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Relationship between ultrasound measurements of body fat reserves and body condition score in female donkeys

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ABSTRACT

Several methods have been developed to monitor body fat reserves of farm animals and body condition scoring (BCS) is generally assumed to be the most practical. Objective methods, such as real time ultrasonography (RTU), are accepted methods for measuring fat reserves in several farm species but there is no published information about the use of RTU to monitor body fat reserves in donkeys. The aim of the present study was to determine the relationship between RTU measurements and BCS in female donkeys (jennies) ($n = 16$) with a BCS of 3–7 on a 9 point scale.

Ultrasound images were captured using an Aloka 500-V scanner equipped with a 7.5 MHz probe and subcutaneous fat (SF, range: 1.0–14.0 mm) and thoracic wall tissue (TD, range: 5.6–21.4 mm) depths measurements were determined. A significant correlation was found between BCS and all RTU measurements ($0.65 < r < 0.86$; $P < 0.01$). Linear regression models described the relationships between RTU measurements and BCS and between log transformed RTU measurements and log transformed BCS. All equations with variables transformed into a logarithmic scale gave better coefficients of determination ($0.42 < r^2 < 0.75$) and an increase of 7–23% in this coefficient. The study suggested that RTU depth measurements have a logarithmic relationship with BCS and that RTU combined with image analysis permits accurate fat and tissue depths measurements to monitor fat reserves in jennies.

Introduction

Changes in body energy reserves have considerable influence on animal productivity, health, reproduction and welfare. Several methods have been developed and used to optimise management of body fat reserves in different farm species, but body condition scoring (BCS) has been widely accepted as the most practical method for assessing body fat reserves in cattle (Lowman et al., 1976; Edmonson et al., 1989), sheep (Russel et al., 1969), and goats (Santucci et al., 1991). The body condition evaluation methods rely on a subjective assessment based on the visual appraisal and palpation of some anatomical landmarks.

For Equidae, the most common condition scoring systems use visual appraisal and palpation of the neck, shoulders, back, ribs, pelvis and rump (Henneke et al., 1983; Pearson and Ouassat, 2000; Vall et al., 2003; Carter et al., 2009). These methods are affected by a certain level of subjectivity (Vecchi et al., 2010) and small BCS changes cannot be realistically detected even by trained observers (Ferguson, 1996; Mottet et al., 2009). This can be overcome with more objective techniques. Some of these are indirect, such as dilution techniques with deuterium oxide or tritiated water, where total body fat is estimated after measurement of total body water, assuming that the triglyceride content is anhydrous. More direct methods include the measurement of the mean diameter of fat cells, bioelectrical impedance, real time ultrasonography (RTU), computed tomography, magnetic resonance imaging and dual-energy X-ray absorptiometry (Allen, 1990; Bewley and Schutz, 2008).

Among the above mentioned techniques, only RTU has the potential to be used in the field to routinely predict body fat reserves (Schröder and Staufenbiel, 2006). The others are not viable for field use due to their cost and laboratory needs. The use of ultrasound measurements to predict body fat has been proved to be very accurate in several meat farm species (Silva and Cadavez, 2012) and horses (Westervelt et al., 1976; Gee et al., 2003). It has been demonstrated, however, that not all points for the collection of images equally reflect body fat reserve changes (Gentry et al., 2004). In fact, recent studies have shown variable changes between fat depots during weight loss in horses and ponies (Argo et al., 2012) and this raises the possibility that one-off measurements of fat depths are not consistently related to body fat content (and hence to BCS) across all breeds, ages and sexes in equids and possibly in other farm species.

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In the horse (as in other animals) body fat is the most variable of the body tissues and increases with BCS, irrespective of the specific BCS system used (Dugdale et al., 2012). The combination of a BCS system with objective measurements has the advantage of detecting slighter variations of fat reserves, particularly in obese animals where it is hard to detect body fat variation with the BCS system (Dugdale et al., 2011a). Ultrasound measurement of subcutaneous fat depth in different body locations can also (with further investigation) be a valuable tool to access the risk for obesity related diseases (Dugdale et al., 2010).

In donkeys, research studying variations in body energy reserves are scarce. This species presents two different challenges for body fat reserve evaluation. On the one hand, donkeys in prosperous areas are prone to be overweight, unlike working animals in poor, marginal areas that are usually thin and frequently show large body condition variations throughout the year (Pearson and Ouassat, 2000; McLean et al., 2009). In the North-East of Portugal, the Asinina de Miranda breed of donkeys are still raised under a traditional management system, where animals are housed during the night, pastured during the day and fed according to seasonal availability of food sources and without particular nutritional supplementation (Quaresma et al., 2005). Predictably, variations in body condition of the animals are found, if proper supplementation is not given (Robinson, 1990; McDonald et al., 2002). It is therefore of importance to monitor BCS of these animals in order to make feeding management decisions (Suagee et al., 2008). In ponies, there is also evidence that there is a seasonal appetite variation in temperate latitudes, endogenously-generated, with a winter constraint in appetite (Dugdale et al., 2011b), which can worsen the seasonal negative body reserves variation.

There are reports of BCS systems in donkeys (Pearson and Ouassat, 2000; Vall et al., 2003). However, the objective of the current study was to investigate the relationship between BCS and the subcutaneous fat and thoracic wall tissue depths assessed by RTU in donkeys.

Material and methods

Animals and management

Sixteen adult, non-pregnant jennies from the Portuguese Asinina de Miranda breed, housed at Vila Real, Portugal (41°17′N 7°44′W) were used in this study. The study was developed over two consecutive years, with two groups of eight different animals for both years. Throughout the 2 years a total of 74 recordings were made in 12 independent sessions. The age of the females ranged from 3 to 17 years old. All animals were routinely vaccinated for equine influenza and tetanus (Proteq-Flu, Merial) and dewormed every 6 months with 200 µg ivermectin (Noromectin Oral Paste) per kg bodyweight (BW).

