

Changes in Bone Strength and Density in Standardbreds from Weaning to Onset of Training

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Summary

Sequential determinations of growth rate and bone quality were made on five 5- to 18-month-old foals. Measurements included growth rate, plasma alkaline phosphatase, radiographic optical density, ultrasound velocity, cortical cross sectional area, bone mineral content, bone mineral density, compact bone density and modulus of elasticity. All foals showed a steady growth rate during the 11-month investigation. Plasma alkaline phosphatase concentrations remained static at 200-350 U/L throughout the study period. Radiographic photodensitometry measurements of the mid-metacarpal cortex exhibited a gradual trend to increased optical density of bone with age. The apparent transverse ultrasound velocity at 6 months was 2607 m/sec and increased by 112 m/sec over the next 10 months, while the transverse cortical ultrasound velocity exhibited less change (3028 to 3081 m/sec) over the same period. The ultrasonic estimate of the cross sectional area of the mid-metacarpal cortex increased from 4.7 to 7.5 cm². Bone mineral content showed a marked increase in values from 4.9 to 7.1 g/cm, whereas those for bone mineral density and compact bone density remained static at 0.98 and 1.87 g/cm³, respectively. Values for modulus of elasticity also remained constant at 17.8 GN/m². These preliminary results provide baseline data for assessing skeletal maturation in young horses and for further studies in evaluating the effects of exercise and nutrition in the post-weaning period. Metacarpal cross sectional area, estimated ultrasonically, produced a good indication of growth rate. Radiographic photodensitometry was not judged to be a sufficiently sensitive method, but ultrasound velocity and single photon absorptiometry appear to be safe, accurate and reliable indicators of bone density and strength in young horses.

Index terms: Horse; foal; skeletal maturity; bone quality; cortical bone; third metacarpal bone; ultrasound velocity; modulus of elasticity; photon absorptiometry; bone mineral; radiographic photodensitometry; plasma alkaline phosphatase.

Introduction

Until recently, skeletal maturity in young horses has been assessed by subjective visual appraisal of the animal and by the radiographic closure of various growth plates (Mason

and Bourke, 1973). This paper presents the results of a study aimed at monitoring the changes in bone quality and bone strength in 5 Standardbred foals kept at the Veterinary Clinical Centre over an 11 month period. Two non-specific means of assessing bone development were used. The growth rate was monitored by weekly measurements of various bodily dimensions. Plasma alkaline phosphatase levels were investigated in an attempt to provide an indication of bone formation or turnover (Krabb *et al.*, 1980).

Radiographic photodensitometry was the first of three more specific and non-invasive techniques used to investigate bone quality. It is simple to perform and requires no special equipment apart from an aluminium alloy stepwedge, an optical densitometer and standard radiographic facilities. This technique has been widely used in human medicine (Mack *et al.*, 1959; Schraer, 1966), although it has been largely superseded by more accurate and sophisticated methods including photon absorptiometry and computed tomography. Radiographic photodensitometry has also been successfully used in horses to measure the progressive mineralization of the third metacarpal bone in weanling foals (Meakim *et al.*, 1981).

The measurement of the velocity of ultrasound through cortical bone provides a means of determination of its modulus of elasticity and its mass density (Abenschein and Hyatt, 1970). The modulus of elasticity (E) is a property of the bone related to its stiffness or strength and ultimately its fracture threshold. It can be determined by measurement of the ultrasound velocity (C) and the bone density (ρ), using the equation: $E = \rho \times C^2$. The technique used for the measurement of apparent transverse ultrasound velocity has been described previously (Pratt, 1980; Jeffcott and McCartney, 1985). With an understanding of the pathway of the ultrasound beam, good estimates of the true transverse cortical ultrasound velocity and the cross sectional area of the cortical bone can be achieved (McCartney and Jeffcott, 1985).

