agriculture

MDPI

Review

Role of Cortisol in Horse's Welfare and Health

Urszula Sikorska [1], Małgorzata Maśko [1,*], Anna Ciesielska [1], Łukasz Zdrojkowski [2,*] and Małgorzata Domino [2]

[1] Department of Animal Breeding, Institute of Animal Science, Warsaw University of Life Sciences (WULS–SGGW), 02-787 Warsaw, Poland; urszula_sikorska@sggw.edu.pl (U.S.); anna_ciesielska@sggw.edu.pl (A.C.)

[2] Department of Large Animal Diseases and Clinic, Institute of Veterinary Medicine, Warsaw University of Life Sciences (WULS–SGGW), 02-787 Warsaw, Poland; malgorzata_domino@sggw.edu.pl

* Correspondence: malgorzata_masko@sggw.edu.pl (M.M.); lukasz_zdrojkowski@sggw.edu.pl (Ł.Z.)

Abstract: This review describes the physiological role of cortisol in the horses' body and the usefulness of cortisol concentration measurements in assessing welfare and diagnosing diseases. Cortisol is examined in terms of its production and functions, along with the concept of circadian cortisol rhythms and potential disruptions in these patterns. The significance of cortisol concentration is emphasized, as it provides insights into stress levels, and the connection between cortisol and stereotypical horse behaviors, raising the question of whether these behaviors signify stress or serve as coping mechanisms. Moreover, cortisol concentration helps in diagnosing various health conditions such as Cushing's Disease, inflammation, and metabolic imbalances. As cortisol concentration is considered a stress indicator that may be affected by the sampling protocol, the matrices for cortisol sampling and their practical applications are summarized. In this review, three major cortisol sampling methods: plasma, salivary, and hair cortisol, are described in detail. Plasma cortisol measurements offer acute stress insights, while salivary cortisol analysis provides a non-invasive method for continuous stress monitoring. Hair cortisol, on the other hand, offers an assessment of long-term stress levels. This text underscores the importance of cortisol control in safeguarding the welfare and health of horses.

Keywords: hormone; cortisol; glucocorticoid; blood; saliva; hair; stress; welfare

Citation: Sikorska, U.; Maśko, M.; Ciesielska, A.; Zdrojkowski, Ł.; Domino, M. Role of Cortisol in Horse's Welfare and Health. *Agriculture* **2023**, *13*, 2219. https://doi.org/10.3390/agriculture13122219

Academic Editor: Eva Voslarova

Received: 19 October 2023
Revised: 28 November 2023
Accepted: 28 November 2023
Published: 30 November 2023

1. Introduction

Since domestication, horses have been subjected to a wide array of management techniques and have been utilized in various roles [1]. These practices encompass human handling, single housing in stalls, and controlled meal feeding, all which can induce stress in these animals, diverting them from their natural habitat [2]. That is why domestic horses during everyday life and use are exposed to many external stressors. Among the stressors that are commonly accepted and considered in everyday routine are those related to use, namely physical exertion, transportation, competitive events, and veterinary procedures [3–6]. Equally frequently considered stressors concern the housing and maintenance of horses and include social isolation [6], heat stress [7], and the effect of poor environmental conditions [8]. However, a group that is much less aware, but no less important, are stressors related to nutrition. One may observe that unbalanced diets and fattening conditions are considered potential stressor [9–12], which should be taken into account when assessing the welfare of horses and carrying out corrective actions to improve the well-being of these sensitive and social animals. Each of these stressors holds the potential to evoke emotional excitement, elevate heart rate, and cause the release of stress hormones [13–17]. Thus, stressed animals may exhibit atypical and aggressive behaviors [2,18–20], an array of health issues [2,18], heightened hormone secretion [18], or diminished performance [19]. Consequently, the assessment and regulation of stress are indispensable for ensuring the safety and welfare of horses [2].

The equine stress response serves as a vital supplementary homeostatic system, mobilizing a spectrum of neurological and hormonal pathways to enhance immune, cardiovascular, musculoskeletal, and metabolic processes when the body confronts disturbance or threat [21]. In response to stress, horses activate the hypothalamic-pituitary-adrenal (HPA) axis, initiating the release of a range of chemicals. These include mineralocorticoids such as aldosterone, glucocorticoids such as cortisol, and adrenal androgens such as dehydroepiandrosterone (DHEA), all of which belong to the class of steroid hormones known as corticosteroids. These corticosteroids play pivotal roles in various physiological processes, encompassing the regulation of fluid and electrolyte balance, maintenance of cardiovascular homeostasis, metabolism of carbohydrates, proteins, and lipids, regulation of immunological and inflammatory responses, and the facilitation of sexual maturation and reproductive functions [22].

The equine stress response, the HPA axis, regulates the body's fundamental physiological functions [3,23]. The anterior pituitary gland responds to the secretion of corticotropin-releasing factor (CRF) by the hypothalamus by producing increased levels of adrenocorticotropic hormone (ACTH), also known as corticotropin. The ACTH circulates in the bloodstream, binding to the adrenal cortex, thereby raising the production and release of glucocorticoids. It is worth noting that any form of stress, whether physical, emotional, environmental, or feeding, can trigger the release of glucocorticoids in horses [24].

The primary glucocorticoid released by horses is cortisol [25,26]. Cortisol belongs to the group of steroid hormones, with a molecular weight of approximately 362.46 g/mol and a chemical formula of $C_{21}H_{30}O_5$ [3,27]. Cortisol significantly influences the metabolism of carbohydrates, proteins, and lipids. It actively promotes gluconeogenesis, the synthesis of glucose from non-carbohydrate sources, thereby ensuring a consistent supply of energy substrates, particularly during periods of fasting or stress [28]. The cortisol concentration increases rapidly in circulating blood after stressor, and can be accurately detected in various collected material [29–32]. It is worth noting that blood [13,33–39] and partially salivary [13,25,32,39–41] cortisol samples reflect an acute stress, whereas salivary [41–43], hair [44–49] and feces [8,17,38,50–53] cortisol samples show a chronic stress. This is why cortisol is considered a biomarker for assessing both psychological and physiological stress, which can be used in the identification, treatment, and prevention of stress-related disorders.

Therefore, this review describes the physiological role of cortisol in the horses' body and the usefulness of cortisol concentration measurements in assessing welfare and diagnosing diseases. As cortisol concentration is considered a stress indicator that may be affected by the sampling protocol, the matrices for cortisol sampling and their practical applications are summarized in detail. We believe that by underlining the importance of cortisol management, this review contributes to a better understanding of equine welfare and health.

2. Cortisol in Horse's Welfare and Health

While cortisol is indispensable for survival and adaptation, chronically elevated concentration can yield detrimental effects [54]. High cortisol concentration can negatively impact heart rate, causing it to increase, although simultaneously, the organism may be stimulated to combat dehydration [55]. Numerous studies have demonstrated a positive correlation between cortisol concentration and various physiological stress indicators in horses, including heart rate, respiratory rate, rectal temperature, eye temperature, and blood lactic acid [13,33,56–59]. Similarly, numerous studies point to the positive correlation between cortisol concentration and stereotypical behavior occurrence [8,60–62]. However, the question is still raised whether such behavior is an indication of stress or a coping mechanism. One may observe that higher plasma cortisol concentration was noted before stereotypical behaviors, such as weaving and crib-biting than following them, hinting at stereotypy as a potential coping mechanism [60]. On the other hand, crib-biting behavior did not have a noticeable impact on the horses' plasma cortisol, beta-endorphin, and adrenocorticotropic hormone concentrations [62]. No differences in plasma cortisol concen-

tration were found between horses displaying stereotypical behaviors and those that do not exhibit such behaviors [61]. Moreover, no differences in plasma cortisol concentration and fecal cortisol metabolites concentration were found in stereotypic and non-stereotypic horses, which confirms the possibility of stress assessment and the relationship between stress and welfare indicators using less invasive, and thus less biased fecal sampling [8].

Cortisol serves as an important diagnostic indicator for various diseases and health conditions in horses, such as stress-related conditions [29,30], equine Cushing's disease (ECD) (also known as Pituitary Pars Intermedia Dysfunction (PPID)) [63,64], inflammatory and infectious diseases [65–67], as well as metabolic imbalances, dehydration, and electrolyte imbalances [55]. The ECD is the most prevalent endocrine condition affecting 20–25% of elderly horses over the age of 15 years [34,54,63,68–70]. ECD is characterized by a long-term elevation in circulating glucocorticoids [63], however, plasma cortisol concentration is typically within the normal range or even lower [71]. Some research suggests that the paradox of normal plasma cortisol concentration in horses with ECD may be best explained by alterations in cortisol metabolism [28]. According to a recent study, the free plasma cortisol fraction was found to be higher in ECD horses than in normal horses of the same age and season [72]. The increased free plasma cortisol fraction could be attributed to a decline in the binding affinity of cortisol-binding globulin. Apart from the obvious role of cortisol in the pathogenesis of ECD, this hormone contributes to the body's stress response to serious illness and is instrumental in regulating the inflammatory reaction [65,67]. One may observe that in relative adrenal insufficiency (RAI) or critical illness-related corticosteroid insufficiency (CIRCI), the cortisol response to illness is inadequate. This can result in cardiovascular collapse and an extensive, uncontrolled systemic inflammatory response, which may lead to serious decrease of general condition or death [66,67].

