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## Soybean Oil in Horses' Diets

Fernando Queiroz de Almeida and Fernanda Nascimento de Godoi  
*Veterinary Institute, Universidade Federal Rural do Rio de Janeiro, Seropédica,  
 Rio de Janeiro,  
 Brazil*

### 1. Introduction

There is interest in the use of oils and fat in horses' diets aiming to increase energy consumption by animals with high energy requirements, provide essential fatty acids, increase the absorption of fat-soluble vitamins, reduction of the caloric increment, increase energy efficiency and reduce dust from ration avoiding upper respiratory tract diseases (Palmquist, 1988).

High-performance athletic horses are usually fed diets with high inclusion levels of grain to reach energy requirements, which can cause intestinal acidosis, gastrointestinal mucosal injury and disorders in the microbial ecosystem, causing colic and laminitis. According to Holland et al. (1996), horses fed diets with oil reduced the activity and excitability.

Horses use non-structural carbohydrates such starch, maltose and sucrose as a primary source of energy, being hydrolyzed and absorbed as glucose in the small intestine. However, intestinal amylase activity is limited in the equine species and, because of the low stomach capacity, providing large amounts of starch in the diet compromises digestion in the small intestine, increasing intake of rapidly fermentable carbohydrate in the colon-ecum, which may contribute to metabolic complications such as endotoxemia, colic and laminitis (NRC, 2007). Critical capacity for overload of hydrolysable carbohydrate digestion is approximately 0.4% of horse body weight (Potter et al., 1992).

It is known that the intake of concentrate containing high levels of fats presents some advantages in metabolic point of view and this kind of diet can reduce the risk of gastrointestinal disturbances, because the intake of fat stimulates the flow of digesta in the jejunum and ileum (Meyer et al., 1997). Oils and fats are used in horse diets to replace the hydrolysable and rapidly fermentable carbohydrates that are present in grains and cereals (Frape, 2004).

Oils and fats addition in the diets of high activities sport horses aim to reach the high energy requirements and, according to NRC (2007), the increase on performance of athletic horses fed diets containing oils is due to better the energy / weight relation, with a reduction in dry matter intake and gastrointestinal tract weight; lower metabolic heat production associated to digestion and exercise; greater physical performance resulting from a lower muscle glycogen use, best performance in short distance running energy from anaerobic glycolysis and acidemia reduction during high intensity exercise.

High fat level diets reduce the activity of lipase in adipose tissue and increase their activity in muscle, increase muscle glycogen stores, increasing the energy of the glycolytic pathway,

with fatigue delay during aerobic exercise with large duration, increase or maintain blood glucose concentration during extensive exercise and slow lactic acid accumulation during anaerobic exercise (Frape, 2004), more over improving respiratory and cardiac recovery post-exercise (Mattos et al., 2006), providing athletic horse better conditions for their performance.

## 2. Effects of oil in horse digestion

The use of oils or fats in horses' diets has been studied for a long time. Bowman et al. (1977) studied the inclusion of corn oil in horses' diets. Oils are easily digestible with the production of 9 Mcal of digestible energy per kg of dry matter, resulting in a readily available source of energy for exercise and digestibility above 90% (Kronfeld et al. 2004; Frape, 2004).

However, there are differences in the absorption of fatty acids and glycerides in the small intestine, emphasizing such factors as the fatty acid chain length- increasing the number of carbons in the fatty acid chain reduces the absorption, the number of insaturations and the presence of a larger number of insaturations in the fatty acid seem to favor its absorption, the distribution order of the fatty acid in the glycerol molecule - a saturated monoglyceride in position 2 has a higher absorption rate, as an example one may cite the free palmitic acid, whose absorption is 12%, and the same fatty acid in the two monopalmitic form would present absorption approximately 55%; animal age - younger animals have lower ability to digest fats than adults, the relationship unsaturated/saturated fatty acids (UFA / SFA) in the diet - experiments show the presence of UFA encourages the absorption of SFA, and the melting point - the digestibility is higher in fats with low melting point, such as vegetable oils, than in saturated animal fat (Meyer, 1995).

Some authors found that high dietary energy density due to the oil addition on horses diets reduces dry matter intake. Marqueze et al. (2001) using diets with 7.8% soybean oil observed the dry matter intake of 1.66% PV, similar to that reported by Kronfeld et al. (2004), from 1.60% BW in several digestibility trials with hiperlypidemic diets. Mattos et al. (2006) observed reduction in dry matter intake in horses fed 3.1 and 6.8% soybean oil diet, 1.74 and 1.6% BW, respectively. Delobel et al. (2008) evaluating diets with 8% linseed oil in adult horses for 90 days, with dry matter intake of 1.2% BW, found that horses remained healthy throughout the period.

Godoi et al. (2009b) evaluating jumping and dressage horses consuming diets without inclusion of soybean oil (control) and with addition of 8.5% and 19.5% soybean oil found that the dietary soybean oil addition increased significantly the diet energy density, with dry matter intake, expressed as a percentage of body weight, 1.80, 1.55 and 1.26% BW, respectively, keeping constant the concentrate:forage ratio in all diets. In these horses the digesta kinetics in the gastrointestinal tract was not affected ( $P > 0.05$ ), with average values for mean retention time (MRT), rate of passage (RP) and transit time (TT) of the digesta liquid phase of 35.7 hours, 2.8% / hour and 7.6 hours, respectively (Table 1).

The inclusion of 8.5 and 19.5% of soybean oil in the diets of horses did not influence the daily fecal production based on natural matter, the water content and feces characteristics, with average values of 2.18% of BW and 71.4% moisture (Godoi et al. 2009a). Results similar to those observed in healthy horses and with varied diet, with daily fecal production from 1 to 3% of BW on natural matter and 75% moisture (Meyer, 1995). Soybean oil inclusion until 19.5% does not alter the feces characteristics (Godoi et al. 2009a).

