Assessment of the nutritional status of lactating donkeys by an integrated approach as a tool for breeding management

ABSTRACT: The assessment of the nutritional status (NS) represents a complex task, because it depends by several factors. This study aims to define which factors mostly affect the lactating donkey's NS, considering parameters that can be easily measured in field conditions; to implement the scoring system for neck adiposity specifically for donkey; and to evaluate the stockperson perception of lactating donkey's NS. Fifty-three healthy lactating donkeys, 7 Martinafranca, 10 Ragusana, 2 Romagnola, and 34 crossbreeds were evaluated. Bodyweight (BW), lengths, girth and abdominal (waist) circumferences, neck length, neck height (NH), neck thickness (NT) and neck circumferences were measured, and body condition score (BCS) and fatty neck score (FNS) were rated. Also stockperson BCS evaluation was assessed. For each animal included in the study, oral cavity observation was performed and month of lactation was recorded.

A Principal Component analysis, performed including all the variables measured, revealed 3 principal components (PC) that together explained 84.37% of the variation of the NS among the lactating donkeys. PC1 revealed meaningful relations between BCS and neck measurements. Age and month of lactation seemed to be more independent factors affecting the NS. NH and NT had the strongest positive association with FNS ($r_s = 0.83$; $P < 0.001$). Mean NC:neck heigh ratio and Mean NC:neck thickness ratio had the strongest negative association with FNS ($r_s = -0.83$; $P < 0.001$) ($r_s = -0.82$; $P < 0.001$), respectively. No correlations were found between BCS and body morphometric measurements. Stockperson evaluation resulted to be influenced by abdominal (waist circumference) ($r_s = 0.41$, $P = 0.002$), in contrast to researchers' assessment.

A significant inverse relationship was highlighted between BCS and the presence of dental abnormalities (Chi squared $P < 0.05$).

The overall adiposity is the most indicator for the NS evaluation: the assessment of FNS and BCS seem to improve the assessment of the NS. Body morphometric measurements are not adequate for the NS assessment of donkeys. NT was a suitable morphometric for assessment of neck regional adiposity. During NS evaluation resulted important to consider the inspection of mouth conditions and the stage of lactation. Breeders need additional training in how to properly evaluate the NS.

Key words: Donkey, nutritional status, body condition, management, fatty neck score

INTRODUCTION

The nutritional status of livestock has a great importance in the breeding farm. It influences their productivity, health, reproduction and welfare (Quaresma et al., 2013). Therefore, the assessment of the nutritional status (NS) is considered a fundamental indicator for the overall physical exam of livestock animals. In particular, several studies have shown its importance in dairy animal breeding (Mulligan et al., 2006). In the last few years the breeding of donkeys for the production of milk has created a lot of interest. This product is of growing relevance and scientific interest in Europe, where it is used for different purposes. It is proven that it can be a useful substitute foodstuff for children affected by cow's milk protein allergy or multiple food intolerance (Monti et al., 2007; Monti et al., 2012) and a role of donkey milk for arteriosclerosis prevention and modulation of the immune response in elderly people has been suggested (Tafaro et al., 2007; Amati et al., 2010). The donkey milk has also been reported as probiotic food (Coppola et al., 2002) and it is widely used in Europe for cosmetic production (Cosentino et al., 2012). In addition, it has been showed that donkey milk possesses interesting antimicrobial that an important role in the improvement of the host defence in the infant newborn and infants which either cannot be nourished by human milk or are allergic to other milks. (Nazzaro et al., 2010). According to D'Alessandro and Martemucci (2011) these potential uses of donkey's milk have shown a positive trend in donkey population, with an increase of the number of animals bred in Italy. Although several data covering the potential of donkey's milk use is available, the information concerning the management and the assessment of lactating donkeys' NS is lacking. It is widely recognized that the assessment of NS of livestock can represent a complex task (Leitch, 1962). It is described by many parameters

and is not simple to recognize which is the most important (Becvarova et al., 2009) since they are linked in a intricate series of interactions.

Among the methods that can be used to evaluate the NS, body condition score (BCS) method is one of the most frequently adopted. Different BCS scoring systems are know and they include the visual appraisal and palpation of the adipose tissue site by use of a 5 or 9-point scale (Burden, 2012; Pearson and Ouassat, 2000). Other indexes from anthropometric measurements are not applicable since body morphometric may vary within the same species even if many are proposed for horses and ponies (Carter et al,., 2009; Pleasant et al., 2013) and donkeys (Mendoza et al., 2015). However more objective techniques to measure subcutaneous fat and BCS correlation such as dilution techniques, computed tomography or dual-energy X-ray absorptiometry (Quaresma et all., 2013) present important limitations as they are expensive and not easily applicable on farm.

Nevertheless, the animal-based indicators such as BCS used to assess appropriate nutrition are an essential component of the equine welfare assessment (Dalla Costa et al., 2014) and for assessing NS which may be an indicator linked to the overall welfare and animal production (Ireland et al., 2012). According to Cappai et al. (2013) it is necessary to emphasize that a complete assessment of the NS is the result of the evaluation of several components like age, physiological status, body morphometry, adiposities site localization and extension, the status of dental board (Du Toit et al., 2008, Du Toit et al., 2009). Also the stockperson plays an important role in maintaining and guaranteeing the NS, since they are essential to determining animal welfare and performance (Hemsworth and Coleman, 2000). According to the Equine health welfare strategy it is the keeper's responsibility to be able to recognize disease and seek for any veterinary assistance (Ireland et al., 2012)

Since nutritional assessment may have a direct impact on both dairy donkey production and welfare further investigations are needed. The objectives of this study focus on measuring and

defining parameters that are related to nutritional assessment that can be used by producers and clinicians. The aims of the study are: 1) to define which factors mostly affect the lactating donkey's NS, considering parameters that can be readily assessed in order to provide economic, simple and rapid tools, for both clinician and breeder for performing a lactating donkeys' NS evaluation; 2) to implement the scoring system for neck adiposity specifically designed for the donkey and evaluate the association between measurements for the assessment of the overall adiposity in donkey; 3) to evaluate the perception of the breeder of the lactating donkey's NS.