It is worth emphasizing that cortisol concentration in horses may be affected by factors other than stress or illness, including physical activity and the time of day. Horses exhibit a circadian rhythm in cortisol release, akin to other species such as humans [73], dogs [74], and rhesus monkeys [75], with peak levels in the morning and troughs in the late afternoon and evening. This circadian rhythm is crucial for maintaining the horse's physiological balance [31,76,77], therefore, even minor disruptions in this pattern can diminish daily oscillations and elevate cortisol concentration [31,32]. Therefore, technique and time of sample collection and storage, analytical method used, and variables beyond stress, such as physical activity, health status, and the time of day should be considered when interpreting equine cortisol concentration.

3. Matrices for Cortisol Sampling

3.1. Blood Cortisol

Blood cortisol concentration offer valuable insights into the horse's acute stress response, serving as a snapshot of their immediate state. An elevation in blood cortisol concentration indicates recent stress or activation of the horse's HPA axis. Thus, blood cortisol concentration is often used in clinical settings to assess acute stress responses [33], to assess welfare [8,38], to diagnose cortisol-related diseases [37], and to evaluate the effectiveness of treatment protocols [36,40]. While measurement of blood cortisol is a useful tool for assessing acute stress responses, it may not provide a complete picture. It is essential to recognize that blood cortisol concentration may not offer a comprehensive assessment of chronic stress, as the pulsatile production of glucocorticoids have to be considered for the evaluation of blood samples [8,78,79].

The blood cortisol concentration is typically measured in plasma or serum. For this reason, a blood sample is obtained most commonly through venipuncture. However, this method is invasive and potentially stressful for the horse, especially if they are anxious or uncooperative during the process. The handling of the animals necessary to obtain blood samples can introduce additional stress, which could have an impact on the final results [8]. The serum or plasma cortisol concentration is measured using laboratory methods such as radioimmunoassay (RIA) [8,35,39], enzyme-linked immunosorbent assay

(ELISA) [13], rapid enzyme immunoassay (EIA) [33], or liquid chromatography–tandem mass spectrometry (LC-MS/MS) [36].

The nominal or basal value of plasma cortisol concentration in horses typically falls within a specific range, although it can vary based on factors such as the horse's age, health status, and circadian rhythm. The literature offers a reference range for basal cortisol concentration in equines, so that according to available reports, healthy adult horses exhibit resting basal cortisol concentration that typically range from 30 to 395 nmol/L [22,35,80,81]. The reference range provided offers a guideline for what is considered normal or nominal basal cortisol concentration in adult, healthy horses. However, it is important to note that individual variations and the clinical context should also be considered when interpreting cortisol values in specific cases. Some of the publications on basal blood cortisol concentrations are shown in Table 1.

Table 1. Studies reporting basal blood cortisol concentrations in horses.

Objectives	Demographic Data	Time of Day and Method	Results	Reference
Correlates of oral and motor stereotypic behaviors and glucocorticoid concentration	55 horses (41 geldings, 14 mares) 5 to 20 years old	Blood sampling between 18:00 and 19:00 RIA method	Plasma cortisol concentration varied from 7 to 112 nmol/L	[8]
The use of plasma sampling for the determination of cortisol concentration in relation to the intensity of exercise in horses during race training	12 horses (6 stallions, 6 mares) 2 to 3 years old	Blood sampling between 7:00 and 9:00 ELISA method	Plasma cortisol concentration varied from 249 nmol/L at rest, 335 nmol/L immediately after exercise, and 281 nmol/L 30 min after exercise	[13]
Plasma cortisol and ACTH concentrations in the warmblood horse in response to standardized treadmill exercise test	10 horses (10 geldings) 3 years old	Time not reported EIA method	Plasma cortisol concentration before exercise varied from 41 to 185 nmol/L	[33]
Database for steroid reference values for domestic Mongolian horses	123 horses (18 colts, 34 stallions, 25 geldings, 17 fillies, 29 mares) 2 months to 17 years old	Blood sampling twice on 10:00 and 14:00 RIA method	Serum cortisol concentration varied in groups, with 64 nmol/L for colts, 158 nmol/L for stallions, 193 nmol/L for geldings, 102 nmol/L for fillies, 171 nmol/L for mares, 140 nmol/L for all horses	[35]
Steroid profiles in equine plasma before ACTH-stimulation test	11 horses (8 geldings, 3 mares) 6 to 14 years old	Time not reported LC-MS/MS method	Plasma cortisol concentration was 138 ± 54 nmol/L before the ACTH-stimulation test	[36]
Determine the use of plasma cortisol concentrations in the diagnosis of the ECD	7 horses (5 geldings, 2 mares) 5 to 15 years old	Blood sampling twice on 06:00 and 18:00 RIA method	Plasma cortisol concentration varied from 251 nmol/L at 06:00 and 142 nmol/L at 18:00	[37]

Table 1. *Cont.*

Objectives	Demographic Data	Time of Day and Method	Results	Reference
Relation between compromised welfare, such as chronic pain and hematological anomalies, and cortisol concentration in domestic horses	49 horses (44 geldings, 15 mares) 5 to 20 years old	Blood sampling twice a day in the morning (between 08:00 and 09:00) and in the evening (between 18:00 and 19:00) RIA method	Plasma cortisol concentration varied from 80 nmol/L in the morning, 42 nmol/L in the evening (day of work), and 35 nmol/L in the evening (day of rest)	[38]
Develop a sensitive and specific RIA to measure cortisol concentration precisely in serum	5 horses (2 geldings, 3 mares) 9 to 17 years old	Time not reported RIA method	Serum cortisol concentration varied from 137.84–256.92 nmol/L at rest and 146–222 nmol/L during catheter placement	[39]

3.2. Salivary Cortisol

The traditional approach to assessing HPA axis activity in horses has primarily involved measuring cortisol concentration in blood. However, in recent years, there has been a growing trend toward utilizing salivary cortisol analysis for this purpose [82,83]. Salivary cortisol is considered the biologically active form of the hormone [78], and there is substantial evidence of a strong correlation between cortisol levels in blood and saliva in horses [13,39]. Total blood cortisol concentration could account for approximately 80% of the variations in salivary cortisol concentration, and vice versa [39]. However, the use of salivary cortisol concentration measurement for the evaluation of acute physiological stress in horses may be limited, as free cortisol is excreted from the salivary gland during oral movements, which makes it available [61]. Prolonged elevations in salivary cortisol concentration may be linked to chronic stress, which, in turn, can contribute to multiple health disorders, including weakened immune systems and metabolic aberrations [42]. Thus, salivary cortisol concentration was proposed in clinical settings to assess stress levels, particularly in exercised horses [25,41–43], during competition [40], during transportation, under environmental pressure [41], and in order to identify horses that have been exposed to chronic stress and require special care to maintain their welfare [42].

The measurement of salivary cortisol concentration offers a non-invasive and convenient method for repeated sampling without causing disruption to the animal [40,84]. This is particularly advantageous in situations where collecting blood samples may be challenging or unfeasible. Additionally, compared to blood sampling, saliva collection does not require the involvement of highly trained and certified personnel [40,84]. Due to ethical concerns, earlier research on stress often focused on evaluating cortisol concentration in saliva, since methods involving blood serum collection may induce fear and stress in animals [40,85]. As a result, the practical and noninvasive technique of measuring cortisol concentration in saliva has been increasingly applied to reduce stress and ensure the welfare of horses and other animals [3,37,40,85]. Various techniques are employed to collect saliva samples from horses. One popular approach involves the use of special sponges or cotton balls that are placed in the horse's mouth for a brief period and then subsequently analyzed for cortisol content [86]. Cortisol in saliva can be measured using a variety of techniques, including RIA [38,40], ELISA [13,25], or EIA [32,41], which make it possible to measure this material precisely. In addition, salivary cortisol research also uses gas chromatography and other mass spectrometry techniques, which ensure the accuracy of the results [32].

Studies reporting salivary cortisol concentrations in horses are summarized in Table 2.

Table 2. Studies reporting salivary cortisol concentrations in horses.

Objectives	Demographic Data	Time of Day and Method	Results	Reference
The use of saliva sampling for the determination of cortisol concentration in relation to the intensity of exercise in horses during race training	12 horses (6 stallions, 6 mares) 2 to 3 years old	Saliva sampling between 7:00 and 9:00 ELISA method	Salivary cortisol concentration varied from 1.63 nmol/L at rest, 2.57 nmol/L immediately after exercise, and 3.82 nmol/L 30 min after exercise.	[13]
Evaluation of salivary cortisol concentration for association with strongyle-type egg shedding levels	200 horses (132 geldings, 3 stallions, 65 mares) 1 to 30 years old	Saliva sampling between 11:00 and 13:00 ELISA method	Salivary cortisol concentration was 2.7 nmol/L	[25]
Estimation of the change of stress level in horses based on saliva cortisol concentration	61 horses (28 geldings, 33 mares) 5 to 20 years old	Saliva sampling at 7:00, 11:00, 14:00, and 16:00 EIA method	Salivary cortisol concentration varied from 2.44 nmol/L in tourist riding group, 3.07 nmol/L in resting group, and 2.76 nmol/L in education horse-riding group.	[32]
Develop a sensitive and specific RIA to measure cortisol concentration precisely in saliva	5 horses (2 geldings, 3 mares) 9 to 17 years old	Time not reported RIA method	Salivary cortisol concentration varied from 0.58–1.77 nmol/L at rest and 0.59–2.21 nmol/L during catheter placement	[39]
Quantification of the stress levels in competition using salivary cortisol concentration	23 horses (11 geldings, 12 mares) 6 to 11 years old	Saliva sampling at 9:00 and just before competition, and 20, 40, and 60 min after RIA method	Salivary cortisol concentration varied from 1.01 nmol/L at rest to 1.56 nmol/L after whole day of competition	[40]
Analyze the salivary cortisol concentration in horses related to stressors	14 horses (9 geldings, 3 stallions, 2 mares) 3 to 7 years old	Saliva sampling at 15:00 EIA method	Salivary cortisol concentration varied from 9 nmol/L to 9.5 nmol/L	[41]

3.3. Blood and Salivary Cortisol in Stimulation or Suppresion Tests

The baseline cortisol measurement [4,31,36,87], the ACTH-stimulation test [4,33,36,39,88–90], and the dexamethasone-suppression test [44,90–95] are commonly used to assess blood cortisol concentration in horses, each serving specific diagnostic or research purposes.

