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ABSTRACT

There is not a welfare protocol for horses reared for meat production in semi-extensive systems. The aims of the study were to develop a specific welfare protocol suitable to be applied at pasture and on stable; and to evaluate whether the welfare items were influenced by the farming system (pasture vs stable). 52 non-animal-based measures (N-ABMs) and 14 animal-based measures (ABMs), classified into 6 thematic areas (training, feeding, facilities, ABMs, biosecurity, health management) were selected by a focus group. The protocol was applied on a total of 429 Catria horses located across 26 pastures during the warm seasons and on 7 stables during the cold seasons. Differences obtained within each horse-unit were calculated by using the diversity index (VARNC) and the distance from the ideal (dfi) index. Chi-square test was used for comparing the relative frequencies (%) of the answers (pasture vs stable). Most the welfare items were classified as adequate in both pasture and stable, yet differences were found within 'training' ($p = 0.02$) and 'feeding' ($p < 0.01$) areas in relation to the welfare items 'inspection of the animals' and 'feeding management'. Weaknesses of both pasture and stable were represented by some welfare items in the 'health management' and 'biosecurity' areas. After proper validation, the welfare protocol developed in the present study could help to fill the existing gap of knowledge on horse welfare assessment for semi-extensive system systems, providing support for official control of veterinarians and enabling the identification of key weakness to address preventive interventions.

HIGHLIGHTS

- A welfare protocol suitable for pasture and stable was developed
- Key weakness can be identified to address preventive interventions

Introduction

More than 500,000 horses are slaughtered in Europe each year to produce meat for human consumption (Faostat, 2023). Spain stands out as the major horse meat producer (17%), followed by Italy (16%), Romania (14%), Poland (11%), and France (8.2%) (Lorenzo et al. 2014; Belaunzaran et al. 2015). Most of the existing scientific literature focuses on meat according to its consumption and nutritional values (De Palo et al. 2013; Belaunzaran et al. 2015). Instead, little knowledge is available on the welfare conditions

on farm of horses intended for meat production. It is reported that horse meat is mainly obtained from young horses which are specifically fattened for this purpose (Tateo et al. 2008; Belaunzaran et al. 2015); yet there are not standardised farming conditions (Lorenzo et al. 2014). These horses are generally reared in confined systems characterised by intensive farming practices, including overcrowding and intensive feeding regimes based on high-starch diets (Raspa et al. 2020b, 2020a). Moreover, it is reported that horses intended for meat production can be reared in semi-extensive systems, a traditional farming

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method used for native breeds (Insausti et al. 2021). Among Italian native breeds used for meat production, Catria horse is an autochthonous breed from Mount Catria, located in central Apennines within the provinces of Pesaro-Urbino and Perugia. According to the Food Agriculture Organisation (FAO, 2024), the Catria breed is currently considered in endangered status. Catria horses were traditionally used in mountain agriculture, whereas now they are mainly utilised as saddle horses for leisure activities (e.g. mountain trekking) and for meat production (Bigi and Perrotta 2012). Catria horses are reared in semi-extensive systems, grazing on the mountainous pastures around Mount Catria and adjacent areas of the Central Apennines during the warmer seasons. In autumn and winter, animals are moved to valley where they are housed in stables (Mantovani et al. 2013; Trombetta et al. 2017).

At European Union (EU) level, currently there are not Directives aimed at safeguarding the welfare of horses reared for meat production. Council Directive 98/58/CE (European Union Council, 1998) and the subsequent Italian Legislative Decree 2001/146 (Gazzetta Ufficiale 2001) on the protection of animals kept for farming purposes, are considered too general and inadequate to protect the welfare of farmed animals including horses reared for meat purpose (Broom 2017). A welfare assessment protocol for horses reared for meat production under intensive farming system has been published (Raspa et al. 2020a) but no tools are available for semi-extensive systems. According to the experience of the Italian National Reference Centre for Animal Welfare (CReNBA), both non-animal-based measures (N-ABMs) (i.e. resource- and management-based measures) and animal-based measures (ABMs) are needed to obtain an effective overall assessment of animal welfare (Bertocchi et al. 2018). Since there are not standardised farming conditions for horses reared for meat production, there is the need to have a specific welfare assessment protocol for horses that can be used both at pasture and on stable and that includes N-ABMs and ABMs to help breeders apply best practices and ensure the welfare of their animals as well as to support the official veterinary controls.

The present study is part of a larger research project aimed at safeguarding horse welfare, breed biodiversity and sustainable animal production in central Italy. In particular, the aims of the study were (i) to develop a specific welfare assessment protocol for horses reared for meat production that included both N-ABMs and ABMs and suitable to evaluate horse welfare on pasture and on stable; (ii) to apply the developed welfare protocol on field, and (iii) to evaluate whether the selected welfare items were influenced by the farming system (pasture vs stable).

It was expected that the data collected could be useful to assist farmers in implementing best practices to ensure the welfare of their animals, as well as to support official veterinary controls.

Materials and methods

The study followed the guidelines of the current European Directive (2010/63/EU) on the protection of animals used for scientific purposes, and was approved by the Ethical Committee of the Department of Veterinary Sciences of the University of Turin (Italy, Prot. 1129 04/21/2021).