MATERIALS AND METHODS

The institutional animal care and use committee statement should appear as the first item in MATERIALS AND METHODS and should specify which publically available animal care and use standards were followed (JAS).

Population Description

This study was conducted during the spring season (May to June). All the lactating donkeys present in dairy farms located in the North West of Italy were evaluated. The animal were kept in six breeding farms authorized from Italian's governerment welfare rules for equids (OR ASL TO-CN) to produce and commercialize milk. All the donkeys included in this study were bred in semi-extensive farms with free access to drinking water and forages. Fifty-three healthy lactating donkeys, 7 Martinafranca, 10 Ragusana, 2 Romagnola, and 34 crossbreeds, of age (median (interquartile range)) 9 (7-12) years, estimated bodyweight (BW) 314.5 (269-350) kg and mean month of lactation 4±3 months were examined.

Morphometric measurements

The following body measurements were taken on each animal, using a soft measuring tape: 1) body length, measured from the point of the shoulder (intermediate tubercle of humerus) to the pin bone (ischiatic tuberosity); 2) body length, measured from the point of the shoulder (intermediate tubercle of humerus) to the point of the hip (tuber coxae) in centimetres (cm); 3) girth circumference, taken around the body, caudal to the elbow (olecranon tuber), two centimetres behind the highest point of the withers; 4) waist (abdominal) circumference, measured two-thirds of the distance from the point of the shoulder to the point of the hip; 5) neck length, from the poll to the highest point of the withers; 6) neck circumference (NC) at 0.25, 0.50, 0.75 of neck length; 7) neck height (NH) at 0.50 of neck length, taken from the dorsal midline of the neck to estimated differentiation between the crest (tissue apparent above the *ligamentum nuchae*) and neck musculature; 8) neck thickness (NT) at 0.50 of neck length, from the estimated differentiation between the crest and the neck musculature, from one side to the other of the neck.

Bodyweight (BW) was calculated using the formula suggested by Pearson and Ouassat (2000):

$$BW(kg) = [girth(cm)^{2.12} \times length(cm)^{0.688}]/3801$$

Four independent trained assessors rated the body condition score (BCS) from 1 (poor) to 5 (obese), using a scoring system previously established (Burden, 2012). The median of four scores rounded to nearest whole or half score increment was used for the analysis. Intra-class correlation coefficients for the reliability of individual assessors scores were 0.85 for BCS and 0.58 for FNS.

The stockpersons were asked to evaluate the body condition score according to a 5 point scale, with the help of a chart representing the different scores (bibliographic reference). The farmers rated the BCS through visual assessment and palpation of animals.

Even if a neck score was proposed by Mendoza et al., 2015 on a 0-4 points scale, The authors developed a new judgment system of the neck fat deposition (fatty neck score) based on 0-5 points scale as already developed for horses. The researchers judged the fatty neck score (FNS) with visual inspection and palpation of the fat deposition between the topline of the neck and the muscular line, as described in Table 1. This scoring system is based on a 6 point scale in the same way as proposed for horses (Carter et al., 2009).

Oral cavity assessment

Observation of oral cavity was performed to assess the presence of quidding and dental abnormality like sharp points and hooks. The same evaluator used a scale for assessment of the mouth conditions where 0 is “normal ”, 1 is “discrete teeth conditions and quidding ”, 2 is “poor ".

Statistical analyses

The analysis was performed with IBM SPSS Statistics 21 software (IBM corp., 2012). Median (interquartiles) and mean values [± SD] were calculated for the following parameters: age, month of lactation and body measurements. A Principal Component Analysis PCA (correlation matrix, no transformation) was performed to reduce the variables to factors: data assumption was checked, Keiser-Meyer-Olkin (KMO) and Barlett’s test were performed to test the suitability of the data for structure detection. Factors with eigenvalues greater than 1 were considered. Ratios of girth:length, girth:BW, waist:length, waist:BW, waist:girth, 0.50 NC:neck length, 0.50 NC:neck height, 0.50 NC:neck thickness, mean NC: neck length, mean NC:neck height, mean NC:neck thickness were calculated. Mean NC was calculated as the average of 0.25 NC, 0.50 NC and 0.75 NC. Possible associations between variables were quantified using Spearman’s rank correlation coefficient (r_s).

Inter-observer reliability of researchers and farmers when assessing BCS was evaluated by intra-class correlations and Kendall's Coefficient of concordance. Chi-squared test was used to investigate possible relations between researchers'-BCS with lactating donkeys' mouth conditions.

RESULTS

A good suitability of data for PCA was valued (KMO = 0.80 and Barlett's test, $P < 0.001$). The PCA was performed including all the variables measured on each animal that represent the most used parameters in the practice to evaluate the NS. It revealed 3 principal components that together explained 84.37% of the variation of the NS among lactating donkeys. Table 2 and Fig. 2 display the loadings of the variables on the first, second and third component and show how the different variables are related to each other. In particular, FNS, NT, BCS and NH present high positive loadings on the first component and seem to be related in the definition of the NS. Always on the first component, the presence of dental abnormalities shows high negative loadings and appears to negatively influence the NS. PC2 and PC3 are characterized by month of lactation and age respectively that probably have a more independent influence on the NS (Table 2).

Median BCS and FNS for lactating donkeys were 2.5 (2–3) and 2.5 (1.5–3) respectively. No significant correlations between morphometric measurements and BCS were found (Table 3), however, our results highlighted a positive and significant correlation between BCS and FNS ($P < 0.001$) (Table 3).

Moreover, FNS was positively associated with NH and NT ($P < 0.001$) and negatively associated with mean NC:neck height and mean NC:neck thickness ($P < 0.001$) (Table 4; Fig. 4). We found also other significant but lower correlations between FNS and 0.50 NC:neck height and 0.50 NC:neck thickness ($P < 0.001$) (Table 4; Fig. 5).

