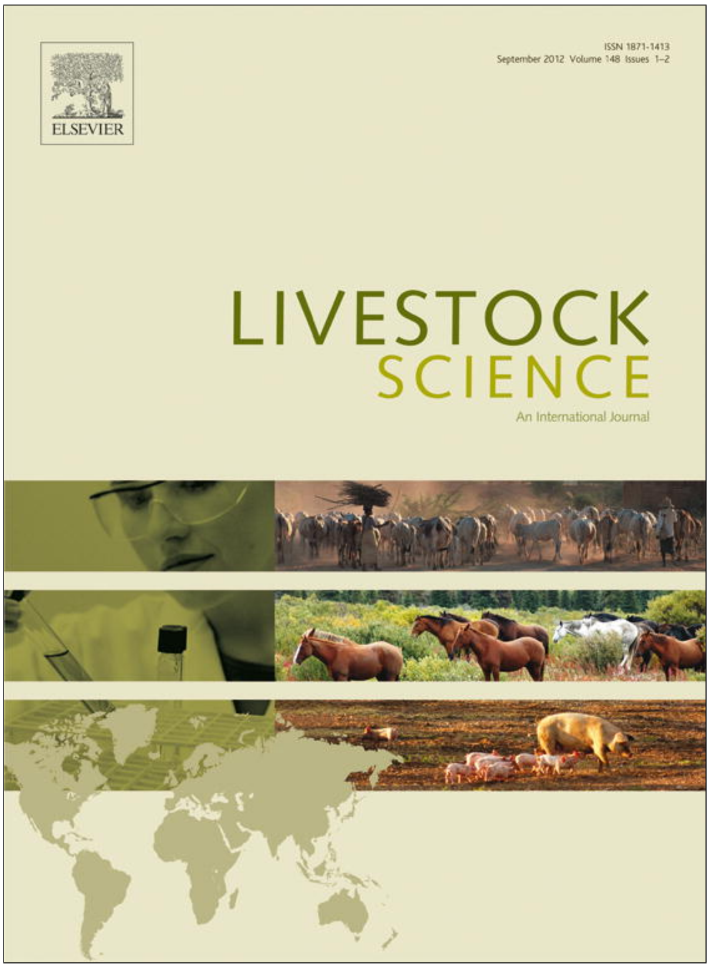
ISSN 1871-1413
September 2012 Volume 148 Issues 1–2
ELSEVIER
LIVESTOCK
SCIENCE
An International Journal

Livestock Science 148 (2012) 168–173

Contents lists available at SciVerse ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Effects of dietary biotin supplementation on performance and hoof quality of Chinese Holstein dairy cows

Bo Chen, Cong Wang, Jian-Xin Liu *

Institute of Dairy Science, College of Animal Science, Zhejiang University, Hangzhou 310058, PR China

ARTICLE INFO

Article history:
Received 24 March 2011
Received in revised form
1 June 2012
Accepted 4 June 2012

Keywords:
Biotin
Milk performance
Hoof quality

ABSTRACT

The objective of study was to determine the effects of supplemental biotin on lactation performance and hoof quality in dairy cows. Forty-five multiparous Chinese Holstein dairy cows (40 ± 6 d in milk) were randomly divided in three groups according to parity, previous lactation milk yield and days in milk. The experimental diets were identical for three treatments except for the level of biotin: 0, 20 and 40 mg/d, respectively. The experiment lasted for 70 d including the first 10 d for adaptation. Milk was sampled weekly and blood samples were taken from coccygeal vein biweekly. Hoof quality was assessed by hardness test, moisture content and light-microscopic examination of hoof structure. Dry matter intake was not different significantly across treatment groups. Milk yield was increased by supplementation of 20 or 40 g biotin per day to 35.3 kg/d from 32.7 kg/d in non-supplementation group. There were no significant differences in milk fat percentage and yield, lactose, total solid in milk, and concentrations of glucose, triglyceride, non-esterified fatty acid in plasma among three groups. Concentrations of milk protein and milk urea nitrogen in 40 mg/d group were higher than those in control, with no significant difference between 20 and 40 mg/d or control groups. However, protein yield was increased linearly by supplementation of biotin with the highest value at 40 mg/d group, mainly attributed to the increased milk protein content. Biotin concentration in plasma was higher in biotin-added groups than in the control. Biotin supplementation did not have effect on moisture content and hardness of hoof, but made hoof more compact than control group. In summary, supplemental biotin increased milk and milk protein yields, with no significant difference in milk performance and hoof quality between supplemental levels of 20 and 40 mg biotin per day.

