An integrated approach towards the nutritional assessment of the Sardinian donkey: a tool for clinical nutritionists

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Abstract

The aims of the current study were to establish: i) a species-specific approach for nutritional assessment during clinical checks on Sarda breed adult donkeys, and ii) a suitable equation for body weight prediction, which at present is lacking. A total of 18 adult donkeys were sampled and the following criteria were recorded: body weight (BW), body condition score (BCS), muscular mass index (MMI), along with somatometric measures (height at withers; chest girth) and metabolic profiles of each individual. The species-specific nutritional assessment based on the integrated approach turned out to correlate significantly (P<0.05) with the body condition score and levels of Na, P and cholesterol in blood. The parametric equation for predicting the BW (y=0.335a+0.443b−7.62, where a= height at withers in cm; b= chest girth in cm) closely fit the determined BW, with a standard error of SE=±5 kg BW.

Introduction

Nutritional assessment is considered to be a fundamental indicator during the veterinary checkup for the overall health status evaluation of the animal, with emaciation and obesity representing the extreme points on the scale of evaluation of the veterinary clinical nutritionist. In between, various physiological and physio-pathological conditions can occur. The effects of the breed, age and exercise can influence the nutritional status. It is necessary to underline that nutritional assessment and that of body condition are based on different concepts: the complete assessment of the nutritional status is the result of several components and should therefore be based on: i) body morphology and the stage of skeletal development (in growing and adult animals); ii) the distribution and the extent of adiposities; iii) the body mass determination; and iv) the muscular condition. In addition, the metabolic profile provides the biochemical information useful to screen out the systemic metabolic pathways. In veterinary practice, at least as far as the donkey species is regarded, nutritional assessment is not systematically included in clinical checks. Moreover, the Body Condition Score (BCS) system and the predictive equation for body weight (BW) estimation based on body measurements are widely developed for horses and have been standardized for different horse breeds (Henneke et al., 1983; Jones et al., 1989; Martin-Rosset,1990; Bergero, 1996; Miraglia et al., 1998). However, to our knowledge, very few species-specific methods and techniques have been developed for the donkey species to date (Pearson and Oussat, 1996; Kay et al., 2000; De Aluja et al., 2005).

The Sarda breed donkey is an autochthonous breed from the island of Sardegna, in the Mediterranean Sea. The distribution and population size of the breed dramatically decreased after World War II, when widespread abandonment of traditional rural activities took place. These donkeys were mainly used for farmwork and as a means of transportation up to the last century; at present, due to their small size (Pinna et al., 1994) and their very tame temperament, they are used for riding and are involved in specific pet therapy programs. Breeders have become progressively interested in these animals and the breed is reared not only in Sardegna, but also in other Italian regions and in other European countries. The Sarda breed occupies the third position in terms of population size in the Official National Registry, yet nevertheless the number of animals reared in the Sardegna region is 6.3% of the total donkeys reared in the whole of Italy, ranking as the 8th of the 20 Italian regions (AIA, 2011), according to the number of registered donkeys per region. This indicates that breeders of the Sarda donkey are not concentrated in Sardegna, but are distributed across the national territory, and furthermore that one out of every two donkeys is reared as a companion animal on the farms. An appropriate technique for nutritional assessment for the donkey species is needed to avoid the risk of malnutrition or nutrient imbalances that could lead to poor animal welfare. Moreover, dietary errors following inappropriate estimates of requirements might even complicate the clinical conditions of diseased animals and give rise to further pathologies.

This investigation was undertaken with the purposes of establishing: i) a species-specific approach for nutritional assessment during clinical checks; and ii) a suitable equation for body weight prediction in Sarda breed adult donkeys.

Materials and methods

Animals and care

Eighteen adult donkeys of the Sarda breed, from the Equine Research Center of the Regional Agency for Research of the Autonomous Region of Sardegna, all recorded in the Official Register as being of the Sarda breed, were chosen as the sample for this study. The inclusion criteria in the trial were: same farming conditions and homogeneous age (ranging from 5 to 7 years).

Key words: Donkey, Nutritional assessment, Body condition score, Metabolic profile, Predictive equation.

Contributions: MGC, MP, data analysis in relation to the nutritional hints, manuscript drafting; WP, manuscript design participation, critical revision.

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All the procedures presented in this study comply with current regulations covering animal experimentation in Italy.

