2014)
Ear Posture	EAD101	Ears Forward – interest, positive engagement	Duration	(Wathan *et al*., 2015)
	EAD103	Ears Flattened – stress, pain, aggression	Duration	(Torcivia and McDonnell, 2020)
Locomotor Activity	—	% Time in spontaneous movement during observation	% (per session)	(Minero *et al*., 2016)
Social Interaction	—	Affiliative Behaviors – approach, mutual sniffing, synchronized movement	Frequency	(Phelipon *et al*., 2025)
	—	Aggressive Behaviors – biting, ear pinning, threats toward conspecifics	Frequency	Adapted from (Wathan *et al*., 2015)
Stereotypies / Stress	—	Crib-biting, pawing, head tossing, vocalizations	Frequency and Duration	(Chen *et al*., 2016)
Depression-like Signs	—	Immobility over 60 sec, social withdrawal, apathy during forage distribution	Duration	(Dantzer *et al*., 2008)
Horse–Human Interaction	—	Voluntary Approach to Human (during passive approach test)	Count per session	(Hausberger *et al*., 2008)
	—	Avoidance / Irritability during passive approach	Occurrence	(Torcivia and McDonnell 2020)
Physiological Parameters	—	Basal Insulin (µIU/mL), Glucose (mmol/L), Plasma Cortisol (nmol/L)	Laboratory values	(Frank *et al*., 2010); (Toribio, 2011)

Physiological markers: The following physiological parameters were measured at each time point (T0, T1, T2): basal insulin, blood glucose, and plasma cortisol (Table 3), Blood samples were collected at 07:00 AM after an 8-hour fast, using a jugular catheter placed 24 hours in advance to minimize stress-related artifacts. Insulin concentrations were quantified using a validated, equine-specific ELISA kit (Mercodia, Sweden), The Mercodia Equine Insulin ELISA kit has a reported sensitivity of 0.02 µIU/mL, with intra-assay and inter-assay coefficients of variation below 5% and 7%, respectively. Validation for equine plasma has been confirmed in previous studies (Frank *et al*., 2010). Blood glucose was measured using a veterinary-calibrated handheld glucometer (AlphaTRAK 2, Zoetis). Plasma cortisol levels were determined by radioimmunoassay, using a method validated for equine plasma (Toribio, 2011; Burns, 2016).

To assess repeatability, duplicate blood samples were collected from each horse at each time point. All assays for insulin and cortisol were performed in triplicate, and mean values were used for statistical analysis. Coefficients of variation (CV) remained below 5% for all measurements.

Statistical Analysis

All statistical analyses were performed using SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Behavioral outcomes were reported as medians with interquartile ranges. Repeated-measures comparisons across the three timepoints (T0, T1, T2) were performed using the Friedman test for ordinal variables. When significant, post-hoc comparisons were conducted using Wilcoxon signed-rank tests with Bonferroni correction. Associations between behavioral and physiological parameters (insulin, glucose, cortisol) were assessed using Spearman's rank correlation. Statistical significance was set at $p < 0.05$.

To account for individual differences in the time each horse remained within the camera's field of view, both behavioral events and facial action units (as listed in Table 3) were standardized using time-corrected frequencies as described by Phelipon *et al*. (2025):

$$Occurrence\ frequency\ (\%) = \frac{Number\ of\ events \times 100}{Time\ visible\ (in\ seconds)}$$

$$Duration\ frequency\ (\%) = \frac{Total\ duration\ of\ the\ behavior \times 100}{Time\ visible\ (in\ seconds)}$$

These standardized metrics ensured fair comparisons across individuals and timepoints. All behavioral and facial expression variables were further analyzed using generalized linear mixed models (GLMMs). Fixed effects included the type of condition (positive or negative), while random effects accounted for inter-individual variability (individual horse) and the order of exposure to conditions, thereby controlling for potential sequence effects.

RESULTS

Locomotor Activity

At baseline (T0), horses exhibited markedly reduced locomotor activity. Median spontaneous movement time was only 4.5% (range: 2.8–6.2%)—well below levels typically observed in healthy horses. Subjects remained immobile for prolonged periods, with some lying down and showing minimal exploratory behavior (Figure 2).