Bone mineral content (BMC) can be accurately estimated by single photon absorptiometry. This method, pioneered by Cameron and Sorenson (1963), has been widely used as a diagnostic and research tool in human medicine (Chesney and Shore, 1982; Wahner *et al.*, 1983), however its application in horses has been somewhat limited (Wentworth *et al.*, 1971; Pezzoli *et al.*, 1977; Tomioka *et al.*, 1985; Jeffcott *et al.*, 1986). Bone mineral content is of interest since the strength of bone largely depends on the degree of mineral reinforcement, in the form of hydroxyapatite, of what is essentially a fibrous scaffold of collagen.

The objective of this study is to gain a better understanding of the development of skeletal maturity and to non-invasively quantify the associated changes in bone strength and density. This is of particular interest since many horses begin serious training long before they are skeletally mature. In view of the tremendous biomechanical forces involved in high level equestrian competition, it is hardly surprising that lameness is such a critical cause of lost performance (Jeffcott *et al.*, 1982). An increased knowledge of skeletal development is hopefully the first step in a long process of investigating the cause of some of these skeletal problems and instituting preventive measures.

Materials and Methods

Animals. A group of 5 Standardbred foals, 4 colts and 1 filly, was used. The foals were prospective racehorses acquired on loan from their owners and were returned for breaking in and training at the end of an 11-month period of examination. The foals

arrived at the Veterinary Clinical Centre immediately after weaning and the first few weeks were spent on handling and familiarization with the surroundings and techniques. They were between 5 and 6 months old when bone measurements began.

During the investigation the foals were kept on a controlled diet aimed at providing a steady growth rate. This ration consisted of lucerne hay and a concentrate pellet. They were initially kept in yards and then later in a small paddock so that their level of exercise was limited to their own activity.

Growth rate. Weekly measurements were made of body weight, height at withers, girth, left humeral length from the greater tuberosity of the humerus to the olecranon and the circumference of the left fore cannon region at the midsite.

Plasma alkaline phosphatase (AP). Weekly blood samples were taken throughout the period of investigation and analyzed for alkaline phosphatase using a Roche Uni-Kit on a Roche Cobas Mira biochemical analyzer.

Radiographic photodensitometry. Lateral radiographs of the left and right third metacarpal bones were taken at monthly intervals to monitor any changes in radiographic optical density with age. An aluminium alloy stepwedge was included in each radiograph after the method of Schraer (1966). A Phillips XC2031 mobile x-ray machine was used with Kodak T-Mat G films and Kodak Lanex Regular rare earth screens. The films were processed using a Dupont Cronex Processor T3. Optical density was measured from a lateral radiograph at three sites in the midregion of the metacarpus using a Sakura PDA-81 optical densitometer (Konishiroku Photo Ind). These sites were the dorsal cortex, the palmar cortex and a site between these over the medullary cavity.

Ultrasound velocity measurements. Five latero-medial readings were taken covering the part of the metacarpal shaft where the cortical surfaces are parallel. Both left and right forelimbs were measured at weekly intervals (Jeffcott and McCartney, 1985; Jeffcott *et al.*, 1987). In addition to measuring the apparent transverse velocity (C_a m/sec), a correction was made to determine the transverse cortical ultrasound velocity (C_b m/sec) which takes into account the pathway of the ultrasonic beam around the medullary cavity (McCartney and Jeffcott, 1985). The C_b consequently provides a better estimate of the modulus of elasticity *in vivo*. A good estimate of the cross sectional area (CSA cm^2) of the cortical bone can also be achieved (McCartney and Jeffcott, 1987).

Single photon absorptiometry. Five measurements of the bone mineral content (BMC g/cm) at the mid-site of both left and right metacarpi were made at monthly intervals. General anesthesia was used for the first 2 sessions on each foal, but later a combination of acepromazine and xylazine provided satisfactory tranquilization to keep the animal still enough for scanning. The technique used has been documented (Jeffcott *et al.*, 1986; 1987) and involves the passage of a beam of photons from a 45 mCi radiation source ($^{241}\text{Americium}$) through the bone in the direction of a scintillation detector. The degree of absorption of photons is related to the bone mineral content and an estimate of the amount of bone mineral in a 1 cm thick transverse slice of the mid-metacarpal shaft is achieved.