Item	Soybean oil inclusion (%)			
	0%	8.5%	19.5%	CV (%)
Dry matter intake (Kg DM/day)	9.0 <sup>a</sup>	7.1 <sup>b</sup>	6.2 <sup>c</sup>	7.4
Dry matter intake (% BW)	1.88 <sup>a</sup>	1.55 <sup>b</sup>	1.26 <sup>c</sup>	9.8
Fecal production (Kg DM/day)	3.4 <sup>a</sup>	2.7 <sup>b</sup>	2.3 <sup>b</sup>	17.2
Fecal production - natural matter (kg/day)	13.3 <sup>a</sup>	9.3 <sup>a</sup>	8.6 <sup>a</sup>	19.6
Fecal production - dry matter (% BW)	0.71 <sup>a</sup>	0.58 <sup>ab</sup>	0.47 <sup>b</sup>	19.6
Moisture (%)	72.5 <sup>a</sup>	69.9 <sup>a</sup>	71.7 <sup>a</sup>	3.1
MRT (hours)	34.3 <sup>a</sup>	40.4 <sup>a</sup>	32.3 <sup>a</sup>	11.8
RP (%/hours)	2.9 <sup>a</sup>	2.5 <sup>a</sup>	3.1 <sup>a</sup>	12.3
TT (hours)	7.3 <sup>a</sup>	10.7 <sup>a</sup>	4.7 <sup>a</sup>	89.1
-----				
Feces characteristics				
Consistency	Standard*	Standard	Standard	
Color	Standard	Standard	Standard	
Strange particle	Absent	Absent	Absent	
Hay	Present	Present	Present	
Grains	Present	Present	Present	

\*One horse produced softer feces than others.  
Means in line followed by different letter differ by SNK test (P<0.05)

Table 1. Dry matter intake, fecal production, mean retention time (MRT), rate of passage (RP) and transit time (TT) of liquid phase of digesta and feces characteristics of horses fed diets with soybean oil

As for the dry matter digestion in the digestive tract of horses fed diets with oil should be considered that the feed management or the meals supply frequency influences the dry matter digestibility, especially due to the division of oil consumption, because it avoid lipids overloads in the small intestine and transport to the cecum-colon. In studies conducted by Kane et al. (1979), with the addition of up to 10% corn oil, Hughes et al. (1995), which added 10% of animal fat in the concentrate, and Bush et al. (2001), which included up to 15% of corn oil in the horses diets, fractionated 2x/day, no significant difference in dry matter digestibility was found. However, authors observed that soybean oil inclusion reduced the dry matter digestibility significantly when soybean oil supplied 37 to 63% of net energy of the ration (Jansen et al., 2000). Jansen et al. (2007) also found a decrease in dry matter digestibility of 82.4% in diets without the inclusion of soybean oil to 73.2% including 15% of soybean oil in the ponei's diet. However, Delobel et al. (2008), evaluating diets with 8% linseed oil inclusion in the concentrate, found a significant increase in dry matter digestibility, with values of 64.1 to 66.5%, with diet fed two times a day. Godoi et al. (2009b) fed horses with diet with 19.5% of soybean oil, diets were fractionated into five different times, roughage were offered separately of concentrate for at least three hours apart and found no effects on digestibility of dry matter (P> 0.05), whose average value was 62.6% (Table 2). This suggests management adopted to avoid the adherence of soybean oil to the hay and that this will lead to the large intestine, thus preventing digestion. These contradictory results may be related to the oil amount used and the feeding management applied in each experiment.

Another aspect to be considered is the concentrate:forage ratio from fat diets. This relationship differs among authors, ranging from diets exclusively with concentrate (Kane et al., 1979) until the ratio of 30:70 (Jansen et al., 2002). Suggesting that feed management, as well as concentrate:forage ratio, will not influence, directly, the dry matter digestibility coefficient, being necessary to consider other factors such as the amount and oil type used in each diet and horses physical activity.

Protein digestibility varies according to protein source, the ingredients and the concentrate:roughage ratio (NRC, 2007). The soybean meal protein has high digestibility, averaging 92.2% (NRC, 2007). Hughes et al. (1995) and Julen et al. (1995), evaluating dietary inclusion of animal fat and using soybean meal to balance diets, observed a significant increase in the digestibility of crude protein.

According Jansen et al. (2000), Bush et al. (2001), Kronfeld et al. (2004) and Jansen et al. (2007), the inclusion of oils or fats in diets for horses does not affect the digestibility coefficient of crude protein. However, Jansen et al. (2002) evaluating diets for adult horses, varying only the energy source, glucose, starch or soybean oil, found a decrease in crude protein digestibility. In studies by Godoi et al. (2009b), the apparent digestibility of crude protein increased in diets 8.5 and 19.5% soybean oil inclusion, in 9.8 and 12.8 percentage points compared to the control diet, respectively (Table 2). This can be explained by the inclusion of soybean meal as protein source in the balance of fat diets, probably because the protein of soybean meal have a higher digestibility than the protein source used in the commercial concentrate.

In relation to digestibility of dietary energy Kane et al. (1979) feeding horses diets with corn oil inclusion equivalent to 15 and 30% of digestible energy diet did not observe differences in energy digestibility, averaging 73.3%. Even as Bush et al. (2001) that using corn oil found no significant difference in energy digestibility. Jansen et al. (2000) found significant reduction of 7.2 percentage points in energy digestibility in diets containing soybean oil. Godoi et al. (2009b) observed that the energy digestibility was not influenced by the inclusion of soybean oil in the diet, with a small increase of 4.1 percentage points in the diet with 19.5% soybean oil inclusion, increasing energy availability for horses (Table 2).

The effects of oil inclusion on fiber digestion in the equine digestive tract are contradictory. The absence of marked effects on fiber constituent digestibility in fat diets was observed by Kane et al. (1979) that, using corn oil at levels up to 30% of digestible energy of diet observed ADF digestibility of 24.1%. Bush et al. (2001), when added up to 15% of corn oil in the concentrate, observed average digestibility of 23% NDF.

However, several authors (Hughes et al. 1995; Julen et al. 1995; Rammerstorfer et al., 1998) observed a significant increase in NDF digestibility, 7.4, 8.9 and 8.7 percentage points respectively, on a diet with 10% of animal fat inclusion in concentrate in relation to control diet for 28 days. Likewise, Delobel et al. (2008) observed a significant increase in NDF digestibility by 2.3 percentage points during the experimental period of 90 days, with a 50% concentrate:roughage ratio. This author justifies that when carbohydrate is replaced by oil or fat, there is a reduction on deleterious effects of starch fermentation on fiber digestion in the cecum-colon, which could explain the increase in NDF digestibility.

Nevertheless, there are reports of reduction in apparent digestibility of the fiber constituents in horses fed large amounts of oil in diets. Jansen et al. (2000), evaluating diets with and without addition of soybean oil equivalent to 37% of net energy in concentrate, with 70:30 and 60:40 concentrate:roughage ratio, respectively, observed a significant reduction in



digestibility of NDF, ADF and cellulose, with average values of 60.8, 50.5, 57.0 and 54.6%, 42.2, 50.2%, respectively.

Jansen et al. (2002), evaluating diets for horses with three different energy sources: starch, glucose and soybean oil, and approximately 70:30, 50:50 and 30:70 concentrate:forage ratio, respectively, observed that the digestibility of the fiber constituents in diets with starch or glucose showed no significant differences, however, the diet with soybean oil provided a significant reduction in digestibility of NDF, ADF and cellulose, 9.4, 13.3 and 16.9 percentage points when compared to other diets. Jansen et al. (2007), using the kinetics in vitro fermentation technique observed that cecum, colon and feces inoculum of horses fed diets with soybean oil inclusion had lower gas production with incubated cellulose and justified by cellulolytic microflora inhibition, because there was a reduction of  $4.1 \times 10^6$  cfu / mL to  $3.6 \times 10^6$  cfu / mL in the bacteria amount in diet with soybean oil. The amount of hay used by Jansen et al. (2000, 2002, 2007) was not similar among diets with and without soybean oil, which produced alterations in concentrate:forage ratio. In addition, the silage was fed with the concentrate and oil. This management may promoted increase of rate of passage in the small intestine carrying fats to large intestine, which could reduce microbial fermentation in the cecum-colon and fiber digestibility of fat diets.