After 30 days of treatment (T1), a moderate increase in locomotor activity was observed in 14 out of 20 horses, particularly during morning observation sessions. Median spontaneous movement rose to 7.3% (range: 5.1–9.8%). By 90 days (T2), locomotor activity had improved significantly in 18 out of 20 horses ($p < 0.01$). Mean time spent in spontaneous movement increased to 12.6% (range: 9.4–14.9%), approaching normal activity levels (Figure 1).

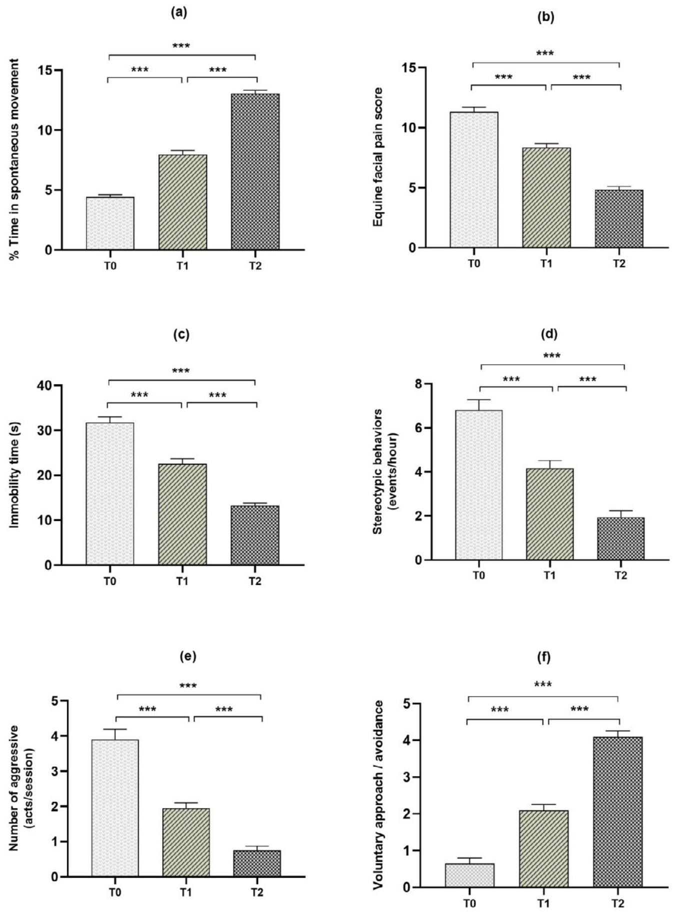

Figure 1: Behavioral and emotional recovery across the 90-day intervention.

Facial Expression of Pain

At T0, facial expression scores using EQUUS-FAP and

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EquiFACS revealed moderate to severe discomfort, characterized by a fixed gaze, ear retraction, nostril tension, and lip compression. The median EQUUS-FAP score was 12 (range: 9–15) (Figure 1).

At T1, stress-related facial cues such as AU17 (lip tightening) and AU145 (ears back) persisted in 12 of 20 horses. While some AUs (e.g., AU101—inner brow raiser) may suggest pain, others may reflect general emotional stress. To distinguish between these, we cross-referenced EquiFACS coding with EQUUS-FAP scores and behavioral indicators (e.g., reluctance to move, immobility, aggression).

At T2, facial indicators of discomfort were markedly reduced. EQUUS-FAP scores declined progressively, from a median of 12 (range: 9–15) at baseline (T0), to 8 (range: 6–11) at day 30 (T1), and 5 (range: 3–7) at day 90 (T2). These improvements were paralleled by more relaxed facial expressions observed via EquiFACS coding, including forward ear orientation, open eyes, and a neutral mouth posture (see Figure 3), indicating a sustained reduction in pain-related facial tension.

