Dual-energy x-ray absorptiometry technology is considered to be the technique of choice to evaluate bone mineral content and density in humans because it allows rapid, inexpensive, noninvasive, precise, and accurate measurement of bone density in almost any part of the skeleton. X-rays at 2 energy levels are differentially impeded by bone and soft tissue; therefore, the type and amount of tissue scanned can be distinguished by use of DEXA.1 By use of human protocols adapted according to the size of the subjects scanned, DEXA has also been increasingly applied to the study of BMD in laboratory animals,2 dogs,3–5 horses,6,7 and farm animals.8–10

Despite the previous reports, the major limitation to the use of DEXA in veterinary medicine is the high cost and consequent lack of availability of such devices, which are routinely used only in specialized veterinary research centers or academic teaching hospitals. Moreover, the lack in veterinary medicine of specific BMD reference values corrected for species, breed, sex, age, and body weight limits the use of DEXA mainly to longitudinal or follow-up studies.4,10

On the other hand, direct conventional or digital radiography has not been considered as a useful tool in evaluating BMD because of the limited resolution and accuracy of radiography itself, which requires at least 30% to 40% of the mineral content to be depleted from the bone for this technology to be applicable, and the intrinsic inability of such a technique to quantify the bone mineral status.2 Different radiographic methods to assess bone density by use of CAIAS have been reported in rats.11–14

### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>BMD</td>
<td>Bone mineral density</td>
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<tr>
<td>CAIAS</td>
<td>Computer-assisted image analysis software</td>
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<td>CV</td>
<td>Coefficient of variation</td>
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<td>DEXA</td>
<td>Dual-energy x-ray absorptiometry</td>
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<td>DICOM</td>
<td>Digital imaging and communications in medicine</td>
</tr>
<tr>
<td>MGV</td>
<td>Mean gray value</td>
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<tr>
<td>ROI</td>
<td>Region of interest</td>
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Objective—To evaluate the precision and accuracy of assessing bone mineral density (BMD) by use of mean gray value (MGV) on digitalized and digital images of conventional and digital radiographs, respectively, of ex vivo bovine and equine bone specimens in relation to the gold-standard technique of dual-energy x-ray absorptiometry (DEXA).

Sample—Left and right metatarsal bones from 11 beef cattle and right femurs from 2 horses.

Procedures—Bovine specimens were imaged by use of conventional radiography, whereas equine specimens were imaged by use of computed radiography (digital radiography). Each specimen was subsequently scanned by use of the same DEXA equipment. The BMD values resulting from each DEXA scan were paired with the MGVs obtained by use of software on the corresponding digitalized or digital radiographic image.

Results—The MGV analysis of digitalized and digital x-ray images was a precise (coefficient of variation, 0.1 and 0.09, respectively) and highly accurate method for assessing BMD, compared with DEXA (correlation coefficient, 0.910 and 0.937 for conventional and digital radiography, respectively).

Conclusions and Clinical Relevance—The high correlation between MGV and BMD indicated that MGV analysis may be a reliable alternative to DEXA in assessing radiographic bone density. This may provide a new, inexpensive, and readily available estimate of BMD.
previous studies, CAIAS was based on gray-level analysis or digital subtraction radiography methods; both methods provided accurate results, with the exception of the study by Specht. Although previous human radiography reports indicate a correlation between grayscale values and BMD as determined in vitro via dental cone-beam computed tomography analysis, the Hounsfield unit scale, radiographic MGVs, and DEXA values, the best of our knowledge, a study that correlates MGV and BMD as determined via DEXA has not been performed in animals.

The purpose of the study reported here was to determine the precision and accuracy of MGV in assessing bone mineral status on digitalized and digital images of conventional and digital radiographs of bovine and equine bone specimens by comparing MGV with BMD values as determined by use of the gold-standard DEXA technique.

Materials and Methods

Bone specimens—The left and right metatarsal bones and the right femur were excised and collected at the slaughterhouse from 11 feedlot beef cattle and 2 horses, respectively. After dissection, the 22 bovine bone specimens were refrigerated at 4°C and all imaging procedures were performed within 36 hours after collection. The 2 equine femurs were immediately defleshed after dissection and sectioned along the transverse plane by means of an electric stainless steel bone saw into 7 and 10 sections, with a mean height of 5 cm. The 17 specimens of equine origin were then boiled in soapy water, and all imaging procedures were performed within 2 months after collection.