1. Introduction

Biotin is an essential nutrient for both rumen bacteria and dairy cows. Biotin functions as a coenzyme for carboxylases, involved in the process of gluconeogenesis, fatty acid synthesis, amino acid catabolism (Wolin and Miller, 1988). Most of cellulolytic rumen bacteria require biotin for growth (Baldwin and Allison, 1983). Biotin can improve efficient fermentation of fiber in rumen (Abel et al., 2001). Several researchers (Bergsten et al., 1999; Midla et al., 1998; Zimmerly and Weiss, 2001) have reported positive productive and metabolic responses in dairy cattle by supplementation of biotin. Biotin is important for intracellular cementing substance and keratinization to improve the hardness of the hoof and horn as well (Geyer, 1998; Higuchi et al., 2003).

Biotin synthesized in the rumen is satisfactory for the requirement by low-producing cows. For the high-yielding cows, a large amount of concentrate mixture is offered to meet the nutrition requirement. Biotin synthesis by ruminal microbes will be reduced in the animals fed on a high concentrate diet (Abel et al., 2001). Thus, it is necessary to supplement biotin in the diet for high-producing dairy

* Correspondence to: Institute of Dairy Science, Zhejiang University, 358 Yuhangtang Road, Hangzhou 310058, PR China. Tel.: +86 571 88982097; fax: +86 571 88982930.
E-mail address: liujx@zju.edu.cn (J.-X. Liu).

1871-1413/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.livsci.2012.06.002

cattle. However, the recommended dose of biotin in diet has not been well established for dairy cows. In most studies, it is suggested that the supplementation amount (20 mg/d) was associated with a positive response (Bergsten et al., 1999; Midla et al., 1998; Zimmerly and Weiss, 2001).

It is reported that biotin was not extensively metabolized in the rumen (National Research Council, 2001). Schwab and Shaver (2005) found that there was a significant increase in biotin in milk and plasma with biotin supplementation, suggesting that biotin supplemented in an unprotected form is not completely degraded or utilized by ruminal microbes. Biotin is unlikely needed for ruminal protection (Majee et al., 2003; Margerison et al., 2002; Midla et al., 1998).

The objective of this study was to evaluate the effect of different levels of supplementary biotin (0, 20 or 40 mg/d) on milk production, blood biochemical parameters and hoof quality in high-yielding dairy cows. The 20 mg per day was chosen because in many studies positive improvement of milk production has been observed at this level. The 40 mg was chosen in order to determine whether a higher amount of supplementation biotin could result in a better response than 20 mg biotin.

2. Materials and methods

2.1. Cows, experimental design and feeding

Forty-five multiparous Chinese Holstein dairy cows (40 ± 6 d in milk) were randomly divided into 15 blocks of 3 cows according to parity, previous lactation milk yield and days in milk and then allocated into three groups. The diets were identical for three treatments (Table 1), and treatments consisted of 0, 20, and 40 mg/d of supplemental biotin, equivalent to 1 and 2 g/d of the 2% commercial product (Xinhua Vitamins Inc., Zhejiang, China). Biotin was dosed in top-dress once daily. Based on visual examination of the orts, all the supplemental biotin was consumed. The experiment lasted for 70 d including the first 10 d for adaptation. Cows kept in tie-stalls were milked and fed three times daily and had free access to drinking water.

2.2. Sample and measurements

2.2.1. Feed intake and feeds

Feed offered and refused was weighed for two consecutive days every other week throughout the trial to calculate dry matter intake (DMI). Forage and concentrates were sampled biweekly and immediately dried in an air-forced oven at 60 °C for 48 h and stored in sealed plastic containers at room temperature until analyzed (Wang et al., 2008). In preparation for analyses, dried forages and concentrates were ground through a 1 mm screen in a Cyclotec mill (Tecator 1093, Hoganas, Sweden). The diet samples were collected weekly for analysis of DM, crude protein (CP), Ca, and P (AOAC, 1990), neutral-detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest et al., 1991).