On the overall, our results suggest that the FNS developed in this study and described in Table 1 is the most explicative variable for describing the NS of lactating donkeys. Generally, the NT is proposed as an index of the neck adiposity. However, in consideration of anecdotal evidence, we proposed to measure not only the NT but also the NH as a parameter that could be associated to the FNS score. Both parameters are well associated to the score proposed ($P < 0.001$). For this reason, a reference range table of the NT according to the FNS score is proposed (Table 1).

The Kendall's coefficient of concordance between researchers' and farmers' scores for BCS was low (0.28) showing a substantial disagreement between their evaluation. We only found a significant but rather low correlation between waist (abdominal) circumference and farmer's-BCS ($r_s = 0.41$, $P = 0.002$) (Table 3).

An inverse relationship was pointed out when testing researchers'-BCS and mouth conditions (Chi squared $P < 0.05$).

DISCUSSION

The NS is a fundamental indicator of health and welfare but, although in recent years the number of dairy donkey farms is increasing, its assessment has not been thoroughly investigated yet in this species. However, the evaluation of the NS is a complicated task, since different variables are used for measure the NS in producing animals. Thus, the mechanism that determines the NS is too complex to be explained only by correlation and univariate analysis. For this reason the PCA could help to identify which components are important to explain the NS. Then, a PCA analysis was performed including all the variables measured in this study that represent the most used parameters in the practice to evaluate the NS under different points of view. According to Table 2 , three principal components were identified and

224 together they describe the 84.37% of the variation among the lactating donkeys included in our
225 study. The component one has the high loading for FNS, NT, BCS and NH. Consequently, it
226 could be identified as the overall adiposity status of the animal. In particular, the FNS is resulted
227 as the main factor that describe the component one. However, it represents a regional fat
228 deposition (Carter et al., 2009). Indeed, donkeys lay down fat stores in localized areas,
229 especially on the neck, and these regional deposits can remain when overall weight is loss
230 (Burden, 2012; Burden and Thiemann, 2015). The second component was described by the
231 month of lactation. This factor should be always considered during the evaluation of the NS of
232 dairy animals. In fact, the lactation period is an high demand period and consequently, if the
233 animals are not adequately supported, they can lose their BW (Heidler et al., 2004; Mulligan
234 et al., 2006; Roche et al., 2007). The third component includes the age of the animals that
235 probably is an independent factor related to the animal and not to the NS (Michela potresti
236 aggiungere un commento relativo ai dati grezzi della tabella?)
237 At the moment the most common method used to assess the NS, even in donkeys, is by
238 evaluating the body condition score (BCS) and different BCS scoring systems, specific for
239 donkeys, have been proposed and developed (Pearson et Ouassat, 2000; Burden, 2012). The
240 BCS systems include both visual and palpation appraisal of adipose tissue and then, a score
241 from 1-5 or 1-9, depending on which scale is used, is assigned (Pearson et Ouassat, 2000;
242 Burden, 2012). The donkeys tend to be predisposed to depositing fat in localized areas: neck,
243 rib cage and rump. However, when utilizing BCS as a source to measure the NS and or welfare,
244 one must keep in mind that there is a certain level of subjectivity. Consequently, for improved
245 evaluation of NS, it is best to combine several parameters along with BCS to evaluate the NS
246 in lactating donkeys. In fact the NS is a complicated task because the status and the needs may
247 change according to the stage of lactation and or gestation and or work level of the animal.

Body morphometrics have been proposed for evaluating the NS of animals (Becvarova et al., 2009). In particular, morphometric measurements are suggested to analyzed the NS of equines (Cappai et al., 2013; Martinson et al., 2014). Also, correlation were previously demonstrated between BCS and morphometric measurements (Carter et al., 2009; Dugdale et al., 2011). However, when measuring the morphometric parameters and comparing the results in this study, we found that there was not a correlation to morphometric parameters and BCS in donkeys. On this regard, it is important not forget that the donkey is not a small horse, although both belong to Equidae family. They are different for many aspects, including, in particular, anatomical variation and physical conformation (Burden and Thiemann, 2015). This variability is not only interspecific, but also intraspecific. In fact, according to Kugler et al. (2008), taking an overview of the donkey population data in Europe, mostly the animals are crossbreeds that cannot be categorized into specific breeds. Also in our study the donkeys were mainly crossbreeds , in contrast to most other livestock breeds were pedigree and high genetic selection exist. All led to a development of much diversity, in particular several differences can be found, especially as far as body size is concerned. Therefore, unlike horses and ponies, the morphometric measurements, although more easily performed in the absence of trained evaluators, cannot provide an objective alternative for lactating donkeys' NS evaluation.

Consequently, in the present study were analyzed other factors that should be considered during lactating donkeys' NS evaluation, in order to provide economic, simple and rapid tools, for both clinician and breeder. The FNS is the other parameter suggests to assess the NS, since it is evaluated during the BCS method. Our results show that FNS is well correlated with the BCS like described in other studies (Carter et al., 2009; Mendoza et al., 2015). However, as previously reported, we should keep in mind that it is an independent measurements of adiposity, since it represents a regional fat deposit (Carter et al., 2009, Burden, 2012, Giles et al., 2015) that it is not well investigated in donkeys. Our findings confirm that it is a regional

fat deposit that correlate with some of the morphological parameters of the neck dimension and for what it should be proposed a specific scoring scale. Either neck high (NH) and neck thickness (NT) are a suitable and objective morphometric measurements for the assessment of neck adiposity. However, in contrast to others studies, the FNS is not positive correlated with the ratio 0.50 NC:Neck height, Mean NC:Neck height, 0.50 NC:Neck thickness, Mean NC:Neck thickness (Table 4; Fig. 4; Fig. 5). On the contrary, it is significantly and negatively correlated with them. This result could be related to the fact that the shape of the neck of donkey is different from that of the horse. The donkey shorter neck and the more protruded manubrium support an heavy skull (Burden and Thiemann, 2015), leading to a remarkably thickness of the cutaneus colli muscle that even covers the middle one-third of the jugular furrow (Burnham, 2002). In our opinion, we used the NT for the evaluation of the FNS since, unlike the other equids, the adipose tissue tend to droop sideway to the crest of the neck (Burden, 2012). Using the population of lactating donkeys included in the present study, in was been possible to develop an objective scale of reference for the FNS, considering its strong association with NT (Table 1).