The baseline cortisol measurement involves the collection of a single blood sample from a resting horse to determine plasma or serum cortisol concentration at a specific moment. This test provides insights into the horse's current cortisol status [4,36]. Typically, the blood is obtained through venipuncture, commonly from the jugular vein, using standard aseptic techniques [87]. The timing of sample collection is crucial. Ideally, the blood sample should be taken from a resting horse in the morning or early afternoon when cortisol concentration tends to be relatively stable [31]. This minimizes the influence of diurnal variations. Reducing stress during sample collection is essential to obtain accurate baseline cortisol concentration. Gentle handling and restraint techniques are employed to prevent the elevation of cortisol concentration due to stress during the procedure.

It is worth noting that while baseline cortisol measurement offers a snapshot of cortisol concentration at a specific moment, other dynamic tests, such as the ACTH-stimulation test or dexamethasone-suppression test, may be required for a more comprehensive evaluation of the horse's endocrine function and stress response.

The ACTH-stimulation test evaluates the horse's ability to respond to stress by administering synthetic ACTH and measuring the subsequent rise in cortisol concentration [4]. This test is valuable for diagnosing conditions such as ECD [36,96]. ACTH-stimulation test can be performed using synthetic ACTH1-24 (Synacthen tetracosactidum) [88–90]. While sometimes a high dosage is used, 1 µg/kg BW, it was found, that in horses maximal increase in secretion rate occurs after administration of only 0.1 µg/kg ACTH. Increased dosage only prolongs cortisol secretion, while its levels remain stable [88,90]. Thus, slightly different time points for sampling are recommended, with 12 h in 1 µg/kg, and in around 9 h in 0.1 µg/kg. Peeters et al. (2011) found changes in cortisol concentration from 189 ± 52 to 357 ± 55 nmol/L after ACTH stimulation [39]. Due to diurnal variations in cortisol secretion, tests should be performed in the afternoon hours, as the concentration variability decreases [89,90]. Low-dose stimulation test, with the administration of 0.01–0.02 µg/kg, is also described as a useful tool in accurately diagnosing RAI/CIRCI, as in some patients adrenal glands fail to secrete an adequate amount of cortisol with higher dosage [22]. Studies evaluating ACTH stimulation test are presented in Table 3.

Table 3. Studies reporting blood and salivary cortisol concentrations in horses after ACTH stimulation.

ACTH Dose and Route of Administration	Timeline	Changes in Concentrations Over Time	Reference
IV, 1 µg/kg BW of ACTH (Synacthen tetracosactidum 0.25 mg/mL equivalent to 25 IU/mL)	Tests performed from 1 p.m. to 4 p.m. Saliva samples were collected before, and 30, 60, 90, 120, 150, and 180 min after ACTH administtration	Salivary baseline 0.83–6.34 nmol/L (2.2 ± 0.83 nmol/L) significant increase after 30 min the peak reached after 122 ± 22 min mean percentage increase: 1620% ± 760	[4]
IV, bolus injection of 250 µg ACTH per horse	Net plasma cortisol measurement every 10 min before administration, starting from 60 min prior; control after 30 min from administration	Plasma baseline: not reported cortisol net increases above 275.86 nmol/L plasma were observed in six out of seven horses. mean percentage increase: not calculable %	[33]
IV, 1 µg/kg BW of ACTH (Synacthen tetracosactidum 0.25 mg/mL equivalent to 25 IU/mL)	Blood collection directly before ACTH administration, and 60 min after	Plasma baseline: 138 ± 54 nmol/L mean percentage increase 60 min after: 196%	[36]
IV, 1 µg/kg BW of ACTH (Synacthen tetracosactidum 0.25 mg/mL)	Collection 30 and 15 min before ACTH injection and the following at 10, 20, 30, 40, 60, 80, 100,120, 140, 160, 180, 200, 220, 240, 280, 320, 260, 400 and 500 min after ACTH injection	Serum baselline: 125–224 nmol/L (148.14 ± 42.69 nmol/L) Significant increase after 10 min The peak reached after 96 ± 16.7 min Return to baseline after 280 min 400 min after ACTH administration concentration dropped to 62.39 ± 16.14 nmol/L Mean percentage increase in serum: 225% Salivary baseline: 0.46–3.14 nmol/L (1.23 ± 1.08 nmol/L) Significant increase after 30 and 40 min (20–30 min later than in serum) The peak reached after 124 ± 8.9 min Return to baseline after 180 min (100 min earlier than in serum) Mean percentage increase in saliva: 2150%	[39]

Table 3. *Cont.*

ACTH Dose and Route of Administration	Timeline	Changes in Concentrations Over Time	Reference
IV, 10 µg and 100 µg of ACTH (cosyntropin)	Collection before 10 µg ACTH injection, 30 min later, 90 min later followed by 100 µg ACTH injection, 30 min and 90 min after second ACTH injection, protocol performed at birth, 12–24 h, 36–48 h, 5–7 days	Plasma baseline at birth: 281.38 ± 63.45 nmol/L, 12–24 h: 99.31 ± 44.14 nmol/L, 36–48 h: 71.72 ± 27.59 nmol/L, 5–7 days: 55.17 ± 22.07 nmol/L Mean percentage increase in plasma after 10 µg of ACTH at birth: 140% ± 20, 12–24 h: 290% ± 100, 36–48 h: 280% ± 160%, 5–7 days: 180% ± 50, 30 min after injection Mean percentage increase in plasma after 100 µg of ACTH at birth: 160% ± 40, 12–24 h: 400% ± 170, 36–48 h: 450% ± 280%, 5–7 days: 320% ± 150, 90 min after injection	[97]
IV, 0.1 µg/kg BW of ACTH (cosyntropin 250 µg/mL)	Collection before ACTH injection, 30 min and 60 min after, performed at birth, 3, 5, 7, 10, 14, 21, 28, 42, 56, and 84 days	Average plasma baseline: 75.31 nmol/L Average 30 min after: 114.21 nmol/L Average 60 min after: 79.72 nmol/L Mean percentage increase in plasma at birth: 192.5% ± 63.1 Mean percentage increase in plasma 3–56 days old: 37.7% ± 9.4 Mean percentage increase in plasma 84 days old: 52.5% ± 18.7	[98]
IV, 0.02, 0.1, 0.25, and 0.5 µg/kg (cosyntropin)	Collection before ACTH injection, 30, 60, 90, 120, 180, and 240 min after	Plasma baseline 76 to 264 nmol/L (mean ± SD, 172.4 ± 44.8 nmol/L) Peak percentage increase after 0.02 µg/kg ACTH: 150% 30 min after injection Peak percentage increase after 0.1 µg/kg ACTH: 190% 30 min after injection Peak percentage increase after 0.25 µg/kg ACTH: 200% 90 min after injection Mean percentage increase after 0.5 µg/kg ACTH: 230% 90 min after injection	[99]

The dexamethasone-suppression test is used to evaluate the decrease in cortisol levels, caused by suppression of the HPA axis by exogenous corticosteroids (dexamethasone) [91]. While it initially was considered as highly specific and sensitive, further studies proved it to be less reliable [100]. Dexamethasone is administered by intravenous or intramuscular injection. Dexamethasone under normal circumstances should suppress the release of ACTH from the pituitary gland and subsequently reduce cortisol production by the adrenal glands [44,92,93]. There are variations of the dexamethasone-suppression test, including the nighttime testing, which differ in the duration of dexamethasone delivery and the interval before cortisol testing. The administered intramuscular dose is around 0.04 mg/kg BW of dexamethasone [90]. Sojka et al. (1993) showed concentrations of cortisol before dexamethasone administration to be 155 ± 8.6 nmol/L, after 12 h it dropped to 1.9 ± 1.4 nmol/L, while after 24 h the concentration was 30.4 ± 16 nmol/L [94]. It is advised, that using dexamethasone suppression test sampling should be around 19 h after administration (up to 24 h), and levels below 27.6 nmol/L are considered normal [90,95]. These tests are typically designed as single-point observation tests, hence the dexamethasone-suppression test data may show the existence of ECD in healthy horses because cortisol production has a circadian rhythm in addition to inter-individual variability [101]. In horses with early ECD, results are reliable when sampling is performed 24 h after dexamethasone administration, as they have a limited capacity for proper reaction [95]. False positive results may be found in the fall when the production of ACTH is increased. However, individuals with cortisol below 5.5 nmol/L after dexamethasone administration in the fall can be assigned as healthy [95].

3.4. Hair Cortisol

Blood cortisol concentration can exhibit fluctuations when horses are subjected to either short- or long-term confinement [102]. Whereas, measuring cortisol concentration in hair is a potential technique for assessing long-term stress [103,104]. One may observe that analyzing hair cortisol concentration provides a more reliable assessment of long-term stress compared to saliva or serum measurements [105]. This is attributed to the belief that hair cortisol concentration serves as an indicator of cortisol secretion and the stress experienced over extended periods [106]. Moreover, horses with higher concentration of hair cortisol tend to exhibit lower compliance in training or work. This means that horses that have experienced higher levels of stress in the past may be less cooperative and more resistant to training. This finding carries significant practical implications for trainers and horse owners. Thus, hair cortisol concentration was proposed in clinical settings to assess stress levels in working horses and horses' ability to perform tasks and achieve success in various areas [107].

