Effect of feeding biotin on milk production and hoof health in lactating dairy cows: A quantitative assessment

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ABSTRACT

Objectives of this study were to critically review randomized controlled trials, evaluate the effectiveness of supplementation with biotin on milk yield and composition and hoof health in lactating dairy cows, explore sources of heterogeneity among studies, and evaluate publication bias. Quantitative assessments can increase the statistical power with which we study the effect of treatments, such as biotin, on outcomes. A total of 9 papers, with 6 production and 3 hoof health studies, met the eligibility criteria for meta-analysis. Eight studies evaluated various hoof lesions in biotin-supplemented cows that did not meet the inclusion criteria. Eleven comparisons were made of milk production responses to biotin treatment. Data extracted included the number of cows in control and treatment groups, measures of variance of responses (standard error or standard deviation) and *P*-values. Other data obtained included the duration of treatment before and after calving, parity, breed of cow, type and dose of biotin, delivery method of supplementation, and types of diets. Biotin increased milk production by 1.29 kg per head per day (95% confidence interval = 0.35 to 2.18 kg) with no evidence of heterogeneity ($I^2 = 0.0\%$). Treatment did not affect milk fat or protein percentages, and a trend to increase fat and protein yields was observed. Milk production and composition results were not influenced by duration of treatment before calving, parity, or diet type. Assessment of biotin supplementation on hoof health indicated that more studies had improved rather than negative or neutral outcomes. The effect of biotin treatment on milk production was relatively large and the effects on fat and protein yields, although not significant, were consistent in direction and magnitude with the milk response. The hoof health responses to biotin should encourage further studies to more effectively define the nature of these responses using consistent criteria for examination of hoof conditions and lameness.

Key words: dairy cattle, biotin, meta-analysis

INTRODUCTION

Although ruminants produce biotin as a result of bacterial fermentation, the requirements for biotin and other B-group vitamins in cattle have been reevaluated (Girard, 1998). Biotin is essential for ruminants and studies have been conducted on biotin synthesis, absorption, and balance in lactating and nonlactating cattle (Miller et al., 1986; Frigg et al., 1993, 1994; Klueenter et al., 1993; Steinberg et al., 1995). Biotin is a cofactor for microbial enzymes involved in propionic acid synthesis (Baldwin and Allison, 1983) and is also present in carboxylase-containing enzymes critical to gluconeogenesis and other metabolic roles in mammals (Mock, 2005).

In vivo studies (Miller et al., 1986; Santschi et al., 2005a,b; Schwab et al., 2006) found low or even negative apparent ruminal synthesis of biotin. However, Zinn et al. (1987) estimated a positive apparent synthesis of biotin in beef cattle. Diets containing more than 50% of DM as grain decreased ruminal synthesis of biotin in vitro compared with diets containing more forage (Abel et al., 2001). Bentley et al. (1954) and Milligan et al. (1967) showed that biotin supplementation increased fiber digestion in vitro, and that ruminal bacteria also require biotin for propionate production. Propionate production by mixed ruminal bacteria was reduced in vitro when biotin was not included in the culture media (Bentley et al., 1954; Milligan et al., 1967). These studies suggest that supplemental dietary biotin may increase fiber digestion or increase propionate production, thereby providing additional nutrients that may be partitioned to milk production by the cow. Consequently, interest exists in milk production responses to supplemental biotin, especially in cattle fed diets containing substantial amounts of concentrate. However, milk production responses to supplemental biotin have not consistently been significant, suggesting the need to quantitatively evaluate responses and to examine the sources of variation in responses.

Many studies have investigated the biological effects of biotin on hoof health, including keratin protein synthesis and formation of the intercellular cement, which influence horn formation of the hoof, hoof strength, and claw integrity (Whitehead, 1988; Sarasin, 1994;

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Hochstetter, 1998; Fitzgerald et al., 2000; Hedges et al., 2001; Koester et al., 2002). There were, however, limited randomized controlled studies on the clinical effect of biotin supplementation to improve hoof health and reduce the risk of lameness in dairy cows.

This study reviewed and collated papers and reports on biotin. Our aim was to quantify the effects of supplementation on milk production and hoof disorders to improve the precision of point estimates derived from pooled data, answer questions not posed by the individual studies identified by the literature search, settle controversies arising from apparently conflicting studies, or generate new hypotheses. We also explored sources of heterogeneity among production studies and evaluated the presence of publication bias.