Furthermore, the findings of the study supports that mouth conditions and, in particular dental disorders, should be considered during the lactating donkeys' NS evaluation. According to Rodrigues et al. (2013) dental disorders, like sharp points and hooks, are recognized as major but often unnoticed and so not treated disorders of equids, including horses and donkeys. Besides, several studies have demonstrated that dental disorders in donkeys are associated with poor body condition score and weight loss (Du Toit et al., 2008, Du Toit et al., 2009), as well as our results confirm, revealing a significant inverse relationship between BCS and mouth conditions.

Interestingly, when the donkey dairy producers were asked to evaluate BCS, we did find that donkeys with a greater waist (abdominal) circumference have received a higher BCS from the

298 producer. Instead this finding was not true for the researchers whose were trained in BCS
299 scoring. Probably the owners are misled by anatomical abdomen conformation of the donkeys.
300 In fact, donkeys are anatomically characterized by pendulous abdomen (Pearson et al., 2001;
301 Burden, 2012). Furthermore, this result suggests that, even though a correlation between
302 morphometric measurements and BCS was not found, the producers may rely on morphometric
303 measurements to evaluate BCS.
304
305 **CONCLUSIONS**
306
307 The difficulty to define and assess the NS it is been further elucidated, highlighting the
308 importance to consider parameters that may be employed in field condition by both clinicians
309 and breeders. According to the PCA analysis the overall adiposity is the most indicator for the
310 NS evaluation. In particular, the assessment of FNS and BCS seem to improve the assessment
311 of the NS. Therefore, although the FNS is an indicator of regional adiposity, showing a well
312 correlation to neck morphometric measurements, it seems play also an important role in the NS
313 definition. For this reason, a score specific for donkeys is proposed to judge this regional
314 adiposity status. Furthermore, in contrast to other studies, the body morphometric
315 measurements are not adequate for the NS assessment of donkeys. Moreover, during the
316 examination of the donkey's NS should be always include the inspection of the mouth
317 condition, taking in account also the month of lactation. Instead, the age should be view as an
318 independent factor that could influence the BCS, especially in older animals that actually were
319 not involved in the present study.
320 The findings indicate also that breeders need additional training in how to properly evaluate
321 the NS because the current points of measurement and assessment may in the end compromise
322 the overall welfare of donkeys.

More study are required including an higher numbers of animals and considering also other variables that could affect the donkey's NS assessment, like feeding and management, to investigate any relationship.

REFERENCES

Amati, L., G. Marzulli, M. Martulli, A. Tafaro, F. Jirillo, V. Pugliese, G. Martemucci, A. G. D'Alessandro, and E. Jirillo. 2010. Donkey and goat milk intake and modulation of the human aged immune response. Curr. Pharm. Des. 16:864-9. doi: 10.2174/138161210790883651

Becvarova, I., R. Scott Pleasant, and C. D. Thatcher. 2009. Clinical assessment of nutritional status and feeding programs in horses. Vet. Clin. North Am Equine Pract. 25:1-21. doi: 10.1016/j.cveq.2009.01.001

Bertino. 2012. Tolerability of donkeys' in 92 highly-problematic cows' milk allergic children. J. Biol. Regul. Homeost. Agents. 26:75-82.

Burden, F. 2012. Practical feeding and condition scoring for donkeys and mules. Equine Vet Educ. 24:589-596. doi: 10.1111/j.2042-3292.2011.00314.x

Burden, F., and A. Thiemann. 2015. Donkeys are different. J. Equine Vet. Sci. 35:376-382. doi: http://dx.doi.org/10.1016/j.jevs.2015.03.005

Burnham, S. L. 2002. Anatomical differences of the donkey and mule. Proc. Annu. Conv. AAEP. 48:102-9

Cappai, M. G., M. Picciau, and W. Pinna. 2013. An integrated approach towards the nutritional assessment of the Sardinian donkey: a tool for clinical nutritionists. Ital. J. Anim. Sci. 12:182-185.

347 Carter, R. A., R. J. Geor, W. B. Staniar, T. A. Cubitt, and P. A. Harris. 2009. Apparent adiposity
348 assessed by standardised scoring systems and morphometric measurements in horses and
349 ponies. Vet. J. 179:204-210. doi: 10.1016/j.tvjl.2008.02.029
350 Coppola, R., E. Salimei, M. Succi, E. Sorrentino, M. Nanni, P. Ranieri, R. Belli Blanes, and L.
351 Grazia. 2002. Behaviour of Lactobacillus rhamnosus strains in ass's milk. Ann. Microbiol.
352 52:55-60.
353 Cosentino, C., R. Paolino, P. Freschi, and M. Calluso. 2012. Short communication: Jenny milk
354 production and qualitative characteristics. J. Dairy Sci. 95:2910-2915. doi:
355 10.3168/jds.2011-5232.
356 D'Alessandro, A. G., and Martemucci G. 2012. Lactation curve and effects of milking regimen
357 on milk yield and quality, and udder health in Martina Franca jennies (Equus asinus). J.
358 Anim. Sci. 90:669-681. doi: 10.2527/jas.2011-4283
359 Dalla Costa, E., L. Murray, F. Dai, E. Canali, and M. Minero. 2014. Equine on-farm welfare
360 assessment: a review of animal-based indicators. Anim. Welf. 23:323-341. doi:
361 10.7120/09627286.23.3.323
362 Du Toit, N., J. Gallagher, F. A. Burden, and P. M. Dixon. 2008. Post mortem survey of dental
363 disorders in 349 donkeys from an aged population (2005-2006). Part 1: Prevalence of
364 specific dental disorders. Equine Veterinary Journal. 40:209-213. doi:
365 10.2746/042516408X267104
366 Du Toit N, F. A. Burden, and P. M. Dixon. 2009. Clinical dental examinations of 357 donkeys
367 in the UK. Part 2: Epidemiological studies on the potential relationships between different
368 dental disorders, and between dental disease and systemic disorders. Equine Vet. J. 41: 395-
369 400. doi: 10.2746/042516408X267104
370 Dugdale, A. H. A., G. C. Curtis, P. A. Harris, and C. Mc. Argo. 2011. Assessment of body fat
371 in the pony: Part I. Relationships between the anatomical distribution of adipose tissue, body

