TÜBİTAK

Research Article

Turk. J. Vet. Anim. Sci.
2010; 34(4): 365-372
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doi:10.3906/vet-0808-28

Effect of extruded fodder on biochemical and haematological parameters of Standardbred horses under training conditions

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Received: 19.08.2008

Abstract: The aim of this study was to examine whether feeding of extruded fodder can improve the energy metabolism of horses under training conditions. The experiment was performed on 12 clinically healthy Standardbred horses (6 horses in the control group and 6 horses in the experimental group). The diet of the horses was based on oats, barley, meadow hay, and a supplement of vitamins and minerals. The diet of the experimental group (6 horses) was supplemented with 1.25 kg of extruded fodder that replaced the corresponding dose of grain. Feeding doses were related to the rate of sub-maximal workload. Selected biochemical parameters of energy and protein metabolism, electrolytes, and haematological parameters were monitored at rest and 5 min after exercise at the beginning and at the end of the 10-week experimental period. The 10 weeks of the training period resulted in a significant decrease in serum glucose concentration, serum total proteins, and plasma creatinine concentration, and a significant increase in plasma urea concentration and haemoglobin in both groups of horses at rest. Post-exercise a significant increase in blood urea levels and a decrease in total proteins in both groups demonstrated that the proteins became sources of energy. A significant increase in the haemoglobin level together with a significant decrease in the creatinine level in the horses of both groups at the end of the experiment indicated an increased aerobic metabolism. Nevertheless, extruded fodder in the horses' diet did not have the anticipated effect.

Key words: Horse, extruded feed, exercise, energy metabolism

Introduction

Nutritional supplements are one of the factors that can improve performance in horses. Energy supplied to the horse via its diet determines its capacity to do work (1). The main sources of energy are non-structural carbohydrates and fats. After a long-term sub-maximal load, an insufficient store of muscle glycogen, in combination with other factors such as increased muscle temperature, neuromuscular exhaustion, and disturbed electrolyte metabolism, results in fatigue (2). The level of muscle energy stores is determined by the concentration of lactic acid in the blood. An increase in the peripheral lactate concentration and a decrease in glycaemia is a

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warning sign of exhaustion in horses, and this is accompanied by the depletion of muscle glycogen (3). Moreover, training increases the oxidative metabolism potential of skeletal muscles and consequently induces a decrease in lactate production during sub-maximal exercise (4). However, in tired horses, during sub-maximal load the lactate accumulation is low and does not exceed the anaerobic threshold (2). Even though carbohydrates and fats are the most important sources of energy, proteins play an increasingly recognised role. In addition, 3%-15% of the total energy reserve is supplied by amino acid catabolism, particularly branched chain amino acid (5). The carbon skeletons are utilised as a source of energy, while amino groups have to be transported from the muscles and used for the synthesis of non-essential amino acids or be excreted in the form of urea (6). Utilisation of proteins as a source of energy leads to an increase in blood urea level depending on workload intensity (7,8).

Extrusion as a specific technology of grain fodder based on the gelatinisation of starch can improve the utilisation of food energy sources (9). The gelatinisation enhances the ability of starch to absorb large amounts of water and thereby improves digestibility of the starch. It also accelerates hydrolytic splitting of starch into simple saccharides by enzymes (10). Feeding of extruded fodder can improve the quality of meat of beef (11), condition score, weight and heart girth in mares (12), and the fitness of older horses (13).

The aim of the present study was to evaluate the effects of extruded fodder on the energy metabolism of horses under training conditions.

Materials and methods

Animals. The experiment was performed on 12 clinically healthy Standardbred horses (10 male, 2 female, age 4-8 years, weight 500 ± 30 kg). Each of these horses had been in a regular training programme prior to the study.

