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Effect of supplementary dietary biotin on hoof growth and hoof growth rate in ponies: a controlled trial

J. D. REILLY*, D. F. COTTRELL, R. J. MARTIN and D. J. CUDDEFORD†

Department of Preclinical Veterinary Sciences, Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Summerhall, Edinburgh EH9 1QH, †Department of Veterinary Clinical Studies, Royal (Dick) School of Veterinary Studies, Veterinary Field Station, Easter Bush, Roslin, Midlothian EH25 9RG, Scotland, UK.

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Summary

The effect of dietary biotin supplementation, at a dose rate of 0.12 mg/kg bwt, on growth and growth rate of the hooves of 8 match-paired ponies was investigated in a controlled feeding trial. Treatment animals had a mean hoof growth at the midline dead centre of the hoof capsule of 35.34 mm after 5 months of biotin supplementation compared to control animals 30.69 mm (P<0.05). Comparison of regression analysis also showed that biotin supplementation produced a significantly higher (P<0.02) growth rate of hoof horn in this trial. Treatment animals had a 15% higher growth rate of hoof horn and 15% more hoof growth at the midline dead centre, after 5 months of biotin supplementation compared to control ponies. No differences were found between feet for growth of horn, but the older animals in the trial had significantly lower hoof growth (P<0.05) than the remaining ponies.

Introduction

Hoof horn 'development', 'structure' and 'integrity' have all been reported to be influenced by dietary changes (Comben *et al.* 1984; Kempson 1987, 1990). However, these were individual case reports that were uncontrolled (Slater and Hood 1997). The methods used for assessment were subjective and no data were provided to support the claims made. Presumably, the reason subjective assessment, as opposed to objective measurement, was used is that there are few features of the hoof which, at first sight, appear suitable for quantitative definition. Objective measurement of defined features of the hoof capsule therefore becomes the challenge in this field of study (Reilly 1995).

Hardness (Buffa *et al.* 1992), tensile strength (Geyer and Schulze 1994), tubule density (Reilly *et al.* 1996), modulus of elasticity (Landeau *et al.* 1983), work of fracture (Bertram and Gosline 1986, 1987), moisture content (Leach 1980), and hoof horn growth (Buffa *et al.* 1992) are examples of quantitative variables that can be measured. They are useful because they give an objective measure of the anatomical, material and physiological characteristics of hoof horn, an understanding of which enables factors governing its function to be unravelled.

The importance of hoof horn growth was recognised by Caulton Reeks (1906) who mentioned that the slow hoof growth found, even in the normal horse, is frustrating for the veterinarian. Geyer and Schulze (1994) expressed the effect of growth rate on the 'renewal time' of the hoof capsule, while Butler and Hintz (1977) acknowledged that the 'rate of hoof growth is of vital importance since it often affects the usefulness of the horse'.

Manipulation of hoof horn growth rate can have practical implications for veterinary treatment and farriery in terms of growing out a lesion, or for producing sufficient horn for nailing in to. A knowledge of hoof horn growth is also an important research prerequisite for meaningful sampling of like material for comparative testing (Reilly *et al.* 1996).

However, there is misuse of the terms 'growth' and 'growth rate'. Growth rate has been used where growth has been calculated or observed (Butler and Hintz 1977; Guthrie and Stoker 1990; Buffa *et al.* 1992), and the 2 terms have been used synonymously. They are different variables.

Hoof growth gives an indication of the total amount of material that has been produced at the end of a given period with units of cm or mm (Dittrich *et al.* 1994; Graham *et al.* 1994; Ott and Johnson 1995).

Hoof growth rate is a time dependant variable and gives an indication of the pace at which that material has been accumulated and, therefore, has units of cm/month or mm/day. (Geyer and Schulze 1994; Josseck *et al.* 1995). The same hoof growth can be achieved by variable periodic growth rates. Hoof growth rate can be given by the slope of a growth curve or by rates that have been calculated on a periodic basis, with growth up to that time being divided by the time relating to that period (Geyer and Schulze 1994; Josseck *et al.* 1995). Reported growth rates for equine hoof horn are given in Table 1

There are few substantiated reports of factors affecting hoof growth rates. Shannon and Butler (1979), and Geyer and Schulze (1994) suggested seasonal differences, with higher growth rates in spring and summer and decreased growth rates in the winter. Geyer and Schulze (1994) found breed differences. Graham *et al.* (1994) and Ott and Johnson (1995) suggested sex differences with male rates higher than female rates in younger horses, although Butler and Hintz (1977) found no differences in growth between the sexes. Butler and Hintz (1977) also concluded that the level of protein intake did not affect hoof growth, but that total dietary intake did, with young ponies on *ad libitum* feed achieving more growth than those on restricted intakes. In studies investigating

*Present address: Royal Army Veterinary Corps, UKSC(G) DASU, BFPO16.