composition and body condition. Equine Vet J. 43:552-56. doi: 10.1111/j.2042-3306.2010.00330.x

Giles, S. L., C. J. Nicol, S. A. Rands, and P. A. Harris. 2015. Assessing the seasonal prevalence and risk factors for nuchal crest adiposity in domestic horses and ponies using the Cresty Neck Score. BMC Vet. Res. 11:13. doi: 10.1186/s12917-015-0327-7.

Heidler, B., J. E. Aurich, W. Pohl, and C. Aurich. 2004. Body weight of mares and foals, estrous cycles and plasma glucose concentration in lactating and non-lactating Lipizzaner mares. Theriogenology. 61:883-893. doi:10.1016/S0093-691X(03)00279-6

Hemsworth, P.H., and G. J. Coleman. 2000. Relationships between human-animal interactions and productivity of commercial dairy cows. J. Anim. Sci. 78:2821-2831.

IBM Corp., 2012. IBM SPSS Statistics for Windows.

Ireland, J. L., P. D. Clegg, C. M. McGowan, S. A. McKane, K. J. Chandler, and G. L. Pinchbeck. 2012. Comparison of owner-reported health problems with veterinary assessment of geriatric horses in the United Kingdom. Equine Vet. J. 44:94-100. doi: 10.1111/j.2042-3306.2011.00394.x

Kugler, W., H. P. Grunenfelder, and E. Broxham. 2008. Donkey Breeds in Europe: Inventory, Description, Need for Action, Conservation. Report 2007/2008. Switzerland: Monitoring Institute for Rare Breeds and Seeds in Europe.

Leitch, I. 1962. The assessment of nutritional status in man: Chairman's opening remarks. Proceedings of the Nutrition Society. In: 152th scientific meeting, 65th Scottish meeting. 29 September 1962, Aberdeen, UK. 22:47-48.

Martinson, K. L., R. C. Coleman, A. K. Rendahl, Z. Fang, and M. E. McCue. 2014. Estimation of body weight and development of a body weight score for adult equids using morphometric measurements. J. Anim. Sci. 92:2230-2238. doi: 10.2527/jas.2013-6689

396 Mendoza, F. J., J. C. Estepa, C. A. Gonzalez-De Cara, R. Aguilera-Aguilera, R. E. Toribio, and
397 A. Perez-Ecija. 2015. Energy-related parameters and their association with age, gender, and
398 morphometric measurements in healthy donkeys. Vet. J. doi: 10.1016/j.tvjl.2015.03.004
399 Monti, G., E. Bertino, M. C. Muratore, A. Coscia, F. Cresi, L. Silvestro, C. Fabris, D.
400 Fortunato, M.G. Giuffrida, and A. Conti. 2007. Efficacy of donkey's milk in treating highly
401 problematic cow's milk allergic children: an in vivo and in vitro study. Pediatr Allergy
402 Immunol. 18:258-264. doi: 10.1111/j.1399-3038.2006.00521.x
403 Monti, G., S. Viola, C. Baro, F. Cresi, P. A. Tovo, G. Moro, M. P. Ferrero, A. Conti, and E.
404 Bertino. 2012. Tolerability of donkeys' in 92 highly-problematic cows' milk allergic
405 children. Journal of Biological Regulators & Homeostatic Agents. 3:75-82.
406 Mulligan, F. J., L. O'Gradya, D. A. Riceb, and M. L. Dohertya. 2006. A herd health approach
407 to dairy cow nutrition and production diseases of the transition cow. Anim. Reprod. Sci.
408 96:331-353. doi:10.1016/j.anireprosci.2006.08.011
409 Nazzaro, F., P. Orlando, F. Fratianni, and R. Coppola. 2010. Isolation of Components with
410 Antimicrobial Property from the Donkey Milk: A Preliminary Study. The Open Food
411 Science Journal. 4:43-47
412 Pearson, A. R., and M. Ouassat. 2000. A Guide to Live Weight Estimation and Body Condition
413 Scoring of Donkeys. Centre for Tropical Veterinary Medicine, Edinburgh, UK. ISBN 0-
414 907146-11-2
415 Pearson, R. A., R. F. Archibald, and R. H. Muirhead 2001. The effect of forage quality and
416 level of feeding on digestibility and gastrointestinal transit time of oat straw and alfalfa
417 given to ponies and donkeys. Br. J. Nutr. 85:599-606.
418 Pleasant, R. S., J. K. Suagee, C. D. Thatcher, F. Elvinger, and R. J. Geor. 2013. Adiposity,
419 Plasma Insulin, Leptin, Lipids, and Oxidative Stress in Mature Light Breed Horses. J. Vet.
420 Intern. Med. 27:576-582. doi: 10.1111/jvim.12056