MATERIALS AND METHODS

Literature Search

The literature search, using PubMed, Google Scholar, Agricola, Science Direct, Scirus, and CAB, contact with workers in the field, and investigation of references in papers identified yielded 205 publications on biotin. The following key words in different combinations were used for our search: transition period, milking period, prepartum, peripartum, postpartum, biotin, vitamin B complex, cattle, cow, dairy, management, milk yield, milk composition, dry matter intake, supplement, hoof health. A total of 34 research papers and reports (journal articles, published abstracts, and reports) had milk production or hoof health data, or both. Of these, 6 randomized clinical studies with production data, containing 11 comparisons, provided suitable information and were considered in the meta-analysis. Eight studies with hoof health information that did not meet the selection criteria for a meta-analysis, were subsequently evaluated to determine the direction of effect. A list of publications reviewed for the study is provided in Table 1.

Inclusion and Exclusion Criteria

Studies were included or excluded in this study based on criteria developed by the authors. Quality assessment criteria included randomization of study groups, blinding to treatment application and analysis of data, statistical analysis, and comparability of treatment groups at entry to each trial. Trials were included in the analysis if the animals utilized were lactating dairy cows and if they reported an adequate description of randomization processes; sufficient data to determine the effect size for production outcomes (e.g., the number of cows in each treatment and control group); a

Table 1. Summary of papers investigated production performance and hoof health outcomes in biotin-supplemented primiparous and multiparous dairy cows before or after calving

Study	Supplementation			No. of farms, parity, and diet ¹		Biotin product, dose, and delivery methods			
	Comparisons (parity/dose)	Start day	Duration (d)	Farms (n)	Parity	Diet	Product	Dose (mg)	Delivery ²
Bergsten et al. (2003)	Low producing High producing	-17	420	1	P, M	PMR	Not reported	20	Mixed
Ferreira et al. (2007)		+136 to +267	14	1	M	TMR	TMR	Rovimix-H2 ³	20
Rosendo et al. (2004)	10 mg 20 mg	+136 to +267	14	1	M	TMR	Rovimix-H2	20	Mixed
		25	70	1	M	TMR	Rovimix-H2	20	Mixed
Zimmerly and Weiss (2001)	10 mg	-14	100	1	P, M	TMR	Rovimix-H2	10	Capsule and mix
Enjalbert et al. (2008)	20 mg	-14	100	1	P, M	TMR	Rovimix-H2	20	Capsule and mix
	Wk 2-6	-15	42	1	P	PMR	Not reported	20	Mixed
	Wk 2-6	-15	42	1	M	PMR	Not reported	20	Mixed
	Wk 7-17	-15	77	1	P	PMR	Not reported	20	Mixed
Hedges et al. (2001) ⁴	Wk 7-17	-15	77	1	M	PMR	Not reported	20	Mixed
	Parity 1 Parity >1	+1	540 (18 mo)	5	P, M	PMR	Rovimix-H2	20	Liquid
Pötzsch et al. (2003) ⁴		+1	90-444	5	P, M	PMR	Rovimix-H2	20	Liquid
	Bonomi et al. (1996)	+1	90-444	5	P, M	PMR	Rovimix-H2	20	Liquid
		+1	150	1	—	TMR	Not reported	10	Mixed

¹Parity: P = primiparous; M = multiparous; PMR = partly mixed ration.

²Mixed = biotin mixed with diet; Capsule and mix = biotin was supplemented to cows in the form of a capsule during the precalving period and mixed with the diet after calving; Liquid = biotin in liquid form.

³Rovimix biotin, DSM Nutritional Products, Basle, Switzerland.

⁴These studies had only the hoof data.

measure of effect amenable to effect size analysis for continuous data [e.g., standardized mean difference (SMD)] and the rate data [e.g., risk ratio (RR)]; a measure of variance (SE or SD) or *P*-value, or both, for each effect estimate or treatment and control comparisons; and the number of cows and incidence of hoof disorders in biotin-supplemented and control groups.