Welfare assessment protocol

The welfare assessment protocol was built by a team of five veterinarians, experts in equine welfare and welfare protocols, during a focus group. The experts took into account the need to develop a welfare protocol suitable for application both on pasture and on stable. The focus group selected 66 welfare items on a multiple-choice checklist which encompassed 52 resource and management-based measures (N-ABMs), and 14 animal-based measures (ABMs). For each welfare item, three different intensities of animal exposure were defined. The baseline level/adequate intensity of exposure was deemed not to affect horse welfare, and the critical/inadequate and beneficial/ideal levels were determined and described for each welfare item (Additional file 1). Since scientific publications of the European Food Safety Authority (EFSA) and Welfare Quality (WQ®) were not available to extract those data; cut-off values were defined on the basis of the existing scientific literature, the minimum EU legal requirements set by the Council Directive 98/58/EC, the Italian Legislative Decree n. 146/2001 and the individual expertise.

Each welfare item had either two possible levels (inadequate, scored 1; adequate, scored 2) or three possible category of answers (inadequate, scored 1; adequate, scored 2; ideal, scored 3).

Moreover, six thematic areas were identified:

- 'Training' (6 welfare items), number, experience and training of stockpersons, animal handling and the animal grouping strategy;
- 'Feeding' (10 welfare items), feeding management (provision of adequate amounts of hay and

concentrates) or pasture management and water provision;

- 'Facilities' (13 welfare items), measures of comfort around resting, environmental conditions and possibility of social interactions among the animals indoors or at pastures;
- 'Animal-based measures' (14 welfare items), directly assessed on the animals as suggested by the Animal Welfare Indicators welfare protocol for horses (AWIN 2017);
- 'Biosecurity' (9 welfare items), preventive veterinary medicine practices such as quarantine measures, facilities for sick animals, pests control, procedures for visitors' entrance, stable disinfection, carcase collection, loading/unloading of animals;
- 'Health management' (14 welfare items) aiming to evaluate the animal's health care, for example the dental and hoof check, the parasite management, and the vaccination programs.

The description of thematic areas and welfare items can be found in the Additional file 1.

Field application

The welfare assessment was carried out on a total of 429 Catria horses that in the warm season (from April to September) grazed on 26 different pastures surrounding Mount Catria, between 800 and 1,700 metres above sea level. During the cold season (from October to March) the 429 horses were housed in 7 different stables providing group indoor housing with access to an outdoor paddock area. The developed welfare assessment protocol was applied on each horse-unit herd by a trained veterinarian between June 2021 and September 2022.

Data analysis

Data analysis was performed by using Python packages 'SciPy' v1.2.1, p &lt (Python Software Foundation).

Comparison of the horse-unit distribution according to the farming system (pasture vs stable)

The relative frequencies (%) for the category of answers – inadequate, adequate, ideal – were computed considering the specific thematic area. A chi-squared test was used to identify differences within each thematic area depending on the farming conditions (stable vs pasture). A p-value < 0.05 was considered significant to infer that the differences were related to the farming conditions. Lastly, an independence assessment of the horse-unit distribution in relation to each welfare item was performed. Since all the measurements were categorical variable, χ-square test was used for comparing the data of the two farming systems.

Calculation of diversity (VARNC) index and distance from ideal (dfi) index

The diversity (VARNC) index and distance from ideal (dfi) index were calculated according to the method described by Fusi et al. (2021).

The variability observed in each horse-unit was evaluated by calculating the VARNC index of diversity according to the following formula:

$$VARNC = \frac{K(N^2 - \sum f_i^2)}{N^2(K-1)}$$

where K is the number of answer categories (either two or three) for each welfare item, N is the total sample size (26 pastures and 7 stables), and f_i is the frequency of ith category for each item.

The VARNC index ranges from 0 to 1. A VARNC index of 0 indicates no dispersion of answers, meaning that all responses fall in a single category (i.e. all horse-units received the same answer). A VARNC index of 1 indicates an equal distribution of answers across categories. The significance of the VARNC index is distributed as a χ-square distribution with $K-1$ degrees of freedom (d.f.). For welfare items with three answer possibilities, the χ-square value at a 5% confidence level is 5.99 (2 d.f.), and for welfare items with two answer possibilities, it is 3.84 (1 d.f.). This calculation was applied to all 66 welfare items selected.

Moreover, the distance of the horse-unit distribution from an ideal condition (all horse-units falling into the highest answer category, $p_{HC} = 100\%$) was calculated using the distance from ideal (dfi) index according to the following formula:

$$dfi = 1 - \frac{K - \sum r_i p_i}{(K-1)}$$

where K is the number of answer categories (two or three) for each welfare item, r is the rank for each category, and p is the percentage of horse-units in each answer category.

The dfi index can range from 0 to 1. A dfi index of 0 indicates that all answers are concentrated in the lowest category (inadequate). A dfi index of 1 indicates that all answers are concentrated in the highest category (ideal condition).

Results

Comparison of the horse-unit distribution according to the farming system (pasture vs stable)

The frequencies (%) of intensity of exposure (inadequate, adequate, ideal) according to the six thematic areas 'training', 'feeding, 'facilities', 'animal-based measures', 'biosecurity' and 'health management' are shown in Figure 1. Significant differences between the two farming systems (pasture vs stable) were found in the 'training' area ($p = 0.02$, Chi-square test) in which 38% of the answers resulted inadequate at pasture compared to 18% of inadequate answers in stable. Moreover, the 'feeding' area was found significantly different between pasture and stable ($p = 0.001$, Chi-square test), with 75% of adequate answers at pasture compared to the 57% of adequate answers in stable. No differences were found between pasture and stable according to the other thematic areas.