Combination of photon absorptiometry and ultrasound velocity data. Monthly estimates of bone mineral density, compact density and modulus of elasticity for the left and right metacarpi were made.

Bone mineral density (BMD g/cm^3). Changes in BMC after birth are largely volumetric since the 1 cm thick slice of bone measured will be constantly increasing in size (Ruff and Hayes, 1984). Despite the physiological importance of this increasing bone

volume, BMC alone provides very little information about the degree of mineralization per unit volume of bone and its density. In order to compensate for growth, a value for bone mineral density can be calculated from the BMC and the ultrasonic estimate of the cortical cross sectional area (McCartney and Jeffcott, 1987):

$$\text{BMD} = \text{BMC}/\text{CSA}$$

Compact bone density (CBD g/cm^3), or specific gravity, can be estimated using a bone model (Greenfield *et al.*, 1981) from BMC, CSA and estimated constants of microscopic bone mineral density ($\rho_m \text{g}/\text{cm}^3$) and collagen density ($\rho_c \text{g}/\text{cm}^3$) by the equation:

$$\text{CBD} = \rho_c + (1 - \rho_c/\rho_m) \text{BMC}/\text{CSA}$$

The modulus of elasticity ($E \text{GN}/\text{m}^2$) or Young's modulus is related to density (ρ) and ultrasound velocity (C) can be estimated in vivo by the equation from McCartney and Jeffcott (1987):

$$E = \text{CBD} \times C_b^2$$

Statistical analysis. Regression analyses were performed for all ultrasonic and photon absorptiometric measurements to estimate relationships with age. Student's one sample t-tests were used to test whether the percentage change per month was significantly different from zero for all seven measurements

Results

All 5 foals exhibited similar trends throughout the period of examination. The mean results, for left and right legs, for one foal (D) are presented to illustrate these trends with time. A summary of the results for all 5 foals is presented later in tabular form. All calculations are based on the assumption of a linear regression, and percentage change per month is calculated at 52 weeks of age.

The foals all exhibited a steady growth rate throughout the investigation as illustrated by the change in girth and mid-cannon circumference for foal D (Fig. 1). Plasma alkaline phosphatase concentrations remained fairly stable in all the foals throughout the period of study with foal D being approximately 150-250 U/L (Fig. 2).

A progressive increase in optical density of the third metacarpal bone was measured by radiographic photodensitometry as seen for foal D (Fig. 3), although in the other foals the trend was not as apparent due to large variations. The dorsal cortex was always slightly more dense than the palmar cortex and both these sites were more dense than the site overlying the medullary cavity.

Foal D showed an increase in C_a of 0.37% per month ($P < 0.001$), which meant a change from 2602 m/sec at 5 months to 2720 m/sec at 16 months (Fig. 4). On the other hand, C_b increased at a much lower rate (0.11% per month, $P < 0.05$) with a change from 3044 to 3084 m/sec over the same period (Fig. 4).

The ultrasonic estimate of the cortical cross sectional area (CSA), critical in the estimation of BMD, CBD and E, also proved to be an excellent indicator of growth rate. The typical pattern of steady increase in CSA is shown for foal D (Fig. 5). In this case CSA increased at 4.73% per month ($P < 0.001$) from 3.83 cm^2 at 5 months to 7.22 cm^2 at 16 months.

BMC at the mid-site of the metacarpal shaft, as measured by single photon absorp-

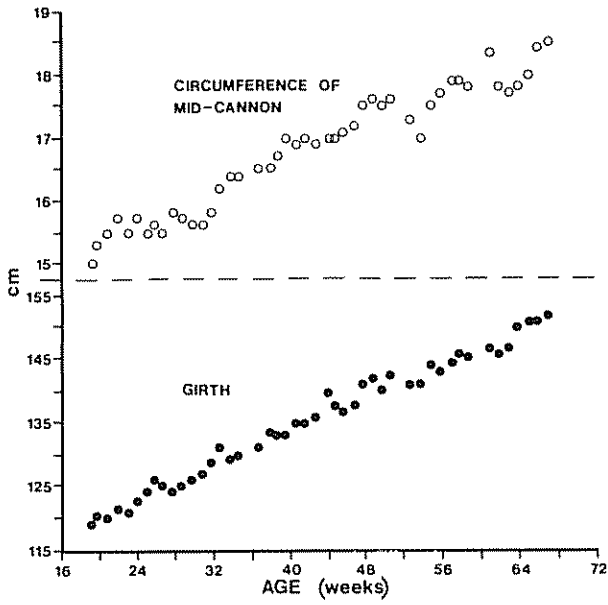


FIGURE 1. Growth rate as measured by change in girth and mid-cannon circumference in Standardbred foal D over an 11 month period

