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Original Research

The Effect of Serum-Based Bioactive Proteins for the Prevention of Squamous Gastric Ulcers in Horses

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ABSTRACT

The objective of this study was to determine the effects of serum-based bioactive proteins on the prevention of squamous gastric ulcer formation in horses in moderate exercise programs over a 21-day period. Horses without ulcers were identified and randomly assigned to treatment or control group. Horses were subjected to a training program which induced squamous gastric ulceration in control horses. In horses treated with 210-g bioactive proteins, the incidence of squamous ulcers was significantly reduced ($P = .0001$) compared to control horses. In horses treated with 80-g bioactive proteins, 66.67% (10/15) of the control horses developed squamous gastric ulceration compared to 33.55% (5/15) of those administered bioactive proteins. In conclusion, dosing horses with bioactive proteins derived from serum was effective for preventing gastric ulcers in horses experiencing stress from exercise or training.

1. Introduction

The high incidence of equine squamous gastric disease (ESGD) in performance horses of multiple disciplines has been identified in numerous publications [1–3]. The exposure of the squamous mucosa to volatile fatty acids and hydrochloric acid can result in cellular damage in a time-dependent manner [4]. The physical aspects of exercise increase abdominal pressure, pushing the acidic contents upward [5]. Exercise beyond a walk for prolonged periods of time such as is seen in race horses and endurance horses can lead to ulceration of the squamous mucosa due to acid exposure. Furthermore, the roughage in the diets of performance horses may be decreased along with sporadic feeding resulting in prolonged periods with less material in the stomach to buffer the continual acid secretion [6].

Mechanisms to prevent ESGD include omeprazole, which is a proton pump inhibitor, dietary and behavioral management, and supplements. Omeprazole has been shown to effectively prevent the formation of squamous gastric ulcers and prevent recurrence in race horses during training [7–9]. Continuous access to grass pasture, splitting the ration into multiple feedings, and decreasing sweet feeds that increase volatile fatty acids can all decrease squamous mucosal ulcers in the horse [10]. Decreasing exercise can also decrease the formation of gastric ulcers, but this is not often a viable option for horses in training.

A number of oral supplements for the prevention of ESGD have been evaluated. Antacids alone are of limited value due to the short duration of their effect [11]. Combinations of pectin-lecithin, antacid and *Saccharomycis cerevisa*, herbs, and coating agents have shown protective effects against ESGD [12–15]. When bovine colostrum containing immunoglobulins and growth factors were supplemented in the diet of race horses, their racing performance and postrace recovery improved [16]. It is well established that plasma-derived proteins from bovine, porcine, and other sources, when added to the diets of

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several species of animals, lead to improvements in appetite, weight gain, intestinal growth, and gut barrier function in a number of intestinal disorders [17–20]. Plasma proteins added to pig diets have been shown to reduce proinflammatory cytokines including tumor necrosis factor α and interleukin 8 in the intestinal tract [21–22]. In horses, improved gait kinematics were observed with the supplementation of serum-based bioactive proteins [23–24]. Bovine serum concentrate added to the drinking water of pigs reduced the morbidity associated with ulcers in growing pigs [25]. Based on the recognized effect of bioactive proteins from either bovine colostrum or plasma proteins in horses and the prevention of gastric ulcers in pigs, the value of bioactive proteins for prevention of ESGD should be considered. The objective of these studies was to evaluate the effect of orally administered bioactive proteins on the prevention of ESGD in horses during a western performance training/showing environment.

2. Material and Methods

The study was completed in two phases. In phase 1, a dose of 210 g of bioactive proteins (contained in 382 g of supplement) was administered to 15 horses and compared to a control supplement (382 g) administered to 15 horses. In phase 2, a dose of 80 g of bioactive proteins (contained in 230 g of supplement) was administered to 15 horses and compared to a control supplement (230 g) administered to 15 horses. The two phases were conducted during separate periods with different horses.

2.1. Horses

From a pool of horses at a private training facility that had been similarly managed for at least the previous 3 weeks, the first 30 qualifying horses with a gastric score of zero (i.e., no squamous or glandular ulcer disease as determined by endoscopic examination) were selected for participation in each phase. Horses included in the studies had never received medications for treatment of gastric ulcers and had not received any nonsteroidal anti-inflammatories within 2 weeks of the start of the study (day 0). Each horse was uniquely identified by registered name, color, unique markings, and identified with a tag attached to the halter. Horses were individually kept in approximately 3.6 $\times$ 3.6 m stalls throughout the study period when not being exercised or trained. All horses were maintained following standard animal use and care guidelines and in accordance with applicable local regulations and informed consent of the owner. The study was approved by the Iowa State University Animal Care and Use Committee.