421 Quaresma, M., R. Payan-Carreira, and S. R. Silva. 2013. Relationship between ultrasound
422 measurements of body fat reserves and body condition score in female donkeys. Vet. J.
423 197:329-334. doi: 10.1016/j.tvjl.2012.12.031
424 Roche, J. R., K. A. Macdonald, C. R. Burke, J. M. Lee, and D. P. Berry. 2007. Associations
425 among body condition score, body weight, and reproductive performance in seasonal-
426 calving dairy cattle. J. Dairy Sci. 90:376-391.
427 Rodrigues, J. B., S. Araújo, F. Sanroman-Llorens, E. Bastos, F. San Roman, and C. Viegas.
428 2013. A clinical survey evaluating the prevalence of incisor disorders in Zamorano-Leonés
429 and Mirandês donkeys (Equus asinus). J. Equine Vet. Sci. 33:710-718. doi:
430 http://dx.doi.org/10.1016/j.jevs.2012.11.012
431 Tafaro, A., T. Magrone, F. Jirillo, G. Martemucci, A. G. D'Alessandro, L. Amati, and E. Jirillo.
432 2007. Immunological Properties of Donkey's Milk: Its Potential Use in the Prevention of
433 Atherosclerosis. Curr. Pharm. Des. 13:3711-3717.
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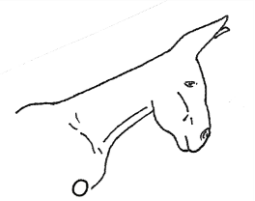
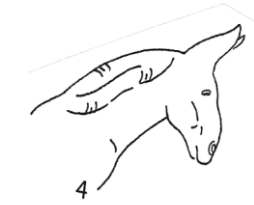
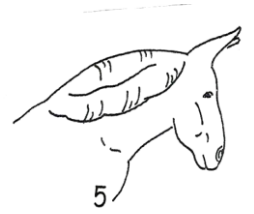
436
437 **Table 1.** Fatty neck scoring system for donkeys
438

Score	Illustrations of individual fatty neck score	Description	Neck thickness range according to FNS (in cm)
0		Neck thin with absence of a visual and palpable crest.	<14

1		Neck still thin with crest no visible, but slight filling felt with palpation.	>14-19
2		Neck with moderate deposition of fat. Noticeable appearance of a crest, with fat deposited fairly evenly from poll to withers. Crest easily cupped in one hand and bent from side to side.	>19-22
3		Neck enlarged and thickened. Crest is palpable from poll to withers and fills cupped hand and begins to make longitudinal fat deposit to both sides of the neck.	>22-27
4		Neck very enlarged and thickened. Crest grossly thickened with fat deposits from poll to withers, forming longitudinal bands of fat on the both neck sides. Crest cannot easily bent from side to side.	>27-34
5		Neck very enlarged and thickened. Crest very thickened with hard fat deposits, rounded along the two sides of the neck.	>34

439

440 **Table 2.** PCA of quantitative data calculated from correlation matrix for lactating donkeys
441 sampled
442

	Components		
	1	2	3
Age	-0.224	0.498	**0.821**[a]
Month of lactation	-0.208	**0.758**	-0.452
BCS[b]	**0.896**	-0.045	0.170

Neck thickness at 0.50	**0.915**	0.291	-0.040
Neck height at 0.50	**0.883**	0.316	-0.100
FNS[c]	**0.944**	0.042	0.043
Mouth conditions	**-0.594**	0.463	-0.036

[a] For each parameter, the higher loadings are bold typed

[b] Body condition score

[c] Fatty neck score

Table 3. Associations of body condition score (BCS) with morphometric measurements of body adiposity

Morphometric measurement	researchers'-BCS (n = 53)	
	r_s[a]	p[b]
BW[c]	0.15	0.13
Girth	0.15	0.27
Waist (abdominal)	0.22	0.11

Girth:Length	-0.02	0.87
Girth:BW	0.13	0.15
Waist:Length	0.11	0.25
Waist:BW	0.13	0.17
Waist:Girth	-0.13	0.17
FNS[d]	0.84	< 0.001

[a] Spearman rank correlation coefficient.

[b] P value for a test of the null hypothesis that the variables are independent.

[c] Bodyweight

[d] Fatty neck score

Table 4. Associations of fatty neck score (FNS) with morphometric measurements of neck adiposity

Morphometric measurement	FNS (n = 53)	
	r_s[a]	p[b]
0.25 NC[d]	0.37	0.007
0.50 NC[e]	0.42	0.002
0.75 NC[f]	0.40	0.003
Mean NC[g]	0.44	0.001

0.50 NC:Neck length	0.35	0.011
Mean NC:Neck lenght	-0.01	0.925
0.50 NC:Neck height	-0.58	<0.001
Mean NC:Neck height	-0.83	<0.001
Neck Height 0.50	0.83	<0.001
0.50 NC:Neck thickness	-0.68	<0.001
Mean NC:Neck thickness	-0.82	<0.001
Neck Thickness 0.50	0.83	<0.001

[a] Spearman rank correlation coefficient.

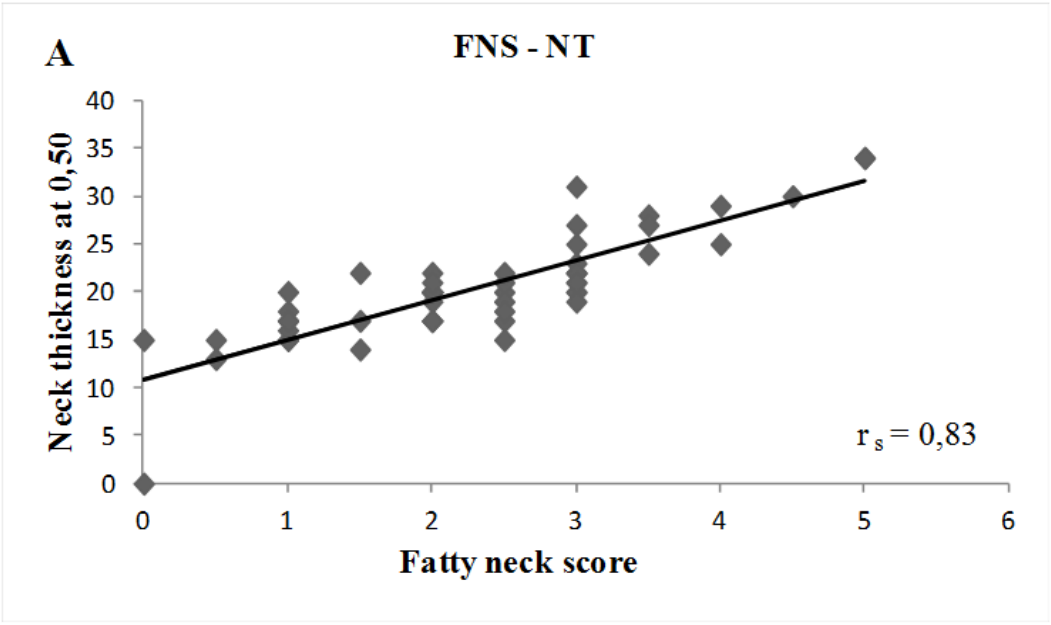
[b] P value for a test of the null hypothesis that the variables are independent.