TABLE 1: Equine hoof growth rates (reported measurements converted to daily rates)

Growth rate (mm per day)	Author
0.19–0.28	Glade and Salzman (1985)
0.200	Kainer (1987)
0.208	Caulton Reeks (1906)
0.248 (left fore), 0.257 (right hind) (Lipizzaners)	Josseck (1991)
0.248 (biotin) 0.25 (control) (Lipizzaners)	Josseck *et al.* (1995)
0.273 (control) 0.333 (biotin)	Buffa *et al.* (1992)
0.286 (Shires & Belgians) 0.143-0.179 (Icelandics)	Geyer and Schulze (1994)
0.287	Knezevic (1959)
0.384 (fed ad lib), 0.254 (limited feed) (8 months old)	Butler and Hintz (1977)
0.445 (diet including proteinate) (yearlings)	Ott and Johnson (1995)
0.427 (control) (yearlings)	Ott and Johnson (1995)
0.4375 (yearlings)	Graham *et al.* (1994)
0.48 (control) 0.42 (biotin) (yearlings)	Dittrich *et al.* (1994)

hoof growth it is therefore important to control for these factors.

The features of the hoof capsule vary at different sites both radially and proximodistally. Whether growth of horn at different radial sites around the hoof capsule takes place at different rates is unclear: Caulton Reeks (1906) and Kainer (1987) suggest that this growth is even, radially, around the coronary band, whereas Geyer and Schulze (1994) reported that the 'palmar/plantar' part of the wall tended to have a lower growth rate in comparison to the dorsal and lateral parts. Josseck *et al.* (1995) also found differences in growth rate between midline, lateral sidewall and palmar/plantar sites.

Therefore, the precise definition of measurement site is important in order to give results a context and to allow repetition of study.

Butler (1976) and Scott and Butler (1980) found that hind hooves grew faster than fore and it can sometimes be unclear which measurement(s) from which site(s) on which feet are contributing to the data presented.

Butler (1976) pooled data from one site on all 4 feet, Graham *et al.* (1994) used a midline site and pooled data from the forefeet only. Geyer and Schulze (1994) used 3 measurement points (dorsal, lateral and palmar) and pooled these data from all 4 hooves in the first year of study and only from the left fore (LF) and right hind (RH) thereafter. Josseck *et al.*(1995) used the same 3 sites as Geyer and Schulze (1994) and used data from LF and RH only. In addition to defining the site(s) for measurement, it is important to state which feet are contributing to the data set.

Apart from objective measurement, Cuddeford (1991), Reilly (1995) and Slater and Hood (1997) have all called for controlled experimentation in this field of study. May (1989) concluded that 'most of the claimed treatments for poor quality hoof horn were empirical and anecdotal'. This remains true and heed must be taken of the recent call for more rigour in setting up experiments involving equids which aim to assess the effects of a therapy. If this is not done then 'therapy becomes lore; medical myths have arisen this way' (Rossdale 1997). This is in danger of becoming the case in this field of study. Unfounded attempts at therapy for hoof horn problems perpetuate medical myths.

More recently controlled studies have been carried out by Buffa *et al.* (1992); Dittrich *et al.* (1994); Josseck *et al.* (1995) and Zenker *et al.* (1995). Coincidentally all these experiments investigated the effects of supplementing dietary biotin. The background leading to the use of biotin has been covered before

