The relationship between the welfare quality and stress index in working and breeding horses

Silvana Popescu\textsuperscript{b,⁎}, Eva A. Diugan\textsuperscript{b}

\textsuperscript{a} Department of Animal Hygiene and Welfare, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Manastur Street, no. 3-5, 400072 Chiş-Napoca, Romania

\textsuperscript{b} Department for Horse Breeding, Exploitation and Amelioration, Beclean Studfarm, The National Forest Administration Romsilva, Petricani Street, no. 9A, 023841 Bucuresti, Romania

\textbf{A R T I C L E   I N F O}

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\textbf{A B S T R A C T}

The aim of this observational study was to investigate if a relationship exists between the calculated overall individual welfare score (as an indicator of the welfare quality) and the neutrophil:lymphocyte (N:L) ratio (as a stress indicator) in working (draught) and reproduction horses (breeding stallions and broodmares), in two different seasons. The welfare of the horses was assessed by a protocol which included health and behavioural parameters. An individual welfare score was computed, the total and differential WBC count was established and the N:L ratio was calculated for each of the 1482 assessed horses. Using all these parameters, comparisons were made between the horse categories (working horses, breeding stallions, broodmares) in two assessment seasons. Finally, the relationship between the individual welfare score and the N:L ratio was studied in light of the significant correlations found. The highest frequency of health problems was recorded in working horses. The human-animal relationship did not present significant differences between the horse categories. The individual welfare scores were significantly lower ($P<0.05$) in the working than in the breeding animals, in both assessment seasons. The N:L ratio was significantly higher ($P<0.05$) in the working horses than in those kept for reproduction. The season had no significant influence ($P>0.05$) on the investigated parameters. All the significant correlations found between the N:L ratio and the individual welfare score were negative. The results obtained indicate poor welfare in the working horses, comparing with the breeding animals. The findings of our study confirmed that the N:L ratio increases in horses with a poor welfare state. The multiple correlations of the N:L ratio with the individual welfare scores of the assessed horses emphasised the efficiency of this parameter in identifying the acute and chronic stress conditions in the living environment of the animals.

1. Introduction

Animal welfare always links back to the five freedoms. Together with the other four freedoms, freedom from fear and distress is imperative for good welfare in animals. Depending on psychosomatic differences, the individuals can perceive the same situation as more or less stressful, thus impairing their welfare to various degrees. It is generally accepted that the same conditions that breed the development of welfare problems can lead to a stress response that is meant to restore the homeostatic balance of the individual (Broom and Johnson, 2000; Etim et al., 2013). Depending on the individual’s ability to cope and on the intensity and duration of the stressor’s action, the organism can adapt or overcompensate, and the stress can increase (Selye, 1955). Some authors (Moberg and Mench, 2000) consider that low levels of acute stress can have positive effects by raising attention and awareness and thus enhancing the learning processes. Yet prolonged exposure to stressors or even short exposure to high intensity stress factors can lead to distress. The distressed animal’s organism cannot replenish its biological resources, thereby accumulating biological costs (Moberg and Mench, 2000) and impairing welfare (Etim et al., 2013). Different physiological measures are used to assess stress levels in horses. Among these, the evaluation of the plasma, faecal or salivary concentration of cortisol and the assessment of the heart rate or heart rate variability are commonly studied. In addition, the use of the leucocyte profile, and especially the neutrophil:lymphocyte ratio, is considered a reliable indicator to measure stress in vertebrates (Davis et al., 2008). The utility of the latter parameter was demonstrated also in horses exposed to acute stress during transportation (Maeda et al., 2011). To our knowledge, no study investigated the relationship between the quality of the welfare and neutrophil:lymphocyte ratio in horses.

⁎ Corresponding author.
E-mail address: silvana.popescu@usamvcluj.ro (S. Popescu).

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The aim of this research was to investigate the relationship between a calculated overall individual welfare score (as an indicator of the welfare quality) and the neutrophil:lymphocyte ratio (as a stress indicator) in working and breeding horses. This study sought to establish whether the N:L ratio increases in horses with a poor welfare state. If so, the N:L ratio would be a good indicator of acute or chronic stress conditions from the living environment of the horses.

2. Material and methods

2.1. Animals

The study was conducted over two years on a sample of 1482 horses, kept in different breeding and housing systems and having different physiological states. The sample horses were of two main types: breeding horses and horses used for work. All the breeding horses were kept in three national stud farms and the working horses were randomly chosen from the same geographical regions, being owned by various private persons. Each horse was assessed twice, in winter and in summer. The breeding animals where assessed in the stud farms, in the barns. Some of the working horses were assessed in their barns and others in the places where they were working (roadsides, woods, lands), attached to their carts. The numbers of animals assessed in winter and in summer differ because the owners sold and acquired working horses in the meantime.

The private working horses (257 in winter, 252 in summer) were of varying age categories and genders (fillies and colts, mares, stallions and geldings). These animals were used for different agricultural works and to carry loads with carts in the rural areas. The great majority of private working horses were of unknown breed parentage or of mixed local breeds. The housing system was broadly similar for the horses used for work. The horses kept in the breeding farms comprised stallions (215 in winter, 221 in summer), and broodmares (265 in winter, 272 in summer) of the Furioso North-Star, Lipizzaner and Romanian Draft horse breeds. The breeding horses were split in two categories, because of their different housing. The stallions were housed tethered, while the mares were generally free (tethered during the night in winter and when fed cereals).

