



Original Research

Effects of β -Hydroxy- β -Methylbutyrate and γ -Oryzanol on Blood Biochemical Markers in Exercising Thoroughbred Race Horses

Piotr Ostaszewski PhD, DVM^a, Agnieszka Kowalska MSc^a, Ewa Szarska PhD^b,
Piotr Szpotański DVM^c, Anna Cywinska PhD, DVM^d, Bożena Bałasińska PhD^a,
Tomasz Sadkowski PhD, DVM^a

^a Faculty of Veterinary Medicine, Department of Physiological Science, Warsaw University of Life Sciences–SGGW, Poland

^b The General Karol Kaczkowski Military Institute of Hygiene and Epidemiology, Warsaw, Poland

^c Equine Veterinary Clinic, Łasiecniki, Poland

^d Faculty of Veterinary Medicine, Department of Preclinical Sciences, Warsaw University of Life Sciences, Poland

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ABSTRACT

In both the horse and the man, nutritional ergogenic aids have been used to improve physical ability in conjunction with an appropriate training regimen. Although training increases physical condition, the ease of taking a nutritional additive to improve training results explains the demand for supplementation, which may increase mechanical energy of work, delay onset of fatigue, or improve neuromuscular coordination. The purpose of this study was to determine the effects of oral supplementation of β -hydroxy- β -methylbutyrate (HMB) and γ -oryzanol (GO) on indices of exercise-induced muscle damage in Thoroughbred race horses. In this 32-week study, the horses were assigned to either a placebo, GO (3.0 g/d), HMB (15 g/d), or GO and HMB treatment groups. The supplements were administered for the first 16 weeks of the study during the training period before the racing season began. Blood samples were taken at baseline, and then during training, before exercise, immediately after exercise, and 30 minutes after exercise. Heart rate and speed were monitored in each exercise session. Hematocrit, glucose, lactate (LA), creatine phosphokinase, and aspartate aminotransferase were measured before and after each exercise session. Analysis of variance showed a significantly greater increase in post-exercise creatine kinase activity in placebo-supplemented group than in the other treatment groups, both in the training period and during the racing seasons ($P < .05$). Blood LA was higher immediately after exercise in the placebo group compared with the supplemented groups. In conclusion, supplementation with HMB and GO resulted in decreased creatine kinase and LA after exercise. These findings support the hypothesis that HMB and GO supplementation helps to prevent exercise-induced muscle damage.

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1. Introduction

β -Hydroxy- β -methylbutyrate (HMB) and γ -oryzanol (GO) are supplements used to enhance the effects of training in exercising humans, dogs, and horses. A metabolite of the amino acid leucine, HMB is produced

endogenously in small amounts and has been shown to improve gains in strength and lean body mass in humans when associated with resistance training [1,2]. The efficacy of HMB has been demonstrated in pathological conditions, where it has been reported to reduce muscle wasting associated with AIDS, trauma, and cancer cachexia [3-5]. More recently, HMB has been shown to decrease protein degradation and increase protein synthesis [6]. In decreasing muscle damage, HMB may also provide a source of cytosolic HMG-coenzyme A for cholesterol synthesis and increase the availability of cholesterol for cell membrane synthesis.

Corresponding author at: Piotr Ostaszewski, PhD, DVM, Faculty of Veterinary Medicine, Department of Physiological Science, Warsaw University of Life Sciences-SGGW, ul. Nowoursynowska 159, 02-776 Warsaw, Poland.

E-mail address: piotr_ostaszewski@sggw.pl (P. Ostaszewski).

This may result in an overall reinforcement of the sarcolemma as well as the provision of valuable substrate for its repair following muscle damage or injurious exercise [7]. This is evidenced by studies demonstrating that HMB leads to decreased markers of muscle damage following mechanically strenuous exercise, including lower activity of creatine kinase (CK), lactate dehydrogenase, and a decrease in muscle protein breakdown as indicated by serum 3-methylhistidine, a direct marker of muscle protein degradation [8–10]. Other studies have shown that HMB reduces cancer-induced muscle weight loss through attenuation of the ubiquitin-proteasome proteolytic pathway [11], suggesting that HMB functions predominantly as an anticatabolic, rather than anabolic compound. However, a recent study has shown that HMB supplementation induces muscle hypertrophy in the extensor digitorum longus and soleus muscles in rats via mammalian target of rapamycin pathway [12], in addition to attenuating the depression in protein synthesis induced by the proteolysis-inducing factor [6]. In geldings fed an alfalfa-based supplement containing 10 g of HMB per day during 6 weeks of low to moderate-intensity training followed by 6 weeks of high-intensity training, HMB supplementation resulted in a 10% improvement in treadmill endurance [13]. This was followed by a study in racing Thoroughbreds where HMB-supplemented horses had reduced serum CK, maintained body weight better, needed less recovery time between races, and had a better win rate. These effects were most likely through a decrease in training and race-related muscle damage and increased aerobic ability, which allowed for a quicker recovery after racing.

GO is a mixture of ferulic acid esters of sterol and triterpene alcohols extracted from rice bran, and is known to be a powerful inhibitor of iron-driven hydroxyl radical formation; it has also been reported to possess antioxidant activity in stabilizing lipids [14]. Because GO is insoluble in water, a GO emulsion is used in supplementing humans, dogs, and horses. There are few studies in the peer-reviewed literature on GO, despite its apparent use as an ergogenic aid. One study looked at resistance-weight-trained male athletes supplemented with 500 mg/d GO or a placebo [15]. However, this study failed to show an effect of GO on training performance.

Thoroughbred horses undergo intensive training starting at a young age. The results of this training and their race performance may be improved by the use of dietary supplementation. The use of supplementation may not only improve performance but also improve muscle recovery after a race or heavy training period. Therefore, the main objective of this study was to evaluate the effect of dietary supplementation of HMB and GO on exercise parameters in horses trained from winter break to their maximal physical performance at the start of the racing season. Our hypothesis is that one or both supplements, either alone or in combination, will decrease muscle damage and improve recovery, and thus improve overall performance.

