The Effect of a Supplement Containing Amino Acids and Fatty Acids on Hoof Growth in Horses

By

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A thesis presented to the Honors College of Middle Tennessee State University in partial fulfillment of the requirements for graduation from the University Honors College

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Abstract

Amino acids influence fibrous structures found in nails, hair, and even hooves. Specific amino acids such as methionine, lysine, and threonine are most known to aid the structural formation of the hoof. The objective of this study was to determine the impact of an amino acid supplement on hoof growth. Twelve horses were fed a control or supplemented diet in a crossover design for 24 weeks, with 12 weeks on each diet. Hoof growth was measured every 2 weeks. The supplement did not impact hoof growth ($P = 0.21$), but horses on the supplement (3.4 ± 0.2) compared to the control group (2.7 ± 0.2) gained muscle mass ($P = 0.030$). There was a seasonal effect on hoof growth with higher growth rates in the spring compared to winter ($P < 0.0001$).

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Introduction

The importance of a healthy hoof for horses cannot be over-emphasized, as implied by the adage, “no hoof, no horse.” Hooves carry the horse’s weight, which ranges from 900 lbs. to over 2,000 lbs., and withstand concussive and shear forces with every step, from a walk to a 40-mph gallop.

The hoof wall is the hard, horny material that is visible on the outside of the hoof (Figure 1). Formed primarily of keratin, the hoof wall protects the sensitive tissue inside the hoof, and it serves as a shock absorber while supporting the weight of the horse. The hoof wall itself does not contain nerves or blood vessels. Similarly to human fingernails, a healthy hoof wall grows about 6 to 10 mm per month at the toe (Reilly et al., 1998) and must be either worn down naturally or trimmed off by a farrier.

The coronary band surrounds the top of the junction of the hoof and the hairline of the lower leg. The coronary band has a rich blood supply and functions as the source of nutrition for the hoof wall, which essentially grows down from the coronary band.

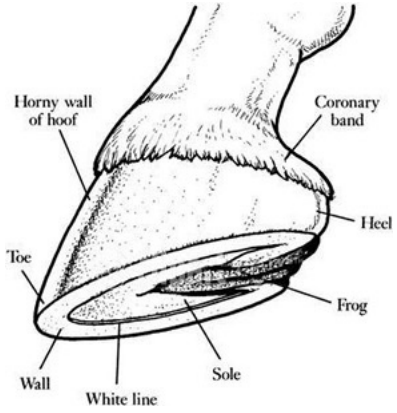


Figure 1. Illustration of external horse hoof structures.

Amino acids, minerals, and vitamins present in supplements are known to influence growth and repair of tissues. In humans, supplements can be used for hair growth, nail growth, and muscle growth. Similarly, supplements have been used to build

stronger hooves in cattle and horses. When certain nutritional elements are not provided through an animal's regular diet, they are commonly introduced through supplements. Hoof growth is influenced not only by genetics but through nutrition as well. Amino acids, minerals, and vitamins are involved in the keratinization process, allowing for proper growth and stronger hooves (Langova et al., 2020). There are a select few amino acids that impact the growth rate of hooves such as Cytosine and Methionine.

Cytosine and methionine are two key amino acids in the structural formation of hooves. Cytosine is produced in the body from methionine, an essential amino acid (Kellon, 2009). Keratin is a structural protein made up of the sulfur-containing amino acids cytosine and methionine; this structural protein makes up the hoof wall (Pollock, 2013). Moreover, if horses or livestock lack methionine or cytosine, then the hoof growth and quality may be affected (Pollock, 2013).

Amino acids are not the only factors that contribute towards hoof growth and quality. In cattle, calcium, zinc, copper, and biotin are essential in maintaining and growing horny hoof tissue (Muelling, 2009). In a study involving the hoof quality of Lipizzaner stallions, biotin was shown to improve and maintain hoof quality (Josseck et al., 1995). A product called *Farrier's Formula* contains Methionine, Zinc, Biotin, Ascorbic Acid, Tyrosine, and Copper; this product was used on a wide range of defective hooves, including those with thin hoof horn and crumbling areas around nail holes (Kempson, 1990). After researching the effects of Farrier's Formula, researchers noticed a significant increase in the improvement of the hoof (Kempson, 1990). Like Farrier's Formula, the key amino acids and minerals needed for hoof growth are found in many

forms of supplementation, including Purina's SuperSport Amino Acid Supplement, Amplify High-Fat Supplement, and Outlast Gastric Support Supplement.