Table 1
Ingredient and composition of basal diet used in the experiment.

Item	Amount
Ingredient, % of DM	
Corn silage	18.9
Grass hay	10.9
Alfalfa meal	6.8
Brewer's grains	11.7
Concentrate[a]	48.2
Calcium carbonate	0.5
Premix[b]	0.5
Salt	0.5
Sodium bicarbonate	0.7
Dicalcium phosphate	1.3
Composition, % of DM	
Crude protein	14.0
Neutral detergent fiber	32.5
Acid detergent fiber	26.3
Crude ash	7.5
Ca	0.83
P	0.60
NE_L[c], Mcal/kg DM	1.65

[a] 7.0 DDGS; 22.3 crush corn; 4.2 wheat; 3.8 wheat bran; 4.4 soybean meal; 4.4 cottonseed meal; 2.1 Sesame meal.

[b] Formulated to provide (per kg of premix) 1,000,000 IU of vitamin A, 200,000 IU of vitamin D, 1250 IU of vitamin E, 14,000 mg of Zn, 100 mg of Se, 180 mg of I, 3000 mg of Fe, 40 mg of Co, 3000 mg of Mn, and 3000 mg of Cu.

[c] NE_L, net energy for lactation.

2.2.2. Milking and composition analysis

Cows were milked three times per day at 07:00, 14:00, and 21:00. Milk samples were collected weekly by milk sampling devices (WAIKATO, New Zealand) at milking, proportional to yield (4:3:3, composite respectively). The samples containing Bromopol (milk preservative; D&F Control Systems, San Ramon, CA, USA) were used for analysis of fat, protein and lactose by infrared analysis (Laporte and Paquin, 1999) with a four-channel spectrophotometer (Milko-Scan, Foss Electric, Denmark) and milk urea nitrogen (MUN) with the diacetyl monoxime-binding assay (Rahmatullah and Boyde, 1980).

2.2.3. Blood sampling and analysis

Blood were sampled approximately 3 h after feeding on the first day from coccygeal vein biweekly. Blood samples were collected from the coccygeal vein, and centrifuged at 3000 × g for 15 min. (Wang et al., 2008). Serum was frozen at −10 °C and later thawed for analysis of glucose (Barham and Trinder, 1972) by automatic biochemistry analyzer (HITACHI 7020), non-esterified fatty acid (NEFA; McCutcheon and Bauman, 1986), triglyceride (Cole et al., 1997), and cholesterol (Deeg and Ziegenhorn, 1983). Test kits were purchased from Diasys diagnostic systems (Shanghai Co., Ltd.). Plasma concentration of biotin was determined with RIDASCREEN Biotin kit (art. no. H1601, R-Biopharm Gmbh, Germany).

2.2.4. Hoof quality evaluation

Hoof quality was assessed by hardness test, moisture content and light-microscopic examination of hoof structure.

Hoof samples were taken from the same five cows of every group to get the apex sole during the hoof trimming at the beginning and end of the studies. Hoof samples for hardness test were sealed and frozen at −20 °C until further processing. The measurements of hardness were made from the surface of each sample using a durometer by ball indentation hardness (Hinterhofer et al., 2005). The hardness of the hoof was determined at the apex sole. After hardness test, samples were dried at 180 °C for 5 h for calculation of their moisture content (Higuchi et al., 2003). Biopsy samples of hoof taken at the similar positions from the five same cows at the beginning and end of study, were fixed in 10% formalin solution for tissue section and then stained by haemtoxylin-eosin (HE).

2.3. Statistical Analyses

The effects of biotin supplementation on DMI, milk production, milk composition, plasma metabolites, hardness and moisture content were determined using the MIXED procedure of SAS software system (SAS and Institute, 2000) with the following model:

$$Y = U + Ti + W + C + W^{*}Ti + eij$$

where Y is the dependent variable, U the overall mean; T_i the effect of treatment; W the week effect, C the cow effect; $W^{*}T_i$ the interaction effect of week and treatment, and e_{ij} the residual effect. Probability values of $P<0.05$ were defined statistically significant results. All terms were considered fixed, except that C and e_{ij} were considered as random.