TABLE 2: Details of ponies in the trial, feed intake and biotin intake

Pair	Animal	Treatment (T) or Control (C)	Description	Weight (kg)	Age (yrs)	Sex	Mean pair bodyweight (kg)	Fresh weight feed per day (split into 2 feeds) (kg)	Total biotin intake basal + supplemented (mg)	Biotin dose rate (mg/kg bwt)
1	Pony 1	C	Chestnut welsh type	245	2	Mare	257.5	3.86	0.386 + 0 = 0.386	0.00158
	Pony 5	T	Dun welsh type	270	2	Mare			0.386 + 30.88 + 31.266	0.00158
2	Pony 2	C	Bay welsh type	257	7	Gelding	246	3.69	0.369 + 0 = 0.369	0.00144
	Pony 6	T	Bay welsh type	235	6	Gelding			0.369 + 29.52 = 29.889	0.1272
3	Pony 3	C	Chestnut shetland	204	8	Mare	208	3.12	0.312 + 0= 0.312	0.00153
	Pony 7	T	Black shetland	212	8	Mare			0.312 + 24.96 = 25.272	0.1192
4	Pony 4	C	Black shetland	170	12	Mare	165	2.48	0.248 + 0 = 0.248	0.00146
	Pony 8	T	Black shetland	160	14	Mare			0.248 + 19.84 = 20.088	0.1256

TABLE 3: Nutritional specification of basal diet

Component	Proportion in diet as fed
Dry matter	86%
Energy	9.6 MJ/kg DM
Crude Protein	10%
Oil	2.75%
Fibre	20%
Ash	8.5%
Vitamin A	5800 iu
Vitamin D3	1000 iu
Vitamin E	9 iu
Vitamin B1	1 mg/kg
Vitamin B2	3.62 mg/kg
Nicotinic Acid	25 mg/kg
Pantothenic Acid	10 mg/kg
Biotin	100 (µg/kg
Vitamin B12	18 (µg/kg
Vitamin K	1 mg/kg
Manganese	11 mg/kg
Zinc	50 mg/kg
Iron	33 mg/kg
Cobalt	0.25 mg/kg
Iodine	0.4 mg/kg
Calcium	1.2%
Phosphorus	0.5%
Copper	13 mg/kg
Selenium	0.2 mg/kg
Molybdenum	1.0 mg/kg

(Buffa *et al.* 1992; Reilly 1995).

Biotin is a water soluble B group vitamin and is an essential co-factor in glucose and fat metabolism. It can have profound effects on other pathways by its influence on many other intermediaries (Whitehead 1981). It is essential for growth (Tagwerker 1983) and maintenance of epidermal tissues (Geyer and Tagwerker 1984). It is readily absorbed after oral administration and its plasma kinetics are well described (Josseck 1991; Lindner *et al.*1992; Josseck *et al.*1995). It is not stored in the body (Buffa *et al.* 1992) and is safe to feed at high doses as it is excreted via the kidneys in excess. Its lack of toxicity is a major reason for its use in equine feeding trials aimed at assessing the effects of dietary manipulation on hoof horn changes.

Even with more recent controlled studies, whether or not dietary biotin supplementation has an effect on the growth of hoof horn has had equivocal results: Bains (1985) and Buffa *et al.* (1992) found that biotin supplementation increased hoof growth, Geyer and Schulze (1994) and Josseck *et al.* (1995) found no difference in growth rate following supplementation; and Dittrich *et al.* (1994) reported a decrease in hoof growth.

Some of the differences in results from previous workers may be attributable to the different dosages used. Buffa *et al.* (1992) gave 7.5 mg and 15 mg of biotin to horses of, presumably, differing bodyweights. Geyer and Schulze (1994) gave 20 mg to Warmbloods as did Josseck *et al.* (1995) to Lippizanners. Dittrich *et al.* (1994) gave 10, 20 and 40 mg, as single doses to horses for which bodyweights and ages were not reported. Assuming a 500 kg bodyweight horse, these equate to dose rates of 0.015 mg/kg bwt for 7.5 mg, 0.03 mg/kg bwt for 15 mg, 0.04 mg/kg bwt for 20 mg and 0.08 mg/kg bwt for 40 mg.

Therefore hoof horn growth studies can be confounded by factors such as age, sex and breed of horse, site of reading on the hoof capsule, time of year and dosage given. These factors must be taken into account during experimental design.

The aim of this experiment was to investigate the effect of dietary biotin supplementation at one dose rate on hoof growth and growth rate at a specified site on the hoof capsule of all 4 feet of ponies used in a controlled matched-pair designed feeding trial.