Purina Animal Nutrition is in the process of developing a new feed supplement, which combines the benefits of the SuperSport Amino Acid Supplement, Amplify High Fat Supplement, and Outlast Gastric Support Supplement. These supplements are all currently available on the market after extensive testing for safety and efficacy. To date, the effect of these supplements on hoof growth in horses has not been examined.

This study aims to determine if a new Purina supplement will cause an increase in horses' hoof growth rate. Supersport contains essential amino acids Methionine, Lysine, and Threonine (*Supersport*, 2013). Lysine and threonine have long been proven to be the most limiting amino acids for foal growth (Graham et al., 1994) and are commonly added to horse feeds. Burns (2021) reviewed the importance of lysine and threonine for healthy horse hooves, although he referenced primarily studies in cattle. As previously mentioned, methionine is important in building hooves. Copper, Zinc, and Calcium are present in the Gastric Outlast and Amplify High-Fat Supplements (*Amplify*, 2013; *Outlast,* 2017). All the elements needed for hoof growth are in each Purina product used for this study. Growth will be measured in millimeters starting at the coronary band throughout the study. Both the coronary band and hoof wall are illustrated in Figure 1 (above).

Thesis Statement

The hypothesis is that the Purina supplement will increase the rate of hoof growth. If this study supports this statement, it will show the importance of this hoof supplement

by allowing horses to perform at the optimal level, preventing lameness, and determining its overall effectiveness. Furthermore, this information is important to the researchers of this supplement and many people within the horse industry, including horse owners, exhibitors, breeders, veterinarians, and other researchers interested in this subject.

Approach/Methods

Horses and Diets. The proposal was submitted and approved by the MTSU Institutional Animal Care and Use Committee (Protocol #22-2007). For this study, a group of 12 horses from the MTSU Horse Science program were used. The clinical characteristics, including breed, age, and exercise level of the horses used for this study are shown in Table 1. All horses were fed a base diet of prairie grass hay and 14% protein feed (Purina Strategy) balanced to meet their minimal nutritional requirements (NRC, 2007). The amount of feed and hay provided in the base diet was balanced to meet caloric requirements as appropriate for the health of each horse, noted by maintenance of Body Condition Score. The Body Condition Score (Henneke et al., 1983) is an assessment of fat deposits (or lack thereof) on the horse's body. Samples of the grass hay and base feed were analyzed by Equi-Analytical laboratory, Ithaca, NY. The analysis of the hay and feed are shown in Table 2, and the analysis of the Supplement is shown in Table 3. In a crossover experimental design, half of the horses were fed the base diet with the added Purina supplement for the first 12 weeks while the remaining horses remained on the base diet as the Control. After 12 weeks, the horse groups switched diets. In this manner, each horse served as its own control.

Horses were housed in 3.0 x 3.4 meter box stalls during weekly lessons with turnout on weekends. Exercise level was maintained throughout the study through regular

lesson use as is typical for the teaching facility. While exercise level varied between horses due to different use in horsemanship lessons, the exercise level of each individual horse remained similar throughout the study.

Hoof Growth Measurements. At the start of the study, the horses had a farrier treatment that consisted of trimming or shoeing as appropriate for proper health and hoof management of that individual horse. The horses were continuously shod, or trimmed, on their front feet throughout the trial. Because the farrier treatment applied to the lower portion of the hoof, it had no impact on the ability to measure hoof growth. A DeWalt cordless drill was used to mark growth at the 20 mm distance from the coronary band. Pictures of each horse's front hooves were taken for record keeping and measurement reference points (Figure 1), and the body condition score (BCS), of the horses was recorded as well. Every other week throughout the study, the distance between the coronary band and drill hole on both front feet were measured in millimeters using a digital steel caliper for new growth. Yellow play-doh was used to distinguish the drill hole and make it more visible when measuring. The results were recorded in a journal before being transferred into Microsoft Excel. Throughout this study, visual observations regarding the brittleness and flakiness of the hooves were noted. The front feet, not the back, were studied throughout the course of the trial.