3. Results and discussion

3.1. Feed intake and milk yield

Biotin supplementation did not have a significant effect on DMI ($P=0.29$, Table 2). The result is consistent with Zimmerly and Weiss (2001), who found no statistically significant change in DMI across treatments (0, 10 or 20 mg/d) for high-yielding dairy cattle (38.1 kg/d). Compared to the control, the cows supplemented with 20 or 40 mg/d biotin produced more milk ($P<0.05$; Fig. 1). Average milk yields increased quadratically ($P<0.05$, Table 2) with the increasing level of biotin supplementation. Supplemental biotin improved fiber digestion in vitro (Bentley, 1954), and increased production of propionic acid (Milligan et al., 1967). All the major cellulolytic bacteria in the rumen require biotin for growth (Baldwin and Allison, 1983). These results indicated that supplemental dietary biotin may increase the supply of nutrients for milk production, even without increased DMI. Yang et al. (2009) found that supplementation of biotin at 20, 30, or 40 mg/d increased milk performance compared to non-supplemented control, but without significant difference among three biotin-added groups. In our previous meta-analysis result (Chen et al., 2011), 20 mg/d of biotin supplementation could increase milk production by 1.66 kg/d. It is indicated that supplemental biotin at 20 mg/d or higher results in

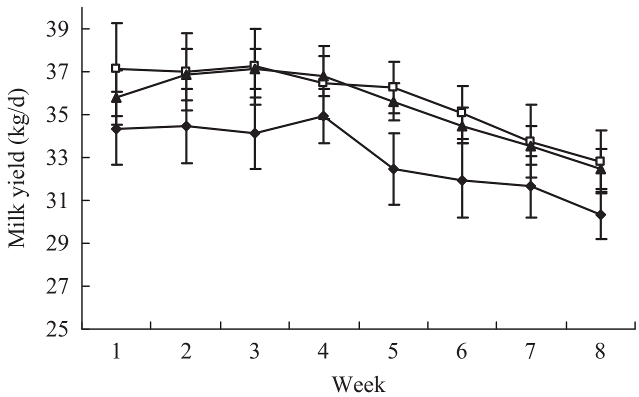

Fig. 1. Average weekly milk yield for dairy cows on 0 (◆), 20 (□) or 40 mg/d of supplemental biotin (▲). Error bars represent SE values; pooled SEM=0.895.

Table 2
The effect of dietary biotin supplementation on milk performance of early-lactating Chinese Holstein dairy cows.

Items	Supplemental biotin (mg/d)			SEM	P		
	0	20	40		Treatment	Liner	Quadratic
DMI[x] (kg/d)	21.3	21.9	21.0	0.386	0.29	1.00	1.00
Yield (kg/d)							
Milk	32.7[a]	35.3[b]	35.3[b]	0.895	0.07	0.01	0.04
FCM[y]	29.5	30.2	31.2	0.715	0.28	0.13	0.58
Milk fat	1.08	1.08	1.13	0.027	0.26	0.32	0.88
Milk protein	0.95[a]	1.03[b]	1.11[c]	0.028	0.002	0.004	0.93
Composition (%)							
Milk fat	3.35	3.20	3.30	0.088	0.45	0.78	0.38
Milk protein	2.92[a]	3.02[a,b]	3.17[b]	0.084	0.13	0.002	0.51
Lactose	4.99	4.90	4.89	0.039	0.16	0.24	0.68
Total solid	11.97[a]	11.71[b]	12.46[a]	0.235	0.07	0.15	0.06
MUN[z] (mg/dL)	11.43[a]	11.61[a,b]	12.14[b]	0.234	0.10	0.18	0.66

[x] DMI, dry matter intake.
[y] FCM, 4% fat-corrected milk; FCM (kg/d)=0.4 × milk (kg/d)+15 × fat (kg/d).
[z] MUN, milk urea nitrogen.
[a,b] Within a row, means without a common superscripts letter differ ($P<0.05$).

similar effect on milk production. The optimal level of supplemental biotin is 20 mg per head per day (Bergsten et al., 1999; Lean and Rabiee, 2011; Midla et al., 1998). The current price of the commercial biotin product (2%) was about 21 USD/kg. Supplementation of biotin at a level of 20 mg/d (equivalent to 1 g/d of 2% product) would cost 2.1 cent of USD.