Nutritional assessment or fifth vital assessment

Until recently, the veterinary clinical check up routinely involved making four vital assessments: body temperature; pulse; breath frequency, and pain. In 2011, the World Small Animal Veterinary Association (WSAVA, 2011) promoted the inclusion of the Nutritional Assessment as the fifth vital assessment during the veterinary clinical check up on cats and dogs. In order to underline the inter-specific importance of the nutritional assessment, we adopted an integrated procedure for the donkey species, based on different parameters. The clinical checks were repeated weekly, for three times (T0=at beginning; T7=intermediate check; T14=end) and consisted of the BCS, the live weight, the body measurements, the muscular condition, the metabolic profile and the development of the equation to predict body weight.

Body condition score

The BCS was measured at T0, T7 and T14, using both the American (1 to 9 point; Henneke et al., 1983) and the French (1 to 5 point; Martin-Rosset, 1990) scales used for the BCS in the horse. The sites of investigation and palpation for the donkey (Figure 1) followed the method proposed by Pinna et al., 2000. On both scales, 1 represents extremely emaciated; whereas 3 and 5, and 5 and 9, represent ideal BCS and extremely fat on the French and American scales, respectively. The BCS was determined by using both the American and the French scales, with no substantial difference between the scores for the evaluation of the adiposities. The adoption of the BCS highlighted the differences between the horse and the donkey, in terms of extent and topography of the sub-cutaneous adipose tissue: the adiposity of the rib region of the horse is extended over a large area and is a distinguishing feature of the overweight horse; on the other hand, in the overweight donkey, high levels of adiposity are more frequently to be found in the tail region, as previously reported by Pinna et al. (2000): three distinct sub-regions of the tail can be observed during the palpation for the BCS of the donkey. On the basis of the BCS, three categories were identified according to a low, intermediate and high scores:

i) BCS, low: BCS<3.5;
ii) BCS, intermediate: 3.5< BCS< 5;
iii) BCS, high: BCS>5.

Live weight

The body weight of each donkey was measured on the scales. Individual weights were recorded (T0) and compared with the predicted body weight in order to calculate the parametric coefficients on the basis of body measurements.

Body measurements

The animals were inspected in order to exclude abnormalities of the development of the skeleton and of the ligaments. The height at the withers (WH) was taken using a measuring stick. For the circumference of the chest (CG) a measuring tape was used. These measurements were expressed in centimetres.

Muscular condition

The development of the muscle mass was evaluated both on stationary and moving animals (T0). Moreover, the symmetry of the muscle masses of the chest, of the forelimb and of the hips was evaluated. The palpation regarded the muscle masses of the temporal region, of the scapulae, of the lumbar vertebrae, of the croup and of the hips. The finger pressure on the muscles during palpation over the integument was expressed on a 1 to 5-point scale, in which 1 represents the condition of hypotrophic muscle mass, 3 represents optimal development and 5 represents the hypertrophic muscle mass condition.

Metabolic profile

Blood samples were collected to evaluate a complete metabolic profile, including glucose, total cholesterol, triglycerides, lactate, total protein, urea, creatinin, total bilirubine, sodium, potassium, chloride, calcium, phosphorous, magnesium, iron, aspartate transaminase also called serum glutamic oxaloacetic transaminase (AST or SGOT), alanine transferase also called serum glutamic pyruvic transaminase (ALT or SGPT), lactate dehydrogenase (LDH), creatinin kinase (CK), alkaline phosphatase (ALKP), γ-glutamytransferase (GGT) and lipase. Blood samples were drawn weekly, starting from T0. Blood was collected by jugular vein puncture and stored in Vacutainer tubes without additives. After centrifugation, the serum was processed for the determination of the concentration of analytes.

Figure 1. The yellow circles indicate the sites for the palpation of adiposities, according to Pinna et al., 2000. Five regions are considered in the donkey (oral to aboral regions): neck, withers, over the ribs, loin area and hooks, base of the tail and pins area. The red lines indicate the body measurements: height at the withers and chest girth.
Analytical methods

The concentrations of the electrolytes in the serum, expressed as mEq/L, were determined by the ion exchange equipment at 37°C. The concentrations of sodium, potassium, and chloride were determined at 25°C. Colorimetric reactions were used for the concentrations of calcium, potassium and magnesium. In order to determine iron concentrations, a colorimetric reaction was measured when Fe^{3+} was reduced to Fe^{2+} bond to a chromogenic ligand.