Cortisol in the blood circulation is gradually absorbed into growing hair, effectively creating a historical chronology of cortisol concentrations [108]. Extensive examination has been carried out on the advantages and drawbacks of different matrices used for determining glucocorticoid concentrations [108–110]. These matrices, including blood serum, saliva, urine, feces, hair, and claws or fingernails, exhibit variations in several characteristics. However, the most significant distinction lies in the time frame that the hormones recovered from them represent. Blood, saliva, urine, and feces primarily reflect recent circulating hormone concentration and are often influenced by daily rhythms or other unpredictable events. In contrast, slow-growing matrices such as hair, claws, or fingernails offer a reflection of glucocorticoid accumulation over extended time periods, providing a more comprehensive view of stress levels in horses [46,107]. Hair stands out as a favorable matrix due to its minimally invasive collection process, resilience under different storage conditions and exposures [46,111,112], and its capacity to provide an extended retrospective perspective on an individual's HPA axis activity [46,108,113]. Each cycle of hair development is comprised of three distinct phases: active growth (anagen), transition (catagen), and rest (telogen) [114]. It is during the anagen phase that systemic cortisol can passively diffuse from blood vessels into the developing hair shaft [105,109]. Additionally, hair cortisol concentration may detect temporal variations in circulating cortisol concentration in longer hairs where cortisol can be measured in several segments [115–118]. Hair is a stable substrate for measuring hormones and allows for storage for extended periods, which are additional benefits of using hair to monitor cortisol concentration [115,119,120]. In studies evaluating cortisol concentration, hair sampling was performed by shaving the neck or abdomen region, tail hair, and mane hair with bulbs or cut with scissors [45–49,104]. Cortisol in hair can be measured using a variety of techniques, including RIA [48], ELISA [45,47] or EIA [46,49], LC-HRMS/MS [45], and ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLCMS/MS) method [44]. Recent studies have utilized hair cortisol as a measure of the health and stress levels of horses, making it a valuable tool in equine welfare research (Table 4).

3.5. Fecal Cortisol

Since the positive correlation between the plasma cortisol and fecal cortisol metabolite concentrations was noted [8], fecal measurements were used for the identification of past potentially stressful situations [121]. It should be noted that after secretion, circulating steroids reach the liver, where they undergo catabolic changes. Then, metabolites are excreted with bile and reach the gastrointestinal tract. Further changes are caused by bacterial enzymes, which may also act outside of the gastrointestinal tract, influencing the result depending on the time of sampling [121,122]. Samples can be obtained from the rectum, or within a short time after defecation, from bedding or pasture [8,38,50–53]. Feces samples, if they cannot be tested straightaway, should be frozen in order to inhibit changes in the concentration of metabolites for reliability, as in some cases bacterial activity

increases metabolite concentration, while sometimes the concentration decreases, as further metabolic processes also take place [50,122,123]. Thus, a separate method, focused on metabolites, must be used instead of evaluating the intact cortisol [51]. In most cases, 11,17-dioxoandrostanes (11,17-DOA) levels are measured with enzyme immunoassay, although antibody selection is influential on the quality of results.

Table 4. Studies reporting hair cortisol concentrations in horses.

Objectives	Demographic Data	Time of Day and Method	Results	Reference
Determination of hair cortisol concentration in horses	47 horses (9 geldings, 38 mares) age not reported	Time not reported Mane hair with bulbs from mid-neck region ELISA and LC-HRMS/MS methods	Hair cortisol concentration varied from 2.0 to 17.9 pg/mg by ELISA and 1.3 to 8.8 pg/mg by LC-HRMS/MS	[45]
Determination of hair cortisol concentrations in feral horses	282 horses (135 stallions, 113 mares) known-age up to 6 years old and a unknown-age	Time not reported Hair collected from tail by hair snags EIA method	Hair cortisol concentration varied from 2.17 pg/mg in stallions to 1.64 pg/mg in mares	[46]
Monitoring of hair cortisol concentrations during a one-year time-lapse	47 horses (26 geldings, 21 mares) 6 to 20 years old	Time not reported Hair collected from neck region by shaving ELISA method	Hair cortisol concentration varied from 290 pg/mg in feale to 230 pg/mg in gelding male	[47]
Verification of the reliability of a hair cortisol assay. Measuring of hair cortisol at birth and at 30 and 60 days old	204 horses (102 foals, 102 mares) birth day to 17 years old	Time not reported hair collected from withers by shaving RIA method	Hair cortisol concentration varied from 18.02 to 156.51 pg/mg at birth to 40.56 pg/mg in the first quartile to 62.88 pg/mg in the third quartile	[48]
Determination of the effect of a relocation period and the multiple factors associated with a rest period on hair cortisol concentrations in horses	13 horses (13 stallions) 5 to 13 years old	Time not reported hair collected from ventral abdomen area by shaving EIA method	Hair cortisol concentration varied from 5.34 pg/mg in control group to 3.09 pg/mg in relocated horses	[49]
Determination of the mane hair and body hair cortisol concentration in horses	153 horses 3 to 28 years old	Time not reported Mane hair cut with scissors, hair from above the scapula obtained with clippers from the left side UHPLCMS/MS method	Hair cortisol concentration varied from 6.0 pg/mg in the mane to 6.1 pg/mg in the body hair	[104]

Cortisol in feces can be measured using an EIA method [8,17,38,51,52]. The 3α,11-oxo-A EIA method seems to be more valuable than Extr-DOA EIA, as it cross-reacts with some other metabolites, is more stable at room temperature, and ACTH stimulation showed the higher amplitude of metabolite concentration between baseline value and following ACTH administration. The throughput of this method is also higher than in previously developed methods [8]. This method has been validated and can be effectively used to evaluate the concentration of fecal metabolites of cortisol [124].

Fecal measurements of cortisol metabolites are informative regarding the situation starting from 24 h from sampling, up to 48, or even 72 h [51,53,121]. Merl et al. (2000) found the peak of concentration occurring 36 h after a stressful event (castration) [122]. It is the time necessary for cortisol release, circulation, catabolism, excretion, and passage through the gastrointestinal tract. Thus, in the identification of potentially stressful situations,

samples can be collected the day after their occurrence, giving an opportunity to evaluate stress post factum [121]. While blood and saliva testing provides data about rapid reaction and instant cortisol increase, feces concentration changes are rather delayed. Another important issue regarding fecal metabolites is that they are less variable, as they are a consequence of the overall stress reaction in a unit of time, rather than ongoing fluctuations of concentration, as it smoothens the pulsatility of cortisol secretion [43,53,123]. Recent studies have utilized fecal cortisol as an important animal welfare indicator considering animal keeping and day-long stress (Table 5).

Table 5. Studies reporting fecal cortisol concentrations in horses.

Objectives	Demographic Data	Time of Day and Method	Results	Reference
Correlates of oral and motor stereotypic behaviors and glucocorticoid concentration	55 horses (41 geldings, 14 mares) 5 to 20 years old	Feces sampling between 12:00 and 13:00, from bedding, directly after defecation EIA method	Fecal cortisol metabolite concentration varied from 2.4 to 37.6 ng/g	[8]
Evaluation of the influence of reproductive status and reproductive procedures on cortisol secretion	50 horses (50 mares) 6 to 16 years old	Feces sampling three times a day at 0:00, 8:00, 16:00 sampling method not reported EIA methods	Fecal 3α,11-oxo-A concentration varied from 3.8 ± 0.6 ng/g in teaching mares to 12.7 ± 4.0 ng/g in maiden mares	[17]
Relation between compromised welfare, such as chronic pain and hematological anomalies, and cortisol concentration in domestic horses	49 horses (44 geldings, 15 mares) 5 to 20 years old	Feces sampling between 12:00 and 13:00, from bedding, directly after defecation EIA method	Fecal cortisol metabolite concentration varied from 5.0 ± 0.3 ng/g in the working day, 4.9 ± 0.4 ng/g in the day of rest.	[38]
Evaluation of the influence of insects on stress reaction	39 horses (8 geldings, 1 stallion, 30 mares) 1 to 21 years old	Feces sampling between 12:00 and 14:00 or between 14:00 and 16:00, from pasture, immediately after defecation EIA method	Fecal 3α,11-oxo-A concentration varied from 38.1 ± 2.8 ng/g to 45.6 ± 2.7 ng/g	[50]
Validation of method for glucocorticoid metabolites measurement in feces	10 horses (5 stallions, 5 mares) 3 to 14 years old	In the morning (hour not reported) and in the evening (hour not reported), feces removed from the rectum EIA method	Fecal 3α,11-oxo-A concentration baseline was 49.91 ± 21.13 ng/g fecal Extr-DOA baseline was 3.48 ± 1.65 ng/g	[51]
Evaluation of exercise and psychological endocrine responses to a new environment and initial training	40 horses (12 geldings, 9 stallions, 19 mares) 2 years old	Fecal sampling twice a day in the morning (between 07:00 and 09:00) and in the evening (between 16:00 and 19:00), from bedding, within 30 min after defecation EIA method	Fecal 11-oxoetiocholanolone concentration varied from 1.3 and 20.1 ng/g	[52]
Quantification of the stress response of transrectal palpation	36 horses (36 mares) 5 to 14 years old	Time not reported Feces removed from the rectum EIA method	Fecal 3α,11-oxo-A concentration was 25.5 ng/g with ranges 16.3–121.4 ng/g	[53]

3.6. Other Cortisol Matrices

In addition to the methods mentioned above, cortisol can also be obtained from various other matrices such as urine, tears, or hooves. Urine cortisol concentration provides insights

into the horse's stress response over a slightly longer duration than blood cortisol, making it valuable for assessing stress associated with specific situations [125], whereas cortisol concentration in tears was reported to rise concurrently with blood cortisol after ACTH stimulation [71]. These studies may often involve a limited number of animals, but the results are frequently reported in the context of the coping theory, focusing on stereotypical behavior as a potential sign of poor welfare. Further research is required to identify whether endogenous cortisol in tears plays a role in ocular disease [71]. Interestingly, it is thought that cortisol is not fully recognized as a primary biomarker for stress in horses [21]. In general, more markers should be evaluated, also to assess a range of stressful stimulus, such as glucose and catecholamines. Such an approach would allow to evaluate the physiological effect of stress reaction. Generally, in stress evaluation more than one stress marker should be measured, as adrenal glands can be stimulated with exercise and excitement, not only with stress reactions [121].