Diversity (VARNC) index and distance from ideal (dfi) index

Considering the welfare items included in the **'training'** area, a certain level of variability (Table 1) was found between the two farming systems for the

following welfare items: 'number of stockpersons' (pasture, 0.84; stable, 0.53), 'inspection of the animals' (pasture 0.84; stable, 0.00) and 'animal grouping strategy in relation to feeding' (pasture, 0.00; stable, 0.91). As shown in Figure 2, the horse-units housed in stable were more often close to the highest answer category (ideal condition) for 'number of stockpersons' (dfi index – pasture, 0.70 vs stable, 0.90) and 'inspection of the animals' (dfi index – pasture, 0.30 vs stable, 0.50). In particular, significant differences (Table 2) were found for the welfare items 'inspection of the animals' (65% of inadequate answers at pasture compared to 0% in stable, $p < 0.01$), and 'animal grouping strategy in relation to feeding' (100% of inadequate answers at pasture compared to 43% of inadequate answers in stables, $p < 0.01$).

The assessment of the differences obtained in the horse-unit distribution by the VARNC index (Table 1) for the welfare items included in the **'feeding'** area revealed a certain level of variability for the 'feeding management' (pasture, 0.37; stable, 0.77), 'cleanliness of water points' (pasture, 0.47; stable, 0.77) and 'source of drinking water' (pasture, 0.47; stable, 0.90). Those welfare items were closer to the ideal conditions in the horse-units housed in stable (Figure 2). In particular, as shown in Table 2, the welfare item 'feeding management' was judged inadequate in 92% of the
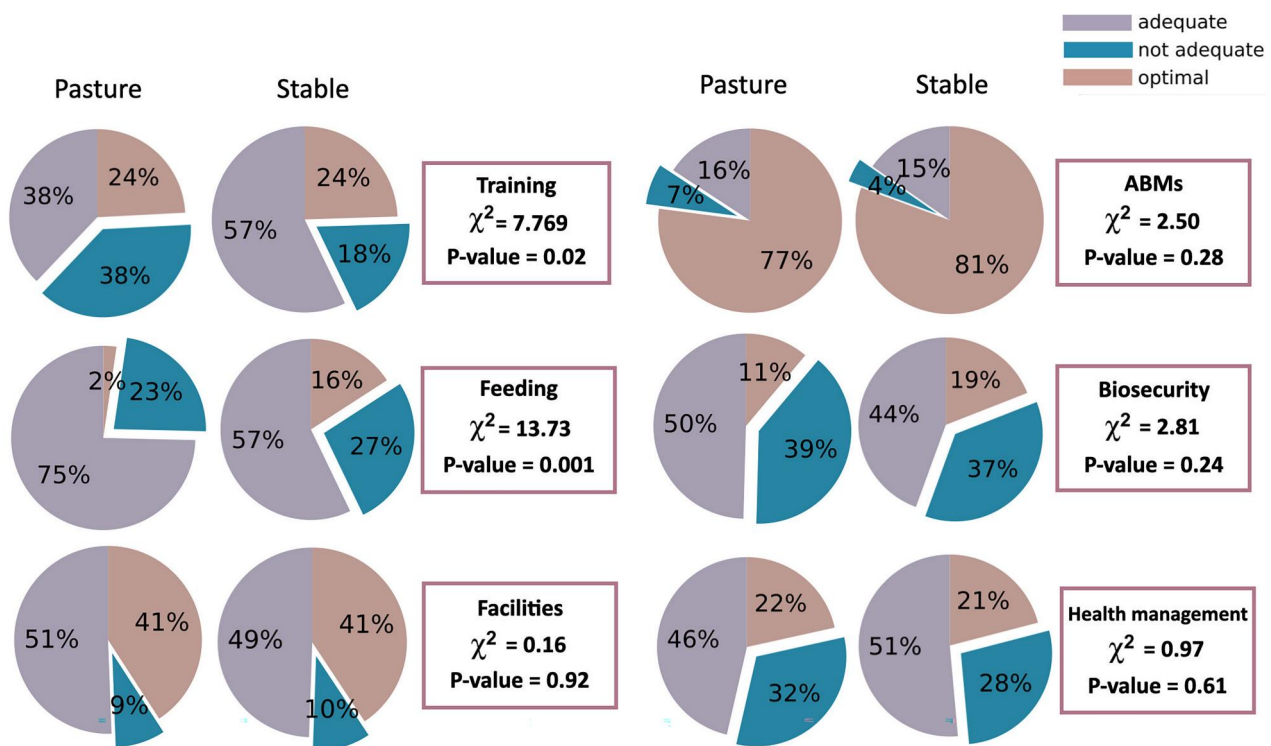

Figure 1. Comparison of the outcomes obtained from pasture vs stable according to the relative frequencies (%) for intensity of exposure – inadequate, adequate, ideal – within the selected thematic areas – training, feeding, facilities, animal-based measures, biosecurity, health management.

surveyed horse-units kept on pasture compared to 29% of inadequate answers collected from the horse-units kept in stables ($p < 0.01$).

Regarding the **'facilities'** area, the VARNC index (Table 1) displayed some degree of variability of the answers for the following welfare items: 'outdoor shelters' (pasture, 0.31; stable, 0.91), 'bedding quantity' (pasture, 0.27; stable, 0.85), 'management of the delivery area' (pasture, 0.00; stable, 0.90), 'environmental conditions' (pasture, 0.00; stable, 0.53), 'possibility of social interactions among the animals' (pasture, 0.00, stable, 0.53) and 'space availability' (pasture, 0.00; stable, 0.53). However, as shown in Table 2, the significant differences between the two farming systems were found for the 'outdoor shelters' that resulted adequate in 88% of the cases for the horse-units kept on pasture, compared to the 43% of the horse-units kept on stable ($p = 0.03$). Moreover, the 'management of the delivery area' resulted significantly different between pasture and stable ($p < 0.01$). In fact, no area (100%) of the surveyed pastures was dedicated to foaling and 57% of the stables did not have specifically designated box.