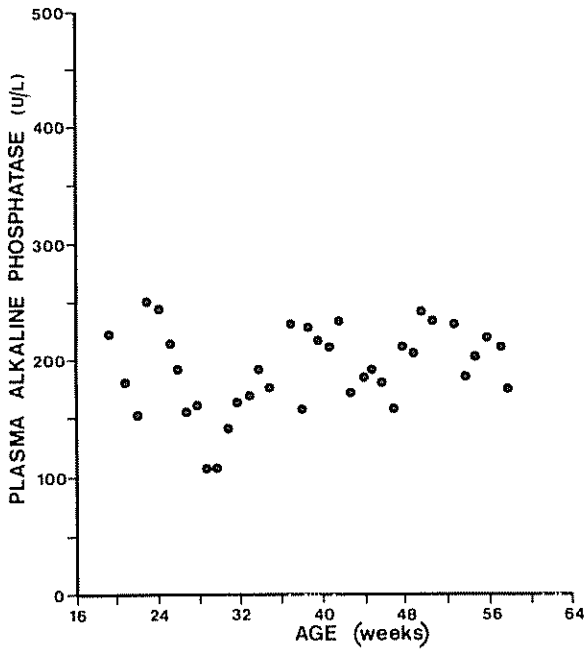


FIGURE 2. Plasma alkaline phosphatase (U/L) concentrations in Standardbred foal D over an 11 month period.

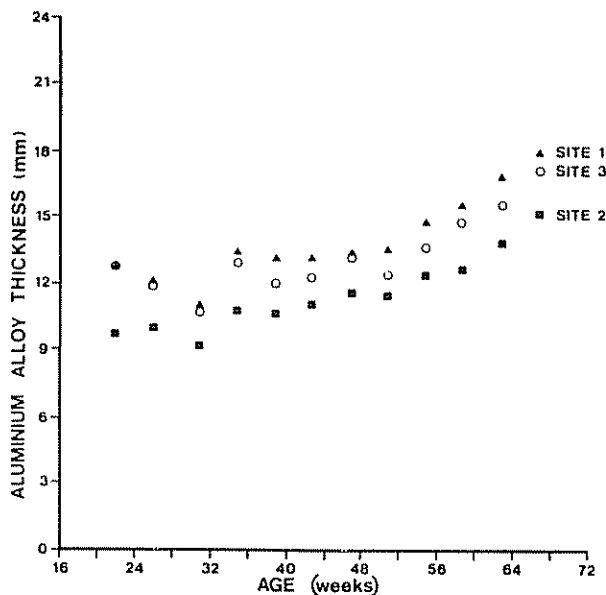


FIGURE 3. Changes in bone density, as measured by radiographic photodensitometry, at the mid-site of the third metacarpal bones of Standardbred foal D. The sites measured were the dorsal cortex (Site 1), the palmar cortex (Site 3), and over the medullary cavity (Site 2). Bone density is expressed as equivalent thickness of aluminium alloy LM4.

tiometry, exhibited an increase (3.65% per month, $P < 0.001$) in foal D from 4.196 to 6.741 g/cm over the period of examination (Fig. 6). This increase was considered to reflect the volumetric increase of the bone and closely matches the increase in CSA. The BMD showed a slight decrease in this foal (-1.25% per month, $P < 0.01$), remaining fairly static at approximately 0.96 g/cm³ (Fig. 7). CBD exhibited an even smaller decline (-0.41% per month, $P < 0.01$) remaining almost constant at 1.86 g/cm³ (Fig. 7), while the estimate of the modulus of elasticity, E , was constant at 17.6 GN/m² (Fig. 8).