Abel et al. (2001) reported that ruminal microbes synthesize more (or degrade less) biotin when the hay replaces barley grain. Biotin synthesis was reduced by 50% when the forage-to-grain ratio decreased from 83:17 to 50:50 (Da Costa Gomez et al., 1998). However, ruminal biotin synthesis was less than 1 mg/d in steers fed either high-corn grain (88% of dietary DM) or high-alfalfa meal (70% of dietary DM) diet (Miller et al., 1986). When the cows were fed on a 35 or 60% forage diet, the apparent synthesis of biotin was negative, suggesting either no ruminal synthesis or that destruction by ruminal microbes was greater than synthesis (Schwab et al., 2006). In the current study, the dietary ratio of forage was about 50%. It is inferred that the diet type has little effect on overall biotin concentration in ruminal contents (Hayes et al., 1966), and supplemental biotin is needed for dairy cattle for all types of rations.

3.2. Milk composition

No significant differences were observed in milk fat content and yield, milk lactose, and the total solid among three treatments ($P>0.05$, Table 3), in consistence with Mehdi et al. (2007). Biotin supplementation linearly increased milk protein yield and percentage ($P<0.05$, Table 3). Compared to the control, milk protein yield in biotin-supplemented group was increased significantly ($P<0.05$), similar to Yang et al. (2009) and Majee et al. (2003). The major cellulolytic microbes in the rumen required biotin for growth (Baldwin and Allison, 1983), and supplemental biotin would increase microbial protein level in the intestines, which was beneficial to synthesize protein in milk (Dakshinamurti and Chauhan, 1988). Furthermore, the MUN concentration in 40 mg/d biotin group was higher than that in the control ($P<0.05$), with no difference between the 20 and 40 mg/d groups ($P=0.11$). The milk protein and MUN content showed similar tendency for the treatments. Interaction effect of week and treatment was not significant on all parameters ($P>0.05$).

3.3. Blood biochemical parameters

No significant difference was observed in the plasma concentrations of glucose, triglyceride and NEFA ($P>0.05$, Table 3) among the three groups, while the concentration of cholesterol was higher in 40 mg/d than that in 20 mg/d group ($P=0.04$). The supplemental biotin should increase glucose production in dairy cows (Dakshinamurti and Chauhan, 1988). However, glucose concentration in the plasma was similar among three groups in the present study, consisted with Zimmerly and Weiss (2001) and Mehdi et al. (2007). Glucose in plasma is only a part of glucose produced by the cow since part of glucose may have been used for milk synthesis. In addition, the plasma concentration of biotin in the 20 and 40 mg/d group was significantly higher than that in the control ($P<0.01$), with no difference between the biotin-added two groups ($P=0.29$).

3.4. Hoof quality

No significant difference was found in hardness and moisture content of hoof among treatments ($P>0.05$, Table 4). An increased hardness was observed from the beginning to end of the experiment in all groups, with no difference between treatments ($P=0.58$). Lischer et al. (2002) reported that the effect of biotin on hoof quality could not be assessed adequately by macroscopic examination in short periods. Sixty days in this study may not be long enough to improve the hoof quality. Precise and detailed assessment of hoof quality could be conducted by light microscopic techniques. The hoof samples, taken from the area of the stratum medium of dairy cow hoof on day 0 showed that there was more interspace in all the samples (Fig. 2a–c), compared to the samples on 60 d (Fig. 2A–C). The evidence in this study proved that biotin promoted keratin content and reduced microcrack (the white parts in the figures) in the hoof of dairy cows.

4. Conclusions

Supplementation of biotin increased milk production and protein percent and yield, but did not exert effect on DMI, milk fat and plasma triglyceride and NEFA. The supplemental biotin increased the plasma level of biotin, with no difference between the 20 and 40 mg/d group. Moisture content and hardness of hoof were not affected by biotin supplementation, but the hoof of dairy cows with supplemental biotin

Table 3
Effects of dietary biotin supplementation on plasma concentrations of glucose, triglyceride, non-esterified fatty acids (NEFA), cholesterol and biotin in lactating Chinese Holstein dairy cows.