Weeks 1-12. The horses were split into group A and group B. Group A was the control group for the first 12-week period while group B was the experimental group; both groups were switched at the beginning of the next 12-week period. The first period began in the winter months, from late December to early March. The experimental group of horses undergoing the treatment were fed the supplementation dressed over their

regular diet. The control group remained on their current diet, and both groups carried out their daily routines. The experimental group was fed once in the morning with 1.1 kg of the hoof growth supplement plus base diet of feed and hay, and once again in the afternoon with another 1.1 kg of the supplement plus base diet of feed and hay, making a total of 2.2 kg of supplement per day. On week six, all the horses underwent a routine farrier treatment (hoof trims or shoes as needed for regular management).

Weeks 12-24. The second period took place from March to May. On week twelve, the diets of the control group and experimental group were switched, and another routine farrier treatment took place. The control group from weeks 1-12 consumed the supplement with their current diet, and the supplement was excluded from the experimental group's diet. The feeding schedule remained the same. The new growth continued to be measured every two weeks, and routine farrier treatments continued to take place on weeks 18 and 24. Body Condition Score was recorded after the summation of the study. After the twenty-fourth week, the effects of the supplementation on hoof quality and growth were concluded, and the results were compared to the hypothesis.

Statistical Analysis. Data were first analyzed for normality and found to be normally distributed. A mixed model with repeated measures was used to compare the effects of Diet on Hoof Growth, using Horse as the individual Subject, Period and Day as repeated effects, with the Period*Day interaction as the error term using SAS (SAS Ver. 9.4, SAS Stat. Inc., Cary, NC).

Muscle mass scores. Photographs of all horses were taken after each period and used for *post hoc* assignment of a subjective muscle mass score from 1 to 5 (1 = lowest to 5 = highest), based on mass and definition of major muscle groups, including gluteal

(hindquarters), pectoral (chest), complexus (crest of neck), and the digital flexor and extensor muscles in the forearm and gaskin. Because muscle mass scoring is subjective, the use of images was chosen instead of live scoring during the study so that the images could be randomized, and muscle scores assigned, with the observer (Hoffman) blind to the dietary treatment associated with the image. Muscle scores were assigned using a system previously described by Graham-Thiers and Kronfeld (2005), as follows:

1 - very little development of muscle mass and definition for the breed of horse

2 - some development of muscle mass and definition for the breed of horse

3 - average development of muscle mass and definition for the breed of horse

4 - above average development of muscle mass and definition for the breed of horse

5 - significant development of muscle mass and definition for the breed of horse

Table 1. Clinical characteristics of the horses used in this study.
Group A horses remained on the Control base diet during the first 12-week period while Group B were fed the base diet plus Supplement. The horse groups switched dietary treatments during the second 12-week period.

Horse	Group	Breed	Age	Exercise Level
Boots	A	Quarter Horse	19	Maintenance
Gracie	A	Quarter Horse	15	Moderate
Penny	A	Quarter Horse	15	Moderate
Raquel	A	Quarter Horse	7	Light
Sadie	A	Quarter Horse	19	Moderate
Sweets	A	Paint Horse	19	Moderate
Bonnie	B	Quarter Horse	6	Light
Dixie	B	Paint Horse	18	Light
Fish	B	Quarter Horse	15	Moderate
Lila	B	Warmblood	23	Light
Smores	B	Quarter Horse	25	Moderate
Sugar	B	Quarter Horse	25	Light

Table 2. Nutrient analysis of the hay, grain concentrate, and supplement.
The Control base diet consisted of hay and grain concentrate fed to maintain body weight and condition throughout the study.