Horse–Human Interactions

At T_0, horse–human interactions were minimal. Most horses avoided voluntary contact with humans. In passive approach tests, subjects showed infrequent engagement (median of 1.1 approaches per session, range: 0–3) and occasional signs of irritability.

At T_1, voluntary approach behavior improved in 9 subjects, with the median number of approaches increasing to 2.4 (range: 1–5), and a reduction in avoidance behaviors during passive contact tests.

At T_2, all subjects tolerated handling better. Twelve horses actively engaged during approach tests. Engagement improved significantly compared to baseline ($p < 0.05$), indicating an enhanced affiliative response (Figure 1).

Aggression

At T_0, aggressive acts were frequent, including behaviors such as biting, ear pinning, and threatening gestures. The median frequency was 3.5 per session (range: 2–6).

- At T_1, aggression decreased slightly, with the median frequency dropping to 2.1 (range: 1–4).
- At T_2, aggression was rare. The median frequency had decreased to 1.0 (range: 0–2).

Depression-Like Signs

At T_0, several horses displayed apathy and signs of anhedonia, characterized by immobility lasting more than 60 seconds, diminished responsiveness, and lack of interest during forage distribution. The mean proportion of time spent immobile was 31.6% (range: 22–43%).

At T_1, immobility time decreased to a mean of 21.7% (range: 16–31%). Horses showed greater curiosity and increased responsiveness to environmental stimuli.

At T_2, depression-like signs significantly declined. The proportion of time spent immobile dropped further to 11.2% (range: 7–17%), with increased interaction with conspecifics and human observers.

Stereotypic Behaviours

Stereotypic behaviors included pawing, crib-biting, head tossing, and vocalizations during anticipation of feeding. Both frequency (events/hour) and duration (seconds/event) were systematically recorded (see Figure 1).

At T_0, stereotypic and stress-related behaviors were common, particularly pawing, crib-biting, and vocalizations in the context of feeding anticipation. The median frequency was 5.8 events per hour (range: 3–10).

At T_1, stereotypic behaviors moderately decreased to a median frequency of 3.9 events per hour (range: 2–7).

At T_2, stereotypies declined by over 60% compared to baseline. The median frequency was further reduced to *2.1 per hour (range: 0–4).*

Behavioral parameters in EMS horses improved significantly from baseline (T0) to post-intervention (T2), including increased voluntary locomotion (a), reduced facial pain expression (b), reduced immobility (c), fewer stereotypic behaviors (d), decreased aggression (e), and improved affiliative responses toward humans (f). These changes support the integration of ethological indicators in EMS welfare assessment.

Collectively, these behavioral metrics strongly support the hypothesis that behavioral recovery parallels metabolic improvement and validates the use of ethological indicators to monitor welfare in EMS horses.

Physiological Data

At T_0: Insulin: All subjects had elevated levels. The mean concentration was 32.5 ± 3.4 μIU/mL (range: 28–39).

Glucose: Mean level: 6.3 ± 0.4 mmol/L (range: 5.7–7.0). Cortisol: Mean plasma concentration was 142 ± 18 nmol/L (range: 119–170).

At T_1: Insulin levels declined from a baseline mean of 32.5 ± 3.4 μIU/mL to 28.4 ± 2.9 μIU/mL. Glucose decreased from 6.3 ± 0.4 mmol/L to 5.9 ± 0.3 mmol/L.

Although values remained above normal thresholds, these changes indicated an early metabolic response to dietary management. Cortisol levels showed a slight reduction, with a mean of 129 ± 13 nmol/L (range: 112–149).

At T_2: Insulin: Dropped below 20 µIU/mL in 17 horses. The group mean was 18.3 ± 2.1 µIU/mL (range: 14–22).

Glucose: Normalized in 16 horses. Mean level: 5.3 ± 0.2 mmol/L (range: 5.0–5.7), within the reference range for fasting horses.

Cortisol: Continued to decline, with a mean concentration of 108 ± 10 nmol/L (range: 96–124).

Normalization thresholds were based on established reference values for healthy adult horses (Geor, 2008; Tadros and Frank, 2013), with fasting glucose considered normal between 4.5–5.6 mmol/L. At T_2, 16 horses fell within this range.