Experimental Design. The diet of the horses was based on oats, barley, meadow hay, and a supplement of vitamins and minerals (NutriHorse Sport – complete feeding additive of biologically active substances for Standardbred and Thoroughbred horses, Biofaktory Praha, s.r.o.). The diet of the experimental group was supplemented with 1.25 kg of extruded fodder (Supplementary extruded fodder – NutriHorse Universal and NutriHorse Energet for Standardbred and Thoroughbred horses, 60% of starch gelatinisation, Biofaktory Praha, s.r.o.), which replaced the corresponding dose of grain (Tables 1 and 2). The horses were fed 3 times per day, while NutriHorse Sport with NutriHorse Universal and NutriHorse Energet were added in the morning and evening dose, respectively. The horses had free access to water and salt blocks (the salt blocks were weighed before and after the experiment and the average was determined). One month before the start of the experiment, all the horses were fed with the control group diet. After this time, 6 horses from the control group continued with this diet and the diet of 6 horses of the experimental group was supplemented with extruded fodder (Tables 1 and 2). Feeding doses were related to the rate of sub-maximal workload. An examination of the diet compounds was performed by an accredited laboratory (Biofaktory s r.o., Czech Republic). The first measurement was made on quietly standing horses on the first day of the study at rest (start/rest) and after 5 min of exercise (start/exercise). The second measurement was taken after 70 days of supplementation with extruded fodder (end/rest) and 5 days after exercise (end/exercise). The exercise began after the taking of blood samples and took place in a covered riding hall with 5 hurdles. The exercise started with a walk at pace of 2 m/s for 2 min. Then the horses jumped the hurdles for 16 min without a rider at a constant speed of 7 m/s, and finally the horses walked at a pace of 2 m/s for 2 min. Blood samples were taken from v. jugularis 1.5 h after the morning feeding. The samples were collected in polyethylene tubes (Vacutainer system) and were immediately processed in the laboratory. The biochemical parameters of energy metabolism (glucose, lactate dehydrogenase, and hydroxybutyrate dehydrogenase) and the parameters of protein metabolism (serum total proteins, albumin, creatinine, urea, and uric acid) were determined by commercial kits (Roche, Switzerland) on an automatic analyser Hitachi 91 (Roche, Switzerland). Lactate concentrations were measured with a Clark electrode by an ECU analyser (VEB MLV Prüfgeräte–Werk, Germany). Ions were tested by ion-selective

electrodes. The haematological parameters were determined by Celltac MEK- 5206K (Nihon Kohden, Japan).

Data Analysis. The data were first subjected to repeated measures, split-plot design analysis of variance using the SAS statistical software package (SAS V91, SAS Institute Inc.) and treatment alignments were done using the least squares significant difference method. For all the statistical analyses, the level of significance was set at $P < 0.05$, and the data were presented as means ± SE.

Results

Parameters of energetic metabolism. The resting values of glycaemia did not differ either between the groups or at the beginning and at the end of the experiment, with the exception of the beginning values in the horses of the experimental group. The level of glycaemia was significantly lower ($P < 0.05$) in both groups after workload in comparison with the resting values at the beginning as well as at the end of the experiment. The ending values in the horses of the control group were found to be significantly lower ($P < 0.05$) in comparison with those in the horses of the experimental group (Table 3). The beginning resting values of lactate, activities of the enzymes lactate dehydrogenase (LD), and hydroxybutyrate dehydrogenase (HBD) in the control group were not significantly different from those in the experimental group of horses. The ending resting activities of LD and HBD enzymes in the control horses were significantly higher ($P < 0.05$) than the resting activities of these enzymes in the experimental group. In accordance with the increase in lactate up to the anaerobic threshold of 4 mmol L^{-1}, the activities of HBD and LD significantly increased ($P < 0.05$) in the control group in contrast to the experimental group, where the activities of HBD and LD did not differ from the resting values (Table 3).

Parameters of protein metabolism. The beginning resting values of serum total proteins (TP), albumin, creatinine, urea, and uric acid were not significantly different between the control and experimental groups. The ending resting values of TP and creatinine significantly decreased ($P < 0.05$) and values of urea significantly increased ($P < 0.05$) in both groups of horses in comparison with the start values. Post-exercise values (start and end of experiment) of TP and creatinine significantly increased ($P < 0.05$) and the concentration of blood urea increased ($P < 0.05$), but not significantly, in comparison to corresponding values. The post-exercise concentrations of uric acid significantly increased ($P < 0.05$) in horses of the experimental group at the beginning and in horses of the control group at the end of the experiment (Table 4).

Electrolytes. The resting concentrations of natrium ions were significantly lower ($P < 0.05$) and calium ions significantly higher ($P < 0.05$) in the horses of experimental group at the start of the experiment. The resting concentrations of chloride ions were not significantly different between the control and experimental groups either at the start or at the end of the experiment. Post-exercise concentrations of Na^+ and Cl^- were higher in comparison to the rest values at the beginning and ending values but were significantly higher ($P < 0.05$) only in the horses of the experimental group at the beginning. Concentrations of K^+ after workload were significantly higher ($P < 0.05$) in the horses of all groups at the beginning and end of the experiment except the beginning values of the horses of the experimental group, because their resting values at the start were significantly higher ($P < 0.05$) than K^+ concentration in the control group (Table 5).