Imaging procedures—Each bovine specimen was radiographed in a dorsoplantar view by means of an x-ray unit operating at 400 mA, 0.025 seconds, and 58 to 61 kVp (in relation to specimen size), with a focus-film distance of 100 cm. A conventional high-detail film-screen radiographic system was used, and the same x-ray processor along with developing and fixing fluids was kept throughout the study. Immediately following the x-ray procedure, each bovine specimen was scanned by means of a DEXA device with a distal proximal direction and by use of the same view as for the x-ray analysis; the ROI was manually selected to include the distal metaphys of each scanned specimen as indicated by a proximal and distal metallic marker (lateral to the specimen), and the BMD (expressed as g/cm²) of each ROI was calculated.

Each equine specimen was radiographed in a craniocaudal view by means of the same x-ray unit operating at 400 mA, 0.025 seconds, and 60 kVp, with a focus-film distance of 100 cm. A computed radiography system was used; image processing was performed by use of software included in the computed radiography image-reading device, and the image settings were not modified after image acquisition. The computed image processing procedure did not alter the gray level values of each pixel.

Image features were as follows: resolution, 2,180 × 2,660 pixels; mean file size, 2.4 Mb; and pixel depth, 16 bit. Each image was stored in DICOM format without compression. Each specimen then underwent a DEXA scan in the same view as for the radiographic procedure. The ROIs were chosen to obtain a quadrangular shape including both medullar and cortical bone; the BMD (expressed as g/cm²) of each ROI was calculated.

Experimental image analysis protocols—The MGV analyses of digitalized and digital radiographic images were obtained by means of an open-source CAIAS. Mean gray value is the sum of the gray values of all the pixels in the selection divided by the number of pixels. The pixel depth is the number of data bits each pixel represents. In 8-bit contexts, the pixel depth is 8 bit, and each displayed pixel can be 1 of 256 possible shades of gray (2⁸ combinations in binary code). Therefore, when operating with 8-bit images, the gray level may range between 0 and 255.

Conventional radiography—A cold-light negatoscope and a digital camera were used to digitalize the x-ray films. The camera was mounted on an adjustable support with the lens oriented orthogonally toward the negatoscope. The distance between the lens and the film was 45 cm, and each photograph was obtained excluding all light sources except the negatoscope. Camera settings were kept constant for the entire study as follows: aperture, 3.6; time exposure, 0.017 seconds; resolution, 2,048 × 1,536 pixels; white balance neon mode; exposition matrix mode; manual focus; iso auto adaption; saturation black and white; macro function on; and autofocus and zoom off. The file format of choice should have been .nef (raw data), but it was discarded because it could not have been read directly by the open-source CAIAS. Therefore, the digital images were stored as 8-bit high-quality .tiff files without compression.

To standardize the brightness and contrast of each digitalized image without altering the gray values of pixels, the CAIAS automatic balancing function was used; this enabled the CAIAS to optimize brightness and contrast on the basis of an analysis of the image histogram. Optimization was done by allowing a small percentage of pixels in the image to become saturated (displayed as black or white).

The CAIAS procedure to evaluate MGV on the digital images comprised the following consecutive steps: automatic balancing of the digitalized image by use of the window and level function; pixel-centimeter calibration of the digital image by use of the straight line tool to draw a line corresponding to the scale bar (a ruler) and then the set scale function (this step is not influenced by relative image magnification); ROI selection by use of the polygonal selection tool to obtain an area, expressed in cm², as similar in size as possible to that of the ROI of each corresponding DEXA scan; applying of the set measurements function, enabling the operator to examine the area of the ROI by use of the area tool and to verify proper image balance by use of the minimum and maximum gray value tool; and the final MGV analysis by use of the measure function.

Digital radiography—16-bit DICOM images were converted into 8-bit DICOM files to obtain the same gray-level scale as in conventional radiography. The
CAIAS analysis procedure was the same as for conventional radiography with the exception of the automatic balancing and the pixel-centimeter calibration, which were not required. Mean 8-bit DICOM file resolution was 1,220 × 1,100 pixels.

Statistical analysis—Statistical analyses were performed by use of commercial statistical software.1 Precision results were expressed for both the MGV determination procedures as percentage CV values (CV = 100 × SD/mean). Accuracy was determined by comparing the MGVs obtained on conventional and digital radiographs and the corresponding BMD values as determined via DEXA. Correlation coefficients (r² = covariance/SD × SD) relating MGVs on conventional and digital radiographs to corresponding BMD values as determined via DEXA were calculated. Furthermore, r² values were also calculated between the ROI areas as determined via CAIAS on conventional and digital radiographs and the corresponding areas as determined via DEXA.