All hormone assays were conducted in triplicate, with intra-assay coefficients of variation below 5%.

To evaluate the progression of behavioral and physiological parameters over the 90-day intervention, repeated assessments were conducted at baseline (T0) and at the end of the study period (T2). The data were analyzed using generalized linear mixed models and non-parametric tests where appropriate. Table 3 summarizes the key changes observed between T0 and T2, highlighting statistically significant improvements in locomotor activity, social behavior, pain expression, and metabolic markers.

Figure 2 illustrates the evolution of key metabolic and stress-related biomarkers—insulin (Figure 2a), glucose (Figure 2b), and cortisol (Figure 2c)—across the three timepoints (T0, T1, T2).

A significant and progressive decline in insulinemia was observed from T0 to T2 ($p < 0.001$), indicating improved insulin sensitivity following the intervention. Similarly, glucose levels showed a gradual and significant decrease over time ($p < 0.001$), reflecting enhanced glycemic regulation.

The most notable decrease was observed in cortisol concentrations, which dropped consistently from T0 to T2 ($p < 0.001$), suggesting a marked reduction in physiological stress.

Together, these metabolic changes align with the behavioral improvements reported earlier and support the effectiveness of the combined nutritional and physical activity program in restoring endocrine balance in horses affected by EMS.

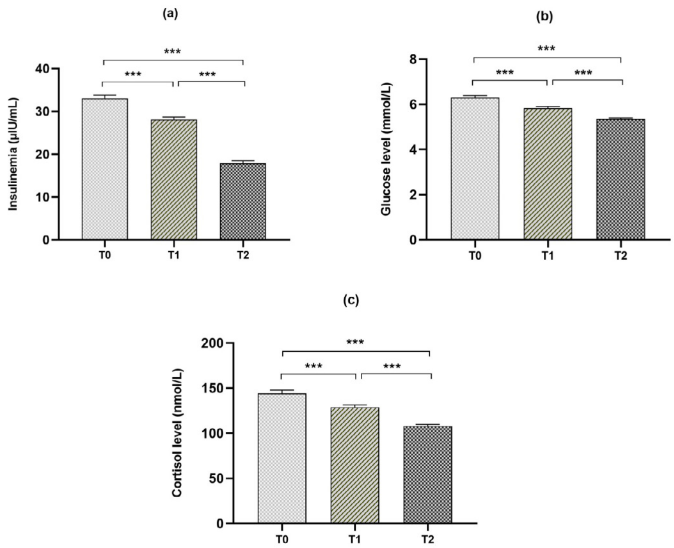

Figure 2: Improvement of metabolic and stress biomarkers following intervention.

Body Condition and Weight Changes

Body condition score (BCS) was assessed using the 9-point Henneke scale at T_0, T_1, and T_2. The median BCS at baseline (T_0) was 8/9 (range: 7–9). After 90 days (T_2), the median BCS had decreased to 6/9 (range: 5–7).

Body weight was monitored weekly using a calibrated livestock scale. On average, body weight decreased by 7.8% over the 90-day intervention period (range: 5.5%–9.6%), consistent with the study's targeted caloric restriction objectives (Figure 3).

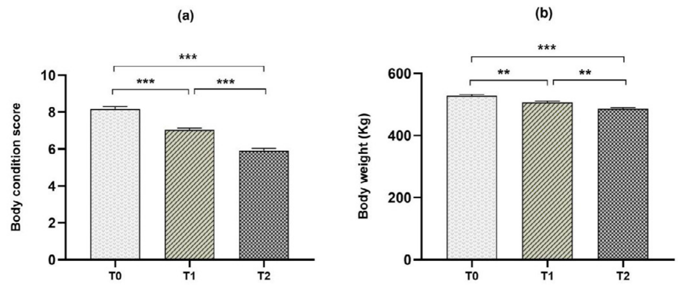

Figure 3: Progressive reduction in body fat and weight in EMS horses.