Materials and methods

Trial design

Four pairs of ponies were matched for sex, age, weight, size and breed type (Table 2). Their previous history was largely unknown but they were selected in a vetting based on general signs of health and lack of obvious hoof capsular defects. Prior to purchase, potential pairs were aged from their dentition by 2 different veterinary surgeons. All animals were treated with anthelmintics on arrival, had their teeth rasped and their feet trimmed for mediolateral balance. No abnormalities were revealed by individual oral glucose absorption tests or by routine haematology and biochemistry. All 8 animals were then loose-housed in a barn on wheat straw bedding with access to *ad lib* water. They were fed a commercial high fibre pony cube diet which contained 100 µg/kg of biotin. The details of the cube diet are given in Table 3. This was fed, from individual feed mangers, as a common basal diet for a period of 12 weeks prior to the supplementation experiment beginning, and gave a period during which controlled basal hoof growth could take place. No further supplement, nor hay, was fed although each of the animals had equal access to eat its bedding. The total daily ration allocated to each individual was calculated on the basis of 0.015 kg food fed/kg bwt. In order to control for the effect of total energy intake on hoof growth (Butler and Hintz 1977), each animal within a pair received a ration that was calculated for the mean bodyweight for the pair (Table 2). In this way, energy, protein, mineral and vitamin intakes were controlled.

After 12 weeks, one animal from each pair was allocated randomly to treatment or control diet. The treatment diet was exactly the same as the basal diet except for the addition of 8 mg biotin/kg food as fed. This was also fed on a mean paired weight basis at a rate of 0.015 kg food kg/bwt. The total daily ration was divided to give 2 equal feeds. The absolute amount of biotin fed to each individual within the treatment group varied (Table 2) but the dose rate was consistent at 0.12 mg/kg bwt. Similarly, the absolute amount of biotin fed to control animals maintained on the basal diet varied, but the dose rate to them was 0.0015 mg/kg bwt (Table 2).

There were no problems with acceptance of the food. Biotin supplementation continued for a further 5 months during which period the extent of new hoof growth was measured.

Measurement of hoof growth

New horn streams distally from the level of the coronary band (CB). An assessment of growth was given by measuring the distance to which a hot-branded mark ('X'), made on the hoof wall of each foot of each pony, had descended, at given times during the trial, with respect to a proximal reference point in the region of the coronet. The 'X' mark was made with the flat filed end of a Philips screwdriver which was heated to red hot and placed approximately 1.5 mm into the dorsal hoof wall. The definitive feature used as a reliable proximal fixed point was the reference hairline (RH) at the coronet. This was revealed by turning back the fringe of hair that normally hangs over the CB (Fig 1).