Haematogical parameters. To determine the level of the workload, the haematological parameters were monitored. The resting values of the number of erythrocytes, haematocrit, and haemoglobin were not significantly different in the control and experimental groups at the beginning of the experiment. Significant changes in the resting haematological parameters were recognised only in haemoglobin at the end of the experiment, which significantly increased ($P < 0.05$) in the horses of both groups compared to the concentrations at the beginning of the study. After the workload a significant increase ($P < 0.05$) in the number of erythrocytes and PCV (packed cells volume) in both groups of horses, as well as in the concentration of haemoglobin in the control group, was found at the end of the experiment (Table 6).

Table 1. Composition of feeding doses for the control and the experimental group of horses weighing 550 kg.

Group	Feed Ingredients (kg)						
	Hay	Oats	Barley	Nutri Horse Sport	Nutri Horse Universal	Nutri Horse Energet	Salt block
Control	8.0	2.8	1.45	0.068	-	-	0.080
Experimental	8.0	2.0	1.0	0.068	0.25	1.0	0.080

Table 2. Composition of the nutrients in the control and the experimental groups of horses weighing 550 kg.

Nutrients	Control group	Experimental group	NRC
Dry matter (g kg^{-1})	10679.8	10704.8	12195.0
DE (MJ kg^{-1})	113.9	115.0	110.7
N - substances (g kg^{-1})	1169.5	1185.1	1059.6
Lysine (g kg^{-1})	44.2	50.8	37.1
Methionine (g kg^{-1})	21.4	23.4	10.0
Fibre (g kg^{-1})	3002,3	2959.5	2195.1
Ca (g kg^{-1})	32.8	42.3	39.0
P (g kg^{-1})	34.5	35.2	26.0
Na (g kg^{-1})	27.7	31.5	36.6
Mg (g kg^{-1})	13.8	20.4	13.0
Fe (mg kg^{-1})	1829.4	1982.4	
Cu (mg kg^{-1})	152.6	166.4	182.9
Vitamin A (U.I.) × 10^3	107.4	121.6	73.2
Vitamin D (U.I.) × 10^3	8.6	9.1	7.3
Vitamin E (mg kg^{-1})	1566.6	1624.1	1355.0

NRC – Nutrient Requirements of Horses (14)

Discussion

There were no differences in the resting concentrations of all parameters measured between the control and experimental groups at the beginning of the experiment, except for the values of glycaemia, potassium, and natrium. However, these differences in concentrations ranged within the values of reference limits (15).

A statistically significant decrease in resting glycaemia at the end of the experiment was found in horses of the experimental group. Vervuert et al. (16) presented the minor effects of mechanical or thermal grain processing for the metabolic reaction. However, they observed, like in our experiments, the effect of grain processing on the glycaemia. It tended to be lower. The glycaemia level significantly decreased in horses of both groups after workload. This decrease

Table 3. Parameters of energy metabolism of the control and experimental groups in rest and after sub-maximal exercise.

	Time	n	Start		End		SE
			Control $\mu+\alpha_i$	Experiment $\mu+\alpha_i$	Control $\mu+\alpha_i$	Experiment $\mu+\alpha_i$	
Glucose (mmol L^{-1})	Rest	6	$3.63^{a,1}$	$4.55^{b,1}$	$3.77^{a,1}$	$3.57^{a,1}$	0.24
	Exercise	6	$3.33^{a,2}$	$3.78^{a,2}$	$1.38^{b,2}$	$1.77^{b,2}$	0.24
Lactate (mmol L^{-1})	Rest	6	$2.93^{a,1}$	$2.72^{a,1}$	$3.37^{a,1}$	$3.08^{a,1}$	0.24
	Exercise	6	$2.78^{a,1}$	$2.72^{a,1}$	$4.08^{b,2}$	$3.87^{b,2}$	0.24
LD (µkat L^{-1})	Rest	6	$10.60^{a,1}$	$11.80^{a,1}$	$12.68^{b,1}$	$11.98^{a,1}$	0.94
	Exercise	6	$12.18^{a,1}$	$12.13^{a,1}$	$13.75^{a,1}$	$12.42^{a,1}$	0.94
HBD (µkat L^{-1})	Rest	6	$4.23^{a,1}$	$4.73^{a,1}$	$5.03^{b,1}$	$4.80^{a,1}$	0.37
	Exercise	6	$4.72^{a,1}$	$4.87^{a,1}$	$5.47^{b,1}$	$5.02^{a,1}$	0.37

[a,b]Statistically significant differences ($P < 0.05$) in the rows between the control and experimental groups and between the same groups at the start and end of the experiment are indicated by different alphabetic superscripts.
[1,2]Statistically significant differences ($P < 0.05$) in the columns between the rest and exercise are indicated by different numerical superscripts.