[d] Neck circumference at 0.25 neck length

[e] Neck circumference at 0.50 neck length

[f] Neck circumference at 0.75 neck length

[g] Average of 0.25 NC, 0.50 NC, 0.75 NC



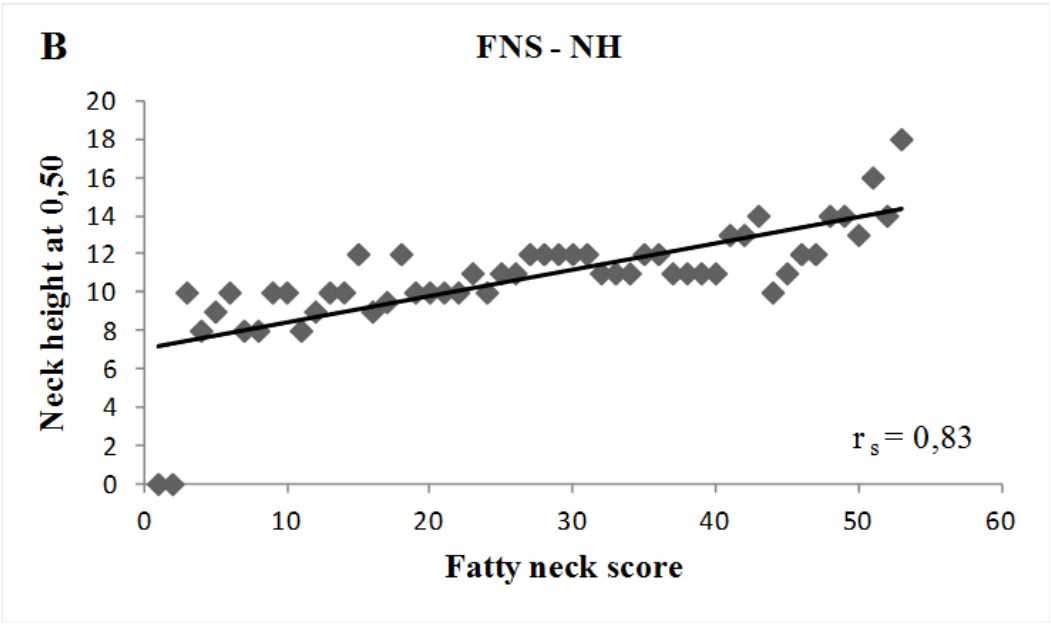


Figure 3. Linear relationship of neck thickness at 0.50 and fatty neck score (A) ($P < 0.001$). Linear relationship of neck height at 0.50 and fatty neck score (B) ($P < 0.001$)