Nutrient, %	**Hay**	**Grain Concentrate**	**Supplement**
Digestible Energy, Mcal/kg	2.07	3.30	4.42
Crude Protein	6.3	16.1	45.3
Estimated Lysine	0.2	1.1	3.5
Estimated Threonine			2.1
Estimated Methionine			1.6
Acid Detergent Fiber	39.6	17.8	21.0
Neutral Detergent Fiber	65.5	35.4	35.0
Water Soluble Carbohydrates	7.8	9.6	9.7
Simple Sugars	7.3	5.5	9.0
Starch	2.0	14.1	17.8
Crude Fat	3.3	6.3	30.0
Calcium	0.43	1.38	4.5
Phosphorus	0.09	0.87	0.6
Magnesium	0.16	0.36	
Copper, ppm	6	91	60
Zinc, ppm	16	348	220
Selenium, ppm	Non-detectible	0.6	0.6
Vitamin A, IU/kg		6,600	6,600
Vitamin D_3, IU/kg			2,200
Vitamin E, IU/kg		275	1,246

Figure 2. The initial hoof measurement setup for each horse in the study to determine hoof growth. The measurement recorded by the caliper is the distance between the coronary band and the bottom of the drilled hole filled with yellow play-doh. The play-doh was used to increase the visibility of the hole, making measurements simpler to read.

Figure 3. General Tool 6” Steel Digital Caliper 147. Manufactured by Penn Tool Co., Inc located at 1776 Springfield Avenue Maplewood, NJ 07040. This is the digital caliper used to record measurements throughout the study on both the control and experimental groups. Measurements were taken in millimeters.

Results

All horses accepted and consumed the supplement willingly without any feed refusals. Horses were aged 17 ± 7 yrs with body weight of 540 ± 65 kg and body condition score (Henneke et al., 1983) of 6.4 ± 0.8. There was no effect of diet on body weight ($P = 0.76$) or body condition score ($P = 0.64$). Body weight and body condition scores throughout the study are shown in Figure 5.

Hoof growth is shown in Figure 4. There was no effect of diet on hoof growth ($P = 0.21$), but there was an effect of period, with hoof growth during the winter months being lower than during the spring ($P < 0.0001$).

Muscle mass scores, shown in Figure 6, were higher ($P = 0.030$) when horses were fed the Supplement (3.4 ± 0.2) compared to the Control (2.7 ± 0.2).

Visual changes in muscle mass and haircoat are shown in Figure 6. Haircoat was noticeably healthier in the supplemented photo compared to the control photo. Muscle mass improved in specific areas of the horse including the crest of the neck, the area where the neck ties into the withers (thoracic vertebrae), the topline (over the back, loin, and hindquarters), and muscling in the forearm and gaskin (muscles in the upper front and hind legs).