Table 4. Parameters of protein metabolism of the control and experimental groups in rest and after sub-maximal exercise.

	Time	n	Start		End		SE
			Control $\mu+\alpha_i$	Experiment $\mu+\alpha_i$	Control $\mu+\alpha_i$	Experiment $\mu+\alpha_i$	
Total protein	Rest	6	$71.9^{a,1}$	$71.8^{a,1}$	$65.8^{b,1}$	$64.6^{b,1}$	1.9
g L^{-1}	Exercise	6	$78.2^{a,2}$	$74.9^{a,2}$	$70.8^{b,2}$	$68.5^{b,2}$	1.9
Albumin	Rest	6	$31.2^{a,1}$	$31.2^{a,1}$	$31.9^{a,1}$	$31.5^{a,1}$	0.58
g L^{-1}	Exercise	6	$32.7^{a,1}$	$32.8^{a,1}$	$33.5^{a,1}$	$33.8^{a,1}$	0.58
Urea	Rest	6	$5.82^{a,1}$	$5.35^{a,1}$	$6.25^{b,1}$	$6.00^{b,1}$	0.23
mmol L^{-1}	Exercise	6	$5.97^{a,1}$	$5.45^{a,1}$	$6.38^{b,1}$	$6.12^{b,1}$	0.23
Creatinine	Rest	6	$117.3^{a,1}$	$115.0^{a,1}$	$93.5^{b,1}$	$102.0^{b,1}$	4.1
μmol L^{-1}	Exercise	6	$123.3^{a,2}$	$123.3^{a,2}$	$100.0^{b,2}$	$106.2^{b,2}$	4.1
Uric acid	Rest	6	$7.00^{a,1}$	$6.67^{a,1}$	$8.00^{a,1}$	$8.00^{a,1}$	2.23
μmol L^{-1}	Exercise	6	$8.83^{a,1}$	$10.17^{a,2}$	$11.67^{a,2}$	$10.67^{a,1}$	2.23

[a,b]Statistically significant differences (P < 0.05) in the rows between the control and experimental groups and between the same groups at the start and end of the experiment are indicated by different alphabetic superscripts.
[1,2]Statistically significant differences (P < 0.05) in the columns between the rest and exercise are indicated by different numerical superscripts.

Table 5. Electrolytes in the control and experimental groups in rest and after submaximal exercise.

	Time	n	Start		End		SE
			Control $\mu+\alpha_i$	Experiment $\mu+\alpha_i$	Control $\mu+\alpha_i$	Experiment $\mu+\alpha_i$	
Na^+	Rest	6	$136.8^{a,1}$	$134.2^{b,1}$	$135.3^{a,b,1}$	$134.8^{a,b,1}$	0.7
(mmol L^{-1})	Exercise	6	$138.3^{a,1}$	$137.5^{a,2}$	$136.5^{b,1}$	$135.8^{b,1}$	0.7
K^+	Rest	6	$3.76^{a,1}$	$4.36^{b,1}$	$3.71^{a,1}$	$3.68^{a,1}$	0.15
(mmol L^{-1})	Exercise	6	$4.50^{a,2}$	$4.27^{a,1}$	$4.40^{a,2}$	$4.54^{a,2}$	0.15
Cl	Rest	6	$104.0^{a,1}$	$102.5^{a,1}$	$105.0^{a,1}$	$105.2^{a,1}$	0.6
(mmol L^{-1})	Exercise	6	$105.5^{a,2}$	$103.8^{a,2}$	$104.3^{a,1}$	$104.5^{a,1}$	0.6

[a,b]Statistically significant differences (P < 0.05) in the rows between the control and experimental groups and between the same groups at the start and end of the experiment are indicated by different alphabetic superscripts.
[1,2]Statistically significant differences (P < 0.05) in the columns between the rest and exercise are indicated by different numerical superscripts.

was accompanied by a significant increase in plasma lactate. Plasma lactate concentration is used as a diagnostic parameter for fatigue and acidosis (17). The decrease in glycaemia accompanied by an increase in lactate indicated an insufficient store of disposable energy supplied by glucose, and it could be partly associated with the onset of peripheral fatigue (2,6). The activity of the skeletal muscles is closely related to the LD level (18). A higher level of workload results in an increased level of LD (19). However, this phenomenon was not observed in our experiment. The LD increase in the experimental group of horses was not statistically significant after workload. Moreover, the levels did not exceed the reference limits 13.9 mkat L^{-1} (20).