2. Material and Methods

2.1. Horses

Twenty-four Thoroughbred racehorses ranging in age from 3–6 years (12 mares and 12 stallions) and weighing

Table 1

Analyzed composition and calculated energy density of hay and concentrates

Item	Hay	Oat Plus Commercial Feed
DM, % of fresh matter	91.50	85.14
Crude protein, % of DM	5.37	14.55
Crude fat, % of DM	3.12	9.08
Crude fiber, % of DM	50.27	14.51
Crude ash, % of DM	8.24	6.10
Net energy, MJ/kg DM	5.00	10.18

DM, dry matter.

520 ± 50 kg were studied at the Sluzewiec Racetrack training center (Warsaw, Poland). The horses were privately owned, and the experimental design and all procedures were approved by the Ethical Committee in Warsaw and by the owners of horses. The horses were selected on the basis of a clinical examination and hematological analysis, and horses with pathological conditions were excluded. The horses were also chosen on the basis of similar racing performance as recorded from the previous year's racing records. During a preliminary period of 2 weeks, all the horses were acclimated to a basal diet and were individually housed on straw in box stalls under identical conditions. Each horse consumed 5 kg of oats, 1 kg of a complete high-quality commercial feed (daily ration) distributed in three feedings, and 6 kg of hay that was administered daily ad libitum. The concentrate/forage ratio was 50/50. Table 1 shows the composition of the feedstuffs. Total net energy intake in the daily ration was 135 MJ/horse.

2.2. Supplement and Placebo: Composition and Administration

The HMB alfalfa-based supplement (150 g/horse/d) was distributed in a pelleted form twice daily and provided for a total of 15 g of CaHMB (Metabolic Technologies Inc, Ames, IA). The placebo for this treatment consisted of 150 g of the pelleted supplement matrix (dehydrated alfalfa meal, cane molasses, soy oil, soy lecithin, caramel flavor) but did not contain HMB. GO was purchased from Oryza oil & Fat chemical Co., Ltd (Aichi, Japan) as a crystalline powder. Three grams of GO was suspended extempore in 20 mL of rice bran oil and was top-dressed on the morning concentrate (once a day). The placebo for this treatment consisted of 20 mL of regular cooking oil and was also given as a top-dress with the morning concentrate.

A groom verified the consumption of both the supplements and the placebo. All treatments were palatable and consumed by the horses, and no adverse reactions were reported. The riders and trainers, as well as the technicians performing blood sample analyses, were blinded to the treatment allocation.

2.3. Experimental Design

After the 2-week acclimation period, four homogenous groups of six horses were chosen, with three mares and three stallions of similar age assigned within each group.

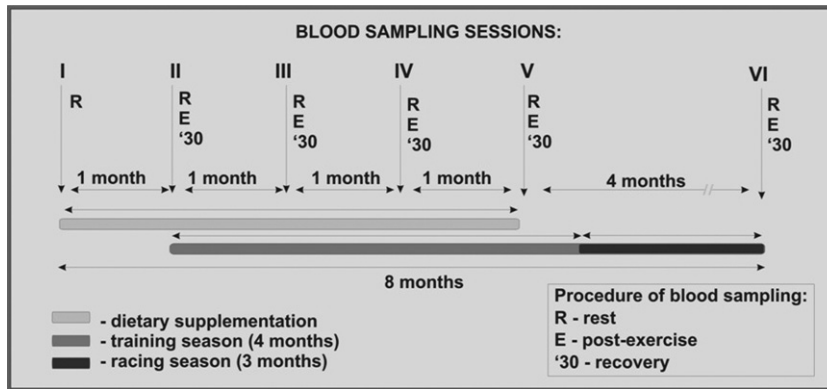


Fig. 1. Experimental design and sampling schedule.

The horses were fed the same diet as during acclimation, and the supplement regimens were begun. The placebo group consumed 150 g of pelleted matrix without HMB, and 20 mL of cooking oil; the GO group consumed 150 g of pelleted matrix without HMB, and 3 g of GO suspended in 20 mL of rice oil; the HMB group consumed 150 g of pelleted HMB supplement and 20 mL of cooking oil; and the GO/HMB group consumed 150 g of pelleted HMB supplement and 3 g of GO suspended in 20 mL of rice oil. Oral administration of selected supplements was initiated 4 weeks before the beginning of training season and lasted for 16 weeks. The blood samples were taken between February and September, and the exercise data were collected between March and September.

Four weeks after horses were assigned to dietary treatments, they began a conditioning program as shown in Fig. 1. During the entire training season, the horses were exercised 6 d/wk on a training course with a sand footing. The same exercise intensity was repeated every day. For the first 3 weeks, the conditioning program consisted of 10 minutes of walking, 15 minutes of trotting at 250 m/min, and 4 minutes of cantering at 600 m/min, followed by 30 minutes of exercise on a horse walker. During the following weeks, only time and intensity of the exercise were changed so that at week 5, galloping time consisted of 5 minutes of cantering at 500 m/min followed by 1 minute of galloping at about 750 m/min. Starting week 8, the intensity was again increased to 5 minutes of cantering at 550 m/min, followed by 1 minute of galloping at 850 m/min. This higher intensity conditioning program was continued until the beginning of the racing season (end of June). After the conditioning program, all horses participated in the races for the next 12 weeks. During this time (racing season), the horses were also exercised, but with the intensity modified to the racing schedule. Exercise during the racing season was designed to maintain the horses' current condition. Additional training was withheld on the day of the races.