Physical examinations were completed, and body weights were obtained 1 day (day 0) before administration of treatment or placebo and again on day 21 of the study. Blood was obtained on days 0 and 21 for complete blood count and serum chemistries. Horses were observed at least twice daily, and any clinical signs of illness and consumption of study material were recorded. Horses were maintained at the same level of activity that they were doing for 1 week (days 1–7) before initiation of stress to allow for consistent consumption of the test supplements. Then, from days 7 to 21, horses were subjected to a 2-week period of physiologic stress induced by a training regimen applicable for each horse. Young horses were broke for being handled and to lead and lunge. They were also trained to load on and off trailers and were taken on short trips around town. The horses from 2 years up were being worked under tack and various levels of training for a finished saddle horse. This includes walk, trot, and canter in each direction in the correct leads and stopping on cue. A 15- to 30-minute session under tack 5 days a week is routine. Horses are also put on a mechanical walker for 30 to 60 minutes daily. Both horses in a replicate were treated similarly.

2.2. Gastroscopy

Gastroscopy was performed on each horse on day 0 to verify the absence of squamous gastric ulcers and glandular erosions (i.e., gastric lesion score, 0) and conducted again on all horses on day 21 at the conclusion of the trial. Horses were sedated with 20 μg/kg of detomidine (Dormosedan; Pfizer Animal Health, Kalamazoo, MI) and lightly restrained with a twitch for the endoscopic examinations. Food was removed from the stalls approximately 8 hours before endoscopy, and all horses were tied 2 hours before endoscopy to prevent intake of any bedding or water. The entire squamous mucosa and most of the glandular portions of the stomach including the pyloric antrum were viewed in all horses with a 3-meter gastroscope (Tele-View USB Video Gastroscope; Advanced Monitors Corporation, San Diego, CA). One endoscopist performed all gastroscopies was blinded to the identification of the horse groups. Gastric lesion severity was a score of 0 which indicated no erosion or ulcer but could include reddening or hyperkeratosis, and scores of 1 to 3 indicated the presence of erosions and ulcers of increasing severity. Reddening and hyperkeratosis and glandular ulcers were scored as present or absent.

2.3. Experimental Protocol

The studies used a randomized complete block design. Replicates of two horses were formed on the basis of similarity of sex, age, and weight, respectively. Age as a blocking factor assured both horses in a replicate were subjected to a similar training program in a model used previously to induce gastric ulcers [26,27]. All horses were the same breed. Within replicates, horses were randomly allocated to one of two study groups, which were randomly assigned as group A (placebo) and group B (treatment). All investigators and participants were blinded to treatment and control groups until completion of the study. The treatments for phase 1 were 382 g/d of pellets containing either 210 g of soybean meal (control; A) or 210 g of bioactive proteins derived from bovine serum isolate (Biothrive, APC Inc, Ankeny, IA; treatment; B). The total 382 g volume was split into two doses per day in individual plastic bags marked only as A or B. For phase 2, 230 g of pellets containing either 80 g of soybean meal (control; A) or 80 g of bioactive proteins (treatment; B) was split into

two doses per day in individual plastic bags marked only as A or B. Each horse was assigned a bucket of their respective individual bag doses to provide a mechanism of accounting for the administration of the materials.

The horses were administered the test material with two pounds of oats twice a day, labeled as A or B; thus, both the researcher and site were blinded to the treatments. Test material was presented by the trainer. They were allowed at least 2 hours to self-administer the grain/test material. Administration was recorded as complete or partial, and an estimate of the amount left was recorded. Horses were fed approximately 2% body weight/d of a mixed orchard grass and alfalfa hay split into two feedings. They had free access to well water via automatic water system in each stall, and a salt block was present in each stall. No other supplements or feeds were allowed during the study period. All horses were observed twice a day at feeding for signs of in appetence, colic, or any other abnormal clinical signs. In addition, if any clinical signs of disease were seen throughout the day during training and handling of the horses, they were recorded.