The jennies were maintained under the same basic management conditions for both years in a 2500 m^2 park with a 50 m^2 shelter for protection from rain, sun and wind, for voluntary use by animals, year-round. The park had no pasture. Animals were fed according to accepted protocols (Smith and Wood, 2008), which consisted of 5–7 kg of hay and straw per jenny twice daily, equating to a dry matter intake between 1.5% and 2% of BW. Clean fresh water was available ad libitum.

Care and handling of the jennies used in this study were carried out in accordance to the guidelines with the European Council Directive 86/609/ECC for the protection of animals used for experimental and other scientific purposes.

Body measurements

BCS evaluation by visual appraisal and palpation was obtained independently by two technicians, but final classification was decided by consensus if there was any discrepancy. The first assessment aimed to include the animal in one of three main categories, namely, thin, medium or fat. Afterwards, each jennet was examined more closely to subdivide the main category into one of three and give it a score 1–3, 4–6 or 7–9 for thin, medium and fat categories, respectively, according to Pearson and Ouassat (2000).

RTU image capture and image analysis

After BCS evaluation, ultrasound examinations were performed in six measurement points (Fig. 1). An Aloka SSD 500 V real time scanner equipped with a linear probe of 7.5 MHz (UST-5512U-7.5, 38 mm) was used. The selected measurement points were determined based on previous studies in horses (Westervelt et al., 1976; Kearns et al., 2002; Gentry et al., 2004; Santos et al., 2009). The choice of this ultrasound array frequency, which allows high capacity to monitor thin structures such as subcutaneous fat in low BCS animals, was supported by the previous work of Silva et al. (2006) in sheep.

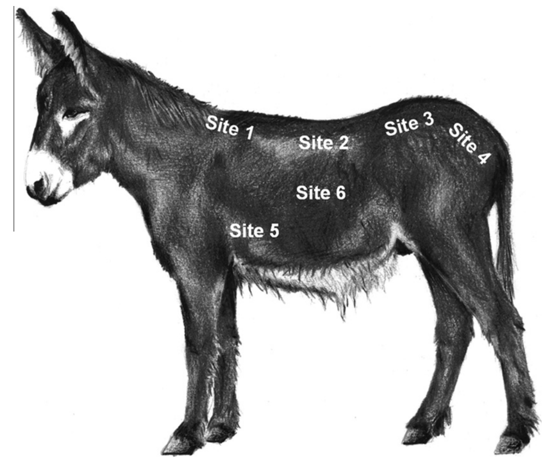

Fig. 1. Measurement sites for RTU image capture (image by Barros). At sites 1 and 2 the probe was placed perpendicular to the backbone at the withers and over the 13th thoracic vertebra; at site 3 the probe was placed along the mid-portion of the rump; at site 4 the probe was placed on the flat area anterior to the tail-head; for sites 5 and 6 the probe was placed at the thoracic cage, over the 6th and 7th, and 12th and 13th intercostal spaces, respectively.

The hair was first clipped close to the skin and alcohol was used as a coupling medium. At sites 1 and 2, the probe was placed perpendicular to the backbone at the withers and over the 13th thoracic vertebrae, respectively; at site 3 the probe was placed along the mid-portion of the rump 4 cm laterally and parallel to the midline; at site 4 the probe was placed on the flat area anterior to the tail-head parallel to the midline; for sites 5 and 6, the probe was placed at the thoracic cage perpendicular to the ribs. At site 5 the probe was placed directly behind the elbow over the 6th and 7th ribs. At site 6 the probe was placed at middle of thoracic cage over the 12th and 13th ribs, midway between the dorsal and ventral midlines.

All images were taken on the left side with the assumption that bilateral variation errors were small enough to consider that only one side needed to be measured (Domecq et al., 1995). The scanner was connected to a video camera (DCR-HC96E, Sony). The video camera started recording and once satisfactory images had been obtained at each site, the probe was moved to the next site. During all RTU image capture sessions, the transition from one site to the other followed the same order.

The recorded video was then analysed and once a satisfactory image was selected the frame was extracted as a 724 × 580 TIFF image file. Measurements were performed over a frame-fixed image with aid of the ImageJ software[1] for image analysis. Image interpretation was always performed by the same operator. From the images obtained at sites 1–4, the subcutaneous fat depths (SF) were determined (SF withers-SFW; SF thoracic-SFTh; SF rump-SFR; and SF tail-head-SFT, respectively). For all SF measurements, with the exception of SFW, where the measurement was taken above the spinous processes, the average of three depths was considered (Fig. 2). This procedure allowed us to overcome small variations in the thickness of the subcutaneous fat along the scanning site.

From the thoracic cage images, the tissue depth (TD) over the 6th and 13th rib (TDOrib6 and TDOrib13, respectively) and the tissue depth between ribs 6–7 and 12–13 (TDBrib6 and TDBrib13, respectively) were determined. The pleura are the lower limit of this measure (Fig. 2). None of those measurements included the skin thickness.

Statistical analysis

All the statistical analyses were performed with the JMP software (version 5.0.1.2. SAS). The SF and TD measurements were analysed by ANOVA using Tukey's test for comparison of group means, with the anatomical site as effect. The relationship between BCS and RTU measurements was assessed by correlation and regression analysis. To perform regression analysis the untransformed variables and both independent and dependent variables log transformed were used. The accuracy of the estimates was based on determination coefficient (r^2), and residual standard deviation (rsd) (MacNeil, 1983). For log transformed regressions, the rsd are presented for comparative purposes.

[1] See: http://imagej.software.informer.com/.