	Supplemental biotin (mg/d)			SEM	P		
	0	20	40		Treatment	Liner	Quadratic
Glucose (mmol/L)	3.75	3.75	3.76	0.06	1.00	1.00	0.99
Cholesterol (mmol/L)	4.42[a,b]	4.71[a]	4.07[b]	0.22	0.12	0.33	0.16
Triglyceride (mmol/L)	0.13	0.16	0.12	0.02	0.44	0.20	0.21
NEFA (mmol/L)	0.30	0.26	0.26	0.025	0.41	0.52	0.73
Biotin (ng/L)	970[a]	1033[b]	1053[b]	12.7	0.01	0.07	0.05

[a,b] Within a row, means without a common superscripts letter differ ($P<0.05$).

Table 4
Effects of dietary biotin supplementation on moisture content and hardness of the hoof in lactating Chinese Holstein dairy cows.

	Supplemental biotin (mg/d)			SEM	*P*		
	0	20	40		Treatment	Linear	Quadratic
Moisture content (%)							
Beginning	37.5	38.2	38.2	0.45	0.53	0.31	0.93
End	37.2	38.0	37.9	0.73	0.58	0.40	0.55
Hardness (N/mm^2)							
Beginning	23.34	23.23	23.24	0.78	0.85	0.93	0.95
End	23.61	23.56	23.67	0.78	0.60	0.96	0.94

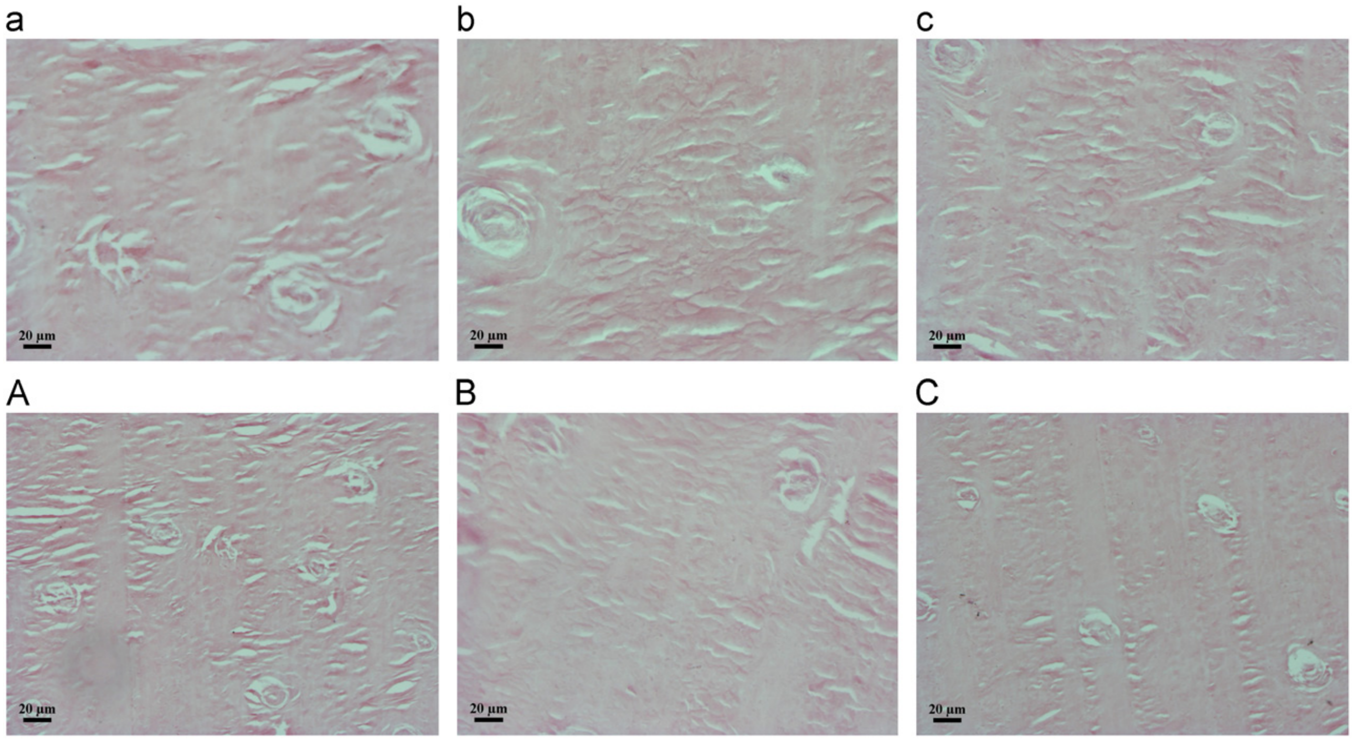

Fig. 2. Light-microscopic appearance of stratum medium of dairy cow hoof. The letters a or A (0 mg/d), b or B (20 mg/d), and c or C (40 mg/d) indicated the samples taken at the beginning (day 0) or end (day 60) of the study from the same cattle, respectively. Significant improvement in hoof structure was observed, compared to the control.