In total, 26 studies were excluded from analysis. Studies that failed to meet the essential criteria of the randomized controlled trial included reviews and studies that had no milk production or hoof health data or had positive controls; the excluded studies are listed in the Appendix. Crossover and Latin square studies were also excluded from the meta-analysis. The gold standard for inclusion in a meta-analysis is a randomized clinical trial, and crossover and Latin square studies provide less rigorous evidence. We exclude Latin square and crossover designs because the randomized, controlled trial is at present the unchallenged source of the highest standard of evidence used to guide clinical decision making (Lavori and Kelsey, 2002). Other reasons for the exclusion of crossover and Latin square studies were the potential for carryover effects (Lean et al., 2009) and the small sample size of these studies that often had multiple phases. Most of these studies reported a standard error or standard deviation of the means overall rather than for each period; of those that reported the measure of variance, it was not always clear how these measures of variance were estimated. Finally, if these studies are included in a meta-analysis, the data set may become heterogeneous because the design of these studies inherently differs from the randomized, controlled trial studies.

Data Extraction

A total of 6 studies (11 comparisons) with production data met the eligibility criteria for meta-analysis. A total of 3 eligible studies (4 comparisons) with hoof health incidence data were available, but this was insufficient to justify a meta-analysis. The other hoof health studies (*n* = 9 papers) reported various hoof disorders or inconsistent measurement metrics (prevalence and incidence), or did not have adequate information to be included in the meta-analysis.

The extracted data also included number of cows in control and treatment groups, measures of variance of responses (SE or SD), and *P*-values. Other information extracted from relevant papers were duration of treatment before and after calving, parity, breed, type and dose of biotin, delivery method of supplementation, and types of diets. A summary of studies and variables measured is given in Table 1. Papers included in this study

reported that Holstein-Friesian cows were enrolled, no bST was used, cows were milked twice daily except in one study (Rosendo et al., 2004), and stated the vitamin and minerals that were part of the diet.

Statistical Analysis

A meta-analysis was conducted on the extracted production outcomes using Stata (Intercooled Stata v.11, StataCorp, College Station, TX).

Continuous Data. Continuous data were analyzed using SMD, which is also called effect size (ES), in which the difference between treatment and control groups means was standardized using the standard deviations of control and treatment groups. The SMD estimates were pooled using the methods of Cohen (1988) for the fixed effect and DerSimonian and Laird (1986) for the random effects models. If the paper reported separate estimates of measure of variance (SE or SD) for each group, these were recorded as such. If the studies reported a common SE or SD, the estimate was used for both control and treatment groups. If the study only reported a *Z*-statistic or *P*-value, estimates of SE or SD were computed using the number of cows in each group. For studies that only reported a *P*-value less or equal to a given value (e.g., *P* < or > 0.05), then the given value was used, and *P*-value and SE were computed similarly to the methods described above. For studies that only reported a nonsignificant effect, *P*-values of 0.15, 0.3, and 0.5 were assigned and compared as described by Sanchez et al. (2004). The *P*-value that produced the smallest estimate of overall SMD was selected for the calculation of the standard error. Fixed and random effects models were conducted for each production outcome to estimate the effect size, 95% CI, and statistical significance of SMD.

Forest Plots. The effects of biotin on production performance of lactating dairy cows are displayed in the forest plots, using the estimated SMD of the included studies (Figure 1). Points to the left of the line represent a reduction in the trait, whereas points to the right of the line indicate an increase in the variable. Each square represents the mean effect size for that study. The upper and lower limits of the line connected to the square represent the upper and lower 95% CI for the effect size. The size of the square reflects the relative weighting of the study to the overall effect size estimate, with larger squares representing greater weight. The dotted vertical line represents the overall effect size estimate. The diamonds at the bottom represent the 95% CI for the overall estimates of fixed effect (using inverse variance procedures) and random effects (DerSimonian and Laird, 1986) models. The solid ver-

Figure 1. Forest plot of standardized mean difference (SMD) and their 95% CI and weights for individual trials determined from the results of 11 comparisons of milk production of cows supplemented with biotin versus other cows. Box sizes are proportional to the inverse variance of the estimates. Summary estimates of treatment effects (diamonds) are shown using (1) a fixed effects approach (I-V specifies a fixed effect model using the inverse variance method), (2) a random effects approach (D+L specifies a random effects model using the method of DerSimonian and Laird, 1986), and (3) the predicted interval of a future trial, with the estimate of heterogeneity being taken from the inverse variance fixed effect model. PMR = partial mixed ration. Color version available in the online PDF.

tical line represents a mean difference of zero or no effect. The first author and year of the publication are provided under “Study.”