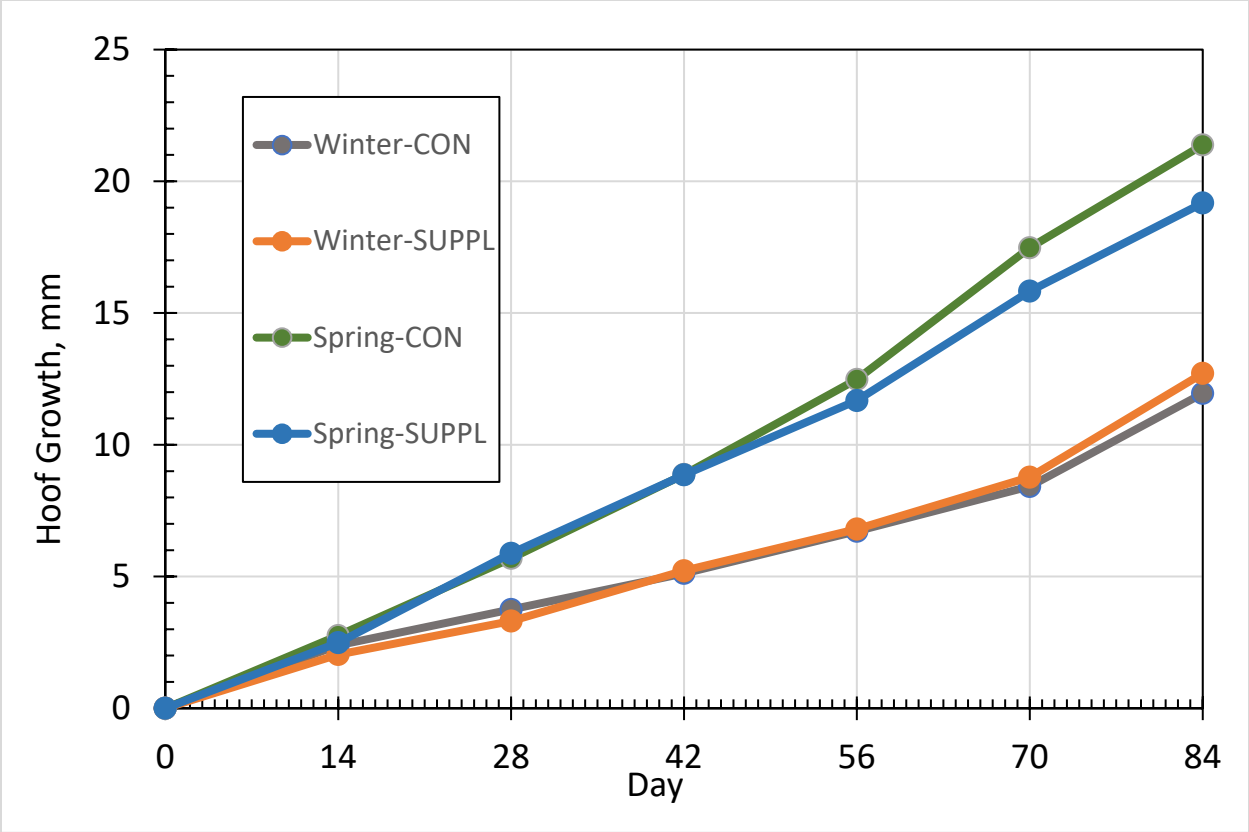
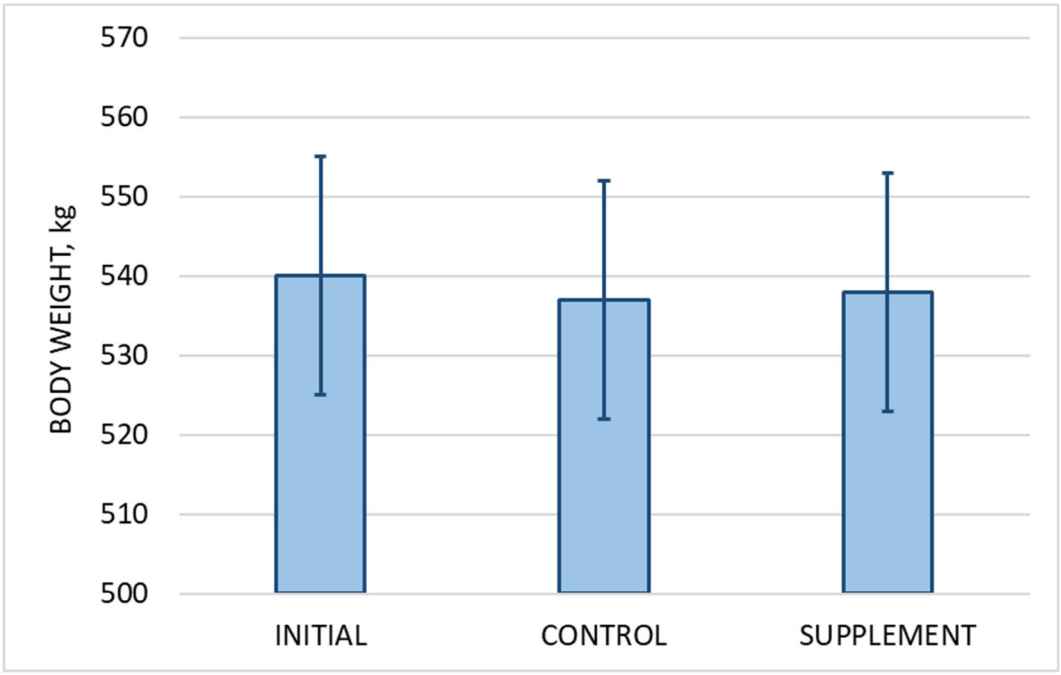


Figure 4. Hoof growth (mm) over time from Day 0 to 84. Hoof growth was measured as the distance from the coronary band to the mark on the hoof.