Godoi et al. (2009b) evaluated the digestibility of fiber fractions, and observed that there was significant reduction in cellulose apparent digestibility in horses fed a diet with 19.5% soybean oil inclusion. This reduction was 18.3 and 11.1 percentage points, while the diet with large amounts of soybean oil was compared with the control diet and 8.5% inclusion of soybean oil, respectively (Table 2). Godoi et al. (2009b) maintained the relation of concentrate:forage similar in all diets and coast-cross hay was provided separately, thus reducing the possibility of carrying fats to the cecum-colon. The lack of significant effect of oil inclusion in the hemicelluloses digestibility may be due to the fact that non-ruminant herbivores digest relatively more hemicelluloses than cellulose (Van Soest, 1994).

Morgado et al. (2009) evaluated the apparent digestibility coefficient of total carbohydrates, non-fibrous carbohydrates and their hydrolysable and rapidly fermentable fractions in horses fed diet with higher levels of soybean oil inclusion observed that the higher level of soybean oil inclusion, 19.5% resulted in significant reduction in apparent digestibility of non-fibrous carbohydrates, at 26.6 percentage points. The apparent digestibility coefficient of rapidly fermentable carbohydrate showed the largest significant reduction of 94.9% in the diet without the soybean oil addition, to 53.2% for in the diet with 19.5% soybean oil inclusion. Hydrolysable carbohydrates are composed of fructans, pectins,  $\beta$ -glucans and galactans that are not digested by equine digestive enzymes, but are fermented by microorganisms in the large intestine. The lowest digestibility value of rapidly fermentable carbohydrates associated with a greater level of soybean oil inclusion may be due to the microfloral change, reducing these carbohydrates digestibility. However, there were no significant differences in neutral detergent fiber (NDF) and acid detergent fiber (ADF) digestibility, which becomes important, the fractionation of non-fibrous carbohydrates (Table 2).

The efficiency of utilization of dietary fiber in horses is related to diet composition, especially by structural carbohydrates and non-structural fractions, the rate of fermentation and rate of passage through the digestive system that is influenced by intake (Drougoul et al. 2000). Changes in forage consumption can modify the digesta rate of passage, exposing the microflora in the large intestine to a change in the amount of fermentable substrates and thus may affect the apparent digestibility of fiber constituents (Hallebeek & Beynen, 2002). The influence of associative effects on nutrients digestibility is related to ingredients quality

and quantity in diets (Palmgren Karlsson et al., 2000). The carbohydrates availability varies between different cereals types and, likewise, the fibrous components percentage varies among different forage and concentrate feeds, which may modify the fermentation in the large intestine. According to NRC (2007) the concentrate:forage ratio, ingredients, feed supply at the same time or separately, among other factors, can alter the intake and digestibility of nutrients.

Coefficient of digestibility (%)	Soybean oil inclusion (%)			CV (%)
	0%	8.5%	19.5%	
Dry matter <sup>1</sup>	62.3 <sup>a</sup>	62.6 <sup>a</sup>	62.8 <sup>a</sup>	8.0
Organic matter <sup>1</sup>	65.8 <sup>a</sup>	64.1 <sup>a</sup>	61.6 <sup>a</sup>	7.8
Crude protein <sup>1</sup>	70.5 <sup>b</sup>	80.3 <sup>a</sup>	83.3 <sup>a</sup>	6.3
Gross energy <sup>1</sup>	63.0 <sup>a</sup>	66.2 <sup>a</sup>	67.1 <sup>a</sup>	8.1
Ether extract <sup>1</sup>	71.8 <sup>b</sup>	89.7 <sup>a</sup>	91.2 <sup>a</sup>	7.7
Neutral detergent fiber <sup>1</sup>	53.9 <sup>a</sup>	48.8 <sup>a</sup>	41.2 <sup>a</sup>	13.3
Acid detergent fiber <sup>1</sup>	41.3 <sup>a</sup>	35.9 <sup>a</sup>	30.7 <sup>a</sup>	19.5
Cellulose <sup>1</sup>	50.1 <sup>a</sup>	42.9 <sup>a</sup>	31.8 <sup>b</sup>	16.8
Hemicelluloses <sup>1</sup>	63.2 <sup>a</sup>	59.2 <sup>a</sup>	51.2 <sup>a</sup>	10.6
Non-fibrous carbohydrates <sup>2</sup>	96.5 <sup>a</sup>	87.3 <sup>a</sup>	69.9 <sup>b</sup>	9.5
Hydrolysable carbohydrates <sup>2</sup>	97.7 <sup>a</sup>	97.3 <sup>a</sup>	91.9 <sup>b</sup>	2.7
Rapidly fermentable carbohydrates <sup>2</sup>	94.9 <sup>a</sup>	76.3 <sup>a</sup>	53.2 <sup>b</sup>	17.8
Total carbohydrates <sup>2</sup>	65.0 <sup>a</sup>	58.4 <sup>a</sup>	48.8 <sup>b</sup>	10.0

Means in line followed by similar letter do not differ by SNK test (P>0.05)

<sup>1</sup> Godoi et al. (2009), <sup>2</sup>Morgado et al. (2009).

Table 2. Apparent digestibility coefficient of nutrients in horses fed diet with higher levels of soybean oil inclusion

Sales & Homolka (2011) in a meta-analysis of 22 papers about use of oil in diets for horses observed no significant effects of fat supplementation in protein and NDF digestibility, that can be explained by the anatomy of the gastrointestinal tract of horses with large bowel fermentation opposed to the ruminants.

Contradictory results reported in the literature are probably related to varying levels of fat, differences in relation to the dietary ingredients, especially in relation to the NDF and ADF. In relation to the contradictions in the results of the fiber constituents digestibility observed among various authors, these may be related to inadequate oils and fats adaptation in horse, and also a short period of replacement of rapidly hydrolysable carbohydrates by oils and fat (NRC, 2007).

Horses fed diets with soybean oil increased number of erythrocytes and reduced mean corpuscular volume (Godoi et al., 2009a). Hemoglobin level of evaluated horses differed only among animals fed control diet and 8.5% soybean oil inclusion, with lower value in the control diet, 9.5 g / dL. Including 19.5% of soybean oil in the diet increased serum levels of triglycerides in the horse.