2.4. Statistical Analyses

All data were entered into a spreadsheet (Excel; Microsoft Corp, Redmond, WA), and statistical analysis was completed with a commercial software program (SAS v.9.2; SAS Institute Inc, Cary, NC). The physical examination data (weight, temperature, pulse rate, respiratory rate, age) were compared between groups at days 0 and 21 with a Student's *t*-test, and sex distribution between groups evaluated with a Fisher's exact test. The primary efficacy variable is the binomial proportion of successes at day 21, where success was defined as a day 21 gastric lesion score of 0 (intact mucosal epithelium, i.e., no ulcers present) and failure as a day 21 gastric lesion score of $\geq$1 (ulcers present). The incidence of squamous ulcers was compared using chi-squared analysis. Similarly, the presence or absence of reddening, hyperkeratosis, glandular erosions, and physical examination data was compared between groups using chi-squared analysis, and all binomial data are presented with the incidence, prevalence coefficient, and the Jeffreys lower and upper 95% confidence intervals. The categorical ulcer scores are presented as the median and interquartile range with differences between groups determined by a median two-sample test. Serum chemistry and complete blood count data and the change in weight of each horse from days 0 to 21 were dependent variables, while treatment was the independent variable analyzed by analysis of variance (PROC GLM of SAS). For all comparisons, a value of $P \leq .05$ was considered significant, and a tendency was considered at $P > .05$ and $P < .10$.

3. Results

3.1. Horses

In phase 1, gastroscopic examinations were performed on 33 horses on day 0 of the trial of which the first 30 qualifying horses (15 horses per treatment) with a gastric score of zero were selected. One horse was diagnosed with a spasmodic colic on day 18 of the study. Flunixin meglumine was administered, and the horse was not administered any grain or test material that day. That horse and the corresponding horse in the block were dropped from further evaluation. No additional health issues were recorded during the study resulting in 28 horses (14 horses per treatment) completing the study. There were 15 females and 13 geldings, with three 4-year olds, seven 3-year olds, 10 2-year olds, and 8 yearlings included in the study. The mean weight was 428.5 kg, with a range of 269.9 to 644.1 kg.

In phase 2, gastroscopic examinations were performed on 34 horses on day 0 of the trial of which the first 30 qualifying horses (15 horses per treatment) with a squamous gastric ulcer score of zero were selected. There were 16 females and 14 geldings, with one 5-year old, four 4-year olds, seven 3-year olds, 10 2-year olds and 8 yearlings included in the study. The mean weight was 469.3 kg, with a range from 367.4 to 630.9 kg. The complete descriptive data for the groups are reported in Table 1. All horses were client-owned American Paint Horses.

3.2. Treatment Self-administration of Test Articles

In phase 1, three horses consistently failed to self-administer the entire allotment of test material "B" (treatment) throughout the study. All three were initially leaving an estimated 1/3 of the test material, which decreased to 1/4 to 1/5 by the completion of the 21-day period. One horse on B did not take 1/3 to 1/4 of the material for the first week, but when exercise was increased, it self-administered the entire portion. None of these horses had squamous gastric ulcers on day 21.

In phase 2, four horses failed to completely self-administer their dose of test material B for the first 7 to 14 days with an estimated 1/4 left. All horses had full self-administration the last week of the study. Only one of these four horses had squamous gastric ulcers on day 21. All horses in both phases self-administered all the control product.

3.3. Gastroscopy

None of the horses enrolled in the study had squamous ulcers or glandular erosions on day 0. In phase 1, on day 21, 14 horses had squamous ulcers, 5 reddening, 17 hyperkeratosis, and there were no horses with glandular ulcers. In the control group, there were eight horses with a gastric ulcer score of 1, three with a score of 2, and one with an ulcer score of 3 for a total of 12 of 14 horses (85.7%) developing ESGD. In the treatment group, there was one horse with a gastric ulcer score of 1 and one horse with a gastric ulcer score of 2 for a total of 2 of 14 horses (14.3%) ESGD. Thus, the primary outcome variable of presence or absence of squamous mucosal ulcers at day 21 was significantly different between groups ($P < .0001$) (Tables 2 and 3) with horses dosed with the bioactive proteins resulting in 14.3% ESGD incidence compared to 85.7% incidence in the controls.

In phase 2, there were 15 horses with squamous ulcers, 1 reddening, 13 hyperkeratosis, and one horse with a glandular ulcer on day 21. In the control group, there were seven horses with a squamous gastric ulcer score of 1, two

Table 1
The mean and standard deviation for the age at the beginning of the study and the physical examination parameters for both days 0 and 21 along with the change in weight during the study period between horses dosed with a placebo supplement (based on soybean meal, control group) or bioactive proteins (BioThrive, treated group) and *P* value as determined by a Student's *t*-test. The sex distribution between groups was compared with a Fisher exact test.