A comprehensive summary of all values determined in the study for the 5 foals is given in Table 1. The average values for the 5 foals, meaned for left and right legs, have been standardized (using the appropriate regression line) to 52 weeks of age and are given with 1 standard deviation. The variation with time for each measurement is expressed as a percentage change per month (28 days) and the level of significance for the change is listed. For the four direct measurements which showed significant variation, Table 2 presents more detailed information. Table 2 lists the mean values (\pm SD) of C_a , C_b , CSA and BMC for the 5 foals standardized for different ages.

Discussion

Despite its obvious importance in equestrian athletics, there has been very little sequential information on bone quality with respect to skeletal maturation in horses. Safe,

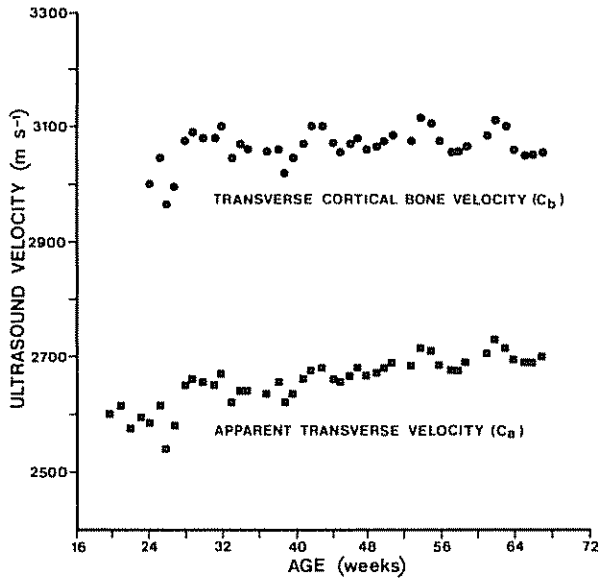


FIGURE 4 Changes in apparent transverse ultrasound velocity (C_a , $m s^{-1}$) and transverse cortical ultrasound velocity (C_b , $m s^{-1}$) with age for Standardbred foal D

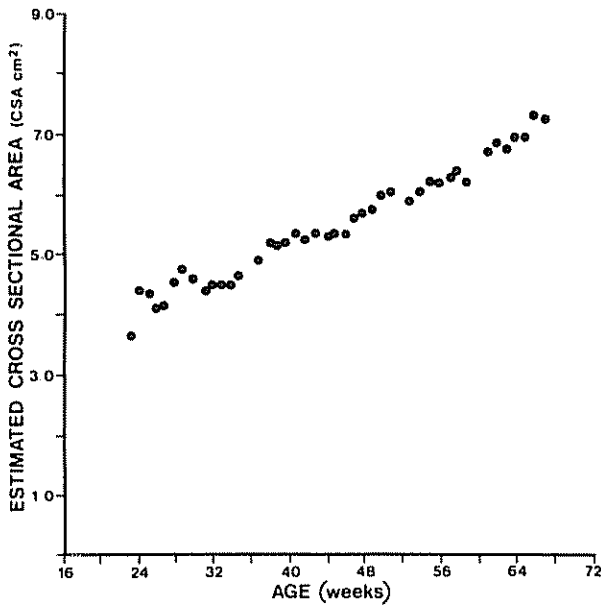


FIGURE 5. Change with age in the ultrasonic estimate of the cortical cross sectional area (CSA cm^2) of the mid-metacarpal site in Standardbred foal D.