The concentration of glucose was determined by indirect colorimetric reactions of glucose-oxidase and peroxidase, at pH levels of 5.0, in the presence of 4-aminophenazin and 1,7-dihydroxyflavahene. The concentration of the total proteins was determined by the alkaline reaction with copper-tartrate. The total cholesterol concentration was obtained through three different sequential reactions of cholesterol-ester-hydrase, cholesterol-oxidase and finally cholesterol-peroxidase. The triglyceride concentration was determined through the enzymatic reactions of oxidase, glycerol-kinase and L-α-glycerophosphate oxidase. The lactate concentration was indirectly determined by the fluorogenic reaction of lactate-oxidase at pH 6.3. The urea concentration was determined by the urease reaction and the production of NH3 at pH 7.8.

All the enzyme concentrations were determined by the use of specific colorimetric reactions at different values of pH, due to the diversity of each biological activity.

Calculations and statistical analysis

The regression equations for predicting the live body weight of Sardinian donkeys were elaborated by means of multiple regression analysis. The accuracy of the parametric equation was evaluated using the coefficient of multiple determination (R²) and the standard error of the estimate (SEE). The goodness of estimation of the equation was also evaluated by the slopes of the regression and the production of SE=±5 kg BW.

A further difference with the horse consists of the adiposity distribution and relative extent: the adiposities of the donkey are mainly visible at the base of the tail, whereas for the horse, the region between the 10th and the 14th rib is more essential to the evaluation of body condition. Such species-specific differences can also explain the different coefficients of the parametric equation for the prediction of the BW in the donkey. Moreover, the different distribution of adiposities in the body of the horse and of the donkey, as emerged from the BCS, adds information to the explanation for such differences. The serum concentrations of the analytes appeared to be within the range for the donkeys. Specific values for BCS, glucose and total cholesterol can be compared to those reported by other authors on different genetic types of the donkey species (Pinna et al., 1994). Typical cholinesterase and lipase

Results and discussion

In relation to the distribution and the extent of adiposities, blood parameters from the individual metabolic profile were associated with the respective category of BCS of the donkey; the data and the statistical significance are summarized in Tables 1, 2, 3 and 4. The muscular mass index was found to be 3±0.5, showing good muscle mass development in all the animals. This was considered to be an indicator also in the donkeys from the low BCS category, the muscle mass development was found to be optimal and no apparent catabolism of muscular tissue had started as no conditions of emaciation or extremely lean animals were reported. In addition, severe nutritional imbalances were excluded, due to the satisfactory clinical development of muscle mass. No instances of muscle mass asymmetry were observed: consequently, no vicariant hypertrophy of muscle mass was found to be present. This finding was also supported by the concentration within the physiological range of ubiquitous enzymes, which could potentially be involved in cases of damage to the muscular tissue. The somatometric measurements are consistent with those reported by Pinna et al. (2000) who underline the small size of this breed in comparison to other Italian donkey breeds (Orlandi et al., 1997).

The equation obtained from the analysis of data concerning eighteen donkeys showed a high association with the determined body weight: actually, R² was 91.2 and the slopes for a and b were significantly different from 0 (P<0.001). Moreover, residuals resulted randomly distributed. The standard error associated with this formula was SEE=±5 kg BW.

The BCS method, though specifically developed for horses, can be applied to the donkey with some adaptation: in particular, some modification is necessary due to the fact that the visual inspection of adiposities is less efficacious than in the horse, due to the different peculiarities of the fur and the thickness of the skin (Pinna et al., 2000; De Aluja et al., 2005). A further difference with the horse consists of the adiposity distribution and relative extent: the adiposities of the donkey are mainly visible at the base of the tail, whereas for the horse, the region between the 10th and the 14th rib is more essential to the evaluation of body condition. Such species-specific differences can also explain the different coefficients of the parametric equation for the prediction of the BW in the donkey. Moreover, the different distribution of adiposities in the body of the horse and of the donkey, as emerged from the BCS, adds information to the explanation for such differences. The serum concentrations of the analytes appeared to be within the range for the donkey species. Specific values for BCS, glucose and total cholesterol can be compared to those reported by other authors on different genetic types of the donkey species (Pinna et al., 1994). Typical cholinesterase and lipase

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Table 1. Average ± standard error of glucose, cholesterol, triglycerides and lactate concentrations in the serum of the three categories with low, intermediate and high body condition score.

| Condition       | Glucose (mmol/L) | Cholesterol (mmol/L) | Triglycerides (mmol/L) | Lactate (mmol/L) |
|-----------------|------------------|----------------------|------------------------|------------------|
| Low BCS         | 4.59±1.10        | 2.08±0.51            | 0.82±0.19              | 2.83±1.6         |
| Intermediate BCS| 4.83±1.71        | 1.61±0.29            | 0.77±0.29              | 2.25±2.50        |
| High BCS        | 6.15±2.33        | 2.05±0.07            | 0.55±0.29              | 1.90±0.71        |

BCS, body condition score. *Values within the same column with different superscripts are significantly different at P<0.01.