Variable	Phase 1				Phase 2			
	Control	Treated	SD	*P* Value	Control	Treated	SD	*P* Value
Age (y)	2.21	2.14	0.97	.8518	2.00	2.07	0.87	.8416
Castrated males	7	6			8	6		
Females	7	8		1	7	9		.7152
Day 0								
Weight (kg)	432.68	426.14	101.58	.8717	476.91	463.70	60.67	.5668
Temp (°C)	37.31	37.36	0.24	.5256	37.65	37.49	0.34	.1974
Pulse (per min)	43.14	43.57	9.27	.9071	45.73	48.13	10.93	.5635
Respiration (per min)	16.57	16.79	4.83	.9109	18.80	18.93	6.89	.9583
Day 21								
Weight (kg)	433.12	424.19	59.64	.8177	480.18	467.03	52.60	.5104
Temp (°C)	37.63	37.59	0.33	.7827	37.49	37.29	0.36	.1353
Pulse (per min)	41.86	44.86	11.44	.506	43.07	40.93	6.53	.3886
Respiration (per min)	18.29	23.43	5.77	**.0175**	27.60	25.87	7.69	.5511
Weight change (kg)	0.49	−1.95	9.68	.5309	3.27	3.33	11.47	.9889

Abbreviation: SD, standard deviation.
P values that is significant or show a tendency is indicated in bold.

with a score of 2, and one with an ulcer score of 3 for a total of 10 of 15 horses (66.7%) developing ESGD. In the treatment group, there were two horses with a score of 1, two horses with a gastric ulcer score of 2, and one horse with a gastric ulcer score of 3 for a total of 5 of 15 horses (33.3%) developing ESGD. The primary outcome variable of the presence or absence of ulcers at day 21 showed a trend ($P = .0719$) for horses dosed with bioactive proteins having 33.3% squamous mucosal ulcer incidence compared to 66.7% incidence in controls.

Table 2
The binomial data of occurrence of squamous gastric ulcers, hyperkeratosis, reddening, and glandular ulcers on day 21 for horses dosed with a placebo supplement (based on soybean meal, control group) or bioactive proteins (BioThrive, treated group) and the *P* value as determined by chi-square tests are shown. The data are presented with the incidence, prevalence coefficient, and upper and lower Jeffreys confidence intervals. A significant difference between groups was seen in phase 1 and a trend identified in phase 2.

Variable	N	Prevalence Coefficient	Lower 95% CI	Upper 95% CI	*P* Value
Phase 1					
Ulcer incidence					
Control	12/14	0.857	0.615	0.969	**.0001**
Treated	2/14	0.143	0.031	0.385	
Hyperkeratosis					
Control	9/14	0.643	0.385	0.849	.7115
Treated	8/14	0.571	0.319	0.797	
Reddening					
Control	4/14	0.286	0.105	0.545	.1387
Treated	1/14	0.071	0.008	0.288	
Glandular ulcers					
Control	0/14	0	0	0.162	1
Treated	0/14	0	0	0.162	
Phase 2					
Ulcer incidence					
Control	10/15	0.667	0.416	0.86	**.0719**
Treated	5/15	0.333	0.14	0.584	
Hyperkeratosis					
Control	8/15	0.533	0.294	0.761	.2849
Treated	5/15	0.333	0.14	0.584	
Reddening					
Control	1/15	0.067	0.007	0.272	.3259
Treated	0/15	0	0	0.152	
Glandular ulcers					
Control	1/15	0.067	0.007	0.272	.3259
Treated	0/15	0	0	0.152	

Abbreviation: CI, confidence interval.
P values that are significant or show a tendency are indicated in bold.

3.4. Clinic Pathologic Data

There were some sporadic significant differences between treated and control groups observed for serum chemistry (Table 4). All 38 variables remained within normal reference ranges, and with the large number of variables, there is an increased likelihood of type I error. In phase 1, the RBC count showed a trend ($P = .0523$), and hemoglobin ($P = .0478$) and hematocrit ($P = .0418$) were all significantly higher in the treated horses compared to the controls. In phase 2, no differences for any analytical parameters were observed between groups.