Soybean oil was found to be palatable and its use is common in compound diets or added to diets with grains in equine nutrition (Meyer & Coenen, 2002). The absence of negative changes in hematological, biochemical and feces indicates that the inclusion of soybean oil in the diets of horses can be used to reduce dry matter consumption, leading to reduced

consumption of rapidly fermentable carbohydrates and lighter digestive tract during exercise, what can improve athletic performance in horses (Godoi et al. 2009a). Zeyner et al. (2002) evaluating fed horses with inclusion of 11.5% of soybean oil in the diet during 390 days, also observed no adverse effects.

In a study with three adult horses fistulated at right dorsal colon with 300 kg body weight, soybean oil was included in different ways. Horses were distributed in randomized complete block design with five treatments and three blocks formed by animals, with each block consisting of an experimental unit. Experimental diets were composed of *coastcross* hay (*Cynodon dactylon*), commercial concentrate, soybean meal and soybean oil in a forage: concentrate ratio of 60:40 on a dry matter basis, defined as: diets without soybean oil and diet with soybean oil on the level of 10% of total diet.

Soybean oil supplied with concentrate in four different ways: 1) one time a day at 07 a.m., 2) two times a day (two equal fractions) at 7 a.m and 17 p.m., 3) three times a day (three equal fractions) at 7 a.m., 13 p.m. and 17 p.m , or 4)four times a day (four equal fractions) at 7 a.m., 13 p.m., 17 p.m. and 19 p.m. Roughage supply was always performed in two equal fractions, at 11 a.m and 21 p.m. Diets were formulated according to nutritional requirements (Table 3) for adult horses at maintenance (NRC, 2007), with daily intake of approximately 2.0% BW, on dry matter basis.

Item (%)	Nutritional composition				Diet without soybean oil	Diet with soybean oil
	Concentrate	Soybean oil <sup>1</sup>	Coastcross hay	Soybean meal		
Dry matter	89.9	99.6	87.9	88.0	88.6	89.6
Crude protein	12.5	-	7.4	46.9	9.2	8.2
Gross energy (Mcal/Kg DM)	3.5	9.4	3.8	4.2	3.8	4.3
Ether extract	3.4	100.0	1.4	2.7	2.1	12.2
NDF <sup>2</sup>	32.7	-	63.5	10.7	52.3	48.3
ADF <sup>3</sup>	16.7	-	30.3	9.6	25.4	23.4
HEM <sup>4</sup>	16.0	-	33.2	1.1	26.9	24.9
Celulose	8.8	-	23.0	6.9	21.9	16.7
NFC <sup>5</sup>	44.3	-	19.3	22.1	28.4	24.1
CHO-H <sup>6</sup>	6.3	-	1.7	1.8	3.3	2.8
CHO-RF <sup>7</sup>	38.0	-	17.6	20.3	25.1	21.3
CHO-T <sup>8</sup>	77.0	-	82.8	32.8	80.7	72.4
Control diet	40.0	-	60.0	-	-	-
Diet with soybean oil	26.9	10.4	62.1	0.6	-	-

<sup>1</sup>Rostagno (2005)  
<sup>2</sup>Neutral detergent fiber, <sup>3</sup>Acid detergent fiber, <sup>4</sup>HEM – hemicelluloses, <sup>5</sup>Non-fibrous carbohydrate;  
<sup>6</sup>Hydrolizable carbohydrates; <sup>7</sup>Rapidly fermentable carbohydrate; <sup>8</sup>Total carbohydrate

Table 3. Nutritional composition and percentage of ingredients in the diet, on a dry matter basis

Animals were previously adapted to soybean oil with inclusion gradually in the diet, during 25 days. First, the trial was conducted in four periods with 17 days, 10 days to diet adaptation, 4 days to feces collection, 1 day to blood collection and 1 day to digesta



collection at right dorsal colon. Next, animals were re-adapted to the diet with decreasing levels of soybean oil during 25 days and then proceeded another trial with a control diet, totaling 135 days. Feces were collected from each animal immediately after defecation, directly from the floor of stalls without bedding, during 24 hours over four collection days. Blood collections were performed on the 16<sup>th</sup> day of each experimental period, with the first sample collected before the morning meal, at 7:00 pm and at 30, 60, 120, 180, 240 and 300 minutes of the postprandial period.

Digesta collection from the colon was performed four hours after first meal of the day, obtaining a aliquot of about 1.5 kg of digesta per animal. A 100g digesta aliquot was immediately used for pH measurement and determination of buffer capacity (Zeyner et al., 2004). Another digesta aliquot from the colon was directed to the analyzing process. Hydrolysable carbohydrates were estimated directly, non-fibrous carbohydrates, rapidly fermentable carbohydrates and total carbohydrates were estimated (Hoffman et al., 2001).

The inclusion of 10% soybean oil, in a single or fractionated form did not affect ( $P > 0.05$ ) dry matter intake (equivalent to 1.73% BW) with fibrous fractions intake of 2.7, 1.3, 1.5 and 1.0 kg to NDF, ADF, hemicelluloses and cellulose, respectively. Average daily intake of hydrolysable carbohydrates observed in this study was 100 g / day, similar to the results observed by Hoffman et al. (2001), in diets with 11% corn oil and intake from 118 to 186 g hydrolysable carbohydrates / day. Soybean oil did not affect the nutrient intake by horses probably due to the large period of adaptation to the diet with soybean oil, and Kronfeld et al. (2004) suggested 4 to 14 days of adaptation, depending on the amount of oil in order to avoid negative effects.

Soybean oil inclusion, either on a single or fractionated did not affect ( $P > 0.05$ ) dry matter digestibility, with a mean value of 69.4%, crude protein with average coefficient of 71.6%, gross energy with an average of 73.2%, and, there was effect ( $P < 0.05$ ) of soybean oil inclusion in the ether extract digestibility coefficient, as well as in intake of 0.1 kg in control diet and 0.7 kg in fat diet, but the fractionation of soybean oil did not influence the digestibility of fat, averaging 94.7% (Table 4). Kronfeld et al. (2004) evaluating different oils and fats sources in diets for horses observed an increase in ether extract digestibility from 55 to 81% when compared to the basal diet.

Digestibility coefficient of NDF, ADF and cellulose were not affected by the inclusion of soybean oil ( $P > 0.05$ ). However, significant increase was observed in hemicelluloses digestibility when horses fed hiperlypidemic diet fractionated into one, two and three times, averaging 63.8, 66.8 and 67.0% respectively. Fractionation of soybean oil did not affect ( $P > 0.05$ ) non-fibrous, hydrolyzable and rapidly fermentable carbohydrates digestibility, with average values of 99.1, 99.0 and 99.1%, respectively. Diet without soybean oil and diet with soybean oil in four fractions, the carbohydrates digestibility was better ( $P < 0.05$ ) than diets with a fractionation of up to three times. In this study, soybean oil inclusion was done four times, to avoid high intake of hydrolysable carbohydrates that can change the microflora of large intestine of horses and consequently decreased digestibility.