Weighted mean differences (**WMD**) are estimated using the mean difference between biotin-supplemented and control groups, and weights of number of observations, where weights were inversely proportional to the variance of observations. Typically, the observations represent averages and the weights are the number of elements that give rise to the average.

Hoof Data. Because of the lack of adequate studies that examined any single hoof lesion, lack of consistent definition of hoof lesions between studies, lack of raw data (number of affected and not affected in both groups), and lack of consistency between papers on hoof health measurements (e.g., incidence, prevalence), we were unable to assess these data quantitatively. Only 3 hoof studies met all study criteria. Consequently, the hoof health studies were only assessed to determine the potential trend of effect and justification of the need for further investigation.

Assessment of Heterogeneity. Variations among the trial-level SMD were assessed using a chi-squared (Q) test of heterogeneity. We used an α -level of 0.10 because of the relatively poor power of the χ^2 test to detect heterogeneity among small numbers of trials (Egger and Davey Smith, 2003). Heterogeneity of results among the trials was quantified using the I^2 statistic (Higgins and Thompson, 2002; Higgins et al., 2003). Negative values of I^2 were assigned a value of zero; consequently, the value I^2 lies between 0 and 100%. An I^2 value $>50\%$ may be considered indicative of substantial heterogeneity.

Meta-Regression. Meta-regression is a technique that can formally test whether evidence exists of different effects in different subgroups of trials (Knapp and Hartung, 2003). Meta-regression analyses were used to explore the source of heterogeneity of response, using the individual ES for each trial as the outcome and the associated standard error as the measure of variance. It is appropriate to use meta-regression to explore sources of heterogeneity even if an initial overall test for het-

Table 2. Standardized mean difference (SMD), significance, weighted mean difference, and heterogeneity of production outcomes

Outcome	SMD (95% CI)			Heterogeneity			Weighted mean difference (kg/d) (95% CI)
	Studies (n)	Random effects model	P-value	Chi-squared (Q)	I ² (%)	P-value	
Milk yield (kg/d)	11	0.30 (0.11 to 0.50)	0.002	8.70	0.0	0.561	1.29 (0.47 to 2.11)
Milk fat (%)	11	0.12 (-0.08 to 0.31)	0.225	7.22	0.0	0.704	0.05 (-0.05 to 0.15)
Milk protein (%)	10	-0.15 (-0.45 to 0.15)	0.332	12.25	26.5	0.200	-0.09 (-0.08 to 0.06)
Milk fat yield (kg/d)	10	0.18 (-0.02 to 0.38)	0.081	5.02	0.0	0.833	0.07 (-0.001 to 0.13)
Milk protein yield (kg/d)	9	0.23 (-0.04 to 0.50)	0.093	6.18	0.0	0.628	0.02 (-0.01 to 0.06)
DMI (kg/d)	5	0.30 (-0.04 to 0.64)	0.085	1.67	0.0	0.795	0.70 (-0.02 to 1.62)

erogeneity is nonsignificant (Thompson and Higgins, 2002). The chi-squared test often has low power (Hardy and Thompson, 1998) and therefore a nonsignificant result does not reliably identify lack of heterogeneity. Furthermore, the test is for general over-dispersion of trial results, and does not address whether heterogeneity relates to particular covariates. A meta-regression was conducted to explore whether the outcomes were influenced by start time (before or after calving), duration of treatment before and after calving, parity of cows in the study (multiparous herds vs. mixed herds), bST treatment, or delivery method (mixed with diet, in a liquid form, or capsule).

Meta-regression analysis was conducted by first screening individual variables using a P -value of ≤ 0.20 . All variables with P -value of ≤ 0.20 were entered into a backward stepwise weighted meta-regression, until all remaining variables were significant at $P < 0.05$.

Publication Bias. The presence of publication bias is commonly investigated using funnel plots. A funnel plot is a simple scatter plot of the intervention effect estimates from individual studies against some measure of each study's size or precision. It is commonly presented as a plot of the effect estimates on the horizontal scale, and the measure of variance on the vertical axis. The name "funnel plot" arises from the fact that precision of the estimated intervention effect increases as the size of the study increases. Effect estimates from small studies will therefore scatter more widely at the bottom of the graph, with the spread narrowing among larger studies. In the absence of bias, the plot should approximately resemble a symmetrical (inverted) funnel. If bias exists, for example, because smaller studies without statistically significant effects remain unpublished, this will lead to an asymmetrical appearance of the funnel plot with a gap in a bottom corner of the graph. In this situation the effect calculated in a meta-analysis will

tend to overestimate the intervention effect (Egger et al., 1997). The more pronounced the asymmetry, the more likely it is that the bias will be substantial.