4. Conclusions

Cortisol plays a pivotal role in assessing the welfare and health of horses, as it is closely tied to their physiological responses to stress in various conditions. The extensive body of research demonstrates the strong correlations between cortisol levels and physiological stress indicators, offering valuable insights into the horse's overall state, exercise effort, and health. While cortisol is crucial for adaptation and survival, chronically elevated concentration can have detrimental effects, making it a critical indicator of specific diseases. Additionally, studying cortisol concentration in different matrices such as blood, saliva, and hair provides versatile tools for monitoring acute and long-term stress, offering non-invasive and retrospective perspectives on equine health and welfare.

Author Contributions: Conceptualization, U.S. and M.M.; writing—original draft preparation, U.S., M.M., A.C., Ł.Z. and M.D.; writing—review and editing U.S., M.M., A.C., Ł.Z. and M.D.; supervision, M.M.; project administration, M.M.; funding acquisition, M.M. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Centre for Research and Development as part of the POIR 2014–2020 project number POIR.01.01.01-00-1001/20.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Hovey, M.R.; Davis, A.; Chen, S.; Godwin, P.; Porr, S.C.A. Evaluating Stress in Riding Horses: Part One-Behavior Assessment and Serum Cortisol. *J. Equine Vet. Sci.* **2021**, *96*, 103297. [CrossRef] [PubMed]
2. Hausberger, M.; Roche, H.; Henry, S.; Visser, E.K. A review of the human-horse relationship. *Appl. Anim. Behav. Sci.* **2008**, *109*, 1–24. [CrossRef]
3. Iqbal, T.; Elahi, A.; Wijns, W.; Shahzad, A. Cortisol detection methods for stress monitoring in connected health. *Health Sci. Rev.* **2023**, *6*, 100079. [CrossRef]
4. Scheidegger, M.D.; Gerber, V.; Ramseyer, A.; Schüpbach-Regula, G.; Bruckmaier, R.M.; van der Kolk, J.H. Repeatability of the ACTH stimulation test as reflected by salivary cortisol response in healthy horses. *Domest. Anim. Endocrinol.* **2016**, *57*, 43–47. [CrossRef] [PubMed]
5. Sauer, F.J.; Hermann, M.; Ramseyer, A.; Burger, D.; Riemer, S.; Gerber, V. Effects of breed, management and personality on cortisol reactivity in sport horses. *PLoS ONE* **2019**, *14*, 0221794. [CrossRef] [PubMed]
6. Lundblad, J.; Rashid, M.; Rhodin, M.; Haubro Andersen, P. Effect of transportation and social isolation on facial expressions of healthy horses. *PLoS ONE* **2021**, *16*, e0241532. [CrossRef]
7. Kang, H.; Zsoldos, R.R.; Sole-Guitart, A.; Narayan, E.; Cawdell-Smith, A.J.; Gaughan, J.B. Heat stress in horses: A literature review. *Int. J. Biometeorol.* **2023**, *67*, 957–973. [CrossRef] [PubMed]
8. Fureix, C.; Benhajali, H.; Henry, S.; Bruchet, A.; Prunier, A.; Ezzaouia, M.; Coste, C.; Hausberger, M.; Palme, R.; Jego, P. Plasma cortisol and faecal cortisol metabolites concentrations in stereotypic and non-stereotypic horses: Do stereotypic horses cope better with poor environmental conditions? *BMC Vet. Res.* **2013**, *9*, 3.

9. Raspa, F.; Tarantola, M.; Muca, E.; Bergero, D.; Soglia, D.; Cavallini, D.; Vervuert, I.; Bordin, C.; De Palo, P.; Valle, E. Does feeding management make a difference to behavioural activities and welfare of horses reared for meat production? *Animals* **2022**, *12*, 1740. [CrossRef]
10. Raspa, F.; Tarantola, M.; Bergero, D.; Bellino, C.; Mastrazzo, C.M.; Visconti, A.; Valvassori, E.; Vervuert, I.; Valle, E. Stocking density affects welfare indicators in horses reared for meat production. *Animals* **2020**, *10*, 1103. [CrossRef]
11. Raspa, F.; Tarantola, M.; Bergero, D.; Nery, J.; Visconti, A.; Mastrazzo, C.M.; Cavallini, D.; Valvassori, E.; Valle, E. Time-budget of horses reared for meat production: Influence of stocking density on behavioural activities and subsequent welfare. *Animals* **2020**, *10*, 1334. [CrossRef] [PubMed]
12. Raspa, F.; Dinardo, F.R.; Vervuert, I.; Bergero, D.; Bottero, M.T.; Pattono, D.; Dalmasso, A.; Vinassa, M.; Valvassori, E.; Bruno, E.; et al. A Fibre-vs. cereal grain-based diet: Which is better for horse welfare? Effects on intestinal permeability, muscle characteristics and oxidative status in horses reared for meat production. *J. Anim. Physiol. Anim. Nutr.* **2022**, *106*, 313–326. [CrossRef] [PubMed]
13. Kędzierski, W.; Strzelec, K.; Cywińska, A.; Kowalik, S. Salivary Cortisol Concentration in Exercised Thoroughbred Horses. *J. Equine Vet. Sci.* **2013**, *33*, 1106–1109. [CrossRef]
14. Schmidt, A.; Hodl, S.; Möstl, E.; Aurich, J.; Muller, J.; Aurich, C. Cortisol release, heart rate, and heart rate variability in transport-naïve horses during repeated road transport. *Domest. Anim. Endocrinol.* **2010**, *39*, 205–213. [CrossRef] [PubMed]
15. Schmidt, A.; Möstl, E.; Wehnert, C.; Aurich, J.; Muller, J.; Aurich, C. Cortisol release and heart rate variability in horses during road transport. *Horm. Behav.* **2010**, *57*, 209–215. [CrossRef] [PubMed]
16. Schmidt, A.; Aurich, J.; Möstl, E.; Muller, J.; Aurich, C. Changes in cortisol release and heart rate and heart rate variability during the initial training of 3-year-old sport horses. *Horm. Behav.* **2010**, *58*, 628–636. [CrossRef]
17. Berghold, P.; Möstl, E.; Aurich, C. Effects of reproductive status and management on cortisol secretion and fertility of oestrous horse mares. *Anim. Reprod. Sci.* **2007**, *102*, 276–285. [CrossRef]
18. Turner, L.C. *Assessment of Horses for Therapeutic Riding Purposes: Comparison of Physiological and Behavioral Parameters*; Honors College, Western Kentucky University: West Lafayette, IN, USA, 2014.
19. Alexander, S.; Irvine, C.H.G. Stress in the racing horse: Coping vs. *not coping*. *J. Equine Sci.* **1998**, *9*, 77–81. [CrossRef]
20. Anderson, M.K.; Friend, T.H.; Evans, J.W.; Bushong, D.M. Behavioral assessment of horses in therapeutic riding programs. *Appl. Anim. Behav. Sci.* **1999**, *63*, 11–24. [CrossRef]
21. Kovac, M.; Vladimirovna Ippolitova, T.; Pozyabin, S.; Aliev, R.; Lobanova, V.; Drakul, N.; Rutland, C.S. *Equine Stress: Neuroendocrine Physiology and Pathophysiology*; IntechOpen: London, UK, 2022.
22. Hart, K.A.; Barton, M.H. Adrenocortical insufficiency in horses and foals. *Vet. Clin. N. Am. Equine Pract.* **2011**, *27*, 19–34. [CrossRef]
23. Kaushik, J.A.; Vasudev, A.; Arya, S.K.; Pasha, S.K.; Bhansali, S. Recent advances in cortisol sensing technologies for point-of-care application. *Biosens. Bioelectron.* **2014**, *53*, 499–512. [CrossRef]
24. Henshall, C.; Randle, H.; Francis, N.; Francis, N. The effect of stress and exercise on the learning performance of horses. *Sci. Rep.* **2022**, *12*, 1918. [CrossRef]
25. Keating, D.L.; Leshman, J.S.; Burk, S.V. Salivary Cortisol, Equine Characteristics, and Management Factors Associated with Strongyle-Type Egg Shedding of Ohio Horses. *J. Equine Vet. Sci.* **2021**, *101*, 103431. [CrossRef]
26. Smith, S.M.; Vale, W.W. The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues Clin. Neurosci.* **2006**, *8*, 383–395. [CrossRef]
27. National Center for Biotechnology Information. PubChem Compound Summary for CID 172464, 1,2,6,7-(3H)Cortisol. Available online: https://pubchem.ncbi.nlm.nih.gov/compound/1_2_6_7-_3H_Cortisol (accessed on 30 September 2023).
28. Morgan, R.A.; Keen, J.A.; Homer, N.; Nixon, M.; McKinnon-Garvin, A.M.; Moses-Williams, J.A.; Davis, S.R.; Hadoke, P.W.F.; Walker, B.R. Dysregulation of Cortisol Metabolism in Equine Pituitary Pars Intermedia Dysfunction. *Endocrinology* **2018**, *159*, 3791–3800. [CrossRef]
29. Lee, H.; Hwang, Y.; Cheon, K.A.; Jung, H.I. Emotion-on-a-chip (EOC): Evolution of biochip technology to measure human emotion using body fluids. *Med. Hypotheses* **2012**, *79*, 827–832. [CrossRef]
30. Debono, M.; Ghobadi, C.; Rostami-Hodjegan, A.; Huatan, H.; Campbell, M.J.; Newell-Price, J.; Darzy, P.; Merke, D.P.; Arlt, W.; Ross, R.J. Modified-release hydrocortisone to provide circadian cortisol profiles. *J. Clin. Endocrinol. Metab.* **2009**, *94*, 1548–1554. [CrossRef]
31. Irvine, C.H.G.; Alexander, S.L. Factors affecting the circadian rhythm in plasma cortisol concentrations in the horse. *Domest. Anim. Endocrinol* **1994**, *11*, 227–238. [CrossRef]
32. Kang, O.D.; Lee, W.S. Changes in Salivary Cortisol Concentration in Horses during Different Types of Exercise. *Asian-Australas. J. Anim. Sci.* **2016**, *29*, 747–752. [CrossRef]
33. Marc, M.; Parvizi, N.; Ellendorff, F.; Kallweit, E.; Elsaesser, F. Plasma cortisol and ACTH concentrations in the warmblood horse in response to a standardized treadmill exercise test as physiological markers for evaluation of training status. *J. Anim. Sci.* **2000**, *78*, 1936–1946. [CrossRef]
34. Ireland, J.; McGowan, C. Epidemiology of pituitary pars intermedia dysfunction: A systematic literature review of clinical presentation, disease prevalence and risk factors. *Vet. J.* **2018**, *235*, 22–33. [CrossRef]