4. Discussion

When bioactive serum proteins were dosed daily at 210 g, the formation of squamous gastric ulcers was

Table 3
The categorical ulcer scores (0–3) are presented as the median and interquartile range (IQR). The *P* value as determined by Median two-sample tests are shown with a significantly lower ulcer score in phase 1 horses treated with bioactive proteins and a trend toward a lower ulcer score phase 2 horse treated with bioactive proteins.

Variable	Median	IQR	*P* Value
Phase 1			
Ulcer score			
Control	1	1–2	
Treated	0	0–0	**.0002**
Phase 2			
Ulcer score			
Control	1	0–1	
Treated	0	0–1	**.0726**

P values that are significant or show a tendency are indicated in bold.

Table 4
The least squares mean and SEM for blood and plasma chemistry analysis in horses dosed a placebo supplement (based on soybean meal, control group) or bioactive proteins (BioThrive, treated group) at the beginning of the study and at the completion of the study on day 21. The *P* values were determined by ANOVA with treatment as the independent variable.

Variable	Units	Phase 1								Phase 2							
		Day 0				Day 21				Day 0				Day 21			
		Control	Treated	SEM	*P* Value	Control	Treated	SEM	*P* Value	Control	Treated	SEM	*P* Value	Control	Treated	SEM	*P* Value
Plasma protein	gm/dL	6.68	6.58	0.09	.418	6.64	6.82	0.09	.1828	6.51	6.56	0.07	.5858	6.71	6.89	0.08	.1398
Fibrinogen	mg/dL	314.29	214.29	26.43	**.0127**	342.86	284.62	35.57	.2495	313.33	320.00	28.51	.8698	300.00	306.67	23.37	.8416
WBC	103	10.84	9.86	0.61	.2684	10.96	10.7	0.81	.8208	8.55	8.76	0.55	.7879	8.43	9.22	0.54	.311
Neutrophil	103	5.27	4.79	0.48	.4853	5.1	5.06	0.49	.9566	3.93	3.91	0.32	.9648	3.78	4.23	0.30	.2913
Lymphocyte	103	4.74	4.36	0.32	.418	5	4.82	0.4	.7472	3.96	4.18	0.31	.618	4.01	4.28	0.38	.6154
Monocyte	103	0.47	0.4	0.05	.3665	0.5	0.47	0.06	.7604	0.32	0.35	0.03	.4878	0.35	0.38	0.03	.5249
Eosinophil	103	0.22	0.19	0.03	.5188	0.14	0.13	0.06	.7532	0.24	0.22	0.03	.6842	0.20	0.21	0.03	.7344
Basophils	103	0.04	0.03	0.01	.1468	0.08	0.15	0.04	.2498	0.03	0.03	0.00	.7364	0.02	0.03	0.00	.1534
RBC	106	9.76	10.28	0.32	.2666	9.12	9.89	0.28	**.0523**	8.56	8.45	0.34	.823	8.16	8.21	0.22	.8735
Hemoglobin	gm/dL	14.04	14.77	0.38	.1839	13.29	14.38	0.38	**.0478**	12.59	12.31	0.40	.6292	12.08	12.05	0.29	.9365
Hematocrit	%	41.31	43.3	1.11	.2137	39.17	42.52	1.08	**.0418**	35.96	34.97	1.13	.5407	34.71	34.45	0.84	.8246
MCV	fl	42.69	42.24	0.83	.7059	43.16	43.01	0.85	.8998	42.27	41.73	0.81	.6393	42.63	42.15	0.83	.6913
MCH	pg	14.51	14.39	0.3	.7884	14.66	14.55	0.31	.8001	14.80	14.69	0.31	.8095	14.81	14.75	0.31	.8911
MCHC	gm/dL	33.95	34.1	0.12	.4034	33.95	33.84	0.17	.6325	34.96	35.22	0.15	.236	34.79	34.97	0.17	.4521
RDW	%	18.78	19.14	0.2	.2121	18.89	19.16	0.2	.3452	19.07	18.90	0.20	.5691	18.84	18.93	0.20	.766
Platelets	103/ul	195.57	202	7.32	.5399	161.64	185.38	14.44	.2475	169.73	188.87	10.22	.1963	177.40	190.20	7.34	.2276
MPV	fl	7.8	7.69	0.19	.695	6.59	6.56	0.15	.8822	8.17	8.03	0.29	.7195	7.63	7.47	0.27	.6811
Sodium	mEq/L	135.43	135.64	0.39	.704	135.71	135	0.56	.3727	136.60	136.33	0.54	.7279	138.00	138.27	0.29	.5186