Contour-Enhanced Funnel Plots. Contour-enhanced funnel plots have been proposed by Peters et al. (2008) to include contour lines corresponding to the statistical significance ($P = 0.01, 0.05, 0.1$). This approach allows the statistical significance of study estimates and areas in which studies are perceived to be missing to be considered. The contour-enhanced funnel plots may help to differentiate asymmetry caused by publication bias from that due to other factors. For example, if studies appear to be missing in areas of statistical nonsignificance, this adds credence to the possibility that the asymmetry is caused by publication bias. Conversely, if the supposed missing studies are in areas of higher statistical significance, this suggests that the observed asymmetry may be more likely caused by factors other than publication bias; for example, variable study quality or a failure to publish findings that were not statistically significant.

Hoof Health Data. Due to substantial amounts of missing information, inconsistencies in reporting hoof lesions in different studies/trials, measures of outcome (incidence vs. prevalence), cattle used (beef vs. dairy), units of observation (animal vs. herd), and doses of biotin, it was not appropriate to conduct a full meta-analysis on hoof data. However, the direction of the effect of biotin in supplemented cattle was assessed by comparing the number of studies with positive and negative outcomes.

RESULTS AND DISCUSSION

Several studies and reviews (reports and abstracts) failed to provide sufficient information on production and hoof health data. We critically reviewed all the

Figure 2. Contour-enhanced funnel plots of production data and DMI for cows supplemented with biotin. Levels of significance for studies (●) within the gray broken lines are 0.01, 0.05, and 0.10; FE = fixed effect.

provided information and reports, and summarized the findings on each study in Table 1. A total of 34 studies investigated the effects of biotin on production performance and hoof health in lactating dairy cows. Of these, 26 studies were excluded for various reasons. Of the 8 remaining, 6 were used in meta-analyses of production outcomes, and 3 studies provided only hoof-health data and were insufficient to justify a meta-analysis of hoof-health outcomes.

Production Outcomes

A summary of 6 studies containing 11 comparisons with biotin and milk production results is presented in Table 2. Some studies contained a summary of a single trial, whereas others studies reported multiple comparisons such as different doses of biotin or parities of cattle. The effect size analysis of biotin treatment on

milk production is presented in Figure 1. An increase in milk production of biotin-supplemented cows was observed ($ES = 0.30$; $95\% CI = 0.11$ to 0.51 ; $P = 0.002$; Figure 1) with no significant evidence of heterogeneity ($I^2 = 0.0\%$, $P = 0.561$; Table 2). The small number of studies included in the meta-analysis may not allow an accurate determination of the effects of starting time of supplementation (before or after calving) or duration of supplementation before and after calving on the milk production response to biotin. The limited number of studies of the effects of biotin on milk production also makes it difficult to accurately evaluate the presence of publication bias using funnel plots; however, it is possible that negative studies with a high standard error were missing from the milk production data (Figure 2). The milk production response to biotin was quite large, being 1.3 kg/d per cow. The milk responses were largely observed with diets based on corn silage and

Figure 3. Forest plot of standardized mean difference (SMD) and their 95% CI and weights for individual trials determined from the results of 10 comparisons of milk fat production of cows supplemented with biotin versus other cows. Box sizes are proportional to the inverse variance of the estimates. Summary estimates of treatment effects (diamonds) are shown using (1) a fixed effects approach (I-V specifies a fixed effect model using the inverse variance method), (2) a random effects approach (D+L specifies a random effects model using the method of DerSimonian and Laird, 1986), and (3) the predicted interval of a future trial, with the estimate of heterogeneity being taken from the inverse variance fixed effect model. PMR = partial mixed ration. Color version available in the online PDF.

alfalfa as the basal forages in TMR diets. Responses were homogeneous across the range of study designs used. No significant effects of stage of lactation, dose of biotin, duration of supplementation, or type of diet were found on responses. However, it should be noted that relatively few studies were analyzed and the statistical power to investigate some of these sources of variation in study design was limited. Consequently, some caution should be applied to extrapolating these responses to all diet and management systems.