35. Haffner, J.; Fecteau, K.; Eiler, H.; Tserendorj, T.; Hoffman, R.; Oliver, J. Blood steroid concentrations in domestic Mongolian horses. *J. Vet. Diagn. Investig.* **2010**, *22*, 537–543. [CrossRef]
36. Kirchmeier, A.; van Herwaarden, A.E.; van der Kolk, J.H.; Sauer, F.J.; Gerber, V. Plasma steroid profiles before and after ACTH stimulation test in healthy horses. *Domest. Anim. Endocrinol.* **2019**, *72*, 106419. [CrossRef]
37. Van Der Kolk, J.H.; Nachreiner, R.F.; Scott, H.C.; Refsal, K.R.; Zanella, K.J. Salivary and plasma concentration of cortisol in normal horses and horses with Cushing's disease. *Equine Vet. J.* **2001**, *33*, 211–213. [CrossRef]
38. Pawluski, J.; Jego, P.; Henry, S.; Bruchet, A.; Palme, R.; Coste, C.; Hausberger, M. Low plasma cortisol and fecal cortisol metabolite measures as indicators of compromised welfare in domestic horses (*Equus caballus*). *PLoS ONE* **2017**, *12*, e0182257. [CrossRef]
39. Peeters, M.; Sulon, J.; Beckers, J.F.; Ledoux, D.; Vandenheede, M. Comparison between blood serum and salivary cortisol concentrations in horses using an adrenocorticotropic hormone challenge. *Equine Vet. J.* **2011**, *43*, 487–493. [CrossRef]
40. Peeters, M.; Closson, C.; Beckers, J.F.; Vandenheede, M. Rider and Horse Salivary Cortisol Levels during Competition and Impact on Performance. *J. Equine Vet. Sci.* **2013**, *33*, 155–160. [CrossRef]
41. Massányi, M.; Halo, M.; Mlyneková, E.; Kováčiková, E.; Tokárová, K.; Greń, A.; Massányi, P.; Halo, M. The effect of training load stress on salivary cortisol concentrations, health parameters and hematological parameters in horses. *Heliyon* **2023**, *9*, 19037. [CrossRef]
42. Olvera-Maneu, S.; Carbajal, A.; Serres-Corral, P.; López-Béjar, M. Cortisol Variations to Estimate the Physiological Stress Response in Horses at a Traditional Equestrian Event. *Animals* **2023**, *13*, 396. [CrossRef]
43. Hughes, T.; Creighton, E.; Coleman, R. Salivary and fecal cortisol as measures of stress in horses. *J. Vet. Behav.* **2010**, *5*, 59–60. [CrossRef]
44. Held, F.; Ekstrand, C.; Cvijovic, M.; Gabrielsson, J.; Jirstrand, M. Modelling of oscillatory cortisol response in horses using a Bayesian population approach for evaluation of dexamethasone suppression test protocols. *J. Pharmacokinet. Pharmacodyn.* **2019**, *46*, 75–87. [CrossRef]
45. Saluti, G.; Ricci, M.; Castellani, F.; Colagrande, M.N.; Di Bari, G.; Vulpiani, M.P.; Cerasoli, F.; Savini, G.; Scortichini, G.; D'Alterio, N. Determination of hair cortisol in horses: Comparison of immunoassay vs. LC-HRMS/MS. *Anal. Bioanal. Chem.* **2022**, *414*, 8093–8105. [CrossRef]
46. Medill, S.A.; Janz, D.M.; McLoughlin, P.D. Hair Cortisol Concentrations in Feral Horses and the Influence of Physiological and Social Factors. *Animals* **2023**, *13*, 2133. [CrossRef]
47. Mazzola, S.M.; Colombani, C.; Pizzamiglio, G.; Cannas, S.; Palestrini, C.; Costa, E.D.; Gazzonis, A.L.; Bionda, A.; Crepaldi, P. Do You Think I Am Living Well? A Four-Season Hair Cortisol Analysis on Leisure Horses in Different Housing and Management Conditions. *Animals* **2021**, *11*, 2141. [CrossRef]
48. Comin, A.; Veronesi, M.C.; Montillo, M.; Faustini, M.; Valentini, S.; Cairoli, F.; Prandi, A. Hair cortisol level as a retrospective marker of hypothalamic-pituitary-adrenal axis activity in horse foals. *Vet. J.* **2012**, *194*, 131–132. [CrossRef]
49. Gardela, J.; Carbajal, A.; Tallo-Parra, O.; Olvera-Maneu, S.; Álvarez-Rodríguez, M.; Jose-Cunilleras, E.; López-Béjar, M. Temporary Relocation during Rest Periods: Relocation Stress and Other Factors Influence Hair Cortisol Concentrations in Horses. *Animals* **2020**, *10*, 642. [CrossRef]
50. Christensen, J.W.; Strøm, C.G.; Nicová, K.; de Gaillard, C.L.; Sandøe, P.; Skovgård, H. Insect-Repelling Behaviour in Horses in Relation to Insect Prevalence and Access to Shelters. *Appl. Anim. Behav. Sci.* **2022**, *247*, 105560. [CrossRef]
51. Flauger, B.; Krueger, K.; Gerhards, H.; Möstl, E. Simplified Method to Measure Glucocorticoid Metabolites in Feces of Horses. *Vet. Res. Commun.* **2010**, *34*, 185–195. [CrossRef]
52. Gorgasser, I.; Tichy, A.; Palme, R. Fecal Cortisol Metabolites in Quarter Horses during Initial Training under Field Conditions. *Wien. Tierarztl. Monatsschrift* **2007**, *94*, 226.
53. van Vollenhoven, E.; Grant, C.C.; Fletcher, L.; Schulman, M.L.; Page, P.C.; Ganswindt, A. Salivary Glucocorticoid and Fecal Glucocorticoid Metabolite Concentrations in Pony Mares during Transrectal Palpation of the Reproductive Tract by Veterinary Students. *J. Equine Vet. Sci.* **2018**, *70*, 7–12. [CrossRef]
54. Möstl, E.; Palme, R. Hormones as indicators of stress. *Domest. Anim. Endocrinol.* **2002**, *23*, 67–74. [CrossRef] [PubMed]
55. Janczarek, I.; Bereznowski, A.; Strzelec, K. The influence of selected factors and sport results of endurance horses on their saliva cortisol concentration. *Pol. J. Vet. Sci.* **2013**, *16*, 533–541. [CrossRef] [PubMed]
56. Casella, S.; Vazzana, I.; Giudice, E.; Fazio, F.; Piccione, G. Relationship between serum cortisol levels and some physiological parameters following reining training session in horse. *Anim. Sci. J.* **2016**, *87*, 729–735. [CrossRef] [PubMed]
57. Yarnell, K.; Hall, C.; Billett, E. An assessment of the aversive nature of an animal management procedure (clipping) using behavioral and physiological measures. *Physiol. Behav.* **2013**, *118*, 32–39. [CrossRef]
58. Arfuso, F.; Acri, G.; Piccione, G.; Sansotta, C.; Fazio, F.; Giudice, E.; Giannetto, C. Eye surface infrared thermography usefulness as a noninvasive method of measuring stress response in sheep during shearing: Correlations with serum cortisol and rectal temperature values. *Physiol. Behav.* **2022**, *250*, 113781. [CrossRef]
59. Becker-Birck, M.; Schmidt, A.; Lasarzik, J.; Aurich, J.; Möstl, E.; Aurich, C. Cortisol release and heart rate variability in sport horses participating in equestrian competitions. *J. Vet. Behav.* **2013**, *8*, 87–94. [CrossRef]
60. Nagy, K.; Bodó, G.; Bárdos, G.; Bánszky, N.; Kabai, P. Differences in temperament traits between crib-biting and control horses. *Appl. Anim. Behav. Sci.* **2010**, *122*, 41–47. [CrossRef]