Considering the **'ABMs'** area, the variability of answers (VARNC index, Table 1) was similar between the two farming systems except for the welfare items 'signs of lameness' (pasture, 0.70; stable, 0.00) and 'annual mortality rate of the foals' (pasture, 0.85; stable, 0.67). As shown in Figure 2, those welfare items were closer to the ideal condition for the horse-units housed on stable compared to those housed on pasture (dfi index 'signs of lameness' – pasture, 0.80 vs stable, 1; dfi index 'annual mortality rate of the foals'- pasture, 0.50 vs stable, 0.80). However, no significant differences were found between the two farming systems (Table 2).

Also for the **'biosecurity'** area, the variability of answers (VARNC index, Table 1) was similar between the two farming systems with the welfare items 'presence of other reared species' closer to the ideal condition for horse-units housed in stable compared to those housed on pasture (Figure 2, dfi index – pasture, 0.20 vs stable, 0.70). In fact, as shown in Table 2, the 73% of pastures were judged inadequate, compared to stables which were never scored as inadequate ($p < 0.01$). Interestingly, considering Figure 2, the presence of 'measures for pests control' and 'measures for the entrance of visitors' resulted far from the ideal condition for both the two farming systems (dfi index 'measures for pests control – pasture, 0.20 and stable, 0.30; dfi index 'measures for the entrance of visitors' – pasture, 0.10 and stable, 0.20).

The **'health management'** area did not reveal differences between the two farming systems even if it should be underlined how 'parasitic management' and 'control and prevention of dental disorders' resulted inadequate for at least 50% of the surveyed pastures (Table 2). In fact, as shown in Figure 2, those welfare items were closer to the highest answer category (ideal condition) (dfi index 'parasitic management' – pasture, 0.40 vs stable, 0.60; and dfi index 'control and prevention of dental disorders'– pasture, 0.30 vs stable, 0.70). Moreover, the welfare item 'health management of the foal' was mainly judged inadequate (in more than the 50% of the cases) for both farming systems (Table 2).

Discussion

There are currently not welfare assessment protocols available at EU or national level for assessing horse welfare either on pasture or on stable; and the present study aimed to fill this gap of knowledge, by developing and testing a specific welfare protocol suitable to be used in both farming conditions. However, in contrast to other livestock species, there are not specific scientific publication by the European Food Safety Authority (EFSA) or Welfare Quality (WQ®) that validate welfare items for horses reared for meat production and kept in semi-extensive systems. The 66 welfare items included in the welfare protocol of the present study were chosen by the focus group of expert veterinarians on the basis of the available scientific literature, the minimum EU and nation legislative requirements and the individual expertise with the aim to use easily assessable welfare items both on pasture and on stable. Consequently, the validation of the proposed welfare protocol represents a limitation of the present study. Yet, it could represent a starting point for the following expert knowledge elicitation and the application of the risk assessment methodology suggested by the European Food Safety Authority (EFSA 2016).

This study also aimed to evaluate whether the selected welfare items were influenced by the farming systems (pasture vs stable). Accordingly, it was possible to recognise the main weakness points of the evaluated horse-units (26 were evaluated on pasture and 7 on stable), which could help address future preventive interventions. Considering the welfare items included in the **'training' area**, it was found that the horse units housed in stables were more often close to the highest answer category (ideal condition) for 'number of stockpersons' and 'inspection of the

Table 1. VARNC index of diversity and chi-squared values for each welfare item of the six thematic areas of the developed welfare assessment protocol for horses. n.s., non-significant differences in the horse-unit distribution. n.a., not applicable.