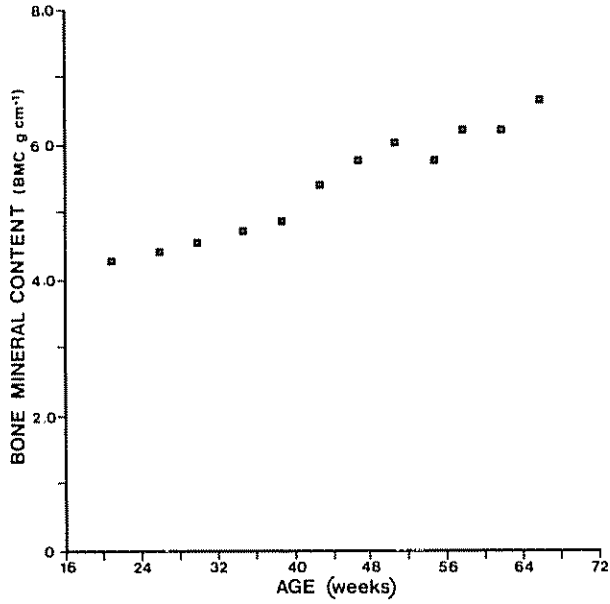


FIGURE 6 Mean results of bone mineral content (BMC g cm⁻¹) at the mid-metacarpal site against age for Standardbred foal D

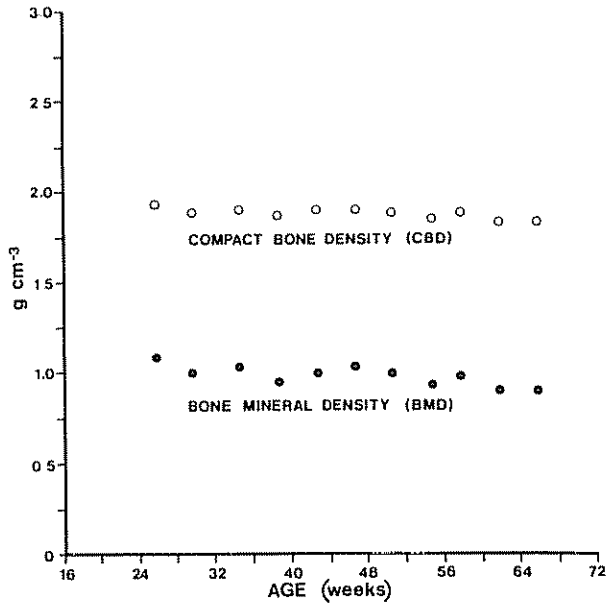


FIGURE 7 Change in bone mineral density (BMD g cm⁻³) and compact bone density (CBD g cm⁻³) in Standardbred foal D as derived from bone mineral content and cortical cross sectional area

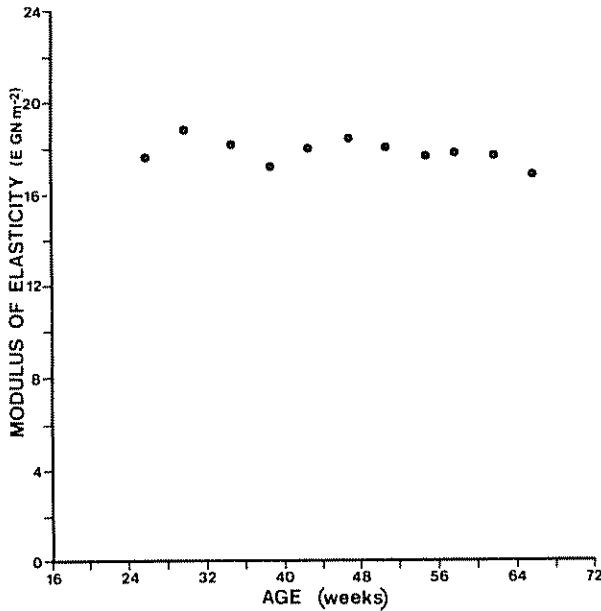


FIGURE 8. Change with age in the modulus of elasticity ($E \text{ GN m}^{-2}$) of the mid-metacarpal site in Standardbred foal D.

reliable and accurate techniques for the objective measurement of bone quality are now available in man (Greenfield *et al.*, 1981; Wahner *et al.*, 1983), and some have been applied to the horse in this study.