According to Hoffman et al. (2001), providing large amounts of hydrolysable carbohydrates in the diets of horses undertakes its digestion in the small intestine, increasing intake of rapidly fermentable carbohydrates in the cecum and colon, and the critical capacity to overload of hydrolysable carbohydrate digestion is approximately 0.4% BW of horses.

Plasma concentrations of glucose ranged during postprandially period in the control diet ( $P < 0.05$ ), showing higher concentrations 30, 60, 120 and 180 minutes after intake. In diets with oil, even single or fractionated forms, glucose levels did not change during this period remained within the reference values (Dukes, 1996), 80 to 120 mg / dL (Table 5). It was

observed lower plasma glucose concentration 300 minutes after intake in horses fed control diet, compared to horses fed diets with soybean oil ( $P<0.05$ ).

Item (%)	Soybean oil in diets						CV (%)
	Control diet	One time	Two times	Three times	Four times	Mean (%)	
Dry matter	70.3 <sup>a</sup>	66.6 <sup>a</sup>	68.9 <sup>a</sup>	69.5 <sup>a</sup>	71.8 <sup>a</sup>	69.4	4.4
Crude protein	73.7 <sup>a</sup>	70.2 <sup>a</sup>	70.7 <sup>a</sup>	71.2 <sup>a</sup>	72.2 <sup>a</sup>	71.6	3.9
Gross energy	69.7 <sup>a</sup>	72.1 <sup>a</sup>	73.9 <sup>a</sup>	74.9 <sup>a</sup>	75.2 <sup>a</sup>	73.2	3.9
Ether extract	59.3 <sup>a</sup>	94.7 <sup>b</sup>	95.2 <sup>b</sup>	95.3 <sup>b</sup>	93.4 <sup>b</sup>	---	3.6
NDF <sup>1</sup>	67.8 <sup>a</sup>	58.4 <sup>a</sup>	61.3 <sup>a</sup>	62.6 <sup>a</sup>	66.2 <sup>a</sup>	63.3	5.5
ADF	62.8 <sup>a</sup>	52.7 <sup>a</sup>	55.5 <sup>a</sup>	57.7 <sup>a</sup>	60.4 <sup>a</sup>	57.8	8.8
Hemicelulloses	71.9 <sup>a</sup>	63.8 <sup>b</sup>	66.8 <sup>b</sup>	67.0 <sup>b</sup>	70.9 <sup>a</sup>	---	3.4
Celulose	70.8 <sup>a</sup>	61.1 <sup>a</sup>	66.2 <sup>a</sup>	67.1 <sup>a</sup>	69.9 <sup>a</sup>	67.0	5.7
NFC <sup>2</sup>	99.2 <sup>a</sup>	99.0 <sup>a</sup>	98.9 <sup>a</sup>	99.2 <sup>a</sup>	99.0 <sup>a</sup>	99.1	1.1
CHO-H <sup>3</sup>	98.9 <sup>a</sup>	99.0 <sup>a</sup>	99.1 <sup>a</sup>	99.2 <sup>a</sup>	98.7 <sup>a</sup>	99.0	1.3
CHO-RF <sup>4</sup>	99.2 <sup>a</sup>	99.0 <sup>a</sup>	99.2 <sup>a</sup>	99.0 <sup>a</sup>	98.9 <sup>a</sup>	99.1	1.3
CHO-T <sup>5</sup>	77.6 <sup>a</sup>	70.8 <sup>b</sup>	72.9 <sup>b</sup>	73.6 <sup>b</sup>	77.1 <sup>a</sup>	---	3.3

Means in the line followed by same letter do not differ with Scott Knott test ( $P>0.05$ )  
<sup>1</sup>Carbohydrates slowly fermentable (CHO-SF) represented by NDF  
<sup>2</sup>Non-fibrous carbohydrates; <sup>3</sup>Hydrolizable carbohydrates; <sup>4</sup>Rapidly fermentable carbohydrates; <sup>5</sup>Total carbohydrates

Table 4. Coefficient of digestibility of nutrients and fiber fractions in horses fed fat diet.

The increase in plasma glucose between 30 and 180 minutes after control diet intake may be related to higher hydrolysable carbohydrate concentration in this diet, leading to increased glucose absorption in the small intestine during the first 180 minutes of the postprandial period. Lower plasma glucose concentration 300 minutes after intake of control diet, compared to diets supplemented with soybean oil, even single or fractionated forms, must also be related to higher concentration of starch in this diet. So, the pronounced increase in plasma glucose after ingestion of control diet stimulated greater insulin release, increasing efficiency in the blood glucose uptake by the tissues, resulting in lower plasma glucose concentration 300 minutes postprandial. Taylor et al. (1995) and Orme et al. (1997); Marqueze et al. (2001), Mattos et al. (2006) and Godoi et al. (2009a) also observed no influence of the intake of hiperlipidemic diets on plasma levels of glucose in the horses. No differences ( $P>0.05$ ) was observed on triglyceride concentration in horses fed control diet or fed diet with soybean oil fractionated one, two and three times a day. Fractionated soybean oil inclusion into four times reduced plasma triglyceride levels 60 and 120 minutes postprandial ( $P<0.05$ ).

In several studies contrasting results were observed in triglyceride levels in horses supplemented with vegetable oils. Harking et al. (1992) evaluating diet inclusion of corn oil equivalent to 10% of digestible energy, fed twice a day and Hallebeek & Beynen (2002) evaluating diet with soybean oil inclusion of 15%, also fed twice a day, observed no changes in plasma triglycerides levels. However, Orme et al. (1997) evaluating the soybean oil in diets fed twice a day, Geelen et al. (2001) with inclusion of soybean oil 15% diet, fed twice a day and Sloet van Oldruitenborgh-Oostebaen et al. (2002) evaluating the addition of 11.8% soybean oil, observed reduction in triglyceride levels. But Godoi et al. (2009a) observed an

increase in serum triglycerides of horses fed diet with 19.5% soybean oil inclusion, twice a day compared to diets without and with 8.5% for soybean oil.