was more compact than that in control. No significant differences were observed in milk performance and hoof quality between the supplemental levels of 20 and 40 mg biotin per day. The optimal level of supplemental biotin is 20 mg per cow per day, and the cost would be 2.1 cent.

Conflict of interest statement

None.

Acknowledgments

This study was supported in part by the earmarked fund for China Agriculture Research System (CARS-37) and National Key Basic Research Program, Ministry of Science and Technology, PR China (Grant no. 2011CB100801).

References

Abel, H.J., Immig, I., Da Costa Gomez, C., Steinberg, W., 2001. Research note: effect of increasing dietary concentrate levels on microbial biotin metabolism in the artificial rumen simulation system (RUSITEC). Arch. Anim. Nutr. 55, 371–376.

AOAC, 1990. Official Methods of Analysis, vol. I, fifteenth ed. Association of Official Agriculture Chemists, Arlington, VA, pp. 69–90.

Baldwin, R.L., Allison, M.J., 1983. Rumen metabolism. J. Anim. Sci. 57 (Suppl. 2), 461–477.

Barham, D., Trinder, P., 1972. An improved color reagent for the determination of blood glucose by the oxidase system. Analyst 97, 142–145.

Bentley, O.G., ohnson, R.R., Vanecko, S., Hunt, C.H., 1954. Studies on factors needed by rumen microorganisms for cellulose digestion in vitro. J. Anim. Sci. 13, 581–593.

Bergsten, C., Greenough, P.R., Gay, J.M., Dobson, R.C., Gay, C.C., 1999. A controlled field trial of the effects of biotin supplementation on milk production and hoof lesions. J. Dairy Sci. 82 (Suppl. 1), 314. (abstr.).

Chen, B., Wang, C., Wang, Y.M., Liu, J.X., 2011. Impact of biotin on milk performance of dairy cattle: a meta-analysis. J. Dairy Sci. 94, 3537–3546.

Cole, T.G., Klotzsch, S.G., McNamara, J., 1997. Measurement of triglyceride concentration. In: Rifai, N., Warnick, G.R., Dominicazk, M.H.