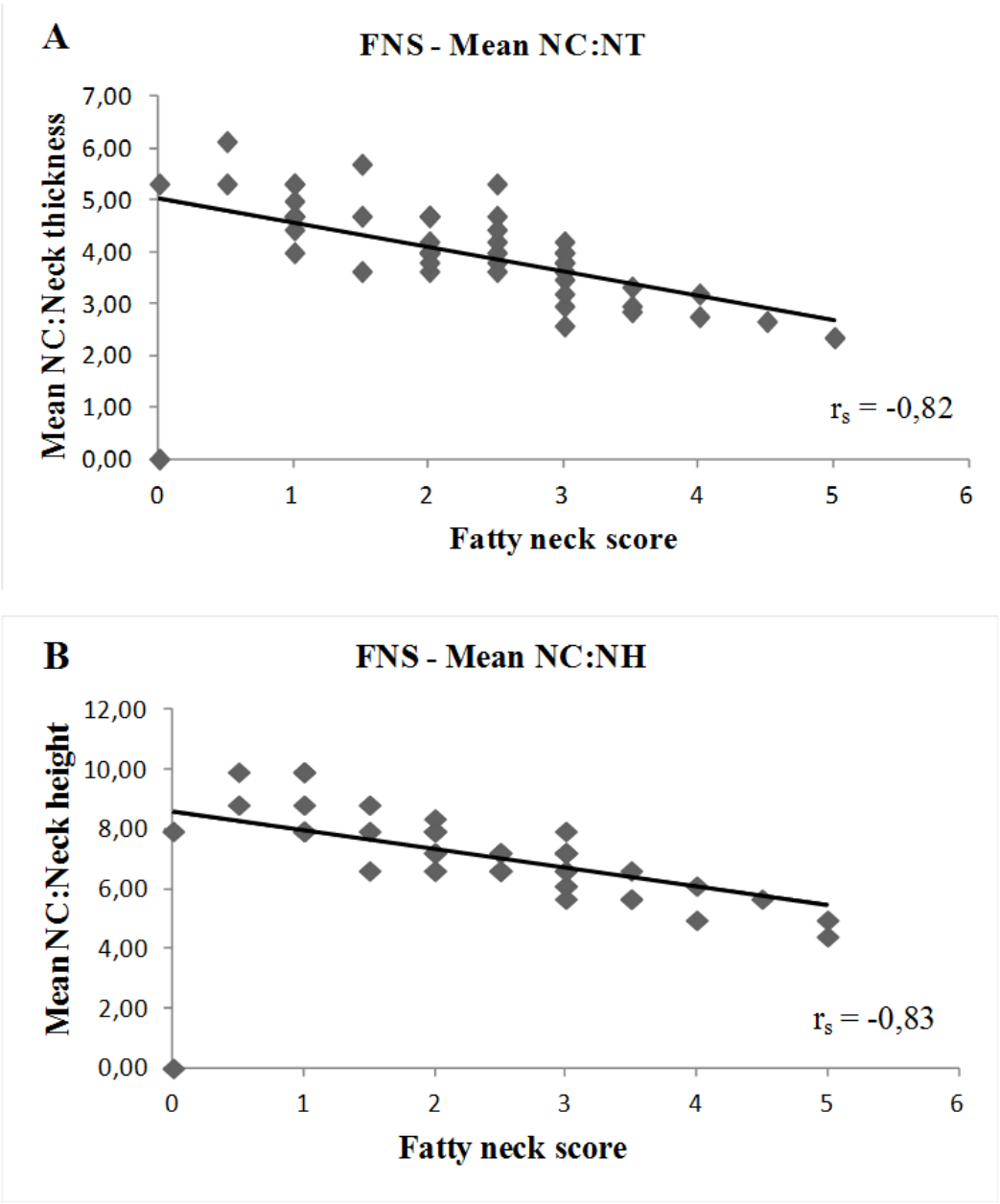


Figure 4. Linear relationship of Mean NC:Neck thickness and fatty neck score ($P < 0.001$) (A). Linear relationship of mean NC:Neck height and fatty neck score ($P < 0.001$) (B).

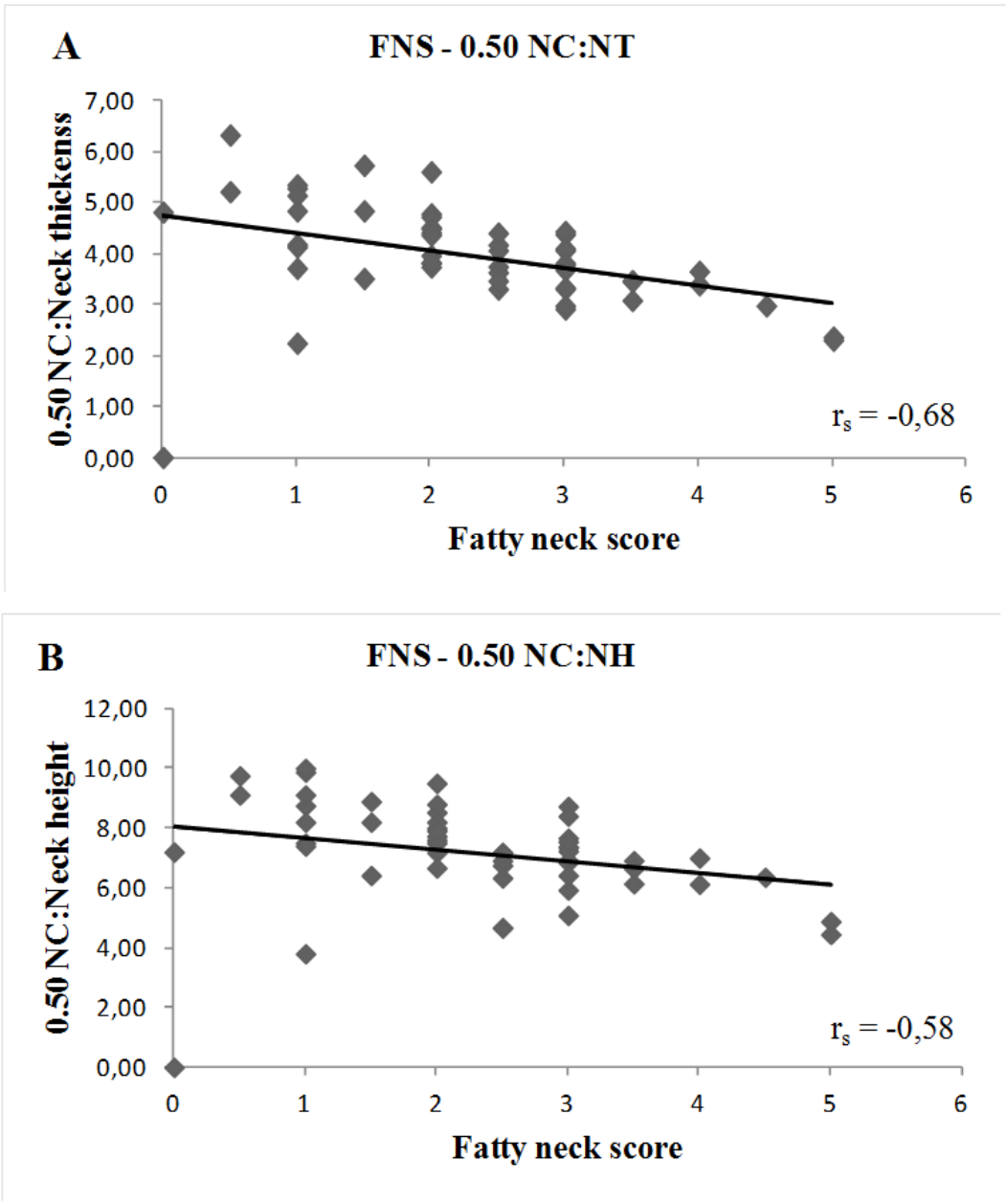


Figure 5. Linear relationship of 0.50 NC:Neck thickness and fatty neck score ($P < 0.001$) (A). Linear relationship of 0.50 NC:Neck height and fatty neck score ($P < 0.001$) (B).