At the end of the experiment, statistically significant decreased levels of total proteins were found in both groups of horses. A long-term

Table 6. Haematological parameters of the control and experimental groups in rest and after sub-maximal exercise.

	Time	n	Start		End		SE
			Control $\mu+\alpha_i$	Experiment $\mu+\alpha_i$	Control $\mu+\alpha_i$	Experiment $\mu+\alpha_i$	
RBC	Rest	6	$6.34^{a,1}$	$6.75^{a,1}$	$6.78^{a,1}$	$6.57^{a,1}$	0.35
T L^{-1}	Exercise	6	$8.06^{a,2}$	$8.77^{a,2}$	$8.35^{a,2}$	$8.38^{a,2}$	0.35
PCV	Rest	6	$0.34^{a,1}$	$0.35^{a,1}$	$0.33^{a,1}$	$0.29^{a,1}$	0.24
L L^{-1}	Exercise	6	$0.42^{a,2}$	$0.46^{a,2}$	$0.42^{a,2}$	$0.42^{a,2}$	0.24
Hb	Rest	6	$8.58^{a,1}$	$7.48^{a,1}$	$12.85^{b,1}$	$15.38^{b,1}$	1.15
g L^{-1}	Exercise	6	$9.57^{a,1}$	$9.58^{a,1}$	$16.00^{b,2}$	$16.25^{b,1}$	1.15

[a,b]Statistically significant differences ($P < 0.05$) in the rows between the control and experimental groups and between the same groups at the start and end of the experiment are indicated by different alphabetic superscripts.
[1,2]Statistically significant differences ($P < 0.05$) in the columns between the rest and exercise are indicated by different numerical superscripts.
RBC – number of erythrocytes
PCV – haematocrite
Hb – concentration of haemoglobin

workload resulted in an increased catabolism of amino acids (5,7,21). Even though proteins are not a nutritionally preferred source of energy in horses (1) we can conclude on the basis of serum TP values that these proteins were used as an alternative source of energy. The increase in TP also corresponds to a significantly increased level of urea at the end of the experiment, because nitrogen from the metabolised amino acids is removed as urea (7,22).

The increasing level of uric acid could be related to the exercise, because intense exercise causes an increase in the plasma antioxidant capacity, which is mainly caused in horses by the increase in the concentration of plasma uric acid as a product of oxidation by free oxygen radicals (23,24).

Immediately after workload, an increase in TP concentration was found in both horse groups at the beginning as well as at the end of the experiment. The increase in serum protein concentration immediately after workload seems to result from an acute shift of fluid from extracellular to intracellular space (25). In both groups of horses, a decrease in the creatinine level was observed, which, together with increased Hb levels, indicated an increase in oxidative metabolism (26). A training period leads to a better oxidation ability of the organism and to an increase in aerobic metabolism.

Significantly increased levels of serum potassium were found after the workload in both groups of horses at the end of the experiment. The increased permeability of membranes was probably due to significantly increased serum potassium concentration after load, but this is a standard change occurring after the end of the exercise (9,27,28). A temporary increase in plasma electrolyte level after load was also reported by Piccione et al. (25).

To determine the level of the workload, the haematological parameters were ascertained. The resting concentration of haemoglobin was significantly higher at the end of the experiment in both groups of horses. This demonstrates a better oxidation capacity of the organism (26). Even though the values of the number of erythrocytes, haematocrit, and haemoglobin increased after exercise, this increase is typical for a routine level of training (29,30).

On the basis of our experiment we can conclude that the observed changes in biochemical parameters were due to the fact that the composition of the diet did not correspond to the required workload and had

lower energy content. The energy supply was reduced in the horses of both groups, and therefore proteins were used as a source of energy. Supplementation of a part of the diet with extruded fodder (soluble carbohydrate-rich meal) with better digestibility and utilisability did not result in the anticipated positive effect on biochemical parameters in horses under training conditions. Feeding of horses before the exercise results in an increased muscle utilisation of blood-borne glucose and carbohydrate oxidation and in decreased lipid oxidation, compared with a meal of insoluble carbohydrate (alfalfa) or not feeding. Carbohydrate feedings did not produce a sparing of muscle glycogen compared with fasting.

Acknowledgement

We thank Mrs Lois Russel for editorial assistance with this manuscript. The project was supported by grant MSM 6046070901.

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