Body Condition Score (BCS)

A progressive and statistically significant reduction in Body Condition Score (BCS) was observed throughout the intervention. At baseline (T0), EMS horses exhibited elevated scores consistent with generalized obesity (mean ± SD = 8.06 ± 0.45). At day 45 (T1), BCS values had moderately decreased (mean = 6.84 ± 0.46), and by the end of the 90-day period (T2), a substantial reduction was recorded (mean = 6.01 ± 0.42).

This decline was consistent across all subjects and reflected the efficacy of the combined dietary and exercise-based intervention in reducing adiposity. These changes in BCS were strongly associated with improvements in behavioral patterns and metabolic markers, suggesting that BCS is a reliable and easily accessible welfare indicator in the field management of EMS horses.

Body Weight

In parallel with the decrease in BCS, a significant reduction in body weight was recorded across the study period. At T0, the average weight of the EMS horses was 107.4 ± 7.3 kg. By T1, weight had declined to 102.5 ± 6.8 kg, and at T2, it further decreased to 97.6 ± 6.4 kg.

All horses exhibited a downward trajectory, with individual weight loss ranging from 6% to 13% of initial body weight. This reduction was statistically significant ($p < 0.001$) and aligned with both the observed behavioral improvements (e.g., increased voluntary locomotion, reduced pain scores) and normalization of metabolic markers such as insulin and cortisol. These findings support the relevance of monitoring body weight alongside BCS as a quantitative measure of intervention success in EMS management.

Figure 3 displays changes in body condition score (BCS; Figure 3a) and body weight (kg; Figure 3b) at baseline (T0), mid-intervention (T1), and post-intervention (T2).

BCS decreased significantly across the three timepoints ($p < 0.001$), reflecting a steady reduction in adiposity consistent with the goals of the weight-loss program. The decline was progressive and uniform, confirming the effectiveness of the combined dietary and exercise intervention in modulating fat reserves.

Body weight also showed a statistically significant reduction from T0 to T2 ($p < 0.01$ to $p < 0.001$), with a sharper drop observed between T1 and T2. Although inter-individual variability was noted, all horses showed measurable weight loss, suggesting the protocol was effective and well tolerated. These findings support the integration of both weight and BCS as robust, field-applicable indicators for monitoring metabolic health and response to EMS interventions.

DISCUSSION

This study demonstrates that a structured intervention combining dietary restriction, low-intensity controlled exercise (mainly in-hand walking and paddock turnout), and welfare-oriented care, defined here as non-pharmacological management promoting physical comfort and emotional stability (e.g., soft footing, social housing, slow feeders, and reduced stressors), leads to marked behavioral and physiological improvements in horses diagnosed with EMS. The observed changes reflect not only a return to metabolic homeostasis but also emotional and cognitive recovery, as evidenced by improved locomotion, reduced facial pain expression, and increased social interaction.

At baseline, EMS-affected horses exhibited behavioral profiles consistent with chronic pain and psychological distress, including low spontaneous activity, stereotypic behaviors, social withdrawal, and facial expressions of discomfort. These findings are in line with previous reports that link systemic inflammation and locomotor discomfort with emotional dysregulation in horses (Frank *et al.*, 2010; Toribio, 2011; Dalla Costa *et al.*, 2014). They also mirror patterns observed in human and rodent models of metabolic syndrome, where chronic low-grade inflammation contributes to depression-like symptoms, reduced motivation, and impaired cognitive function (Gregor and Hotamisligil, 2011; Capuron *et al.*, 2011).

The nutritional analysis of the hay confirmed its appropriateness for EMS horses, supporting the validity of the dietary component of our intervention. This control helps minimize confounding factors related to dietary sugar or starch intake.

Although laminitis is widely accepted as a painful condition, not all horses in this study displayed overt signs of active laminitis during the evaluation period. The presence of pain-related behavior and negative affective states in horses without clinical laminitis suggests that EMS alone—through mechanisms such as inflammation or insulin dysregulation—may contribute to discomfort and emotional disturbance. This distinction highlights the importance of investigating EMS-related behavioral changes independently of laminitis.