Soybean oil in diet	Postprandial (minutes)							Mean	CV (%)
	0	30	60	120	180	240	300		
Glucose (mg/ dL)									
Control	84.0 <sup>b</sup>	96,3 <sup>a</sup>	105.7 <sup>a</sup>	105.0 <sup>a</sup>	95.0 <sup>a</sup>	85.7 <sup>b</sup>	83.7 <sup>bB</sup>	-	1.6
One time	99.3	102,1	101.8	94.2	97.5	98.5	94.8 <sup>A</sup>	98.3	
Two times	98.7	100,4	102.1	105.1	106.5	106.9	113.9 <sup>A</sup>	104.8	
Three times	94.4	105,6	106.5	97.8	102.5	106.1	96.3 <sup>A</sup>	101.3	
Four times	98.0	101,7	99.0	88.3	102.0	97.3	93.7 <sup>A</sup>	97.1	
Mean	94.9	101,2	103.0	98.1	100.7	98.9	-		
CV (%)	4.4								
Triglycerides (mg/ dL)									
Control	32.0	32.0	33.3	29.7	24.3	22.3	30.0	29.1	6.0
One time	22.1	21.6	21.6	20.3	22.7	22.5	24.8	22.2	
Two times	30.2	30.0	30.0	27.2	25.9	22.3	23.1	26.9	
Three times	26.5	21.7	21.7	24.2	30.9	23.4	22.7	24.4	
Four times	25.0 <sup>a</sup>	29.3 <sup>a</sup>	19.3 <sup>b</sup>	22.0 <sup>b</sup>	24.3 <sup>a</sup>	23.3 <sup>a</sup>	35.0 <sup>a</sup>	-	
Mean	27.2	26.9	25.2	24.7	25.6	22.8	27.1		
CV (%)	19.3								

Means in line followed by same lowercase letters do not differ by the Scott Knott test (P <0.05). Means in columns followed by same uppercase letters do not differ by the Scott Knott test (P <0.05)

Table 5. Mean values of plasma glucose and triglycerides in horses fed fat diets.

According to Orme et al. (1997) the reduction in the triglycerides concentrations in fat diets are associated with increased postprandial lipoprotein lipase activity and postprandial plasma cholesterol. These authors observed a 50% increase in lipoprotein lipase activity in horses, after intake of a diet with inclusion of soybean oil. In the present study, the largest interval between meals with soybean oil, observed in the diet where the oil inclusion was fractionated into four times, may have promoted the lipoprotein lipase activity increase, leading to reduction in plasma triglycerides concentration at 60 and 120 minutes postprandial. Horses fed diets with soybean oil did not increase (P>0.05) plasma cholesterol, HDL and LDL, compared to control diet. Serum cholesterol levels remained within reference values of 75 to 150 mg / dL (Kaneko et al., 1997) (Figure 1). Absence of cholesterol concentration changes may be related to the maintenance state of horses used in this study. Orme et al. (1997) evaluated horses that were submitted to aerobic training for 10 weeks and reported cholesterol concentrations increase in horses fed diet with soybean oil. According to these authors, cholesterol concentrations increase in horses may arise as greater feed intake result or as increased cholesterol biosynthesis. Cholesterol dietary content commonly given to horses probably is minimal, since the ingredients were grains, forages, by-products of grains and vegetable oils have low cholesterol contents. Thus, the increase in cholesterol observed by Orme et al. (1997) should be result of increased endogenous synthesis of cholesterol, due to increased acetyl CoA production via triglycerides β -oxidation. Thus, the greatest energy demand needed for muscle activity

during exercise, increased the triglycerides  $\beta$ -oxidation for energy generation. Geelen et al. (2001) and Hallebeek & Beynen (2002) also observed any changes in cholesterol concentrations in horses fed fat diets.

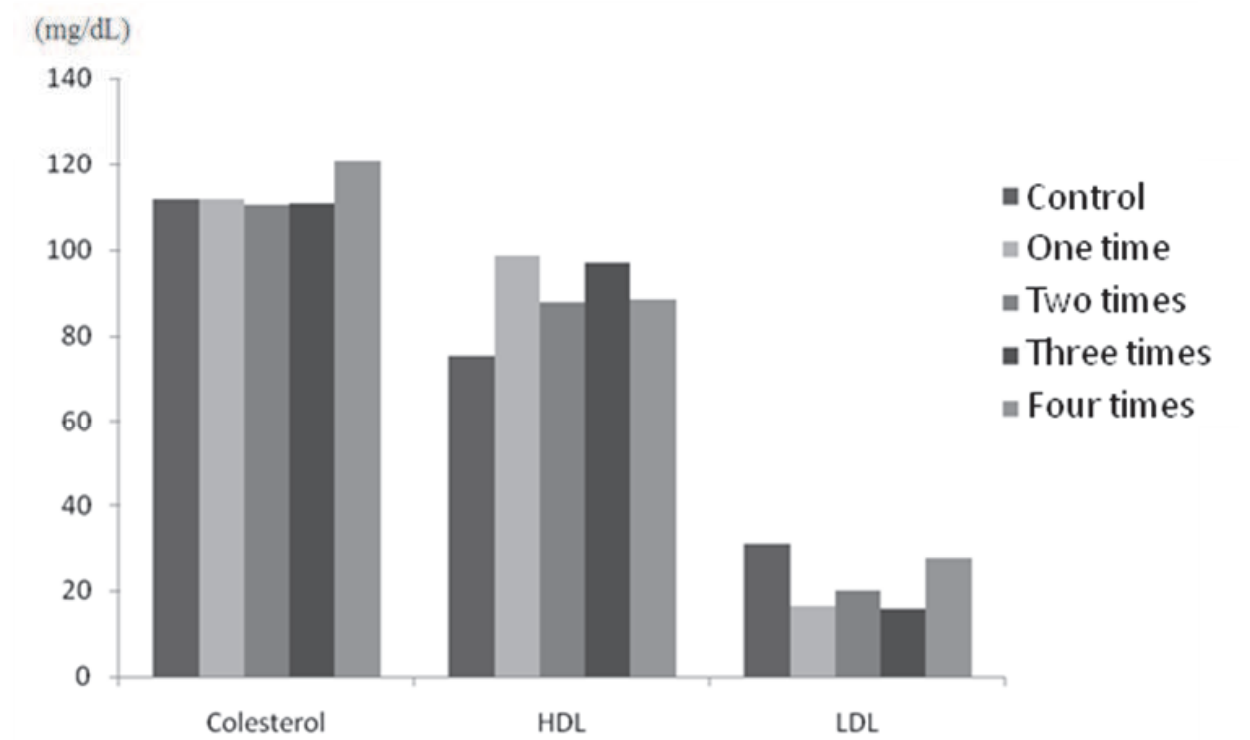


Fig. 1. Mean values of plasma cholesterol, HDL and LDL cholesterol, mg / dL, horses fed diets with soybean oil.

Item (%)	Soybean oil in diets					Mean (%)	CV (%)
	Control diet	One time	Two times	Three times	Four times		
Water	91.9	91.5	89.9	90.5	94.1	91.6	3.3
Dry matter	6.9	8.1	11.4	10.8	6.9	8.8	26.8
NDF <sup>1</sup>	66.8	62.9	67.4	68.6	64.3	66.0	8.6
ADF	35.6	36.7	40.0	40.1	35.9	37.6	7.0
Hemicelulloses	31.2	26.2	27.4	28.5	28.3	28.3	13.4
Celulose	21.2	22.3	24.3	23.8	21.1	22.5	11.1
NFC <sup>2</sup>	14.5	15.7	11.0	9.8	14.3	13.1	30.6
CHO-H <sup>3</sup>	2.0	2.2	2.3	2.6	2.4	2.3	13.7
CHO-RF <sup>4</sup>	12.5	13.4	8.7	7.2	11.9	10.7	37.6
CHO-T <sup>5</sup>	81.3	78.6	78.4	78.3	78.6	79.0	3.5

<sup>1</sup>Carbohydrates slowly fermentable (CHO-SF) represented by NDF; <sup>2</sup>Non-fibrous carbohydrates; <sup>3</sup>Hydrolizable carbohydrates; <sup>4</sup>Rapidly fermentable carbohydrates; <sup>5</sup>Total carbohydrates

Table 6. Mean values of water content and chemical digesta composition from the dorsal colon of horses fed diets with soybean oil.