The training was monitored using heart rate (HR) monitors RS800 on Polar Equine Wearlink W.I.N.D. (Polar Electro Oy, Finland) and G3 GPS. HR monitor consists of a receiver and two electrodes placed under the girth. Model RS800 allows simultaneous control of the HR values and speed of the horse. Twenty-two of the horses successfully completed the entire study. One horse from the GO group

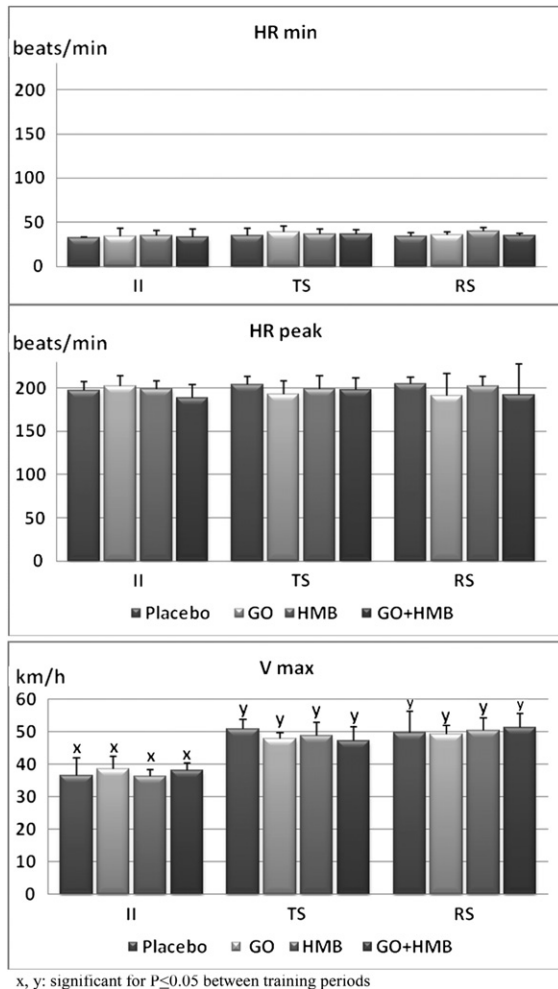
was excluded after exercise session III and one horse from HMB group after exercise session IV due to lameness.

2.4. Blood Sample Collection and Processing

Peripheral venous blood samples were taken by external jugular venipuncture every 4-week intervals, with the final sampling at 16 weeks while the horses were in the racing season. The first session had only one blood sample taken in the morning, whereas the remaining sessions consisted of three samples taken at the following intervals: R—rest (before exercise), E—exercise (after exercise), and '30—half an hour later after being in an exercise walker (recovery). The blood samples were aspirated into 20-mL syringes and immediately transferred into sterile ethylenediaminetetraacetic acid tubes for hematological tests and into plain tubes for serum analyses. Glucose and lactate (LA) concentrations were determined immediately by ejecting a drop of full blood onto single-use Accu-Chek Active and BM-LA test strips (Roche Diagnostics Corp, Indianapolis). The ethylenediaminetetraacetic acid-treated blood samples were kept in refrigerator (4°C) and analyzed within 6 hours after collection. Hematocrit (Hct) was counted with an automated hematology analyzer (Abacus Diatron, Hungary). The samples taken for serum were promptly centrifuged, and the serum samples immediately frozen and kept at -20°C until analyzed. The serum samples were analyzed for creatine phosphokinase (CK) and aspartate aminotransferase (AST) activity by a kinetic method, using a reagent kit (Pointe Scientific, Inc., Canton).

2.5. Statistical Analysis

Results are expressed as means \pm standard errors of mean. Exercise session II ($n = 6$ for each group) was considered as beginning of training, and the data from exercise sessions III, IV, and V were pooled for each group and referred to as the training season. The last exercise session (VI) was referred to as the racing season. The data were analyzed for differences between blood indices and HRs at various stages using a repeated-measures analysis of variance, and pairwise comparisons were made using Tukey test. Probabilities of $P < .05$ were considered statistically significant.



x, y: significant for $P \leq 0.05$ between training periods

Fig. 2. Minimum and peak heart rate (beats/min) as well as maximum speed (km/hr) determined in Thoroughbred racing horses at the beginning of training (sampling session II; $n = 22$), during the training season (sampling sessions III, IV, and V; $n = 66$), and during the racing season (sampling session VI; $n = 22$). For 16 weeks, the horses were supplemented with an oral placebo ($n = 6$), γ -oryzanol (GO, $n = 5$), 3-hydroxy-3-methylbutyrate (HMB, $n = 5$), or GO + HMB ($n = 6$).

3. Results

3.1. Exercise Monitoring

Resting and peak HR as well as maximum speed did not differ between placebo and experimental groups (Fig. 2). Resting HR during all exercise sessions was in the range of 32–37 beats/min. Average peak HR was approximately 200 beats/min for all exercised horses and was not affected by the length of training. Maximal speed increased from average 38 km/hr at the beginning of training to about 50 km/hr at the end of the racing season. These data confirm that training exertion was similar and intense in all treatments.

3.2. Supplements Versus Placebo at Rest

After 4 weeks of dietary supplementation, no change in resting hematocrit (Hct) values was observed among the

groups (Table 2). Hct was, on average, approximately 41% in all groups. Resting blood glucose and LA levels also remained unchanged during the study (Table 3, Fig. 3, respectively).

There were no differences in serum CK activity among treatment groups at the beginning of the dietary supplementation (Fig. 4). However, after 4 weeks of training, resting serum CK was significantly ($P < .05$) decreased in the GO + HMB-supplemented horses compared with the placebo-supplemented horses. During the remainder of the training and racing seasons, resting serum CK was not significantly different from pretraining values for any of the groups.

Resting AST activity was the same among the groups at the beginning of an experiment (sampling session I, Fig. 5). After 4 weeks of training, resting AST activity had decreased in all groups, and a significant 30% decrease was observed in the HMB-supplemented horses ($P < .05$). During the remainder of the training season, pre-exercise AST activity was still significantly lower in horses receiving HMB when compared with the beginning of supplementation ($P < .05$). During the racing season, pre-exercise AST activity was increased in the GO-supplemented horses when compared with the training season ($P < .05$).