| N	Welfare Item	Pasture		Stable	
		VARNC index	Chi-square	VARNC index	Chi-square
Training					
1	N of stockpersons	0.84	2.46 n.s.	0.53	3.57 n.s.
2	Experience and training of stockpersons	0.27	22.15	0.00	n.a.
3	Inspection of the animals	0.84	2.46 n.s.	0.00	n.a.
4	Type of handling	0.00	n.a.	0.00	n.a.
5	Animal grouping strategy - feeding	0.00	n.a.	0.91	1.14 n.s.
6	Animal grouping strategy - sociability	0.00	n.a.	0.53	3.57 n.s.
Feeding					
7	Feeding management (presence of a balanced ration)	0.37	18.61	0.77	1.28 n.s.
8	Pasture management	0.00	n.a.	n.a.	n.a.
9	Hay feeding	–	n.a.	0.53	3.57 n.s.
10	Concentrates feeding	–	n.a.	0.53	3.57 n.s.
11	Available space at feed bunk	–	n.a.	0.00	n.a.
12	Feed quality and storage	–	n.a.	0.00	n.a.
13	Water availability	0.27	22.15	0.00	n.a.
14	N of drinkers per horses	–	n.a.	0.53	3.57 n.s.
15	Cleanliness of water points	0.47	15.38	0.77	1.28 n.s.
16	Source of drinking water	0.47	15.38	0.90	1.14 n.s.
Facilities					
17	Housing conditions	0.00	n.a.	0.00	n.a.
18	Outdoor shelters	0.31	35.61	0.91	1.14 n.s.
19	Hygienic quality of bedding or pastures	0.61	19.92	0.67	4.57 n.s.
20	Bedding quantity	0.27	22.25	0.85	2 n.s.
21	Management of the delivery area	0.00	n.a.	0.90	0.14 n.s.
22	Environmental conditions	0.00	n.a.	0.53	3.57 n.s.
23	Environmental temperatures	0.00	n.a.	0.00	n.a.
24	Environmental humidity	0.00	n.a.	0.00	n.a.
25	Lighting	0.00	n.a.	0.00	n.a.
26	Freedom of movement	0.00	n.a.	0.00	n.a.
27	Possibility of social interactions among animals	0.00	n.a.	0.53	3.57 n.s.
28	Space availability	0.00	n.a.	0.53	3.57 n.s.
29	Space availability – delivery area	0.00	n.a.	0.00	n.a.
Animal-based measures (ABMs)					
30	Avoidance distance test	0.00	n.a	0.00	n.a
31	Body condition score	0.86	7	0.91	1.14 n.s.
32	Consistency of manure	0.00	n.a	0.00	n.a
33	Haircoat cleanliness	0.00	n.a	0.00	n.a
34	Integument alterations	0.63	n.a	0.53	3.57 n.s.
35	Mane condition	0.00	n.a	0.00	n.a
36	Tail condition	0.00	n.a	0.00	n.a
37	Signs of lameness	0.70	15.3	0.00	n.a
38	Presence of discharges	0.00	n.a	0.00	n.a
39	Coughing	0.00	n.a	0.00	n.a
40	Abnormal breathing	0.00	n.a	0.00	n.a
41	Stereotypies	0.00	n.a	0.00	n.a
42	Annual mortality rate (adults)	0.69	7.53	0.85	2 n.s.
43	Annual mortality rate (foals)	0.85	7.69	0.67	4.57 n.s.
Biosecurity					
44	Presence of other reared species	0.64	18.53	0.78	1.28 n.s.
45	Rearing	0.49	26.38	0.53	3.57 n.s.
46	Quarantine measures	0.31	35.61	0.67	4.57 n.s.
47	Facilities for sick animals	0.47	15.38	0.78	1.28 n.s.
48	Measures for pests control	0.70	7.53	0.78	1.28 n.s.
49	Measure for the entrance of visitors	0.63	9.84	0.53	3.57 n.s.
50	Measures of stable disinfection	n.a.	n.a.	0.53	3.57 n.s.
51	Collection of carcase	0.90	0.61 n.s.	0.90	0.14 n.s.
52	Loading/unloading of animals	0.80	3.84	0.90	0.14 n.s.
Health management					
53	Frequency of veterinary checks	0.27	22.15	0.00	n.a.
54	Knowledge of main viral diseases	0.47	15.38	0.53	3.57 n.s.
55	Knowledge of main bacterial diseases	0.80	3.84	0.78	1.28 n.s.
56	Knowledge of main protozoan diseases	0.80	3.84	0.78	1.28 n.s.
57	Knowledge of main parasitic diseases	0.98	1 n.s.	0.97	0.28 n.s.
58	Vaccination programs	0.80	3.84	0.53	3.57 n.s.
59	Parasitic management	0.95	2.15 n.s.	0.97	0.28 n.s.
60	Control and prevention of hoof disorders	0.75	12.53	0.53	3.57 n.s.
61	Control and prevention of dental disorders	0.80	3.84	0.77	1.28 n.s.
62	Health management of the foal	0.89	0.61 n.s.	0.90	1.14 n.s.
63	Age at weaning	0.00	n.a.	0.00	n.a.
64	Abortions	0.27	22.15	0.53	3.57 n.s.
65	Choice of antimicrobial treatments	0.55	12.46	0.53	3.57 n.s.
66	Choice of antiparasitic treatments	0.90	4.69 n.s.	0.91	1.14 n.s.
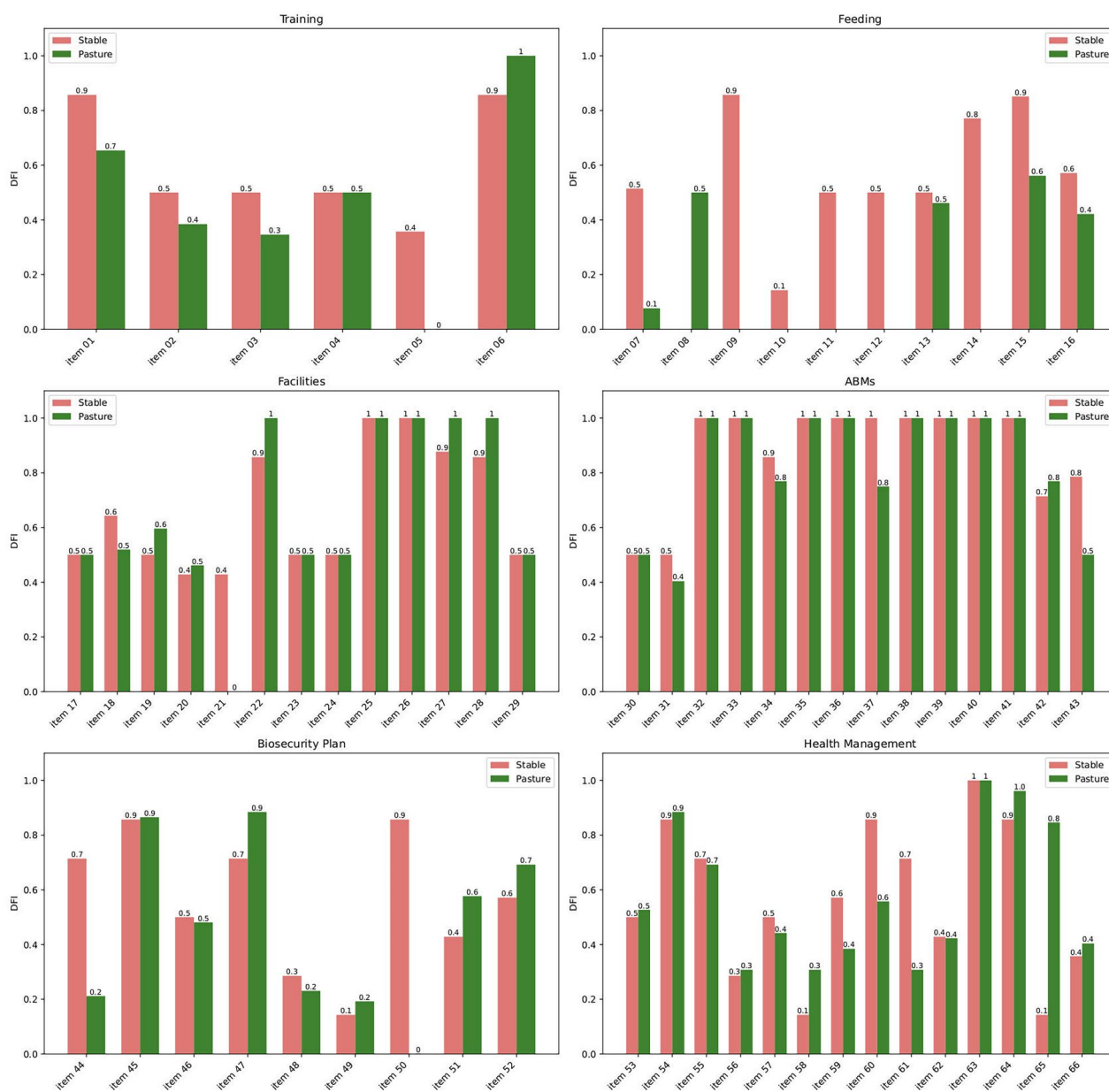
Figure 2. Distance estimate of the horse-unit distribution from an ideal condition (all horse-units grouped into the highest answer category) using the distance from ideal (dfi) index.