Results
Mean ± SD MGVs obtained via 10 repeated measurements on the same digitalized and digital radiographs were 128.86 ± 0.13 and 152.91 ± 0.14, respectively. The CV values calculated for evaluation of technique precision in determining MGV via conventional and digital radiography were 0.10% for MGVs determined on conventional radiographs and 0.09% for MGVs determined on digital radiographs.

All MGV and BMD values obtained on each specimen along with the values of the corresponding ROI areas as assessed via CAIAS and DEXA techniques on conventional radiographs (n = 22) and digital radiographs (17) were considered. Correlation coefficients relating MGVs on conventional radiographs and BMD (r² = 0.910; P < 0.01) and MGVs on digital radiographs and BMD (r² = 0.937; P < 0.01) were determined. Likewise, r² values were calculated between the ROI areas measured via DEXA and the corresponding areas measured via CAIAS on conventional (r² = 1; P < 0.01) and digital (r² = 0.799; P < 0.01) radiographs.

Discussion
The use of CAIAS in radiographic image processing and analysis has been recently reported for measurement of trabecular and cortical bone thicknesses in humans18 and assessing bone healing and the amount of callus formation after canine tibial osteotomies.19 Further reported applications in the diagnostic imaging field are associated with computed tomography sections of muscular tissue20 and ultrasonographic evaluation of uterine endometrial changes.21

The x-ray–based MGVs determined in the present study were precise (CV, 0.10 and 0.09 for conventional and digital radiography, respectively) and accurate (r² = 0.910 and 0.937 for conventional and digital radiography, respectively) in relation to BMD values as determined via DEXA on ex vivo bone specimens. The high correlation between the MGV and DEXA data in the present study reflects the great accuracy and reliability of MGV analysis as an alternative to DEXA for assessing bone density.

Compared with conventional radiography, the smaller CV and the higher r² obtained by use of the digital technique was probably attributable to the fewer steps necessary to attain initial image analysis via CAIAS. In fact, the chemical processing of conventional film and the subsequent digitalization of the radiographic images are likely to negatively influence the final image grayscale determination. For this reason, it is not unlikely to have an even higher correlation when images obtained via direct radiography are used.

The r² between areas as determined via DEXA and the corresponding areas as measured via CAIAS revealed a lower correlation for digital analysis (0.799) than for conventional analyses (1). This was likely attributable to the higher variability of the ROI dimensions in the digital radiography study group. In the conventional radiography study group, the outline of each ROI matched exactly the corresponding specimen edge, whereas in digital radiography, the ROIs were included in the entire ROI determined on the equine specimens. Nevertheless, despite the lower precision of ROI selection with both DEXA and CAIAS analysis, the correlation among areas still was significant.

Moreover, we should stress that, in comparison to the equine specimens, the bovine metatarsal bones were all imaged without the removal of any soft tissue, mimicking an in vivo analysis. For this reason, we propose that MGV is a reliable diagnostic tool for assessing bone mineral status, at least in long bones, in regular follow-up analysis of a patient or in a longitudinal research plan. The complementary MGV analysis of digitalized or digital images of bone radiographs should give more information both in ex vivo or in longitudinal studies of the effects of diet and drug treatments as well as orthopedic (including bone-healing process), developmental, and metabolic diseases. In such contexts, strict standardization of the radiographic procedure (position, view, radiographic parameters, ROI determination, and preprocessing or postacquisition image manipulation in digital radiography) is mandatory for the entire duration of the study to avoid any bias occurring in the image gray scale. A potential limitation of the technique is related to the high influence that radiographic settings could exert on brightness and contrast of each digital and digitalized radiographic image. For example, it has been reported that in diagnostic imaging of dogs with hyperadrenocorticism, direct radiographic assessment of bone density is unreliable because of artifactual osteopenic effects of high kVp settings necessary in obese subjects.22 It appears clear that, similar to results obtained via DEXA technology,1 an appreciable variation of tissue thicknesses that occurs during a follow-up study could alter the image gray levels and consequently the MGVs. Further investigations into accuracy and precision are required to test the effects of greater soft tissue thicknesses on MGVs (eg, in MGV evaluation of vertebral bodies) as well as reliability of MGVs in flat bones. Nevertheless, this technique may provide a new, inexpensive, and readily available tool for radiographic analysis of bone mineral status in veterinary species.

a. Model TS 9600, MT Medical Technology, Biassono, Italy.
References