Plasma alkaline phosphatase concentrations provide only a crude indication of osteoblastic activity, unless the bone-specific isoenzyme can be assayed (R. A. Melick, personal communication). It may be that plasma osteocalcin (or bone Gla protein) is a more sensitive indicator since various studies have suggested that it is released into the circulation during bone formation (Nishimoto and Price, 1979; Slovik *et al.*, 1984; Melick *et al.*, 1985). In this study plasma alkaline phosphatase concentrations remained fairly stable which is probably a reflection of a constant rate of bone formation, consistent with the steady growth rate observed.

The rate of increase in bone density as measured by radiographic photodensitometry was inconsistent. However, the main problem with this technique is that the bone density measured radiographically must necessarily increase as the foal grows. The bone will increase in size and therefore contain more total mineral and consequently will appear more dense on the radiograph. Thus, much of the apparent increase in density measured by this technique is probably only a reflection of volumetric increase, and not of true bone density. This technique was consequently not judged to be sufficiently accurate or informative.

Despite the gradual increase (0.38% per month, $P < 0.001$) in C_a , consistent with the observations of Jeffcott *et al.* (1987), C_b increased at a lower rate (0.16% per month) with a lower level of significance ($P < 0.05$). This is probably due to the changing geometry of the bone being considered, since C_b is corrected for the pathway around

TABLE 1. The mean values (\pm SD) of the ultrasound, photon absorptiometric and derived measurements for the 5 foals.¹

Measurement	Mean (at 52 weeks)	% Change/month (at 52 weeks)	Level of significance
Apparent transverse velocity (C_a m s ⁻¹)	2674 \pm 50	0.38 \pm 0.05	P < 0.001
Transverse cortical ultrasound velocity (C_b m s ⁻¹)	3060 \pm 60	0.16 \pm 0.11	P < 0.05
Cortical cross sectional area (CSA cm ²)	6.36 \pm 0.25	4.08 \pm 0.82	P < 0.001
Bone mineral content (BMC g cm ⁻¹)	6.227 \pm 0.307	3.23 \pm 0.41	P < 0.001
Bone mineral density (BMD g cm ⁻³)	0.98 \pm 0.02	-0.81 \pm 0.44	P < 0.05
Compact bone density (CBD g cm ⁻³)	1.87 \pm 0.01	-0.21 \pm 0.21	NS
Modulus of elasticity (E GN m ⁻²)	17.8 \pm 0.7	-0.07 \pm 0.44	NS

¹Both left and right legs are standardized to 52 weeks of age. The variation with time for each measurement is expressed as a percentage change per month (28 days) and the level of significance for this change is listed.

the medulla. This suggests that the change in the speed with which sound is conducted by the bone *per se* is not nearly so great as is estimated by C_a .

The increase in BMC at the mid-metacarpal site was considered to be volumetric and this suspicion was confirmed when the BMD was found to be fairly constant throughout the period of study. The CBD, or specific gravity, was also constant. This suggests there was no real change in the degree of mineralization of the bone over the period of investigation. There was also negligible change in the elastic bone strength or modulus of elasticity at the mid-site of the third metacarpal bone.

The total strength of any cylinder is a function of its cross sectional area and the

TABLE 2. The mean values (\pm SD) of apparent transverse velocity (C_a), transverse cortical ultrasound velocity (C_b), cortical cross sectional area (CSA) and bone mineral content (BMC) for all 5 foals standardized to different ages.

AGE (months)	C_a (m s ⁻¹)	C_b (m s ⁻¹)	CSA (cm ²)	BMC (g cm ⁻¹)
6	2607 \pm 46	3028 \pm 41	4.68 \pm 0.47	4.927 \pm 0.404
8	2630 \pm 48	3039 \pm 46	5.24 \pm 0.38	5.362 \pm 0.371
10	2651 \pm 49	3049 \pm 53	5.80 \pm 0.30	5.791 \pm 0.339
12	2674 \pm 50	3060 \pm 60	6.36 \pm 0.25	6.227 \pm 0.307
14	2696 \pm 52	3070 \pm 67	6.92 \pm 0.23	6.656 \pm 0.276
16	2719 \pm 54	3081 \pm 74	7.48 \pm 0.25	7.092 \pm 0.246

elastic strength of the material of which it is made (Case and Chilver, 1971). If the cannon bone is considered as being essentially a cylinder, it can be seen from these results that its cross sectional area is increasing while the elastic strength (E) of the bone itself remains constant. Thus the total weight bearing capacity of the whole bone has increased, solely due to the increase in cross sectional area, presumably to compensate for the increasing mass of the animal.