animals' (Figure 2). In particular, the 'inspection of the animals' was judged inadequate in 65% of the surveyed pastures, whereas at least one daily inspection (adequate answer) was performed in stables (Table 2). This finding was likely due to the easier possibility of inspecting animals in stables compared to mountain pasture areas. Indeed, each farmer had horses located across various pastures, hence the daily inspection was not done. The concept of minimum inspection requirements for animals was set in 1976 by the Convention for the Protection of Animals kept for Farming Purpose, and then transposed into the EU Directive 98/58/EC and the national welfare legislations. This concept is not only a matter of the

frequency with which animals are observed but also aims at interventions to safeguard animal welfare when needed, for example, during foaling, or if animals are injured or sick (Veissier et al. 2008). Recognising animals in pain is a significant welfare issue in both intensive and extensive farming systems. Ideally, regular monitoring of animals to detect early signs of pain is crucial for ensuring their survival and welfare. However, the identification of potential indicators of pain can be more challenging in animals kept in extensive conditions (Temple and Manteca 2020). This is particularly true for species like equines, which exhibit subtle pain signals due to their evolution as prey animals (Goodwin 1999). Additionally, the

Table 2. Outcomes from audits in 26 pastures and 7 stable horse-units. Chi-square test performed to compare distribution among categorical variables (answers to items) scored: inadequate (1), adequate (2), ideal (3).