3.3. Supplements Versus Placebo After Exercise

Exercise produced a significant increase in Hct levels in all the groups ($P < .05$); however, no differences were observed between the placebo and the supplemented groups (Table 2). On average, a 29% increase was observed compared with the resting values at the beginning of dietary supplementation, a 36% increase was observed during training season, and a 29% increase was observed during racing season. Exercise did not affect blood glucose concentration in any group except at the beginning of training (sampling session II), where horses from HMB-supplemented group had elevated postexercise (E) glucose level (Table 3, $P < .05$). Thirty minutes after exercise, the glucose level in the HMB-supplemented group was still significantly higher than in GO + HMB group ($P < .05$). During the training season, exercise resulted in a significant increase in glucose level only in HMB-supplemented group ($P < .05$). Exercise also resulted in a significant increase in blood LA concentration in all groups (Fig. 3) during the beginning of training ($P < .05$), except in the HMB-supplemented group. As the training season progressed, the exercise-related increase in LA became significant in the HMB-supplemented group ($P < .05$). In all horses, 30 minutes after exercise, blood LA was only slightly elevated when compared with the corresponding pre-exercise values. During the training season (sampling sessions III, IV, and V), the postexercise (E) increase in blood LA was significantly lower in GO, HMB, and GO + HMB groups when compared with the placebo group ($P < .05$). Exercise sessions performed at the beginning of training, after 4 weeks of dietary supplementation, induced an increase in serum CK activity in all groups. However, only in the GO+HMB supplemented group was the increase significantly different 30 minutes following the end of exercise ($P < .05$). During the training season, a significant exercise-related increase in CK was

Table 2

Hematocrit (%) determined in jugular venous blood of 22 horses at the beginning of training (sampling session II; $n = 22$), during the training season (sampling sessions III, IV, and V; $n = 66$), and during the racing season (sampling session VI; $n = 22$) at rest before (R), immediately after exercise test (E), and 30 minutes later ('30); for 16 weeks, horses were treated with an oral placebo ($n = 6$), GO ($n = 5$), HMB ($n = 5$), and GO + HMB ($n = 6$)

Hematocrit (%)			Placebo	GO	HMB	GO + HMB	Group Effect	Exercise-Time Effect (o, p, r)	Time Period Effect
Nontraining period	Beginning of supplementation	R	41.15 ± 1.92	41.58 ± 2.90	40.55 ± 3.18	42.20 ± 7.00	n.s.	—	n.s.
Training period	Beginning of training	R	42.36 ± 4.38 _o	42.05 ± 3.89 _o	43.93 ± 4.11 _o	44.45 ± 5.12 _o	n.s.	*	n.s.
		E	54.55 ± 7.57 _p	54.62 ± 6.13 _p	58.15 ± 6.31 _p	55.07 ± 5.76 _p	n.s.	*	n.s.
		'30	42.98 ± 2.74 _o	44.60 ± 4.55 _o	46.13 ± 5.61 _o	45.73 ± 4.56 _o	n.s.	*	n.s.
	Training season	R	42.06 ± 2.74 _o	40.45 ± 3.09 _o	42.36 ± 2.99 _o	42.58 ± 4.63 _o	n.s.	*	n.s.
		E	58.03 ± 6.31 _p	55.28 ± 5.23 _p	57.01 ± 6.06 _p	56.22 ± 5.40 _p	n.s.	*	n.s.
		'30	46.26 ± 4.63 _r	44.93 ± 3.26 _r	45.12 ± 4.60 _o	46.34 ± 4.62 _o	n.s.	*	n.s.
	Racing season	R	45.38 ± 9.75	43.65 ± 2.30 _o	45.30 ± 6.75 _o	44.00 ± 3.96 _o	n.s.	*	n.s.
		E	51.55 ± 11.57	54.93 ± 6.55 _p	55.65 ± 3.51 _p	58.32 ± 7.02 _p	n.s.	*	n.s.
		'30	46.63 ± 4.13	46.27 ± 3.18 _{op}	46.75 ± 4.83 _{op}	49.68 ± 5.38 _o	n.s.	*	n.s.

GO, γ -oryzanol; HMB, β -hydroxy- β -methylbutyrate.

Data are shown as means ± SEM.

For general comparison, *P* values of group supplements or time effect are indicated in separate columns. Differences between the placebo and experimental treatment groups receiving supplements are significant ($P \leq .05$) if the first superscript is different (a, b, or c for within-line comparisons); differences between sampling time (R, E, '30) during each exercise session are significant ($P \leq .05$) if the first superscript is different (o, p, or r for within column comparisons); differences between training periods (beginning of training, training, and racing seasons) are significant ($P \leq .05$) if the first superscript is different (x, y, or z for within column comparisons).

* Significant for $P \leq .05$; n.s., nonsignificant.

observed in the placebo, GO, and GO + HMB groups ($P < .05$), but not in the HMB-treated horses. The placebo-supplemented group had the largest postexercise increase, 45% above pre-exercise values (Fig. 4, $P < .05$), which was also significantly higher than the HMB- and GO-supplemented groups 30 minutes after exercise ($P < .05$). Additionally, during the racing season (sampling session VI), CK activity remained significantly greater than that of the placebo-supplemented group 30 minutes after exercise ($P < .05$).

Exercise did not significantly affect serum AST activity in any treatment group; however, placebo-supplemented horses tended to have increased postexercise AST activity during the beginning of training as well as during a training season (Fig. 5). During the training season, horses supplemented with both GO and HMB had significantly lower AST

activity 30 minutes after exercise than the placebo-supplemented horses (sampling sessions III, IV, and V, Fig. 5). During the racing season, serum AST activity 30 minutes after exercise was significantly higher in horses fed GO when compared with the same horses during the training season ($P < .05$).