Definition of recording site

Measurements were made at the midline dead centre (MDC) for

TABLE 4: Hoof horn growth data for all ponies by period of trial animal and hoof

Animal and Hoof	RHG_0 (mm)	RHG_1 (mm)	Period 1 G_n (RHG_1-RHG_0) (mm)	RHG_2 (mm)	Period 2 cum. G_n (RHG_2-RHG_0) (mm)	RHG_3 (mm)	Period 3 cum. G_n (RHG_3-RHG_0) (mm)	RHG_4 (mm)	Period 4 cum. G_n (RHG_4-RHG_0) (mm)	RHG_5 (mm)	Period 5 cum. G_n (RHG_5-RHG_0) (mm)
Control animals											
Pony 1 LF	19.0	26.0	7.0	32.0	13.0	39.0	20.0	45.0	26.0	51.0	32.0
RF	16.0	21.5	5.5	24.0	8.0	33.0	17.0	38.5	22.5	47.0	31.0
LH	27.5	30.5	3.0	35.0	7.5	45.0	17.5	52.0	24.5	60.0	32.5
RH	24.0	27.5	3.5	32.0	8.0	44.0	20.0	49.5	25.5	57.0	33.0
Pony 2 LF	16.5	23.0	6.5	29.0	12.5	40.0	23.5	46.5	30.0	50.0	33.5
RF	15.0	18.0	3.0	22.0	7.0	34.0	19.0	39.0	24.0	47.0	32.0
LH	20.0	28.0	8.0	33.0	13.0	45.5	25.5	50.0	30.0	58.0	38.0
RH	25.0	30.0	5.0	34.0	9.0	43.5	18.5	50.0	25.0	54.0	29.0
Pony 3 LF	22.5	31.5	9.0	43.0	20.5	52.5	30.0	55.5	33.0	64.0	41.5
RF	23.5	30.0	6.5	35.0	11.5	46.5	23.0	51.5	28.0	53.0	29.5
LH	28.0	33.0	5.0	39.0	11.0	48.0	20.0	58.0	30.0	63.0	35.0
RH	26.5	34.0	7.5	43.0	16.5	56.0	29.5	60.0	33.5	64.0	37.5
Pony 4 LF	18.0	22.0	4.0	26.5	8.5	36.0	18.0	40.0	22.0	47.5	29.5
RF	12.5	16.0	3.5	20.0	7.5	24.0	11.5	29.5	17.0	35.0	22.5
LH	20.5	22.5	2.0	26.0	5.5	31.0	10.5	34.0	13.5	37.0	16.5
RH	21.0	23.0	2.0	25.0	4.0	31.0	10.0	34.0	13.0	39.0	18.0
Treatment animals											
Pony 5 LF	29.0	34.5	5.5	42.0	13.0	55.5	26.5	61.5	32.5	68.0	39.0
RF	18.5	25.0	6.5	31.0	12.5	42.5	24.0	51.0	32.5	61.0	42.5
LH	26.5	30.5	4.0	36.0	9.5	49.0	22.5	58.0	31.5	65.0	38.5
RH	32.0	37.0	5.0	42.0	10.0	55.5	23.5	63.0	31.0	70.0	38.0
Pony 6 LF	32.5	37.0	4.5	42.0	9.5	53.0	20.5	64.5	32.0	68.5	36.0
RF	16.0	23.0	7.0	31.0	15.0	41.5	25.5	48.0	32.0	52.0	36.0
LH	33.0	38.0	5.0	44.0	11.0	59.5	26.5	64.0	31.0	67.0	34.0
RH	34.0	38.5	4.5	45.0	11.0	55.5	21.5	63.0	29.0	69.0	35.0
Pony 7 LF	23.0	33.0	10.0	41.0	18.0	49.5	26.5	57.0	34.0	65.0	42.0
RF	17.5	24.5	7.0	32.5	15.0	41.5	24.0	48.5	31.0	58.0	40.5
LH	24.0	27.5	3.5	33.5	9.5	43.5	19.5	50.0	26.0	57.0	33.0
RH	24.5	30.5	6.0	39.0	14.5	50.0	25.5	56.5	32.0	66.0	41.5
Pony 8 LF	22.5	27.5	5.0	33.0	10.5	39.0	16.5	44.0	21.5	52.0	29.5
RF	18.0	23.0	5.0	26.0	8.0	29.0	11.0	36.5	18.5	43.0	25.0
LH	21.5	26.0	4.5	30.0	8.5	39.0	17.5	43.0	21.5	51.5	30.0
RH	23.0	26.5	3.5	30.0	7.0	35.5	12.5	43.0	20.0	48.0	25.0

cum: cumulative.

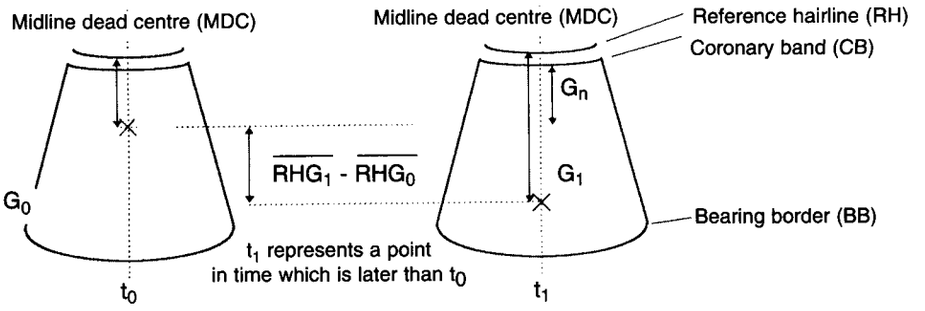
each hoof. The plane of the MDC is that which bisects the foot and is given in Reilly *et al.* (1996). The MDC was given by extending a line that bisected the frog onto the dorsal hoof wall. This then followed the line of the visible dorsal wall hoof tubules to the coronet and was marked with chalk before branding. The initial individual brand marks on the feet of each pony were made below the distal extremity of the periople (XG_0 in Fig 1). Each mark was made at a variable point proximodistally on each hoof, but the distance from it to the RH gave the initial baseline reading for each foot from each animal (RHG_0, column 1, Table 4). The brandmark was not made any higher as the procedure might have damaged periople as well as horn that we were subsequently interested in harvesting for use in other tests. New horn growth from the coronary band, G_n was, therefore, assumed to equal G_1 minus G_0, the difference in measurements from the hairline (Fig 1). The linear distance between the RH and the branded mark was spanned with a pair of dividers. These were then placed against a steel ruler to give a growth reading to the nearest 0.5 mm. Initial growth measurements for the allocated treatment and control groups were made for the last 26 days of the 12 week basal feeding period prior to the start of biotin supplementation. Subsequent growth measurements were made periodically. Period intervals were 32–45 days (Fig 2).