Milk fat percentage did not differ between biotin-fed and control cows (ES = 0.12, 95% CI: -0.08 to 0.31; $P = 0.225$). Milk fat yield (kg/d) tended to be greater in biotin-fed cows (ES = 0.18, 95% CI: -0.02 to 0.38; $P = 0.081$, Figure 3). No evidence of significant heterogeneity in milk fat percentage and yield responses was found (Table 2). Meta-regression analysis showed that fat percentage results were not influenced by duration of treatment before and after calving, delivery method of biotin, parity, or other factors. Little evidence of publication bias was found in milk fat percentage data, but some evidence of publication bias was observed in

milk fat yield outcomes (Figure 2). Small studies that reported a reduction in milk fat yield were more likely to be published than small studies with an increase in milk fat yield. Because of the small sample size, this finding should be interpreted with caution.

Milk protein percentage did not differ between biotin-fed and control cows (ES for protein (%) = -0.15, 95% CI: -0.45 to 0.15; $P = 0.332$), and milk protein yield (kg/d) tended to be greater in biotin-fed cows (ES for protein yield = 0.23, 95% CI: -0.04 to 0.50; $P = 0.093$, Figure 4). Responses to biotin treatment for milk protein percentage and yield data (Table 2) were homogeneous. Meta-regression analysis showed that milk protein percentage results may be influenced by diet [coefficient: -0.78 ± 0.28 , $P = 0.023$; TMR (reference group) vs. partial mixed ration (PMR)]. The effect of biotin supplementation on milk protein percentage appeared to be greater in cows fed a TMR diet compared with those on a PMR diet. Due to the small number studies in the subgroups (TMR vs. PMR), these results require further validation. Evidence was also found of publication bias in milk protein percentage and yield.

Figure 4. Forest plot of standardized mean difference (SMD) and their 95% CI and weights for individual trials determined from the results of 9 comparisons of milk protein production of cows supplemented with biotin versus other cows. Box sizes are proportional to the inverse variance of the estimates. Summary estimates of treatment effects (diamonds) are shown using (1) a fixed effects approach (I-V specifies a fixed effect model using the inverse variance method), (2) a random effects approach (D+L specifies a random effects model using the method of DerSimonian and Laird, 1986), and (3) the predicted interval of a future trial, with the estimate of heterogeneity being taken from the inverse variance fixed effect model. PMR = partial mixed ration. Color version available in the online PDF.

Small studies that reported a reduction in milk protein percentage and milk protein yield may have been more likely to be published than small studies with an increase in milk protein percentage and milk protein yield; again the small sample size suggests that this finding should be interpreted with caution. Milk fat percentage and yield were increased, but not significantly, by treatment. The increase in milk fat percentage did not approach significance, but milk fat yield tended to increase, a result consistent with milk production responses. Similarly, milk protein yield tended to increase, but milk protein percentage was lower with treatment, although not significantly so. It is yet to be determined whether effects of biotin on production are solely mediated by increased feed intake or by increased efficiency of production. The findings support studies that suggest a need for inclusion of biotin in lactating cow diets.

Daily DMI (kg/d) in cows supplemented ($n = 5$ comparisons) with biotin tended to be greater than that in the control cows ($ES = 0.30$; $95\% \text{ CI} = -0.04$ to 0.64 ; $P = 0.085$). No evidence of significant heterogeneity (I^2

$= 0.0\%$, $P = 0.795$; Table 2) was found among studies. The small sample size of the DMI data indicated that it was not appropriate to conduct a meta-regression or examine the presence of publication bias (Figure 2). No significant association was found between the effect size of biotin on milk production and DMI (coefficient: 0.53 , $95\% \text{ CI} = 0.24$ to 3.48 ; $P = 0.604$).

The production responses to biotin were consistently positive, supporting studies that suggest lactating cows require supplementation with B vitamins (Girard, 1998) and studies showing low or even negative apparent ruminal synthesis of biotin (Miller et al., 1986; Santschi et al., 2005a,b; Schwab et al., 2006).