4. Discussion

The present study was the first study to determine whether dietary supplementation with either HMB or GO alone, or in combination, would affect indirect markers of muscle damage and fatigue in Thoroughbred horses during 16 weeks of training in preparation for the racing season. Currently, little data are available on the effects of these supplements in horses, with only one study describing the

Table 3

Glucose (mmol/L) determined in jugular venous blood of 22 horses at the beginning of training (sampling session II; $n = 22$), during the training season (sampling sessions III, IV, and V; $n = 66$), and during the racing season (sampling session VI; $n = 22$) at rest before (R), immediately after exercise test (E), as well as 30 minutes later ('30); for 16 weeks, horses were treated with an oral placebo ($n = 6$), GO ($n = 5$), HMB ($n = 5$), and GO + HMB ($n = 6$)

Glucose (mmol/L)			Placebo	GO	HMB	GO + HMB	Group Effect (a, b)	Exercise Time Effect (o, p)	Time Period Effect
Nontraining period	Beginning of supplementation	R	4.90 ± 0.50	5.17 ± 0.67	5.05 ± 0.72	4.92 ± 0.48	n.s.	—	n.s.
Training period	Beginning of training	R	4.83 ± 0.56	4.80 ± 0.62	4.67 ± 0.28	4.57 ± 0.51	n.s.	n.s.	n.s.
		E	4.93 ± 0.49	4.57 ± 1.08	5.33 ± 0.98	4.67 ± 1.00	n.s.	n.s.	n.s.
		'30	4.75 ± 0.34 ^{ab}	4.55 ± 0.31 ^{ab}	5.00 ± 0.46 ^a	4.43 ± 0.24 ^b	*	n.s.	n.s.
	Training season	R	4.70 ± 0.39	4.67 ± 0.54	4.72 ± 0.36 _o	4.66 ± 0.47	n.s.	*	n.s.
		E	5.04 ± 0.62	4.99 ± 0.73	5.38 ± 1.01 _p	4.80 ± 0.98	n.s.	*	n.s.
		'30	4.76 ± 0.64	4.53 ± 0.56	4.66 ± 0.66 _o	4.32 ± 0.32	n.s.	*	n.s.
	Racing season	R	5.15 ± 0.48	5.12 ± 0.32	4.71 ± 0.51 _{op}	4.71 ± 0.39	n.s.	*	n.s.
		E	5.36 ± 0.52	5.79 ± 0.31	5.78 ± 0.81 _o	5.07 ± 0.89	n.s.	*	n.s.
		'30	4.99 ± 0.87	4.91 ± 0.48	4.27 ± 0.38 _p	4.45 ± 0.21	n.s.	*	n.s.

Data are shown as means ± SEM.

For general comparison, *P* values of group supplements or time effect are indicated in separate columns. Differences between the placebo and experimental treatment groups receiving supplements are significant ($P \leq .05$) if the first superscript is different (a, b, or c for within-line comparisons); differences between sampling time (R, E, '30) during each exercise session are significant ($P \leq .05$) if the first superscript is different (o, p, or r for within column comparisons); differences between training periods (beginning of training, training, and racing seasons) are significant ($P \leq .05$) if the first superscript is different (x, y, or z for within column comparisons).

* Significant for $P \leq .05$; n.s., nonsignificant.

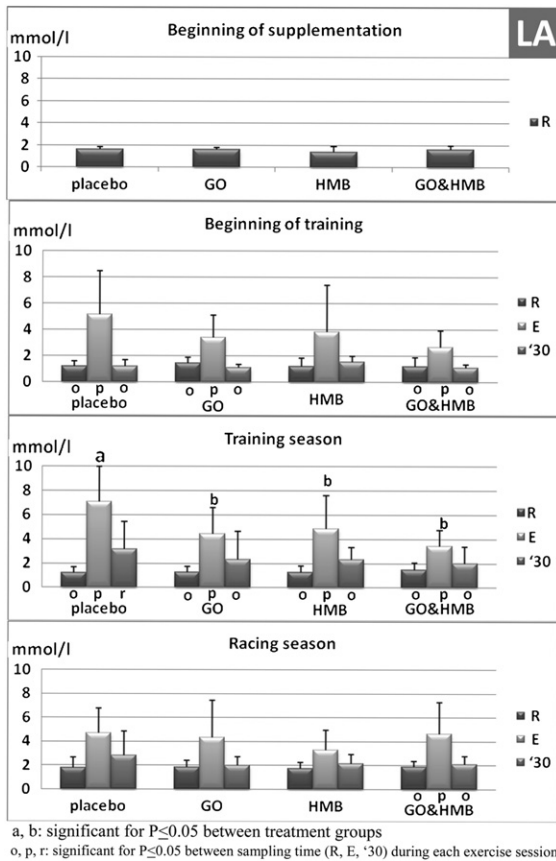


Fig. 3. Blood lactate (mmol/L) in 22 horses at the beginning of training (sampling session II; $n = 22$), during the training season (sampling sessions III, IV, and V; $n = 66$), and during the racing season (sampling session VI; $n = 22$) at rest before (R), immediately after exercise test (E), and 30 minutes later ('30). For 16 weeks, the horses were treated with an oral placebo ($n = 6$), GO ($n = 5$), HMB ($n = 5$), or GO + HMB ($n = 6$).

effect of HMB on the physiological response to exercise in horses. Miller et al. [13] fed horses an alfalfa-based supplement twice daily containing a daily dosage of 10 g of CaHMB. In our study, we administered 15 g of CaHMB daily, split between the morning and evening rations. This dosage provided ~ 30 mg CaHMB/kg $bw d^{-1}$, as the horses weighed approximately 500 kg, and is closer to the recommended dosage of 38 mg/kg $bw d^{-1}$ for humans [1]. Because there were no data concerning the daily requirement for GO in horses, we decided to use 3 g of GO administered once daily. This was similar to the 500-mg GO/d dosage previously administered to humans [15].