At baseline, 12 horses exhibited active laminitis signs. By day 90, 9 of these showed clinical improvement (AAEP lameness score ≤ 1; reduced hoof sensitivity). Radiographic monitoring revealed reduced P3 rotation in 6 cases and no further progression in the others. These improvements were likely facilitated by weight loss, improved insulin regulation, and reduced systemic inflammation.

After 90 days of non-pharmacological treatment, most horses displayed significant improvements across all measured domains. Behavioral outcomes such as locomotion, facial expression, social interaction, and stereotypy frequency all improved notably. These results suggest a return to desirable emotional and behavioral patterns, consistent with pain relief, emotional rebalancing, and improved welfare (Phelipon *et al.*, 2025). Similar recovery patterns have been observed in laboratory rodents and humans following anti-inflammatory or metabolic interventions (Kleinert *et* *al*., 2018; Cryan *et al*., 2019).

Our results support a growing body of evidence suggesting that metabolic and inflammatory stressors impact central motivational and affective circuits in mammals. As a prey species, horses have evolved to hide signs of pain, and therefore behavioral indicators may be subtle and easily overlooked (Chen *et al*., 2016). These findings reinforce the value of ethological tools like EquiFACS and the EQUUS-FAP scale for detecting subtle signs of discomfort.

We acknowledge that EquiFACS is not a diagnostic tool nor a scale of emotional valence. It provides a descriptive coding of facial muscle activity (Action Units) without assumptions about internal states. Interpretation was supported by combining EquiFACS with validated pain tools (EQUUS-FAP) and behavioral context.

The observed decline in pain-related facial expressions, reflected by the drop in EQUUS-FAP scores, supports a reduction in discomfort. While EquiFACS identifies muscle activity, EQUUS-FAP allows inference about pain based on specific facial configurations validated in clinical contexts (Dalla Costa *et al*., 2014).

Importantly, behavioral indicators strongly correlated with physiological markers. For example, locomotor activity was negatively associated with insulin concentrations, and facial pain scores correlated positively with cortisol levels. These associations support the use of behavior as a proxy for internal metabolic and emotional states and underscore the value of behavioral monitoring in the clinical management of EMS. Given that EMS horses may display pain or stress-related behaviors, behavior-based assessments are essential for tracking recovery and ensuring welfare (Phelipon *et al*., 2025). Our observations align with the work of (Torcivia and McDonnell, 2020), who highlighted the subtlety and consistency of pain-related behaviors in horses. The prolonged immobility, reduced exploration, and facial tension we observed at baseline support the notion of chronic low-grade discomfort as described in their laminitis-associated pain ethograms.

The success of this intervention—achieved without pharmacological treatment—demonstrates the effectiveness of holistic and welfare-based strategies in managing EMS. It also emphasizes the importance of treating the horse as a biopsychosocial individual, in which physiological, emotional, and behavioral components are interdependent. This integrative perspective could inform the management of other chronic equine disorders with overlapping physiological and behavioral dimensions.
The absence of a control group and the modest sample size reflect real-world constraints, including limited facility space, funding responsibilities for feed and housing, and reliance on the voluntary cooperation of horse owners. Additionally, all daily care and protocol implementation was carried out directly by the lead author, a licensed veterinarian, ensuring consistent application of the intervention but also highlighting the logistical limitations inherent in field-based longitudinal studies. Nevertheless, the absence of an EMS-free control group receiving the same care limits causal attribution, as some improvements may be attributable to enhanced general welfare rather than EMS resolution specifically.

The 90-day duration was selected based on previous research indicating that behavioral and metabolic adaptations to dietary and exercise interventions in horses typically occur within this time frame (Toribio, 2011; Gregor and Hotamisligil, 2011; Burns, 2016). After the study period, horses were returned to their respective owners or institutions, where continued follow-up was encouraged but not standardized, limiting long-term outcome data. Future studies should explore the long-term stability of behavioral improvements post-intervention, the inclusion of cognitive bias testing to further assess affective changes, microbiome analyses to examine gut-brain axis modulation, and breed-specific responses to behavioral and metabolic rehabilitation. Additionally, integrating automated facial expression detection via artificial intelligence may enhance the early identification of subtle pain indicators, offering a promising tool for clinical practice (Rak-Pasikowska *et al*., 2024).