(Eds.), Handbook of Lipoprotein Testing, AACC Press, Washington, pp. 115–126.
Da Costa Gomez, C., Al Masri, M., Steinberg, W., Abel, H.J., 1998. Effect of varying hay/barley proportions on microbial biotin metabolism in the rumen simulating fermenter (RUSITEC). Proc. Soc. Nutr. Physiol., 7. (abstr.).
Dakshinamurti, K., Chauhan, J., 1988. Regulation of biotin enzymes. Ann. Rev. Nutr. 8, 211–233.
Deeg, R., Ziegenhorm, J., 1983. Kinetic enzymatic method for automated determination of total cholesterol in serum. Clin. Chem. 29, 1798–1802.
Geyer, H., 1998. The influence of biotin on horn quality of hooves and claws. In: Proceedings of the 10th International Symposium on Lameness in Ruminants. Lucerne, pp. 181–201.
Hayes, B.W., Mitchell, G.E., Little, G.O., Bradley, N.W., 1966. Concentrations of B-vitamins in ruminal fluid of steers fed different levels and physical forms of hay and grain. J. Anim. Sci. 25, 539–542.
Higuchi, H., Maeda, T., Kawai, K., Kuwano, A., Kasamatsu, M., Nagahata, H., 2003. Physiological changes in the concentrations of biotin in the serum and milk and in the physical properties of the claw horn in Holstein cows. Vet. Res. Commun. 27, 407–413.
Hinterhofer, C., Apprich, V., Ferguson, J.C., Stanek, C., 2005. Elastic properties of hoof horn on different positions of the bovine claw. Deutsch. Tierärztliche Wochenschr. 112, 142–146.
Laporte, M.F., Paquin, P., 1999. Near-infrared analysis of fat, protein, and casein in cow's milk. J. Agric. Food Chem. 47, 2600–2605.
Lischer, C.J., Koller, U., Geyer, H., Mulling, C.H., Schulize, J., Ossent, P., 2002. Effect of therapeutic dietary biotin on the healing of uncomplicated sole ulcers in dairy cattle—a double blinded controlled study. Vet. J. 163, 51–60.
Lean, I.J., Rabiee, A.R., 2011. Effect of feeding biotin on milk production and hoof health in lactating dairy cows: a quantitative assessment. J. Dairy Sci. 94, 1465–1476.
Majee, D.N., Schwab, E.C., Bertics, S.J., Seymour, W.M., Shaver, R.D., 2003. Lactation performance by dairy cows fed supplemental biotin and B-vitamin blend. J. Dairy Sci. 86 (2), 106–2112.
Margerison, J.K., Winkler, B., Penny, B., 2002. The effect of supplementary biotin on milk production in Holstein cows. In: Proceedings of the XXII World Buiatrics Congress. Hannover, Germany, p. 219.
McCutcheon, S.N., Bauman, D.E., 1986. Effect of chronic growth hormone treatment on responses to epinephrine and thyrotropin-releasing hormone in lactating cows. J. Dairy Sci. 69, 44–51.
Mehdi, G., Salimi, M., Nikkhah, A., Zali, A., 2007. Effects of supplemental dietary biotin on performance of Holstein dairy cows. Pak. J. Biol. Sci. 10, 2960–2963.
Midla, L.T., Hoblet, K.H., Weiss, W.P., Moeschberger, M.L., 1998. Supplemental dietary biotin for prevention of lesions associated with aseptic subclinical laminitis (pododermatitis aseptica diffusa) in primiparous cows. Am. J. Vet. Res. 59, 733–738.
Miller, B.L., Meiske, J.C., Goodrich, R.D., 1986. Effects of grain and concentrate level on B-vitamin production and absorption in steers. J. Anim. Sci. 62, 473–483.
Milligan, L.P., Asplund, J.M., Robblee, A.R., 1967. In vitro studies on the role of biotin in the metabolism of rumen microorganisms. Can. J. Anim. Sci. 47, 57–64.
National Research Council, 2001. Nutrient Requirements of Dairy Cattle, seventh rev. ed. National Academy of Sciences, Washington, DC, USA.
Rahmatullah, M., Boyde, T.R., 1980. Improvements in the determination of urea using diacetyl monoxime; methods with and without deproteinisation. Clin. Chim. Acta 107 (1–2), 3–9.
SAS Institute, 2000. SAS User's Guide. Statistics, version 8.01. SAS Institute Inc., Cary, NC.
Schwab, E.C., Schwab, C.G., Shaver, R.D., Girard, C.L., Putnam, D.E., Whitehouse, N.L., 2006. Dietary forage and nonfiber carbohydrate contents influence B vitamin intake, duodenal flow, and apparent ruminal synthesis in lactating dairy cows. J. Dairy Sci. 89, 174–187.
Schwab, E.C., Shaver, R.D., 2005. B-vitamin nutrition for dairy cattle. Penn State Dairy Cattle Nutrition Workshop.
Van Soest, P.J., Bobertson, J.B., Lewis, B.A., 1991. Methods of dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.
Wang, C., Liu, J.X., Zhai, S.W., Lai, J.L., Wu, Y.M., 2008. Effect of ratio of rumen degradable protein to rumen undegradable protein on nitrogen conversion of lactating dairy cow. Acta Agric. Scand. Anim. Sci. 58 (2), 100–103.
Wolin, M.J., Miller, T.L., 1988. Microbe–microbe interactions. In: Hobson, P.N. (Ed.), The Rumen Microbial Ecosystem, Elsevier Applied Science, New York, pp. 343–359.
Yang, K., Gao, Y.X., Cao, Y.F., Li, J.G., 2009. Effects of dietary biotin supplement on performance and blood biochemical parameters in lactating cows. Chn. J. Anim. Nutr. 21, 853–858.
Zimmerly, C.A., Weiss, W.P., 2001. Effects of supplemental dietary biotin on performance of Holstein cows during early lactation. J. Dairy Sci. 84 (498–506), 733–738.