N	Welfare Item	Pasture			Stable			p-value
		1	2	3	1	2	3	
Training								
1	N of stockpersons	35%	0%	65%	14%	0%	86%	0.56
2	Experience and training of stock persons	0%	96%	4%	0%	100%	0%	0.98
3	Inspection of the animals	65%	35%	0%	0%	100%	0%	<0.01*
4	Type of handling	0%	100%	0%	0%	100%	0%	1
5	Animal grouping strategy - feeding	100%	0%	0%	43%	43%	14%	<0.01*
6	Animal grouping strategy - sociability	0%	0%	100%	0%	14%	86%	0.47
Feeding								
7	Feeding management	92%	8%	0%	29%	71%	0%	<0.01*
8	Pasture management	0%	100%	0%	–	–	–	n.a
9	Hay feeding	–	–	–	0%	14%	86%	n.a
10	Concentrates feeding	–	–	–	86%	14%	0%	n.a
11	Available space at feed bunk	–	–	–	0%	100%	0%	n.a
12	Feed quality and storage	–	–	–	0%	100%	0%	n.a
13	Water availability	4%	96%	0%	0%	100%	0%	0.98
14	N of drinkers per horses	–	–	–	0%	86%	14%	n.a
15	Cleanliness of water points	0%	88%	12%	0%	71%	29%	0.60
16	Source of drinking water	12%	88%	0%	43%	57%	0%	0.17
Facilities								
17	Housing conditions	0%	100%	0%	0%	100%	0%	1
18	Outdoor shelters	4%	88%	8%	14%	43%	43%	0.03*
19	Hygienic quality of bedding or pastures	4%	73%	23%	14%	71%	14%	0.55
20	Bedding quantity (to evaluate in stable)	–	–	–	29%	57%	14%	n.a
21	Management of the delivery area	100%	0%	0%	57%	43%	0%	<0.01*
22	Environmental conditions	0%	0%	100%	0%	14%	86%	0.47
23	Environmental temperatures	0%	100%	0%	0%	100%	0%	1
24	Environmental humidity	0%	100%	0%	0%	100%	0%	1
25	Lighting	0%	0%	100%	0%	0%	100%	1
26	Freedom of movement	0%	0%	100%	0%	0%	100%	1
27	Possibility of social interactions among animals	0%	0%	100%	0%	14%	86%	0.47
28	Space availability	0%	0%	100%	14%	0%	86%	0.47
29	Space availability – delivery area	0%	100%	0%	0%	100%	0%	1
Animal-based measures (ABMs)								
30	Avoidance distance test	0%	100%	0%	0%	100%	0%	1
31	Body condition score	54%	12%	35%	43%	14%	43%	0.87
32	Consistency of manure	0%	0%	100%	0%	0%	100%	1
33	Haircoat cleanliness	0%	0%	100%	0%	0%	100%	1
34	Integument alterations	19%	8%	73%	14%	0%	86%	0.69
35	Mane condition	0%	0%	100%	0%	0%	100%	1
36	Tail condition	0%	0%	100%	0%	0%	100%	1
37	Signs of lameness	19%	12%	69%	0%	0%	100%	0.24
38	Presence of discharges	0%	0%	100%	0%	0%	100%	1
39	Coughing	0%	0%	100%	0%	0%	100%	1
40	Abnormal breathing	0%	0%	100%	0%	0%	100%	1
41	Stereotypies	0%	0%	100%	0%	0%	100%	1
42	Annual mortality rate (adults)	0%	23%	77%	14%	29%	57%	0.13
43	Annual mortality rate (foals)	46%	8%	46%	14%	14%	71%	0.30
Biosecurity								
44	Presence of other reared species	73%	12%	15%	0%	12%	71%	<0.01*
45	Rearing	8%	12%	81%	14%	0%	86%	0.58
46	Quarantine measures	8%	88%	4%	14%	71%	14%	0.48
47	Facilities for sick animals	12%	88%	0%	29%	71%	0%	0.60
48	Measures for pests control	77%	23%	0%	71%	29%	0%	0.90
49	Measure for the entrance of visitors	81%	19%	0%	86%	14%	0%	0.90
50	Measures of stable disinfection	n.a.	n.a.	n.a.	14%	86%	0%	n.a
51	Collection of carcase	42%	0.58%	0%	57%	43%	0%	0.78
52	Loading/unloading of animals	31%	69%	0%	43%	57%	0%	0.88
Health management								
53	Frequency of veterinary checks	0%	96%	4%	0%	100%	0%	0.98
54	Knowledge of main viral diseases	12%	88%	0%	14%	86%	0%	0.90
55	Knowledge of main bacterial diseases	31%	69%	0%	29%	71%	0%	0.90
56	Knowledge of main protozon diseases	0%	69%	31%	0%	71%	29%	0.90
57	Knowledge of main parasitic diseases	42%	27%	31%	29%	43%	29%	0.69
58	Vaccination programs	69%	31%	0%	86%	14%	0%	0.69
59	Parasitic management	46%	31%	23%	29%	29%	43%	0.54
60	Control and prevention of hoof disorders	12%	65%	23%	14%	86%	0%	0.37
61	Control and prevention of dental disorders	69%	31%	0%	29%	71%	0%	0.12
62	Health management of the foal	58%	42%	0%	57%	43%	0%	0.96
63	Age at weaning	0%	0%	100%	0%	0%	100%	1
64	Abortions	4%	0%	96%	14%	0%	86%	0.89
65	Choice of antimicrobial treatments	15%	85%	0%	0%	86%	14%	0.09
66	Choice of antiparasitic treatments	35%	50%	15%	43%	43%	14%	0.92

*statistical significance: p-value < 0.05.

difficulty in identifying pain and injuries is compounded by less frequent human contact and handling in pasture compared to stable (Armbrecht et al. 2019). Within the 'training' area, the welfare item 'animal grouping strategy in relation to feeding' represented another critical aspect at pasture, accounting for 100% of inadequate answers compared to 43% of inadequate answers in stables (Table 2). This latter finding was in agreement with the outcomes of the welfare item 'feeding management' of the **'feeding' area**. The 'feeding management' was described as feeding horses with rations which satisfy their nutritional requirements according to their physiological stage. On pasture, the horses were only fed through grazing, and no other feedstuff or supplement was provided. Moreover, animals were not fed according to their physiological status and this implied that there were not appropriately balanced diets for the mineral-vitamin content necessary to support the various stages of lactation and pregnancy of the broodmares or the growth of the foals. However, the presence of pasture represents a beneficial aspect for the horses since in nature they spend at least the 60% (equal to 16-18 h of the day) grazing while freely and slowly moving (Davidson and Harris 2007). Also on stable the Catria horses could express this behaviour, since the animals had the possibility to access outdoor paddock areas in which they could satisfy their physiological and ethological need for grazing. However, it is interesting to note that the 86% of the horses kept in stable were fed with inadequate concentrate amounts (Table 2), overloading the recommended safe level of 1 g of starch/kg bodyweight/meal (Harris and Shepherd 2021). This is a critical aspect for horse welfare since it is well known that diets rich in starch represent a risk factors for the onset of gastrointestinal (Colombino et al. 2022), metabolic (Pollitt and Visser 2010) and behavioural disorders (Bulmer et al. 2015).