Hoof Health

Very few studies were available that met the rigorous standards needed for meta-analytic evaluation. All studies that examined the effect of biotin on hoof health are provided in Table 3. It was difficult to obtain some of the original reports or papers and only limited data were given in the abstracts. Our assessments of all

Table 3. Effects of biotin on the incidence or prevalence of various hoof disorders in dairy and beef cattle

Study	Cattle	Comparison	Outcome	Incidence/ prevalence	Cattle (n)		Dose (mg/d)	Control (%)	Biotin (%)	P-value
					Control	Treatment				
Bergsten et al. (2003)	Dairy		Sole hemorrhage	Incidence	40	42	20	50.0	24.0	0.041
			Double soles		40	42		28.0	31.0	0.731
			Horizontal grooves		40	39		43.0	24.0	0.072
Campbell et al. (2000)	Beef	June 1994 Oct. 1994 June 1995 Oct. 1995 June 1996	Heel horn erosion	Prevalence	40	40		53.0	52.0	0.991
			Vertical fissures		79	79	10	0.0	0.0	>0.05
					79	79		10.0	17.0	>0.05
					64	68		27.0	13.0	0.05
					64	68		33.0	15.0	0.01
Distl and Schmid (1994)	Dairy		Digital dermatitis and sole bruising	Incidence	—	—	—	—	—	0.03
Distl and Schmid (1994)	Dairy		Heel warts	Incidence	—	—	—	40.3	29.0	<0.05
Fitzgerald et al. (2000)	Dairy		All digital damage	Incidence	1,396 (10 herds)	1,313 (10 herds)	20	18.5	6.9	<0.05
			Locomotion score, digit damage (type of lesion was not recorded)					8.0	1.1	<0.05
Midla et al. (1998) Hagemeister and Steinberg (1996)	Dairy		White line separation	Prevalence	49	51	20	20.3	9.5	<0.05
			Reduction in sole ulcer and heel erosion		—	—	10	3.3	0.7	<0.05
			White line disease		447	453	20	43.0	48.4	0.04
			White line disease		113	338	20	3.04	1.93	>0.05
Pöttsch et al. (2003)	Dairy	5 herds Primiparous Multiparous		Incidence	118	329		8.48	15.48	<0.05
Hochstetter (1998)	Dairy		Digital dermatitis and sole hemorrhage	Incidence	—	—	—	—	—	—
Lischer et al. (1996)	Dairy		Sole ulcer	Prevalence	12	12	20	0.10	0.3	>0.05

compliant and noncompliant comparisons from which data could be obtained showed that the direction of effect with biotin supplementation was consistently and often, significantly, positive. Many of the studies reported positive biological effects of biotin treatment on hoof strength (Distl and Schmid, 1994; Hagemeister and Steinberg, 1996; Midla et al., 1998; Campbell et al., 2000; Fitzgerald et al., 2000; Hedges et al., 2001; Bergsten et al., 2003; Pöttsch et al., 2003). The marked inconsistency of study designs and study outcomes used to evaluate the effects of biotin on hoof health prevented the use of meta-analytic methods on these data. In contrast to the milk data, a large range of diets and management systems was represented in the lameness studies. These findings support contentions that dairy cows increase milk production following supplementation with biotin. More studies, using consistent study designs and definitions of hoof disorder, are needed to quantify the effects of biotin on lameness, in general, and on specific causes of lameness.

CONCLUSIONS

This experiment provided quantitative estimates of the milk and milk solids production responses to biotin. The ES estimates can be compared with other treatments to rank different interventions in lactating cattle. The study also provided an exploration of sources of variation in studies that may have influenced responses.

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Appendix

Table A1. List of studies excluded and reasons for exclusion

Study	Reason for exclusion
Mülling et al. (1999)	No production or hoof health data
Weiss (2001)	Review
Tomlinson and Socha (undated)	Review
Offer et al. (2004)	Lameness in cattle
Abel et al. (2006)	No production or hoof health data
Bunge (2006)	No production or hoof health data
Lischer et al. (2002)	No production or hoof health data
Mülling (2009)	Review
Higuchi et al. (2004)	No production or hoof health data
Girard (1998)	Review
Shearer (2005)	Review
Seymour (2001)	Review
Midla et al. (1998)	Digit data
Girard (1997)	No data
Socha et al. (1999)	Review
Packington (2004)	Review
Distl and Schmid (1994)	German language
Campbell et al. (2000)	Beef
Majee et al. (2003)	4 × 4 Latin square design
Ganjkhanelou et al. (2007)	Crossover design
Ganjkhanelou et al. (2007)	Crossover design
Ferreira and Weiss (2007)	Crossover design