4.1. Supplements Versus Placebo at Rest and After Exercise

The theory of intense training is that a single exercise session will lead to fatigue and cellular damage, which in turn results in a short-term adaptive response [16]. When exercise is performed regularly, and the training stimulus is increased gradually, the adaptation that occurs during the recovery period of a single exercise session leads to an overall improvement in performance. When training is too vigorous and/or rest periods between training sessions are too short, performance is reduced because of an imbalance

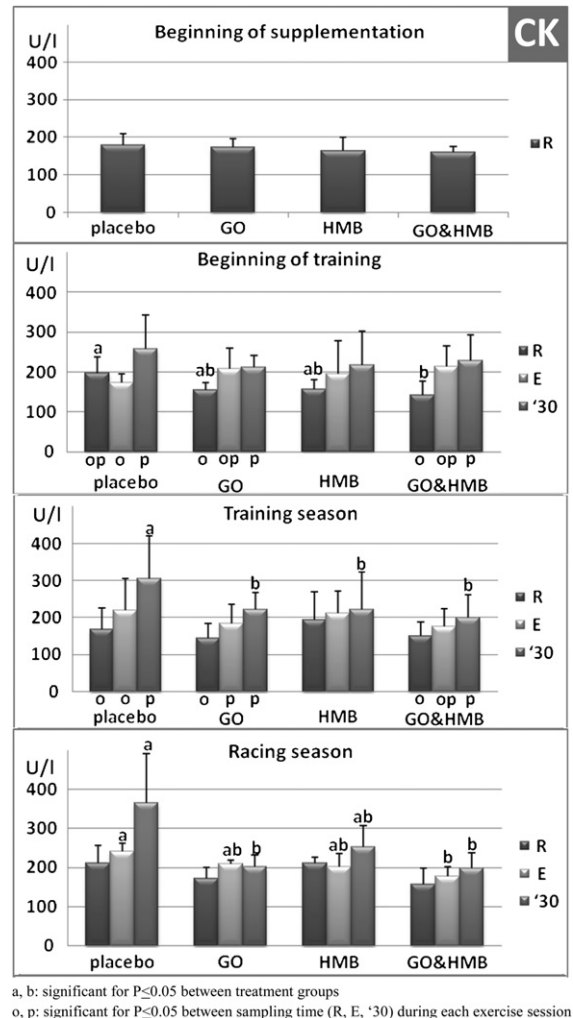
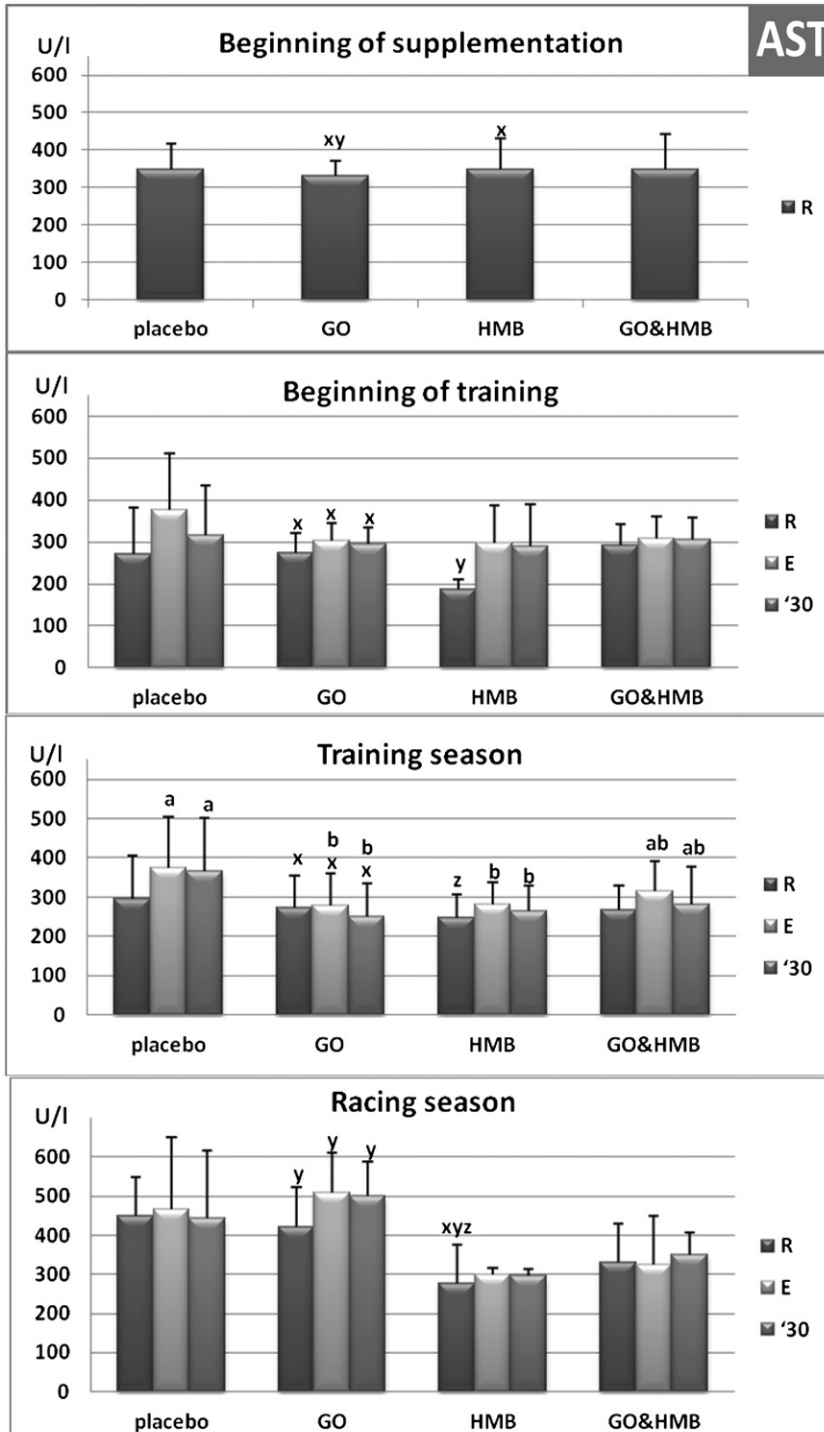