All the procedures involving the animals were carried out with the consent of the horse owners, in accordance with the ethical guidelines of the Romanian National Animal Protection Law (Law 205/2004 amended by Law 9/2008).

2.2. Welfare assessment and the individual welfare score

The welfare assessment of the horses was largely based on a protocol (using mainly the five freedoms) published in another study (Popescu and Diugan, 2013) which included behavioural and health related welfare indicators.

Each horse was observed from a distance of 3–5 m for 60 s to assess their general alertness. The horses were classified as ‘alert’ (i.e. responsive to the stimuli of the environment: eyes open, showing movements of the ears, head, tail and/or skin to keep away flies) or ‘depressed’ (i.e. showing decreased responses to external stimuli: head lowered, eyes half closed, complete or partial cessation of tail and skin movements to avoid insects, reduced ear movements).

In order to assess the behavioural responses of the horses towards humans the assessor approached the horse, walked beside it and attempted to touch its chin. The approach was made at an angle of about 20% and the horse’s response was recorded at the moment the assessor stopped at about 30 cm from the head of the animal. Then the assessor walked alongside the horse towards its rear and back again, maintaining about 30 cm from the horse’s body, while recording any sign of the animal’s attention. The attempt of physical contact was made by raising a cupped hand towards the horse’s chin and touching it if the animal allowed the contact. The responses of the horses towards humans for each test were categorised as follows: ‘aggressiveness’ (any attempt to kick or bite, the latter including also teeth bearing with the head turned towards the human person, with or without previous wrinkling around the mouth and nostrils, ear flattening, head jerk, hitting/stomping the ground); avoidance/fear (adoption of a tense, immobile body position, with the head up, tense muscles, the lips held tight, eyes wide open or active avoidance: moving a step away or turning the head away from the human, with or without nose wrinkling and ear flattening); ‘indifference’ (immobile and relaxed or depressed body position, no movement of the head towards or away from the human, with or without the ears moving, relaxed lips, possibly eyes half closed) or ‘friendliness’ (movement of the head towards the human, with relaxed face, normally open eyes, ears turned forward, no wrinkling around the mouth or nostrils) (Popescu and Diugan, 2013).

The health related indicators were assessed in non-invasive manner, by observation and clinical exam, in order to identify systemic health problems and disorders of the different body systems and organs. The assessment system was the one used by Popescu and Diugan (2013). The only differences consisted of a slightly modified classification of the body condition score (BCS) and the inclusion of two more parameters. Thus, the BCS was assessed according to the description of Carroll and Huntington (1988), on a scale from 0 (meaning extremely thin) to 5 (meaning extremely fat). These scores were grouped into three categories: unacceptable body conditions, meaning body conditions that endanger the health and/or the life of the animal (score 0, 1 and 5 on the original scale), improper body conditions (scores 2 and 4 on the original scale) and good body conditions (score 3 on the original scale). The two newly introduced parameters were the number of times the horses’ teeth were checked (according to the declaration of the owner) and the hair quality of the mane/tail. The latter parameter was adapted from the Welfare Quality Protocol for horses (Wageningen, 2011). The hair of the mane/tail was considered abnormal if it was dull, matted, dirty, broken or missing and normal if it was complete, clean and shiny.

Even if not a direct health indicator, the body hygiene of the horses was also assessed. Faecal soiling under the tail was taken as an indicator of diarrhoea and the presence of manure on the side of the rump was considered to show improper cleaning of the sleeping surfaces in the barn. The health indicators were noted as present/absent or with severity grades (Popescu and Diugan, 2013). Lesions at the corners of the lips can show improper usage or fit of a bit; body lesions at harness contact points are usually caused by poor harness fitting, inadequate harnesses and seem to be aggravated when the body condition of the animals is poor; and lesions at the points of the hip can occur when the horses’ resting places are hard, abrasive and/or covered in little or no bedding (Popescu and Diugan, 2013), so these were considered as marker lesions.

As regards the order in which the indicators were evaluated, freedom from fear and distress was assessed at the start, to record the first reaction of the animal in the presence of the unknown assessor. The BCS was then evaluated, followed by the parameters listed to investigate the freedom from discomfort and the freedom from pain, injury or disease. Of the latter, the gait was evaluated at the end of the assessment. The scores for some parameters (water provision, dental check, company of its own kind and access to unrestricted free exercise) were based solely on the declaration of the horses’ owners or caregivers.

In order to assess the quality of the horses’ welfare, a score was calculated for each studied animal. Scores were assigned to each parameter (see Table 1), so that the negative aspect always received a low score and the positive aspect a high score. Finally, for each evaluated horse an individual welfare score was computed by adding up the scores obtained for each parameter. This way, when the individual welfare score was calculated, a higher score was obtained when the welfare of the animal was more appropriate. The theoretical minimum score for an assessed horse was 0, a practical impossibility for a living animal. The maximum possible score was 50.
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