Table 2. Average ± standard error of total proteins, urea, creatinine and total bilirubine concentrations in the serum of the three categories with low, intermediate and high body condition score.

| Condition       | Total proteins (g/L) | Urea (mmol/L) | Creatinine (μmol/L) | Total bilirubine (μmol/L) |
|-----------------|----------------------|---------------|---------------------|---------------------------|
| Low BCS         | 66.9±3.56            | 4.30±0.75     | 99±13.6             | 6.13±0.35                 |
| Intermediate BCS| 68.3±3.26            | 4.46±0.79     | 91.4±13.5           | 6.05±0.67                 |
| High BCS        | 72±7.07              | 4.50±1.84     | 95±32.5             | 7±1.14                    |
concentrations and their meaning for the donkey were not found in the literature. The former is surely an ubiquitous enzyme, therefore involved in a multiplicity of metabolic pathways; the latter plays a role in fatty substrates.

The three classes of BCS were composed of animals which did not significantly differ according to their body condition, nevertheless, some blood analyte concentrations differed significantly from the BCS group: this occurrence implies a correlation between analyte concentrations in the blood and the clinical nutrition, with particular regard to when the concentration of some analytes highlights the mobilization of energy storage in a clear way. It is important to note that a statistically significant (P<0.01) difference in total cholesterol concentrations was observed in donkeys in the low and intermediate BCS categories.

### Conclusions

Nutritional assessment during the clinical check up of the donkey should be undertaken on a routine basis, in light of its clinical relevance as reported in this trial. The proposal to include nutritional assessment as a fifth vital assessment also for the donkey species has its basis in the integrated method related to a species-specific approach. A specifically developed parametric equation for the prediction of body weight in the donkey would be useful in veterinary practice to prevent nutritional imbalances and pathologies, as well as to avoid further complications in diseased animals.

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### Table 3. Average ± standard error of sodium, potassium, chloride, calcium, phosphorus, magnesium and iron concentrations in the serum of the three categories with low, intermediate and high body condition score.

| BCS    | Sodium, mmol/L | Potassium, mmol/L | Chlorine, mmol/L | Calcium, mmol/L | Phosphorus, mmol/L | Magnesium, mmol/L | Iron, mmol/L |
|--------|----------------|-------------------|------------------|-----------------|-------------------|------------------|--------------|
| Low    | 135±1.7<sup>a</sup> | 5.18±0.36        | 103±1.41         | 3±0.06          | 1.75±0.22         | 0.85±0.08       | 11.5±2.89    |
| Intermediate | 137±2.95<sup>b</sup> | 4.76±0.57        | 105±3.06         | 3.04±0.12       | 1.31±0.37         | 0.91±0.12       | 13.3±2.78    |
| High   | 142±7.07<sup>ab</sup> | 4.60±0.44        | 106±0.71         | 3.22±0.13       | 0.96±0.12<sup>b</sup> | 1±0.28          | 15.4±5.23    |

<sup>BCS, body condition score. Values within the same column with different superscripts are significantly different at P<0.05.</sup>

### Table 4. Average ± standard error of metabolic elements concentrations in the serum of the three categories with low, intermediate and high body condition score.

| BCS    | AST, U/L | ALT, U/L | LDH, U/L | CK, U/L | ALKP, U/L | GGT, U/L | LIP, U/L | ChE, U/L |
|--------|----------|----------|----------|---------|-----------|----------|----------|----------|
| Low    | 273±47<sup>a</sup> | 20.2±1.7<sup>a</sup> | 706±75<sup>a</sup> | 160±56  | 239±30    | 36.6±4   | 96.4±40  | 6069±650 |
| Intermediate | 283±44<sup>b</sup> | 20.7±2<sup>b</sup> | 617±93<sup>b</sup> | 143±171 | 203±48    | 38.4±14  | 99±44    | 5658±1170 |
| High   | 440±191<sup>b</sup> | 25±7<sup>b</sup> | 950±639<sup>b</sup> | 215±156 | 230±32    | 40.5±7.8 | 143±30   | 6342±580 |

<sup>AST, aspartate transaminase; ALT, alanine transferase; LDH, lactate dehydrogenase; CK, creatinin kinase; ALKP, alkaline phosphatase; GGT, -glutamyltransferase; LIP, lipase; ChE, cholinesterase; BCS, body condition score. Values within the same column with different superscripts are significantly different at P<0.01. Values within the same column with different superscripts are significantly different at P<0.05.</sup>