Fig. 4. Serum creatine phosphokinase (CK) activity in 22 horses at the beginning of training (sampling session II; $n = 22$), during the training season (sampling sessions III, IV, and V; $n = 66$), and during the racing season (sampling session VI; $n = 22$) at rest before (R), immediately after exercise test (E), and 30 minutes later ('30). For 16 weeks, the horses were treated with an oral placebo ($n = 6$), GO ($n = 5$), HMB ($n = 5$), or GO + HMB ($n = 6$).

between training stress and recovery. If the next training session is applied without sufficient time for recovery, decreases in the performance occur in the form of earlier onset of fatigue within each session [16,17]. In our study, the total length of the training program before competition was 16 weeks. This was longer than the 4–6 weeks recommended for horses aged >3 years [16], but we wanted to achieve a moderate pace in improvement in performance. The horses were exercised 6 d/wk, with the intensity of training increasing as the study progressed. The data indicated that our methods used for training the Thoroughbreds under field conditions were satisfactory for maximization of physiological adaptations within the animal's body. HRs at rest ranged from 32 to 37 beats/min and were similar to the values obtained by Harris et al. [18]. Peak HRs during cantering/galloping were approximately 200 beats/min and remained the same during both the



a, b: significant for $P \leq 0.05$ between treatment groups
 x, y, z: significant for $P \leq 0.05$ between training periods

Fig. 5. Serum aspartate aminotransferase (AST) activity in 22 horses at the beginning of training (sampling session II; $n = 22$), during the training season (sampling sessions III, IV, and V; $n = 66$), and during the racing season (sampling session VI; $n = 22$) at rest before (R), immediately after exercise test (E), and 30 minutes later ('30). For 16 weeks, the horses were treated with an oral placebo ($n = 6$), GO ($n = 5$), HMB ($n = 5$), or GO + HMB ($n = 6$).

training and the racing seasons. At this value, most horses are close to the point of onset of blood LA accumulation, and it is suggested as a reference point for comparison of cardiovascular capacity [19,20]. This sustained peak HR was

accompanied by an increase in maximal speed, which in turn indicates a physiological adaptation to the exercise. Because the horses in the present study were in a familiar environment and were handled and ridden by the regular

full-time staff, the risk of excitement influencing the HR was minimized.

In our experiment, increases in LA concentrations were evident after all training sessions. Thus, the intensity of exercise during training sessions in our study was high and may be compared with the values reported in the literature [20,21]. This observation was confirmed by changes in Hct levels, which reflect mobilization of splenic erythrocytes. Hct levels increased significantly after all training sessions and did not differ between horses from either the placebo or experimental groups. The extent of the increase in Hct values depends on exercise intensity and has a linear relation to the speed of the exercise, up to a maximum of 60%-65% [22]. Our study saw similar significant exercise-related increases in Hct levels.

Changes in blood glucose concentration are also dependent on the intensity of exercise. Although plasma glucose concentration decreases during prolonged exercise (>3 hours), studies with short intense exercise have shown both decreases and increases, depending on exercise intensity and training and feeding status of the horse [23]. The exercise-associated increase in hepatic glucose output is mainly mediated through a decrease in the insulin:glucagon ratio, whereas the rate of uptake and utilization in exercising muscle is restrained by increases in circulating epinephrine resulting in an elevation in blood glucose concentration [24]. In our study, postexercise blood glucose concentration remained within reference ranges in horses; however, HMB supplementation significantly increased blood glucose concentration during the training and racing seasons. This may suggest a glucose-sparing effect of HMB. Anaerobic energy production is essential for the production of muscular tension when the demand for energy is greater than can be provided aerobically, or when oxygen is in short supply. The largest source of anaerobic energy during the intensive exercise of short duration is from the glycolytic pathway. Although the yield of adenosine-5'-triphosphate from 1 mole of degraded glucose is only 2-3 moles, muscle has a high glycolytic capacity, and the two end products of the glycolytic reactions, pyruvate and hydrogen, combine to form LA. After cessation of exercise, the rate of oxygen consumption remains elevated, and blood LA concentration continues to increase [23]. There is a point where LA efflux mechanisms are probably saturated and rapid accumulation of intracellular LA leads to muscular acidosis [25]. Further high-intensity contractions cause loss of intracellular K with the accumulation of extracellular K⁺, which is associated with muscle fatigue. Therefore, LA itself cannot be considered as an indicator of fatigue, as has been stated in some earlier studies [26]. LA, however, contributes to fatigue by increasing muscular acidosis. Usually, the highest LA concentrations are seen 2-10 minutes after exercise. Blood LA concentration has been used as an indicator of training intensity and performance [27]. Blood LA assessment in the athletic horse is necessary to evaluate the onset of LA accumulation as an indicator of the aerobic capacity [28] and is useful for assessing fitness in equine athletes [29]. In equines, LA may be stored in red blood cells [30], and so measuring LA in the whole blood is necessary for determining total LA production. We used the Accusport technique to determine plasma LA, which was then converted to whole blood LA

using an internal conversion factor. The present study showed a significant increase in blood LA at the beginning of exercise in all treatment groups except the groups supplemented with HMB. Throughout the training, post-exercise blood LA remained lower in GO, HMB, and GO + HMB groups when compared with the placebo group, and continued to be lower during racing season. These data support the concept of a protective role of HMB and/or GO in counteracting the accumulation of LA; however, from the present study, it is impossible to tell whether this was the result of a concomitant reduction of intramuscular acidosis or a shortage of fuel, especially depletion of glycogen stores. In fact, 30 minutes after exercise, blood LA concentrations returned to near pre-exercise values in all horses indicating fast LA removal from blood.