There is an association between ultrasound velocity and exercise (Jeffcott *et al.*, 1986a), and so the level of exercise for this group of foals was intentionally limited. The gradual increase in C_a associated with growth is very similar to the trend found in a group of Thoroughbred weanlings at pasture by Jeffcott *et al.* (1987). Expansion of this research will have important clinical applications. The measured bone quality of any foal could be compared with what is normal for the age group. There is also the possibility of defining critical points of bone quality as an indication of when training should commence, and what levels and types of work are appropriate. These baseline data are also critical to further research into the effects of exercise and nutrition on bone quality in the post weaning period.

Single photon absorptiometry and ultrasound velocity measurements, used alone and in combination, are safe and informative techniques for the objective assessment of skeletal maturation in horses. They are already important research tools and these results, and the work of others (Pezzoli *et al.*, 1977; Pratt, 1980; Rabin *et al.*, 1983; Hasegawa *et al.*, 1985; Tomioka *et al.*, 1985; Jeffcott *et al.*, 1986; 1987), show their clinical importance in the *in vivo* determination of bone quality in the horse.

Acknowledgments

Funding for this project was provided by grants from the Rowden White Fund and the Australian Equine Research Foundation. We are most grateful to Dr. Tony Britt, Mr. Frank Britt, Mr. Warwick Wallis and Dr. Julie Tilbrook for providing us with the Standardbred weanlings, and also to Mr. Ron McCartney for his indispensable help and advice. The diligent technical assistance of Miss Tracy Waters is gratefully acknowledged, as is the constant work of Mr. Frank Griffiths and Mr. John Elso in the care and feeding of the foals.

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NUTRITION

Nutrition of the Equine Athlete

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The title of a well-known book for racehorse owners and trainers, "Feeding to Win" (Wagoner, 1973), misrepresents the possibilities of feeding in relation to the performance of horses: nutrient intake above requirements cannot improve performance beyond the physical capability of the animal. We cannot expect miracles from feeding in general or from giving single nutrients to badly conditioned or trained horses, but if feeding is inadequate we should not be surprised if a horse's performance is far less than could be achieved. In this context, "Feeding not to lose" would be a better expression for the possibilities of feeding than the first mentioned title. Feeding should be based on requirement figures for the animals and the suitability of various feedstuffs. In the first part of this paper, requirement figures for exercising horses will be summarized (as far as recent results have been published since the review of Hintz (1983)), while in the second part some practical recommendations about ration calculation and feeding techniques of performance horses will be given.

Requirements

Nutrients for which work creates little or no increase in demand

The nutrients which fall into this category are protein, calcium, phosphorus, most fat-soluble vitamins and most trace elements (Table 1).

Protein. If energy intake or energy stores are adequate, exercising horses need only small amounts of protein above maintenance requirement, as shown by Harvey *et al.* (1939). These findings have been confirmed in several investigations. Recently Patterson *et al.* (1985) reported no consistent effects of exercise on blood and urine characteristics in exercising horses ingesting less than 0.5 g digestible crude protein (DCP) per kilogram body weight per day. They suggested that no supplemental protein is required for exercise beyond maintenance. In their low protein group, however, the horses needed more time to recover pulse and respiratory rate after a 1.6 km racetrack workout than horses ingesting nearly 1 g DCP/kg BW/day. In exercising yearling foals (300 kg BW) fed 380 g DCP/day, Orton *et al.* (1985) saw no depression in growth rate when compared to foals with an intake of 780 g DCP/day. An additional protein supply (above maintenance) should however be provided to exercising horses for the following reasons (Meyer, 1983a; Freeman *et al.*, 1985): endogenous fecal nitrogen losses increase with higher dry matter (DM) intake (about 3.6 g N per 1 kg DM);