61. Pell, S.M.; McGreevy, P.D. A study of cortisol and beta-endorphin levels in stereotypic and normal Thoroughbreds. *Appl. Anim. Behav. Sci.* **1999**, *64*, 81–90. [CrossRef]
62. Hemmann, K.; Raekallio, M.; Kanerva, K.; Hänninen, L.; Pastell, M.; Palviainen, M.; Vainio, O. Circadian variation in ghrelin and certain stress hormones in crib-biting horses. *Vet. J.* **2012**, *193*, 97–102. [CrossRef]
63. Schott, H.C. Pituitary pars intermedia dysfunction: Equine Cushing's disease. *Vet. Clin. N. Am. Equine Pract.* **2002**, *18*, 237–270. [CrossRef] [PubMed]
64. Kirkwood, N.C.; Hughes, K.J.; Stewart, A.J. Pituitary Pars Intermedia Dysfunction (PPID) in Horses. *Vet. Sci.* **2022**, *9*, 556. [CrossRef] [PubMed]
65. Marik, P.E. Mechanisms and clinical consequences of critical illness associated adrenal insufficiency. *Curr. Opin. Crit. Care* **2007**, *13*, 363–369. [CrossRef] [PubMed]
66. Fernandez, E.F.; Watterberg, K.L. Relative adrenal insufficiency in the preterm and term infant. *J. Perinatol.* **2009**, *29*, 44–49. [CrossRef] [PubMed]
67. Hart, K.; Barton, M.; Vandenplas, M.; Hurlay, D.J. Effects of Low-Dose Hydrocortisone Therapy on Immune Function in Neonatal Horses. *Pediatr. Res.* **2011**, *70*, 72–77. [CrossRef] [PubMed]
68. Durham, A.; McGowan, C.; Fey, K.; Tamzali, Y.; Van der Kolk, J. Pituitary pars intermedia dysfunction: Diagnosis and treatment. *Equine Vet. Educ.* **2014**, *26*, 216–223. [CrossRef]
69. Horn, R.; Stewart, A.J.; Jackson, K.V.; Dryburgh, E.L.; Medina-Torres, C.E.; Bertin, F.R. Clinical implications of using adrenocorticotropic hormone diagnostic cutoffs or reference intervals to diagnose pituitary pars intermedia dysfunction in mature horses. *J. Vet. Intern. Med.* **2021**, *35*, 560–570. [CrossRef]
70. McGowan, T.W.; Pinchbeck, G.P.; McGowan, C.M. Prevalence, risk factors and clinical signs predictive for equine pituitary pars intermedia dysfunction in aged horses. *Equine Vet. J.* **2013**, *45*, 74–79. [CrossRef]
71. Ambrojo, K.S.; Corzano, M.M.; Poggi, J.C.G. *Action Mechanisms and Pathophysiological Characteristics of Cortisol in Horses*; IntechOpen: London, UK, 2018.
72. Breuhaus, B.A. Thyroid Hormone and Thyrotropin Concentrations and Responses to Thyrotropin-Stimulating Hormone in Horses with PPID Compared with Age-Matched Normal Horses. *J. Equine Vet. Sci.* **2019**, *75*, 35–40. [CrossRef]
73. Weitzman, E.D.; Fukushima, D.; Nogeire, C.; Roffwarg, G.; Gallagher, T.F.; Hellman, L. Twenty-four hour pattern of the episodic secretion of cortisol in normal subjects. *Endocrinology* **1971**, *33*, 14–22. [CrossRef]
74. Pryczek, T.; Stefaniak, T. Wykorzystanie oznaczania kortyzolu i jego pochodnych w ocenie stresu u psów służbowych. *Życie Weter.* **2013**, *88*, 136–141.
75. Smith, C.J.; Norman, R.L. Influence of the gonads on cortisol secretion in female rhesus macaques. *Endocrinology* **1987**, *121*, 2192–2198. [CrossRef] [PubMed]
76. Cordero, M.; Brorsen, B.W.; McFarlane, D. Circadian and circannual rhythms of cortisol, ACTH, and α-melanocyte-stimulating hormone in healthy horses. *Domest. Anim. Endocrinol.* **2012**, *43*, 317–324. [CrossRef] [PubMed]
77. Murphy, B.A. Circadian and Circannual Regulation in the Horse: Internal Timing in an Elite Athlete. *J. Equine Vet. Sci.* **2019**, *76*, 14–24. [CrossRef] [PubMed]
78. Palme, R. Monitoring stress hormone metabolites as a useful, non-invasive tool for welfare assessment in farm animals. *Anim. Welf.* **2012**, *21*, 331–337. [CrossRef]
79. Mormede, P.; Andanson, S.; Auperin, B.; Beerda, B.; Guemene, D.; Malnikvist, J.; Manteca, X.; Manteuffel, G.; Prunet, P.; van Reenen, C.G.; et al. Exploration of the hypothalamic-pituitary-adrenal function as a tool to evaluate animal welfare. *Physiol. Behav.* **2007**, *92*, 317–339. [CrossRef]
80. Alberghina, D.; Statelli, A.; Monteverde, V.; Vazzana, I.; Cascone, G.; Panzera, M. Serum Cortisol and Its Correlation with Leucocyte Profile and Circulating Lipids in Donkeys (*Equus asinus*). *Animals* **2022**, *12*, 841. [CrossRef]
81. Burden, F.A.; Hazell-Smith, E.; Mulugeta, G.; Patrick, V.; Trawford, R.; Brownlie, H.W.B. Reference intervals for biochemical and haematological parameters in mature domestic donkeys (*Equus asinus*) in the UK. *Equine Vet. Educ.* **2016**, *28*, 134–139. [CrossRef]
82. Sheriff, M.J.; Dantzer, B.; Delehanty, B.; Palme, R.; Boonstra, R. Measuring stress in wildlife: Techniques for quantifying glucocorticoids. *Oecologia* **2011**, *166*, 869–887. [CrossRef]
83. Waran, N.; Randle, H. What we can measure, we can manage: The importance of using robust welfare indicators in Equitation Science. *Appl. Anim. Behav. Sci.* **2017**, *190*, 74–81. [CrossRef]
84. Contreras-Aguilar, M.D.; Hevia, M.L.; Escribano, D.; Lamy, E.; Tecles, F.; Cerón, J.J. Effect of food contamination and collection material in the measurement of biomarkers in saliva of horses. *Res. Vet. Sci.* **2020**, *129*, 90–95. [CrossRef] [PubMed]
85. Strzelec, K.; Kankofer, M.; Pietrzak, S. Cortisol concentration in the saliva of horses subjected to different kinds of exercise. *Acta Vet. Brno* **2011**, *80*, 101–105. [CrossRef]
86. Moeller, B.A.; McCall, C.A.; Silverman, S.J.; McElhenney, W.E. Estimation of Saliva Production in Crib-Biting and Normal Horses. *J. Equine Vet. Sci.* **2008**, *28*, 85–90. [CrossRef]
87. Holst, J.P.; Soldin, S.J.; Tractenberg, R.E.; Guo, T.; Kundra, P.; Verbalis, J.G.; Jonklaas, J. Use of steroid profiles in determining the cause of adrenal insufficiency. *Steroids* **2007**, *72*, 71–84. [CrossRef] [PubMed]
88. Sauer, F.J.; Bruckmaier, R.M.; Ramseyer, A.; Vidondo, B.; Scheidegger, M.D.; Gerber, V. Diagnostic Accuracy of Post-ACTH Challenge Salivary Cortisol Concentrations for Identifying Horses with Equine Glandular Gastric Disease. *J. Anim. Sci.* **2018**, *96*, 2154–2161. [CrossRef] [PubMed]