CK is a muscle-specific enzyme with a relatively short half-life in serum, that is, ~2 hours. Increased serum CK activity is used as an indicator of muscle damage or injury. Resting plasma level in horses should not exceed 200 U/L. The CK activity increases and decreases through larger ranges earlier at the beginning of training season, and tightens into a narrower range when the horse is attending good fitness levels required for racing. Several studies have shown a direct relationship between the levels of HMB achieved after supplementation and improved nitrogen retention. In humans, Nissen et al. [8] demonstrated a dose-dependent response to oral administration of HMB given at either 1.5 or 3 g/d, with the higher dosage resulting in a greater decrease in serum CK. Nissen et al. also observed a decrease in serum 3-methylhistidine, an indicator of muscle protein breakdown. Dietary supplementation of 3.0 g HMB/d in individuals undergoing intense endurance exercise resulted in decreased CK and LA dehydrogenase after a prolonged 20-km run [9]. In our studies, 15 g of CaHMB/d was administered to horses and resulted in a significant attenuation of the exercise-related increase of plasma CK, an indicator of an alteration in membrane permeability. Taken together, the aforementioned studies indicate a benefit of having more HMB available to the muscles during intense training in horses.

AST has a much longer half-life than CK, approximately 1 week, and therefore reflects muscle changes over several days or even weeks. The activity of AST in horses is much higher than in other animals; AST is less specific for muscle than for CK, as AST is found in many tissues and organs. Muscle use affects AST level, and an increase in plasma AST activity has been observed in response to exercise [31]. This increase is related either to overt damage or to a change in the muscle fiber membrane, causing a transient increase in permeability. Increased AST has been shown to occur without any tissue destruction. Moreover, working horses have approximately 60% higher AST activity compared with horses that are at rest for several days [32]. At the beginning of our study, resting serum AST activity was typical for race horses (300-360 U/L). Four weeks of dietary supplementation resulted in a significant decrease of resting AST activity in horses receiving HMB. We observed trends for a postexercise increase in AST activity only in horses receiving the placebo supplement, which may indicate exercise-associated minor muscle damage in that group. In contrast, a significant decrease in resting AST activity in horses fed HMB may suggest the potential effectiveness of

this leucine metabolite to reduce muscle damage or protein breakdown.

In our study, GO and HMB administered either together or separately did not counteract the postexercise increase in plasma CK activity during the training season. This increase was, however, significantly less than in exercised horses from placebo group. Similar differences were observed during the racing season. Therefore, both GO and HMB may attenuate muscle CK leakage; however, their mechanism of action may be different. Oxidative stress is a detrimental imbalance in the oxidative–antioxidative system in cells and may damage DNA and cell membranes, particularly in muscles during strenuous exercise. Increased oxidation during exercise may be related to muscle enzyme leakage and microtrauma, hydration status, and animal welfare. Therefore, the use of GO as a potent antioxidant for human athletes as well as for horses is widespread. So far, two studies in horses have failed to demonstrate any reduction in CK after supplementation with the antioxidants vitamin C [33] and α -tocopherol [34]. Similarly, Piercy et al. [35] found no attenuation in the CK increase in exercising sled dogs as a result of feeding an antioxidant supplement containing vitamins C, E, and β -carotene. The results of the present study would be the first to indicate that GO, a powerful inhibitor of reactive oxygen species formation, may reinforce cell membrane and decrease its permeability with less CK released into the plasma. The results of the present study would seem to be in agreement with our observations that horses receiving GO had significantly lower postexercise total antioxidant status and thiobarbituric acid reactive substance level than horses from other groups (unpublished data).

In contrast to GO, HMB is not considered as an antioxidant but attenuates the loss of muscle mass and function in various conditions such as resistance exercise training, cancer, and AIDS [3,4,8]. It is also a substrate for cell membrane cholesterol synthesis, and therefore HMB may help stabilize cell membranes during intense exercise. Based on our present study, we are unable to say whether GO and HMB administered together had any additive effect. They both act through different mechanisms; however, average number of starts per horse during the racing season amounted 6.8, 4.3, 6.4, and 8.3 for placebo, GO, HMB, and GO + HMB treatments, respectively. These data indicate that HMB/GO-supplemented horses maintained better condition during the racing season amounted and had an overall 45% win rate compared with 24% for HMB, 16% for GO, and 25% for placebo groups. Thus, horses receiving both GO and HMB dietary supplements performed better during the racing season.

A significant increase in AST activity was observed only in GO-supplemented horses during the racing season compared with training season. In contrast, during training and racing seasons, AST activity remained the same in the HMB and GO + HMB-supplemented groups. AST is less specific for muscle, and its increase may indicate not only muscle damage but also hepatocyte damage. If GO had an adverse effect on the liver, our study showed that HMB may prevent that adverse side effect. Moreover, only HMB treatment resulted in a postexercise increase in blood glucose level, indicating glucose-sparing effect and possible gluconeogenesis in the liver.

Recently, Kreider et al. [36], in the official position paper of the International Society of Sports Nutrition, rated HMB in the second highest category of possible effective supplements for muscle building and performance enhancement in human athletes. Although HMB has gained the reputation of an effective dietary supplement for training humans, more studies are needed to confirm the effectiveness of its supplementation in sport horses. In the same paper, based on available literature, GO was classified as an apparently not effective dietary supplement.

5. Conclusion

This field study, performed on 22 trained Thoroughbred horses, is the first showing that dietary supplementation with GO and HMB may significantly improve training results by decreasing muscle damage caused as a result of the intensity of the training. The current study has shown that GO does not significantly affect performance-related physiological parameters in training Thoroughbred race horses; however, when GO is supplemented with HMB, the training results in increased performance outcomes, such as decreased muscle damage and improved recovery. Further studies investigating the effects of ergogenic supplements in training and racing horses should be conducted to validate the use of these ergogenic supplements in horses.

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