To avoid bias in a nonblinded experiment, data were collected from one group of ponies first and stored, and then data were collected from the other group on the same day. In this way, direct comparison between treatment and control pairs was not allowed during data collection, and analysis of the data did not take place until some months after the experiment had finished.

The data set for hoof growth readings during the course of the trial is given in Table 4.

Statistical analysis

Growth is a continuous variable and would be expected to have a normal distribution. To test this an n-scores probability (Minitab Corp) was plotted for treatment and control data. A correlation of 0.976 for n = 32 (8 horses 4 feet each) was given. This meant that the hypothesis for normal spread of the data could be accepted at a probability of P = 0.01. Therefore, parametric tests; students



Growth is given by: $\overline{RHG_1}$ - $\overline{RHG_0}$ and is equal to the growth from the coronary band, G_n.

Hoof horn growth rate is given by: $\frac{\overline{RHG_1} - \overline{RHG_0}}{t_1 - t_0}$

Fig 1: Measurement of hoof horn growth and hoof horn growth rate at midline dead centre.

t test and analysis of variance were used to assess the data using the Minitab software package.

Results

Hoof horn growth

The cumulative mean growth of horn from the MDC of the feet of all 8 ponies is plotted by period of trial in Figure 2.

The growth of horn for treatment and control groups in the 26 days before supplementation began was compared by *t* test. (Period 1 in Fig 2). There was no significant difference between the 2 groups (P = 0.62).

By the end of the trial treatment animals had achieved a mean hoof growth at the MDC of 35.34 mm (s.d. ± 5.63 s.e. ± 1.41) and the control group 30.69 mm (s.d. ± 6.78 s.e. ± 1.70). This difference was significantly different (P<0.05) by Student's *t* test.

Therefore, there was a treatment effect on hoof growth after 5 months of biotin supplementation.

Hoof horn growth rate

The slopes of the growth curves (i.e. growth rates), for treatment and control groups were assessed by comparison of regression. The whole data sets contributed to the regression lines.

The regression equation for control growth was:

$$y = 6.5906x - 1.6969$$

The regression equation for treatment growth was:

$$y = 7.6969x - 2.6656$$

The s.d. for control hoof growth rate was 1.263 and for treatment hoof growth rate was 1.107.

Comparison of regression analysis showed that biotin supplementation produced a significantly higher growth rate of hoof horn in this trial (P<0.02).

The mean rate of hoof growth over the whole of the trial, by extrapolation from Figure 2, was:

0.164 mm/day for nonsupplemented ponies
and 0.189 mm/day for biotin supplemented ponies

When expressed in percentage terms treatment animals had a 15% higher growth rate of hoof horn and 15% more hoof growth, at the midline dead centre, by the end of the trial period.

Differences in total growth of hoof horn between feet and between ponies

Analysis of variance (ANOVA) was carried out on total growth, by foot, and by individual pony, for treatment and control animals.

This showed that there was no difference between feet for all 8 ponies but that there was a difference between individuals for total growth (P<0.05). The 2 older animals (*pony 4* and *pony 8* which comprised pair 4) both had significantly reduced hoof growth compared to the rest by the end of the trial.