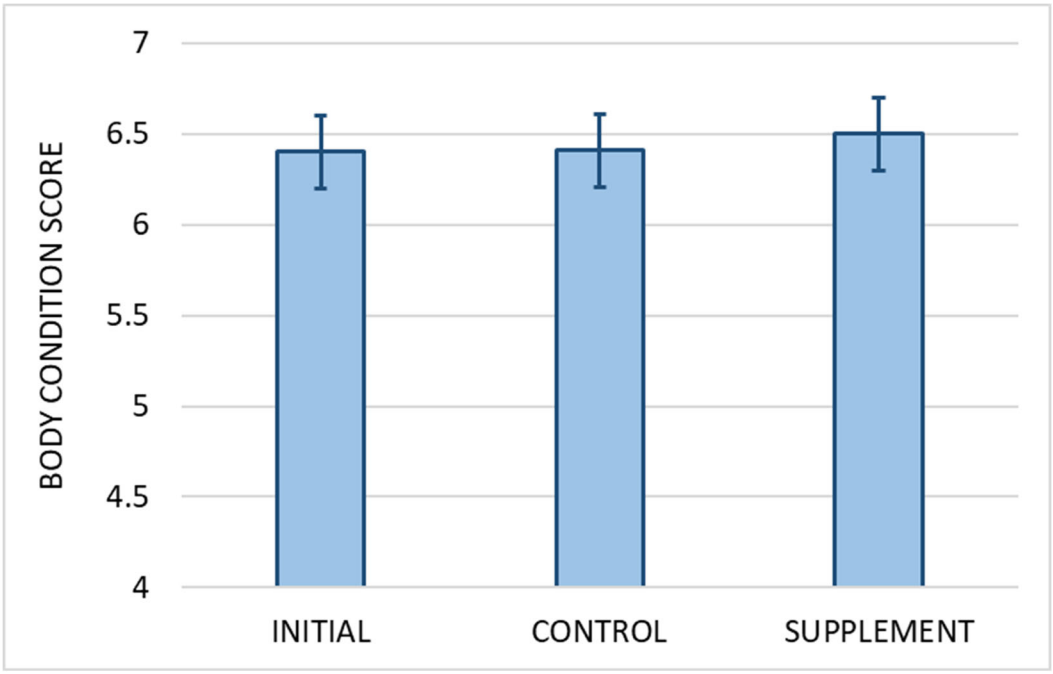


Figure 5. Body weight (kg, top) and body condition score (bottom, Henneke et al., 1983), where a score of 1 is emaciated and 9 is morbidly obese, while a score of 5 is considered optimal fitness for athletic performance. All horses were in the normal range of healthy body weight and body condition score throughout the study. There was no effect of diet on body weight ($P = 0.76$) or body condition score ($P = 0.64$).