Regarding the **'facilities' area**, the two farming systems resulted different according to the welfare items 'outdoor shelters' and 'management of the delivery area' (Table 2). In fact, even if the 'outdoor shelters' resulted adequate in 88% of the cases for the horse-units kept on pasture, the 43% of the stables had outdoor shelters judged as ideal, being easily accessible, numerically adequate and suitable to protect horses from adverse environmental conditions. This is not surprising since on pasture the shelters were mainly natural (trees and shrubs). Interestingly, it is reported that on pasture horses prefer to use artificial shelters during temperatures exceeding the thermal neutral zone as well as during cold, rainy, or windy conditions,

and when bothered by insects (Snoeks et al. 2015). This suggests that natural shelters maybe be not sufficient enough to protect animals from adverse climate conditions. Moreover, the 'management of the delivery area' resulted different between pasture and stable. No area of the surveyed pastures was dedicated to foaling (100%) and 57% of the stables did not have specifically designated box (Table 2). This is reflected in the outcomes related to the 'health management of the foal', that was found to be inadequate in more than the 50% of the cases for both stable and pasture. Interestingly, within the **'ABMs' area**, even if no statistical differences were found between pasture and stable (Table 2), the 'annual mortality rate of the foals' was $\geq 4.5\%$ in 46% of pastures, while in stables it was lower than the 2.5% in the majority of cases. This finding could be related to the interactions of multiple factors on pasture such as the inadequate 'health management of the foal' and the inadequate 'inspection of the animals' by the stockpersons. Also the presence of wild predators such as wolves may play a role on foal mortality at pasture, even if it is reported that livestock losses due to predators could be relative low (Temple and Manteca 2020). The ABMs used in the present study were adapted from the welfare protocol intended for horses reared for meat production in intensive farming systems published by Raspa et al. (Raspa et al. 2020a). However, the VARNC index (Table 1) revealed that the variability of answers was very similar between the two farming systems for most of the evaluated ABMs, except for the welfare items 'signs of lameness' and 'annual mortality rate of the foals'. An expert opinion elicitation should be performed in order to judge the appropriateness of the ABMs used in the welfare protocol developed in this study. In fact, Bertocchi et al. (2018) used this approach to characterise the ABMs included in the welfare assessment protocol they developed for dairy cow. In particular, the authors found that the observation of lameness, the annual mortality rate of calves and the annual mortality rate of adult cows were the most appropriate ABMs.

The cut-off values of the welfare items included in the 'biosecurity' and 'health management' areas were determined by the focus group to describe the health status of the two farming systems (pasture and stable). Considering the **'biosecurity' area**, the 73% of pastures had multispecies grazing, whereas stables were characterised by the presence of one single reared species (Table 2). Multispecies grazing is reported to be advantageous from an ecological point of view since it can increase vegetation use as a result of the complementarity of dietary choice (Fleurance et al.

2022). However, multispecies grazing can increase health risks especially when animals come from different stables with different health background. Interestingly, the presence of 'measures for pests control' and 'measures for the entrance of visitors' resulted to be the main welfare issues for both the pasture and the stable, being far from the ideal condition (Figure 2). In fact, more than the 70% of the answers collected resulted inadequate for both pasture and stable (Table 2), representing an important weakness to be addressed in order to prevent the spread of pathogens among animals.

Within the **'health management' area** the welfare items 'parasitic management', 'control and prevention of dental disorders' and 'health management of the foal' resulted inadequate for at least 50% of the surveyed pastures (Table 2). This condition is related to the intrinsic feature of the pasture that does not allow to properly treat and monitor animals according to consistent daily inspections (see the 'training' area). Farmers were also interviewed about the strategies used to avoid parasites (e.g. rotational grazing strategy) that represent a pasture-specific risk responsible for diseases transmission as babesiosis or anaplasmosis (Aubé et al., 2022).

The proposed welfare assessment protocol resulted easily applicable both on pasture and on farm, yet further validation in field involving more stables and evaluating inter– and intra–observer reliability as well as on-pasture and on-stable feasibility is needed. The findings obtained from the first application of the developed protocol revealed that Italian Catria horses raised for meat production on Mount Catria and neighbouring areas of the Central Apennines could be characterised by an adequate level of animal welfare both on pasture and on stable. However, some weakness points were identified in both farming systems. In particular, the main welfare issues were related to 'inspection of the animals' and 'feeding management' on pasture; and 'concentrates feeding' on stable. For both pasture and stable, 'management of the delivery area' and biosecurity measures in terms of 'presence of other reared species', 'measures for pests control', 'measures for the entrance of visitors', 'health management of the foal' could represented the critical welfare items needing improvement.

Conclusions

The present study allowed to develop and test a welfare assessment protocol for horses suitable to be applied both on pasture and on stable. The protocol is based on non-animal-based measures (N-ABMs) and animal-based measures (ABMs). Yet, further steps are needed to validate the protocol by expert knowledge elicitation and by the application of a risk assessment methodology. This first application of the proposed welfare protocol revealed that Italian Catria horse breed were characterised by some differences between the two breeding systems (pasture vs stable). Differences were found within the 'training' and 'feeding' thematic areas, yet a low dissimilarity was found according to the animal-based outputs. Weakness points for the pasture could be represented by the low frequency of animal inspections and by the absence of balanced rations that take into account the nutritional requirements of horses according to their physiological status. However, horses kept on stable were fed with high amounts of starch in their diet exposing them to the risk of gastrointestinal disorders. Weakness points for both pasture and stable could be some welfare items related to the health management ('health management of the foal', 'hoof care', 'dental care' and 'parasite management') and the biosecurity area ('measures of pests control' and 'measures for the entrance of visitors'). After proper validation, the welfare protocol developed in the present study could help to fill the existing gap of knowledge on horse welfare assessment for semi-extensive systems and to identify the main weakness points to be addressed with preventive interventions.

Ethical approval

The study followed the guidelines of the current European Directive (2010/63/EU) on the protection of animals used for scientific purposes, and was approved by the Ethical Committee of the Department of Veterinary Sciences of the University of Turin (Italy, Prot. 1129 04/21/2021).

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The authors report there are no competing interests to declare.

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Data availability statement

Data available upon reasonable request from the corresponding author.

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