Potassium	mEq/L	4.09	3.96	0.08	.3106	4.19	4.28	0.1	.4992	4.20	4.14	0.08	.6046	4.05	3.85	0.08	**.0846**
Chloride	mEq/L	94.5	94.21	0.49	.6808	95.57	94.57	0.41	**.0956**	97.53	98.40	0.39	.1258	97.33	97.80	0.37	.381
Bicarbonate	mEq/L	31.57	32	0.45	.5058	30.36	30.5	0.43	.818	30.07	30.00	0.35	.8933	30.73	30.87	0.35	.788
Calcium	mg/dL	11.54	11.44	0.1	.4432	12.21	12.26	0.06	.5627	11.46	11.52	0.08	.6061	11.55	11.41	0.11	.3889
Phosphorus	mg/dL	6.01	5.89	0.24	.7229	4.74	4.86	0.17	.6231	6.09	5.97	0.24	.7297	5.70	5.80	0.23	.7601
Magnesium	mg/dL	1.59	1.66	0.03	.1808	1.78	1.93	0.05	**.041**	1.94	1.93	0.04	.8368	1.63	1.66	0.03	.5741
BUN	mg/dL	16.71	16.57	0.77	.8972	18.29	18.64	0.59	.6738	13.33	13.40	0.81	.954	14.00	13.93	0.61	.9387
Creatinine	mg/dL	1.53	1.48	0.05	.4863	1.29	1.2	0.06	.3109	1.65	1.63	0.07	.8386	1.45	1.41	0.06	.5885
Glucose	mg/dL	73.57	77.43	2.32	.2499	99.79	103.71	3.67	.4554	69.53	64.67	2.09	.1107	71.87	67.40	2.43	.2049
Total protein	gm/dL	6.22	6.21	0.1	.9609	6.11	6.31	0.11	.2045	6.23	6.31	0.07	.392	6.02	6.09	0.06	.4488
Albumin	gm/dL	2.84	2.86	0.04	.6756	2.82	2.94	0.04	**.0438**	2.99	2.93	0.04	.2946	2.87	2.83	0.03	.4944
AST	IU/L	350.21	356.14	23.2	.858	337.64	370.29	19.5	.2473	375.33	382.87	18.87	.7798	422.20	332.93	53.60	.2489
Creatine kinase	IU/L	218.71	476.43	127.94	.1662	150.14	141.07	15.96	.691	200.07	186.20	17.25	.5743	231.10	200.20	38.06	.5705
Alk Phos	IU/L	203.5	202.93	18.5	.9827	185.07	185.21	15.42	.9948	181.93	189.07	15.08	.7404	163.60	176.07	10.99	.4292
GGT	IU/L	25.93	25.57	2.29	.9132	22.07	23	1.59	.6835	27.47	29.07	2.15	.6034	24.73	25.93	1.87	.6527
Total bilirubin	mg/dL	1.7	1.77	0.14	.6924	1.13	1.4	0.13	.1538	1.75	1.93	0.16	.4418	1.42	1.49	0.11	.6618
Anion gap		13.36	13.5	0.42	.8096	13.86	14.36	0.53	.5079	13.13	12.53	0.47	.371	13.73	13.53	0.30	.6448
Lipemic indice		20	20	0	1	20	20	0	1	20.00	20.00	0.00	1.00	20.00	20.00	0.00	1.00
Hemolytic indice		15.14	15.43	0.2	.331	15.93	16.57	0.85	.5983	15.00	21.67	4.71	.3259	15.00	15.00	0.00	1.00
Icteric indice		2.64	2.86	0.23	.5122	2	2.21	0.15	.3265	2.53	3.00	0.32	.3152	2.13	2.20	0.12	.6994

Abbreviations: ANOVA, analysis of variance; AST, aspartate aminotransferase; BUN, blood urea nitrogen; GGT, gamma-glutamyl transpeptidase; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; RBC, red blood cell; SEM, standard error of the mean; RDW, red blood cell distribution width; WBC, white blood cell.
P values that are significant or show a tendency are indicated in bold.

prevented resulting in significantly fewer ulcers (14.3 vs. 85.7%) developing in treated horses compared to controls during an exercise and training program. When administered at 80 g per day, there was a trend toward fewer squamous ulcers developing in the treated horses compared to the controls during an exercise and training program in which 67% of control horses developed gastric ulcers, whereas only 33% of treated horses developed gastric ulcers. The incidence of ESGD was lower in the phase 2 controls compared to phase 1 controls which may indicate slightly less physiologic stress associated with exercise and training during phase 2.