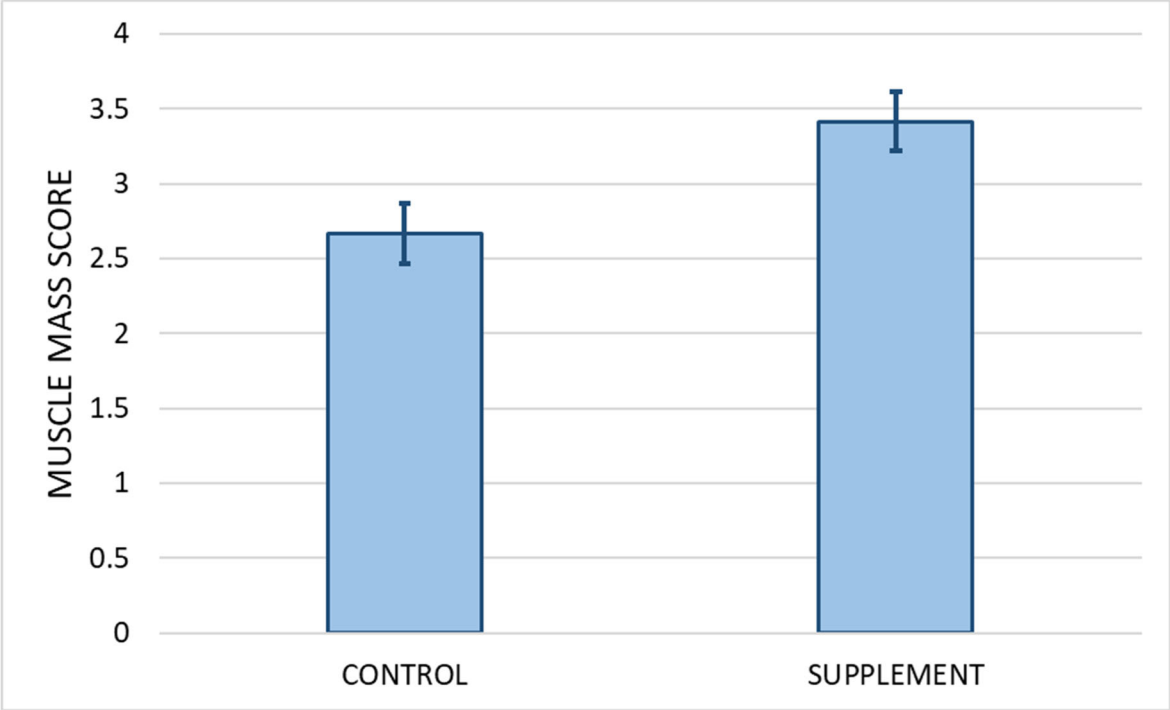


Figure 6. Muscle mass scores were higher (P = 0.030) in horses when fed a supplement containing additional essential amino acids compared to a control diet that met minimum daily requirements.

Figure 7. Example images of one horse to demonstrate visual changes in muscle mass and haircoat quality after 12 weeks of consuming a supplement containing essential amino acids and fatty acids. All horses had improved muscling when supplemented, most notably over the topline, including the crest of the neck, where the neck ties in with the withers, over the back, and through the loin, as well as in the forearm and gaskin muscles of the upper front and hind legs.

Discussion

In this study, there was not a significant amount of hoof growth that was influenced by the supplement alone. Visually, there were no differences in hoof quality or brittleness. In cattle, methionine supplementation increased hoof growth during the spring and summer (Clark and Rakes, 1982) but the rapid growth produced lower hoof quality. In this study, there was a noticeable difference in hoof growth during different seasons. The hoof growth rate was higher in the spring and lower in the winter. A previous study concluded that there was indeed a seasonal impact in the overall hoof growth; the front hooves were longer in the spring months and shorter in the winter months (He et al., 2019). In cattle, Clark and Rakes (1982) also noted greater hoof growth during spring and summer compared to fall and winter. While hoof growth was not significantly impacted due to supplementation in this study, there was a difference in muscle mass.

Muscle mass increased in the horses when they were consuming the supplement. Previous research conducted to determine the effects of amino acid supplementation in horses demonstrated that dietary amino acids increased muscle mass during 14 weeks of supplementation; the two primary amino acids that were studied were lysine and threonine (P. M. Graham-Thiers et al., 2005). Both lysine and threonine were present in the supplement that was used in this study. In another study, muscle mass scores and forearm circumference increased after 56 days in horses fed a supplement containing added lysine, threonine, and methionine (Vineyard et al., 2013).

Although a difference in muscle mass was recorded, there was no measured or visual difference in the weights or body condition scores of the horses. They maintained a consistent weight and BCS throughout the study. Maintaining body weight and BCS was

expected because the base diet of hay and grain concentrate was balanced to meet expected the calorie requirements for each horse. While the supplement added some calories, the increased calorie intake was not enough for the horses in this study to gain weight or BCS. These results agree with Vineyard et al. (2013), who also reported no change in body weight or BCS.