One limitation of this study is the lack of repeated baseline measurements across multiple days to capture intra-individual variability. However, a14-day acclimatization phase before T0 helped stabilize environmental and management conditions, thereby minimizing short-term physiological fluctuations. Future research should consider multiple baseline recordings to enhance data reliability.

These findings support the hypothesis that EMS-related systemic inflammation may disrupt emotional regulation via neuroimmune pathways, akin to mechanisms observed in other mammals (Cryan *et al*., 2019).

CONCLUSIONS AND RECOMMENDATIONS

Our findings suggest that EMS significantly alters not only metabolic and locomotor functions but also the emotional and behavioral well-being of affected horses. Through a structured 90-day intervention involving dietary regulation, controlled physical activity, and welfare-centered care, we observed marked improvements in spontaneous movement, facial pain expression, social engagement, and physiological stress markers. These findings support the integration of ethologically validated behavioral assessment tools as complementary indicators of recovery and welfare in EMS management protocols. Rather than focusing exclusively on metabolic markers, management strategies should include behavioral metrics that reflect both physical and psychological dimensions of health.

Integrating behavioral assessment into EMS management offers a more holistic view of therapeutic outcomes, bridging clinical endocrinology and equine welfare science. This approach aligns with current international guidelines on equine well-being and contributes to a broader understanding of the behavioral impact of chronic metabolic disorders in horses and across species.

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NOVELTY STATEMENT

This study is the first to comprehensively demonstrate that a combined dietary restriction and tailored exercise program can significantly improve behavioral, metabolic, and emotional outcomes in horses affected by Equine Metabolic Syndrome (EMS). By integrating validated ethological tools and EquiFACS facial coding, the work reveals behavioral biomarkers that correlate with endocrine normalization, offering a novel perspective on welfare assessment. Furthermore, the focus on Barb and Arab-Barb horses—a population rarely represented in EMS research—provides new insights into breed-specific responses and contributes to evidence-based, accessible therapeutic strategies suitable for field conditions.

AUTHOR'S CONTRIBUTIONS

Hannane Hmimia: Conceptualization, funding acquisition, experimental design, fieldwork coordination, data collection, data interpretation, manuscript drafting, and critical revision.
Mouloud Lamtai: Statistical analysis, figure preparation, and assistance in data interpretation.
Aboubaker El Hessni and Abdelhalem Mesfioui: Supervision of the research project, critical revision, and final approval of the manuscript.

Highlights

- Behavioral changes in Equine Metabolic Syndrome (EMS) horses are detectable using validated ethological tools.
- EquiFACS and EQUUS-FAP coding revealed reduced facial tension over time.
- Improved voluntary locomotion and social behavior correlated with insulin and cortisol normalization.
- Weight loss and stress reduction were associated with improved behavioral patterns.
- Behavioral assessment is a useful tool for monitoring welfare in horses with endocrine disorders.

Ethics Statement

All procedures were conducted in compliance with international veterinary ethical guidelines. Owner consent was obtained, and the protocol was supervised by a licensed equine veterinarian (Dr. Hannane Hmimia).

To ensure animal welfare, all horses were monitored daily by a licensed veterinarian (first author). No systemic analgesics were administered during the observation period to avoid interference with behavioral or physiological measures (e.g., cortisol, facial activity). However, any horse displaying signs of severe or persistent pain was excluded from data collection and received appropriate medical care and analgesia.

This decision was made with careful ethical consideration, balancing the need to observe natural behavioral patterns with the responsibility to prevent suffering. Environmental and social enrichment, soft ground, slow feeding systems, and daily turnout were implemented to minimize discomfort during the study.

Conflict of Interest

The authors declare that they have no competing interests or financial relationships that could have influenced the outcome of this research.

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