89. Freymond, S.B.; Bardou, D.; Briefer, E.F.; Bruckmaier, R.; Fouché, N.; Fleury, J.; Maigrot, A.-L.; Ramseyer, A.; Zuberbühler, K.; Bachmann, I. The Physiological Consequences of Crib-Biting in Horses in Response to an ACTH Challenge Test. *Physiol. Behav.* **2015**, *151*, 121–128. [CrossRef] [PubMed]
90. van der Kolk, J.H.; Fouché, N.; Gross, J.J.; Gerber, V.; Bruckmaier, R.M. A Comparison between the Equine and Bovine Hypothalamus-Pituitary-Adrenocortical Axis. *Domest. Anim. Endocrinol.* **2016**, *56*, S101–S111. [CrossRef]
91. Dogra, P.; Vijayashankar, N.P. *Dexamethasone Suppression Test*; StatPearls Publishing: St. Petersburg, FL, USA, 2023.
92. Dybdal, N.O.; Hargreaves, K.M.; Madigan, J.E.; Gribble, D.H.; Kennedy, P.C.; Stabenfeldt, G.H. Diagnostic testing for pituitary pars intermedia dysfunction in horses. *J. Am. Vet. Med. Assoc.* **1994**, *204*, 627–632.
93. Frank, N.; Andrews, F.M.; Sommardahl, C.S.; Eiler, H.; Rohrbach, B.W.; Donnell, R.L. Evaluation of the combined dexamethasone suppression/thyrotropin-releasing hormone stimulation test for detection of pars intermedia pituitary adenomas in horses. *J. Vet. Intern. Med.* **2006**, *20*, 987–993.
94. Sojka, J.E.; Johnson, M.A.; Bottoms, G.D. The Effect of Starting Time on Dexamethasone Suppression Test Results in Horses. *Domest. Anim. Endocrinol.* **1993**, *10*, 1–5. [CrossRef]
95. McFarlane, D. Diagnostic Testing for Equine Endocrine Diseases. *Vet. Clin. N. Am. Equine Pract.* **2019**, *35*, 327–338. [CrossRef]
96. Sternberg, Z.; Grewal, P.; Cen, S.; DeBarge-Igoe, F.; Yu, J.; Arata, M. Blood pressure normalization post-jugular venous balloon angioplasty. *Phlebology* **2015**, *30*, 280–287. [CrossRef] [PubMed]
97. Hart, K.A.; Heusner, G.L.; Norton, N.A.; Barton, M.H. Hypothalamic-Pituitary-Adrenal Axis Assessment in Healthy Term Neonatal Foals Utilizing a Paired Low Dose/High Dose ACTH Stimulation Test. *J. Vet. Intern. Med.* **2009**, *23*, 344–351. [CrossRef] [PubMed]
98. Wong, D.M.; Vo, D.T.; Alcott, C.J.; Stewart, A.J.; Peterson, A.D.; Sponseller, B.A.; Hsu, W.H. Adrenocorticotropic Hormone Stimulation Tests in Healthy Foals from Birth to 12 Weeks of Age. *Can. J. Vet. Res.* **2009**, *73*, 65–72. [PubMed]
99. Stewart, A.J.; Behrend, E.N.; Wright, J.C.; Martin, L.G.; Kemppainen, R.J.; Busch, K.A.; Hanson, R.R. Validation of a Low-Dose ACTH Stimulation Test in Healthy Adult Horses. *J. Am. Vet. Med. Assoc.* **2011**, *239*, 834–841. [CrossRef] [PubMed]
100. Spelta, C. Equine Pituitary Pars Intermedia Dysfunction: Current Perspectives on Diagnosis and Management. *Vet. Med. Res. Rep.* **2015**, *2015*, 293–300. [CrossRef] [PubMed]
101. Ekstrand, C.; Ingvast-Larsson, C.; Olsen, L.; Hedeland, M.; Bondesson, U.; Gabrielsson, J. A quantitative approach to analysing cortisol response in the horse. *J. Vet. Pharmacol. Ther.* **2016**, *39*, 255–263. [CrossRef]
102. Zhang, M.; Li, X.; Zhang, X.; Liu, H.; Li, J.; Bao, J. Effects of confinement duration and parity on stereotypic behavioral and physiological responses of pregnant sows. *Physiol. Behav.* **2017**, *179*, 369–376. [CrossRef]
103. Roth, L.S.V.; Faresjö, Å.; Theodorsson, E.; Jensen, P. Hair cortisol varies with season and lifestyle and relates to human interactions in German shepherd dogs. *Sci. Rep.* **2016**, *6*, 19631. [CrossRef]
104. Sauveroche, M.; Henriksson, J.; Theodorsson, E.; Svensson Holm, A.C.; Roth, L.S.V. Hair cortisol in horses (*Equus caballus*) in relation to management regimes, personality and breed. *J. Vet. Behav.* **2020**, *37*, 1–7. [CrossRef]
105. Heimbürge, S.; Kanitz, E.; Otten, W. The use of hair cortisol for the assessment of stress in animals. *Gen. Comp. Endocrinol.* **2019**, *270*, 10–17. [CrossRef] [PubMed]
106. Hanis, F.; Chung, E.L.T.; Kamalludin, M.H.; Idrus, Z. Discovering the relationship between dietary nutrients and cortisol and ghrelin hormones in horses exhibiting oral stereotypic behaviors: A review. *J. Vet. Behav.* **2020**, *39*, 90–98. [CrossRef]
107. Jolivald, A.; Ijichi, C.; Hall, C.; Yarnell, K. The mane factor: Compliance is associated with increased hair cortisol in the horse. *Appl. Anim. Behav. Sci.* **2023**, *258*, 105819. [CrossRef]
108. Russell, E.; Koren, G.; Rieder, M.; Van Uum, S. Hair Cortisol as a Biological Marker of Chronic Stress: Current Status, Future Directions and Unanswered Questions. *Psychoneuroendocrinology* **2012**, *37*, 589–601. [CrossRef] [PubMed]
109. Meyer, J.S.; Novak, M.A. Minireview: Hair Cortisol: A Novel Biomarker of Hypothalamic-Pituitary-Adrenocortical Activity. *Endocrinology* **2012**, *153*, 4120–4127. [CrossRef]
110. Novak, M.A.; Hamel, A.F.; Kelly, B.J.; Dettmer, A.M.; Meyer, J.S. Stress, the HPA Axis, and Nonhuman Primate Well-Being: A Review. *Appl. Anim. Behav. Sci.* **2013**, *143*, 135–149. [CrossRef] [PubMed]
111. Cattet, M.; Stenhouse, G.B.; Boulanger, J.; Janz, D.M.; Kapronczai, L.; Swenson, J.E.; Zedrosser, A. Can Concentrations of Steroid Hormones in Brown Bear Hair Reveal Age Class? *Conserv. Physiol.* **2018**, *6*, coy001. [CrossRef]
112. Del González-de-la-Vara, M.R.; Valdez, R.A.; Lemus-Ramirez, V.; Vázquez-Chagoyán, J.C.; Villa-Godoy, A.; Romano, M.C. Effects of Adrenocorticotropic Hormone Challenge and Age on Hair Cortisol Concentrations in Dairy Cattle. *Can. J. Vet. Res. Rev. Can. Rech. Vet.* **2011**, *75*, 216–221.
113. Stalder, T.; Steudte, S.; Miller, R.; Skoluda, N.; Dettenborn, L.; Kirschbaum, C. Intraindividual Stability of Hair Cortisol Concentrations. *Psychoneuroendocrinology* **2012**, *37*, 602–610. [CrossRef]
114. Harkey, M.R. Anatomy and Physiology of hair. *Forensic Sci. Int.* **1993**, *63*, 9–18. [CrossRef]
115. Duran, M.C.; Janz, D.M.; Waldner, C.L.; Campbell, J.R.; Marques, F.J. Hair Cortisol Concentration as a Stress Biomarker in Horses: Associations with Body Location and Surgical Castration. *J. Equine Vet. Sci.* **2017**, *55*, 27–33. [CrossRef]
116. Russell, E.; Kirschbaum, C.; Laudenslager, M.L.; Stalder, T.; de Rijke, Y.; van Rossum, E.F.C.; van Uum, S.; Koren, G. Toward standardization of hair cortisol measurement. *Ther. Drug. Monit.* **2015**, *37*, 71–75. [CrossRef] [PubMed]

117. D'Anna-Hernandez, K.L.; Ross, R.G.; Natvig, C.L.; Laudenslager, M.L. Hair cortisol levels as a retrospective marker of hypothalamic-pituitary axis activity throughout pregnancy: Comparison to salivary cortisol. *Physiol. Behav.* **2011**, *104*, 348–353. [CrossRef] [PubMed]
118. Anielski, P. Hair analysis of anabolic steroids in connection with doping control—Results from horse samples. *J. Mass Spectrom.* **2008**, *43*, 1001–1008. [CrossRef]
119. Webb, E.; Thomson, S.; Nelson, A.; White, C.; Koren, G.; Rieder, M.; Van Uum, S. Assessing individual systemic stress through cortisol analysis of archaeological hair. *J. Archaeol. Sci.* **2010**, *37*, 807–812. [CrossRef]
120. Webb, E.C.; White, C.D.; Van Uum, S.; Longstaffe, F.J. Integrating cortisol and isotopic analyses of archeological hair: Reconstructing individual experiences of health and stress. *Am. J. Phys. Anthropol.* **2015**, *156*, 577–594. [CrossRef]
121. Mercer-Bowyer, S.; Kersey, D.C.; Bertone, J.J. Use of Fecal Glucocorticoid and Salivary Cortisol Concentrations as a Measure of Well-Being of New York City Carriage Horses. *J. Am. Vet. Med. Assoc.* **2017**, *250*, 316–321. [CrossRef]
122. Merl, S.; Scherzer, S.; Palme, R.; Möstl, E. Pain Causes Increased Concentrations of Glucocorticoid Metabolites in Horse Feces. *J. Equine Vet. Sci.* **2000**, *20*, 586–590. [CrossRef]
123. Yarnell, K.; Hall, C.; Royle, C.; Walker, S.L. Domesticated Horses Differ in Their Behavioural and Physiological Responses to Isolated and Group Housing. *Physiol. Behav.* **2015**, *143*, 51–57. [CrossRef]
124. Seeley, K.E.; Proudfoot, K.L.; Edes, A.N. The Application of Allostasis and Allostatic Load in Animal Species: A Scoping Review. *PLoS ONE* **2022**, *17*, e0273838. [CrossRef]
125. Chandler, K.J.; Dixon, R.M. Urinary cortisol:creatinine ratios in healthy horses and horses with hyperadrenocorticism and non-adrenal disease. *Vet. Rec.* **2002**, *150*, 773–776. [CrossRef]