There there wasn't any effect of soybean oil inclusion in horses' colon pH ( $P>0.05$ ), averaging 6.46. This is below the value cited by Dukes (1996) of 7.09, but was similar to that reported by Santos et al. (2009), evaluating digesta pH in segments of the gastrointestinal tract of horses fed diets with roughage: concentrate similar to the present study found the average pH in the right dorsal colon of 6.41.

Water in the right dorsal colon contents did not differ ( $P>0.05$ ) in horses fed control diet and diet with soybean oil, averaging of 91.6% (Table 6). Lopes et al. (2004) observed lower values of water in the right dorsal colon contents, with a value of 89.6%, when horses were fed concentrate diets, compared to horses fed only hay diet, of 94.2%. Santos et al. (2009) observed mean water concentration in horse right dorsal colon content of 93.4%, a value similar to that observed in this study.

### 3. High fat diets and performance of horses

Fat animal adding to diet of athletes occurred in 1973, aiming to prevent rhabdomyolysis in racing dogs (Kronfeld et al., 1998). From this date, studies with horses were also developed with the same intention. Subsequently, fat inclusion in athletic horses' diets began to be studied in order to reduce muscle fatigue. The possible delay of fatigue, obtained with fat addition in athletic horses' diets may mean the exercise speed maintenance for longer periods or increasing the exercise speed (Meyers et al., 1989).

Horses adapted to physical exercise and fed diets supplemented with oils show a greater ability to oxidize fatty acids as an energy source, saving hepatic glycogen content and providing greater amount of blood glucose, reducing caloric increment and yielding lower respiratory quotients, and producing less  $\text{CO}_2$  when compared to diets containing only carbohydrates. With the increase in free triglyceride concentration, horses slow the anaerobic pathway use with consequent delay in lactate production (Pagan, 2001).

Horses can efficiently digest diets containing up to 30% of digestible energy as fat (Kane et al., 1979).

The addition of vegetable oils or animal fat is an excellent way of increasing dietary energy without increasing the consumed food volume (Cirelli, 1993). This alternative has improved the performance of athletic horses (Harkins et al., 1992), being especially beneficial for horses exercised under high temperatures conditions, due to the low caloric increment being approximately 3% more heat is produced during ATP formation by glucose oxidation, when compared to free fatty acids oxidation (Kohn et al., 1996). Horses fed diets supplemented with oil had large oxidation capacity of free fatty acids, sparing muscle and hepatic glycogen stores during the exercise (Pagan, 2001).

The main benefit of lipids introduction in the daily horses feeding is providing much energy when you've already reached the maximum rate of dry matter intake (Lawrence, 1990). Oil addition of 250 and 500 g in horses diet with average body weight of 400 kg and submitted to medium intensity exercise, increased performance of athletic horses. Equines that consumed oil in quantities of 500 grams daily showed better recovery with better post-test heart rate and hematocrit values (Mattos et al., 2006).

Godoi et al. (2010) evaluated physiological, hematological and biochemical parameters of Eventing horses during a training period consuming a diet with 10% soybean oil inclusion and subjected to physical effort tests. The trial lasted 82 days, performing three physical effort tests: in the beginning, the 60<sup>th</sup> and 82<sup>th</sup> day. There was effect of training duration,



improving horses conditioning, observed in the lactate concentration reduction and increased glucose concentration in the last physical effort test. Soybean oil inclusion only changed the concentration of  $\gamma$ -glutamyl transferase (GGT) and creatinine according to the time of diets consumption (Table 7).

Meyers et al. (1989) and Marquez et al. (2001) found no effect on heart rate before and 20 minutes after exercise in horses receiving diet with or without soybean oil inclusion. However, Mattos et al. (2006), evaluating the performance of horses fed for 30 days with diets containing 0, 3.1 and 6.8% soybean oil inclusion and exercised at a trot for two hours, found that horses consuming a diet with greater soybean oil inclusion had lower levels of heart rate immediately after and 15 minutes after the exercise.