Discussion

Experimental design

Restriction of experimental error in feeding trial design is desirable and is aided by controlling the extent of variability between animals. Experimental variabilty can be reduced by matching animals as nearly as possible (Roberts 1975). This has long been a principle in agricultural experiments. For example with dairy cows, matching by date of calving, parity, size, and previous milk yield are important and has been done for experiments leading to foot measurements in cattle (Reilly and Brooks 1990). For this experiment, because of the species used, and the nature of the measurements, it was more appropriate to match for breed, age, sex, size and weight. These are similar to the important factors to match for in beef trials (Roberts 1975). 'Growth' of horn is therefore analogous to 'weight' of beef animal in such trials, and 'growth rate' of horn is analogous to 'growth rate' of the beast.

In order to control for the vagaries of horse feeding in terms of variable inputs from hard feed, pasture or hay for example, a commercial cube was chosen as the basal diet and as the carrier for biotin supplementation. Although the plasma kinetics for feeding biotin in other forms are known, this is not so for this experiment, as was the case with Buffa *et al.* (1992), because of the prohibitively high cost of plasma biotin analysis. Initial hoof growth measurement between the potential treatment and control groups was carried out in only the last 26 day period of basal feeding as assessment prior to then may have recorded the effects of nutritional and environmental factors affecting horn growth prior to purchase. The fact that there was no difference between the 2 groups meant that they could be confidently allocated without any carry over of covariate confounding factors. This acted as a form of internal control.

Hoof horn growth

Both CB and RH have been used as reference points in the assessment of hoof horn growth which is subject to potential measurement error. Geyer and Schulze (1994) used the CB and Butler and Hintz (1977), Graham *et al.* (1994) and Josseck *et al.*

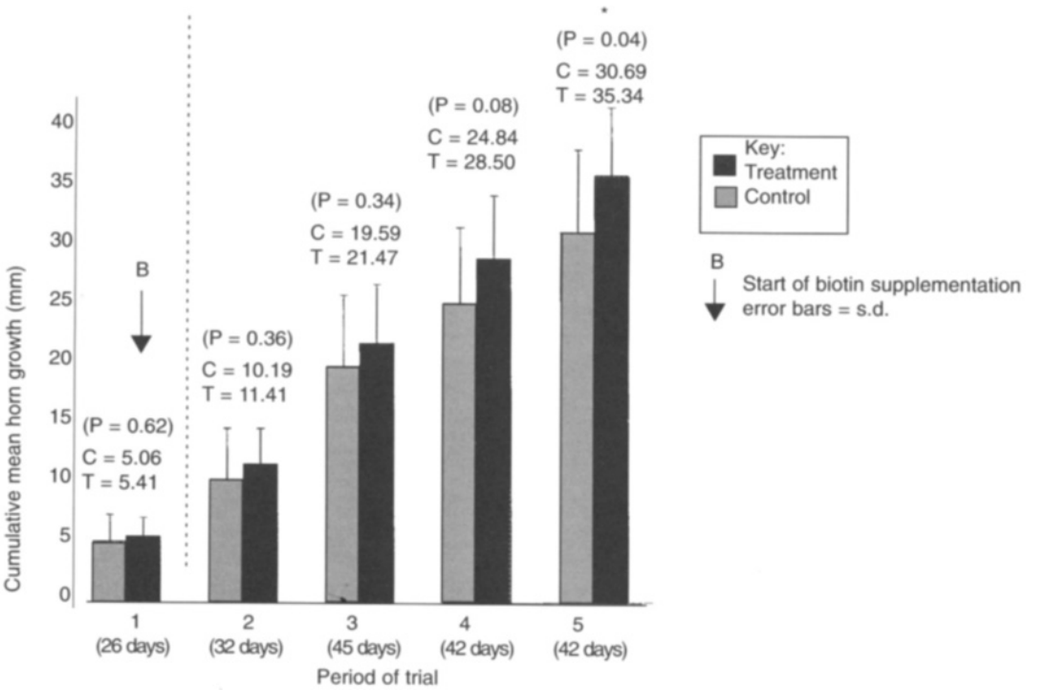


Fig 2: Cumulative mean hoof growth (all feet) by period of trial.

(1995) all used RH. The dividers and steel ruler method was used to improve the accuracy of measurement in this work, but the repeatability of measurements also needed to be addressed. A pilot study established that the repeatability of measurements to RH was within 1% whereas for the CB it was >4%. The latter method was therefore rejected.