The two phases were completed approximately 6 months apart. While horses were treated similarly, they may have been at different stages of training than the horses 6 months later or the trainer may not have pushed them quite as hard at a different time of the year. Although there is potential for some small differences in the training programs and physiologic stress between the two phases, these data suggest there is a dose response for bioactive proteins in the prevention of gastric ulcers in horses.

The model used in this study was similar to what has been previously described [26,27] and is consistent with normal husbandry practices. This model replicates typical breaking, exercise, and training scenarios and therefore is useful for supplement prevention evaluations compared to feed restriction models [13,28]. Young horses are routinely subjected to prolonged exercise throughout breaking and training programs. During these times in particular, the addition of a supplement to the horse may be a feasible mechanism to ESGD in horse training programs. With this model, horses typically do not develop glandular erosions, which was seen here and in previous studies [26,27]. While we did see a change in the glandular erosions, reddening, and hyperkeratosis scores, they were not significantly different.

The mechanism of how bioactive proteins can prevent ESGD in the horse is unknown. The exposure of the squamous mucosa to hydrochloric, valeric, and other volatile fatty acids initiate cellular damage, cellular swelling, and barrier disruption in the nonglandular portion of the stomach [29,30]. In a model of intestinal inflammation in rats, plasma-derived proteins significantly decreased intestinal permeability and maintained the integrity of the intestinal mucosa [31]. The data indicate that the plasma-derived proteins promote repair of the tight junctions and reduce permeability and inflammation in the intestine. In addition, oral plasma proteins reduce expression of proinflammatory cytokines in multiple models of intestinal inflammation [21,22,32], indicating the ability of bioactive proteins to impact the immune response to reduce inflammation and subsequent damage to tissue during stress events. The prevention of ESGD in the horses treated with 210 g of bioactive protein a day is likely the result of less inflammation within the gastric mucosa as seen in other species.

Another potential mechanism of action is repair of tissue. In the swine ulcer treatment study with plasma proteins, an increase in tissue repair was seen in pigs with ulcers [25]. Growth factors including insulinlike growth factor 1 and epidermal growth factor are present in plasma, serum, and colostrum. It has been suggested that improved athletic performance in humans supplemented with bovine colostrum is associated with an increase in insulinlike growth factor 1 [33]. Receptors for epidermal growth factor are present in [34] equine gastric mucosa with the highest concentrations in the basal epithelial layers where cell proliferation occurs. Thus, the complex mixture of bioactive proteins including growth factors may be playing a role in tissue repair if damaged tissue has occurred.

In these studies, the control material was a similar sized pellet with soybean meal as a protein source. This provided similar ingredients with the differences being limited to the bioactive proteins in the treated horses and the soy-based protein in the control horses. There were some initial palatability issues in a few of the treated horses. We think this is because the horses have hot been previously exposed to the taste or smell of a non–plant-derived protein source previously. It appears that after about a week of exposure to the test material, the horses that initially limited their self-administration began to fully self-administer the test product similar to the control product. Although this difference in self-administration was noted with the treated horses, it did not impact the effect of dosing bioactive proteins to horses to prevent gastric ulcers in horses during an exercise and training program.

The cause of the higher respiratory rate in the treated horses (23.43) compared to the control horses (18.29) at day 21 of phase 1 is unknown. Environment was not a factor because horses within a replicate were housed in the same location. While the clinicopathologic data remained within reference range, at the higher dose, there was an indication of higher hemoglobin ($P = .0478$) and hematocrit ($P = .0418$) in the treated horses compared to the controls. This could be incidental or it could be related to the treatment. In a previous study, there were lower hemoglobin and RBC counts in horses with gastric ulcers compared to those without ulcers [1]. Thus, the higher hemoglobin and hematocrit values, albeit within normal ranges, could indicate lower incidence of ulcers as noted in the treated horses compared to the control horses.

In conclusion, the novel results of these studies indicate that dosing horses with bioactive proteins derived from serum was effective in preventing ESGD in horses experiencing physiologic stress from exercise or training programs. These data suggest there is a dose response for bioactive proteins in the prevention of gastric ulcers in horses and relates to the level of stress the horse is experiencing.

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