Item	Physical effort test					P
	At rest	Immediately after	10 min after	20 min after	120 min after	
Beginning						
Heart rate (bpm)	33.5 <sup>d</sup>	102.5 <sup>a</sup>	75.8 <sup>b</sup>	54.3 <sup>c</sup>	36.4 <sup>d</sup>	0.000
Body temperature (°C)	37.3 <sup>c</sup>	38.7 <sup>a</sup>	38.5 <sup>a</sup>	38.3 <sup>b</sup>	37.4 <sup>c</sup>	0.000
Glycose (mg/dL)	88.5 <sup>a</sup>	84.5 <sup>a</sup>	88.3 <sup>a</sup>	95.5 <sup>a</sup>	89.7 <sup>a</sup>	NS
Lactate (mmol/L)	0.6 <sup>d</sup>	6.2 <sup>a</sup>	4.5 <sup>b</sup>	3.7 <sup>c</sup>	0.9 <sup>d</sup>	0.000
γ-Glutamyl transferase (U/L)	11.1 <sup>b</sup>	14.2 <sup>a</sup>	14.0 <sup>a</sup>	13.9 <sup>a</sup>	12.3 <sup>b</sup>	0.001
Aspartate aminotransferase (U/L)	343.6 <sup>a</sup>	399.1 <sup>a</sup>	374.5 <sup>a</sup>	343.6 <sup>a</sup>	355.5 <sup>a</sup>	NS
Creatine kinase (U/L)	147.2 <sup>a</sup>	209.1 <sup>a</sup>	101.4 <sup>a</sup>	212.4 <sup>a</sup>	196.6 <sup>a</sup>	NS
Creatinin (mg/dL)	1.3 <sup>a</sup>	1.3 <sup>a</sup>	1.4 <sup>a</sup>	1.2 <sup>ab</sup>	0.9 <sup>b</sup>	0.001
60 <sup>o</sup> Day						
Heart rate (bpm)	40.1 <sup>d</sup>	132.5 <sup>a</sup>	67.0 <sup>b</sup>	57.0 <sup>c</sup>	40.1 <sup>d</sup>	0.000
Body temperature (°C)	37.6 <sup>d</sup>	38.9 <sup>ab</sup>	38.7 <sup>a</sup>	38.4 <sup>b</sup>	37.9 <sup>c</sup>	0.000
Glucose (mg/dL)	83.5 <sup>ab</sup>	75.8 <sup>b</sup>	84.2 <sup>a</sup>	86.9 <sup>a</sup>	88.0 <sup>a</sup>	0.043
Lactate (mmol/L)	0.9 <sup>e</sup>	6.1 <sup>a</sup>	4.0 <sup>b</sup>	2.6 <sup>c</sup>	1.2 <sup>d</sup>	0.000
γ-Glutamyl transferase (U/L)	22.9 <sup>a</sup>	29.8 <sup>a</sup>	26.6 <sup>a</sup>	27.1 <sup>a</sup>	22.9 <sup>a</sup>	NS
Aspartate aminotransferase (U/L)	127.7 <sup>b</sup>	145.7 <sup>b</sup>	151.8 <sup>b</sup>	169.2 <sup>b</sup>	243.7 <sup>a</sup>	0.049
Creatine kinase (U/L)	84.3 <sup>a</sup>	93.1 <sup>a</sup>	127.7 <sup>a</sup>	114.0 <sup>a</sup>	112.2 <sup>a</sup>	NS
Creatinin (mg/dL)	1.0 <sup>c</sup>	1.0 <sup>bc</sup>	1.0 <sup>ab</sup>	1.1 <sup>a</sup>	1.1 <sup>ab</sup>	0.006
82 <sup>o</sup> Day						
Heart rate (bpm)	40.0 <sup>d</sup>	130.7 <sup>a</sup>	75.3 <sup>b</sup>	59.3 <sup>c</sup>	42.0 <sup>d</sup>	0.000
Body temperature (°C)	37.6 <sup>b</sup>	39.3 <sup>a</sup>	39.2 <sup>a</sup>	39.1 <sup>a</sup>	37.6 <sup>b</sup>	0.000
Glucose (mg/dL)	90.8 <sup>a</sup>	94.3 <sup>a</sup>	96.1 <sup>a</sup>	109.1 <sup>a</sup>	96.6 <sup>a</sup>	NS
Lactate (mmol/L)	0.5 <sup>d</sup>	4.1 <sup>a</sup>	2.9 <sup>b</sup>	1.6 <sup>c</sup>	0.4 <sup>e</sup>	0.000
γ-Glutamyl transferase (U/L)	13.3 <sup>a</sup>	15.9 <sup>a</sup>	14.6 <sup>a</sup>	14.3 <sup>a</sup>	10.7 <sup>a</sup>	NS
Creatine kinase (U/L)	136.7 <sup>b</sup>	193.2 <sup>a</sup>	232.3 <sup>a</sup>	180.6 <sup>a</sup>	207.1 <sup>a</sup>	0.014
Creatinine (mg/dL)	2.1 <sup>b</sup>	2.2 <sup>a</sup>	2.4 <sup>a</sup>	2.4 <sup>a</sup>	2.2 <sup>a</sup>	NS

Values, within a line, followed different letters are different, in function collect time, by Friedman test (P<0.05).

Table 7. Heart rate, body temperature and blood biochemical of horses fed with soybean oil inclusion in diet and submitted to exercise tests at beginning, 60° and 82° day

According to NRC (2007), many factors might be related to contradictory results observed in literature, such as type and amount of oils and fats used equines diets, experimental duration, intensity variation and duration of physical efforts, besides reduced number of equines used for treatment and difference in physical conditioning of these animals. According to Hodgson & Rose (1994), Boffi (2006), Mattos et al. (2006) and Brandi et al. (2008), the beneficial effect in diet intake with soybean oil in horse performance is more evident when submitted to exercise of low intensity and long duration.

Some of the nutritional strategies to prevent postprandial hyperglycemia and insulin responses include reducing starch intake (Vervuert et al., 2009) or the replacement of starch by fat (Treiber et al., 2005). Vervuert et al. (2010), evaluating horses fed three different diets containing ground corn, ground corn with soybean oil or corn with fish oil, observed that there was no effect of corn oil or fish oil on serum glucose and insulin in the postprandial period, suggesting that, to avoid postprandial hyperglycemia and hyperinsulinemia, a feed strategy aiming the reduction of starch intake would be better than fat intake. However, linseed oil addition (0.5 mL / kg BW) in diet composed by grains did not affect the postprandial glycemic response, but reduced insulinic concentrations by almost 50% (Fayt et al., 2008).

Increasing the level at 7.8% of oil in the diet did not significantly influence ( $P>0.05$ ) heart rate, respiratory rate, glucose and lactate levels before and after exercise in horses of Quarter Horse race in moderate exercise intensity (227m/min). Muscle glycogen concentration was higher ( $P<0.025$ ), before exercise, in horses fed diets with soybean oil. The increasing concentration of glycogen in horses conditioned to consume a diet with soybean oil can mean a greater energy supply for muscle activity during exercise (Marqueze et al., 2001).

Soybean oil inclusion in diets possibility energetic demand supply with decreased of dry matter intake, avoiding gastrointestinal disturbs. These are beneficial factors justified utilization of lipid sources in diets of horses in any sportive activity.

#### 4. Conclusions

Oils and fats addition in horses' diets should be used in order to raise energy dietary concentration by increasing the availability of blood glucose during postprandial period. It is expected the dry matter intake reduction, ether extract digestibility increasing and greater availability of polyunsaturated fatty acids beneficial to horses athletes without occurrence of diarrhea or changes in feces characteristics.

Soybean oil inclusion in diets of horses should be fractionated into at least four schedules during the day, and mustn't exceed amounts greater than 20%, avoiding nutrients digestibility losses, particularly of fiber, such as hemicelluloses and cellulose, and non-fiber carbohydrates and its fractions.

Fractionation of the soybean oil addition in the diet increases  $\beta$ -oxidation triglycerides with reduction in plasma concentration, does not alter plasma cholesterol, HDL and LDL concentrations, and increases buffering capacity of colon digesta pH witch does not influence the liquid phase passage kinetics in the digestive tract.

Further researches should be conducted to assess the lipids interactions with other nutrients in the small intestine and large intestine of horses with the goal of developing safer diets for athletic horses which result in increased athletic performance.

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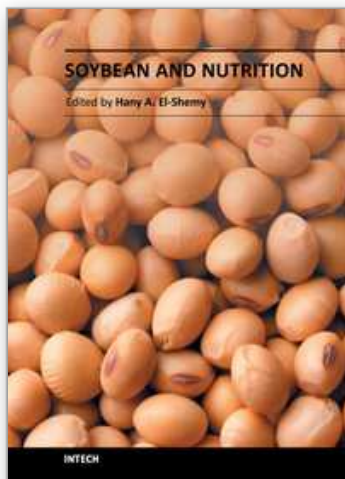
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Phone: +86-21-62489820  
Fax: +86-21-62489821

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