The effect of biotin on hoof horn growth in this trial is in agreement with the findings of Bains (1985) and Buffa *et al.* (1992). Can biotin be considered, then, a therapy? It can in so far as it has produced a change that will alter the 'renewal time' (Geyer and Schulze 1994) of the capsule for these ponies. From the probability of the differences in growth between control and treatment groups over time (Fig 2) it is possible that the effect on the renewal time of the capsule would be more marked should supplementation have continued for longer. In addition, a small but significant difference in mean growth of 4.65 mm in a treated foot compared to a nontreated foot over a period of 5 months, may just be sufficient to make a difference in new horn for nailing in to in an overwintered animal, or to push away an old lesion which, as a result of the rather subtle mechanism of hoof wall growth and repair (Reilly *et al.* 1996), does not rely on total capsular renewal to be replaced. In situations where total capsule regrowth is required, then biotin supplementation may help reduce 'renewal times' following hoof capsule avulsions or perhaps after major resections subsequent to laminitis or onychomycosis. This, however, assumes a similar response in resected horn which cannot be inferred from the results of this work. Further work is required to investigate the effects of biotin as a therapy under these circumstances.

Hoof growth differences between feet and between individuals

There was no difference in growth between feet in this trial. This does not agree with Butler (1976), and Scott and Butler (1980). However, these authors reported growth in foal groups as they aged from 8 months. The ponies in this trial were more mature. There is no clear evidence as to whether or not growth decreases with older age within the equid population, other than foals and yearlings appearing to have higher growth rates when a comparison between different workers' results is made from Table 1.

In this trial, there was a difference between individuals by ANOVA, with the 2 older ponies showing a significantly decreased growth of horn compared to the others. Further work is required from trials designed to investigate the relationship between age of animal and response to biotin supplementation.

Hoof horn growth rates

The mean growth rates in this trial of 0.164 mm/day (control) and 0.189 mm/day (treated) over a winter period (October–February) are in agreement with a generalised figure, for mature horses, of 0.2 mm/day (Table 1) and with Glade and Saltzmann (1985). This is lower than those recorded by Buffa *et al.* (1992) but that investigation was conducted in South Africa, in a dry season and with horses that were exercised and shod.

Pollitt (1990) showed an inferred difference in hoof growth rate by dorsopalmar depth into the dorsal hoof wall, with an autoradiographic technique. Measuring the rate of descent of a branded mark on the outside of the hoof capsule may only indicate part of what is happening within the hoof wall. Evidence for 'zonation' by dorsopalmar depth of the wall has been given by Reilly *et al.* (1996).

How biotin exerts its effect is unclear. Buffa *et al.* (1992) concluded that it influenced the amount or proportion of keratin molecules in hoof horn, resulting in increased growth rate. Kempson (1989), citing Marston (1946), implied that a response to biotin supplementation was brought about 'through modification of the division and maturation of proliferative cells'. To what extent biotin has effects during the cornification

and/or keratinisation processes cannot be concluded until measurement is undertaken at the cellular/molecular level. Otherwise, it can be postulated only that alleviation of a rate limiting step in the enzymatic functions of biotin may explain the responses in this trial.

This was a biotin super supplementation experiment to a group of ponies that did not have overtly problem affected feet. The lack of rationale for a response to super supplementation in horses that should not theoretically be biotin deficient has been discussed by Buffa *et al.* (1992). In that report the horses had 'poor hoof horn' by visual inspection, in this case the ponies did not. They were also not being worked, nor unduly stressed, and a response to supplementation has, therefore, been shown in a group of animals that should not have been biotin deficient and for which confounding factors had been attempted to be minimised. Control ponies were receiving approximately 0.3 mg of biotin/day (Table 2) and the Treatment animals were receiving approximately 30 mg of biotin/day. This is equivalent to approximately 60 mg/day if extrapolated to a 500 kg horse. The 'requirement' for biotin is not known but the control animals were receiving an acceptable level of biotin for maintainance according to Harris *et al.* (1995) who recommend 0.1 mg/kg of diet. Therefore, the results from this trial can be regarded as a supraoptimal response (Cuddeford 1991) in ponies with normal feet. It is unknown whether a higher dose rate of biotin would effect bigger differences. Further work is required to be able to assess fully the response of the equid to supraoptimal supplementation with keratogenous factors. Future approaches to experimental work should, ideally, include double blinding and cross over design.

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