Although there was no change in BCS or body weight, there was visual change in haircoat quality. While haircoat quality could not be quantified, there was a visually noticeable difference in haircoat quality when horses were fed the Supplement compared to the Control baseline diet (Figure 7). Benefits of fatty acids supplemented in the form of fats or oils in equine diets have been long been documented (Potter et al., 1992). Anecdotally, horse owners have noted improved shine and quality of haircoat in horses supplemented with fats and oils (Warren and Vineyard, 2013).

Conclusions

Compared to a baseline diet that provided appropriate nutrients for horses' health and exercise level, the provision of a supplement containing amino acids lysine, threonine, and methionine, fatty acids, and marine-derived calcium in a crossover experimental design for twelve weeks had no effect on hoof growth in horses. When consuming the supplement, the horse did increase subjective muscle mass scores and visual appearance of haircoat quality. Horses demonstrated faster hoof growth in spring compared to winter, with no differences in visual hoof quality. Rather than growth rate alone, future research may explore effects of supplementation on hoof quality at the molecular level, as well as tensile and compressional strength.

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Appendices

Appendix A: CITI Program Completion Certificate

CITI PROGRAM

Completion Date 31-Aug-2021
Expiration Date 30-Aug-2024
Record ID 44622224

This is to certify that:

Fallon Marshall

Has completed the following CITI Program course:

Not valid for renewal of certification through CME.

Working with the IACUC
(Curriculum Group)
Investigators, Staff and Students
(Course Learner Group)
1 - Basic Course
(Stage)

Under requirements set by:

Middle Tennessee State University

CITI
Collaborative Institutional Training Initiative

Verify at www.citiprogram.org/verify/?w5d8778b5-29ec-42f0-b655-87d5cd73a155-44622224

Appendix B: IACUC Approval

IACUC
INSTITUTIONAL ANIMAL CARE and USE COMMITEE
Office of Research Compliance,
010A Sam Ingram Building,
2269 Middle Tennessee Blvd
Murfreesboro, TN 37129

MIDDLE TENNESSEE STATE UNIVERSITY

IACUCN001: PROTOCOL APPROVAL NOTICE

Wednesday, October 06, 2021

Senior Investigator	**Rhonda Hoffman** (ROLE: Faculty Advisor)
Co-Investigators	John Haffner and Fallon Marshall
Investigator Email(s)	*rhonda.hoffman@mtsu.edu; john.haffner@mtsu.edu; fpm2e@mtmail.mtsu.edu*
Department	Agriculture
Protocol Title	***Effects of Purina "Greatest Hits" Supplement on Horse Hool Growth***
Protocol ID	**22-2007**

Dear Investigator(s),

The MTSU Institutional Animal Care and Use Committee has reviewed the animal use proposal identified above under the ***Designated Member Review (DMR) mechanism*** and has approved your protocol in accordance with PHS policy. A summary of the IACUC action(s) and other particulars of this this protocol is tabulated as below:

IACUC Action	**APPROVED for one year**	
Date of Expiration	**10/31/2022**	Approval Date:: 10/4/2021
Number of Animals	TWELVE (12)	
Approved Species	**Equus caballus (MTSU Horses)**	
Category Subclassifications	☐ **Teaching** ☐ Classroom ☐ Laboratory	☒ **Research** ☐ Laboratory ☒ Field Research ☐ Field Study ☒ Handling/Manipulation ☐ Observation
	Comment: NONE	
Approved Site(s)	Housing: MTSU Horse Science Center	
Restrictions	**1. Satisfy DMR requirements AND annual continuing review.** **2. Follow CDC guidelines and MTSU requirements to counter COVID-19 infection**	
Comments	NONE	

This approval is effective for three (3) years from the date of this notice (10/4/2024). This protocol **expires on 104/22** The investigator(s) MUST file a Progress Report annually regarding the status of this study. Refer to the schedule for Continuing Review shown below; NO REMINDERS WILL BE SENT. A continuation request (progress report) must be approved

IACUCN001 Version 1.3 Revision Date 04.15.2016