Article

Investigating the Effects of Feeding Two Sources of Omega-3 on Feed Intake, Digestibility, and Blood Metabolites of Horses

Hamid Ghaem Maghami Farahani , Mehdi Dehghan-Banadaky * , Elham Paknia ,
Valiollah Mahdavi-Zafarghandi

Posted Date: 22 March 2023

doi: 10.20944/preprints202303.0386.v1

Keywords: Horse; Fat powder; Flaxseed oil; Feed intake; Digestibility; Blood metabolites

Article

Investigating the Effects of Feeding Two Sources of Omega-3 on Feed Intake, Digestibility, and Blood Metabolites of Horses

H. Ghaem Maghami-Farahani [1], M. Dehghan-Banadaky [1]*, E. Paknia [1] and V. Mahdavi-Zafarghandi [1]

[1] Department of Animal Science, University of Tehran, Tehran 3158777871, Iran; Authors' email: farahani.maghami@ut.ac.ir, *dehghanb@ut.ac.ir, elhampaknia@ut.ac.ir vzafarghandi@ut.ac.ir
* Correspondence: Author: Mehdi Dehghan-Banadaky, Department of Animal Science, University of Tehran, Tehran 3158777871, Iran

Abstract: To investigate the impacts of various sources of omega-3 on feed intake, digestibility, and blood metabolites in horses, this research was conducted. In this survey, 6 medium-sized Turkmen horses, aged 14 to 16 years, were used. The horses had no movement and health problems. This research was carried out in the form of a rotating design and included 3 periods, and each treatment had 2 repetitions in each period. In each experimental period, horses were randomly assigned to each of the 3 experimental treatments. The experimental treatments included C) Omega-3-free supplement (control treatment), R) Persialin flaxseed oil, and P) Persialin fat powder. At the beginning of each period, after the withdrawal period for the previous period, blood and feces samples were taken as the covariate factors, then the experimental treatments were consumed for 14 days, and after the adaptation to 100 grams of supplementing fat powder or flax seed oil, blood and feces samples were taken at the end of the course. Fat supplements were added to the concentrate 1.5 hours before feeding the midday hay. The palatability was affected by the P treatment and was significantly lower than the R and C groups ($P<0.05$). But the difference between the two treatments (R and C) was insignificant in any of the periods. The digestibility of acid detergent insoluble fiber (ADF), crude protein (CP), ether extract (EE), and organic matter were not affected through any of the experimental treatments. Blood metabolites including glucose, cholesterol, triglyceride, total protein, and blood urea nitrogen were not influenced by experimental treatments. The serum albumin concentration of horses was affected by the R treatment in the first and third periods, but no significant difference was observed between the treatments in the second period.

Keywords: Horse; Fat powder; Flaxseed oil; Feed intake; Digestibility; Blood metabolites

---

Introduction

These days, one of the most important topics in the nutrition of sports horses is how to enhance the energy level in the feeding ration. Grains can be used in the ration to provide energy over the animal's maintenance level, but the consumption of grains in the ration as an energy source has limitations [1]. Supplying excess energy through a high percentage of grains such as barley or corn in concentrate exposes the animal to dangerous metabolic diseases such as colic, laminitis, and gastrointestinal ulcers that will harm the animal's health and sport activities [2–14].

In numerous surveys, it has proposed that fat supplements in concentrate, using to provide excess energy, for the maintenance of sports horses [9,8,15–21] or workhorses and ponies [22–26] should be consumed. By adding a fat supplement to the diet, the energy level of the ration is concentrated and the animal receives required energy through a smaller meal [18,27–30], and there is no necessity to consume high levels of forage or grains to meet the animal's energy needs, and this improves sports horses' performance [21,25,31]. Consuming a fat source in the horse nutrition increases glycogen levels in skeletal muscles and liver [21,25,26,32] that stored glycogen will be used throughout competitions or activities. In fact, intense exercise [25,33–35], and the severe reduction of glycogen resources lead to fatigue and will have detrimental impacts on horses' performance [36]. Therefore, it is recommended to add this form of fat supplement (oil) to the concentrate like soybean oil and flaxseed oil, which are known excellent sources of omega-3 [4,37].

The consumption of fat supplements at the optimal level, especially supplements rich in omega-3 such as flaxseed oil and fish oil, in addition to providing fat-soluble vitamins, reducing food dust, making the body coat shiny, and improving hair growth in horses [18], can improve clotting parameters, support immune function, and enhance fertility rate [2,38], influence some health criteria including the production of prostaglandins and leukocytes that have anti-inflammatory properties and can improve inflammatory responses by increasing resistance to the membrane of red blood cells affecting the osmotic pressure and reduce the blood cortisol level during stress [16,17,39–45].

Based on some studies' results, adding fat supplements in the form of oil to the daily feed of horses increased feed intake. Therefore, it is assumed that adding oil to feed improves palatability [4]. Melo et al. [46] showed that consuming oils rich in omega-3 and omega-6 fatty acids increases their antioxidant capacity and subsequently improves horses' performance and also has shown n-3 PUFA have the potential to mitigate the pain and inflammation caused by arthritis, as well as possibly slowing joint degradation [47–49]. The advantage of flaxseed oil over other vegetable oils is due to its high content of omega-3, especially polyunsaturated fatty acids (PUFA), especially alpha-linolenic acid (ALA) [4,50,51]. In addition, flaxseed oil has a very favorable omega-6 to omega-3 ratio, which does not exceed the ratio of 2:1 [52]. Flaxseed oil also reduces the TC/HDL ratio, which is an excellent index for reducing the risk of the arteriosclerosis and other cardiovascular disorders [53]. Enriching the diet with omega-3, for example using flaxseed or flaxseed oil, may improve the blood lipid profile playing an vital role in preventing heart diseases [51,54] and increase tissue sensitivity to insulin [17,55]. Flaxseed or flaxseed oil has also been used as a laxative [56], treatment of inflammation [2], and allergic skin diseases [57]. Flaxseed oil and marine fatty acids supplementation decrease platelet aggregation in different species [58,59].

Horses have a low capability to elongate fatty acids, and this issue is crucial in response to inflammatory activities. There are speculations about flaxseed that, as a source of long-chain omega-3 fatty acids, may reduce the symptoms of laminitis by inhibiting inflammatory mediators [39]. Moreover, in a study due to the consumption of sesame oil, the concentration of red blood cells, hemoglobin and hematocrit increased following the improvement of the level of omega-3 in the blood [60]. Therefore, there are several reasons for horse breeders to be attracted in using different flaxseed products in horse ration [15].

The major objective of this study was to investigate the difference between two sources of flaxseed calcium fat powder and flaxseed oil added in the amount of 10% (100 grams per 1 kg of concentrate) per day on feed intake, nutrients digestibility, and evaluate the possible improvement of inflammatory responses due to the consumption of flaxseed as a source of omega-3 fatty acids and its effects on blood metabolites in horses.

Experimental methods

Animal welfare

Entire experimental procedures, rearing, and management schedules in addition to sampling methods throughout the study were performed based on the fundamentals and guidelines of the Iranian Council of Animal Care [61].

Materials and Methods

This study was implemented at Azmoun riding club located on the east side of Azadi sport comlex in Tehran, Iran from August to November 2018. Six adult, medium-sized, castrated Turkmen stallions were allocated to this study. The horses had no movement or health problems and were kept in individual boxes with standard dimensions. All boxes were equipped with automatic water trough and separated mangers. To equalize the initial conditions between experimental treatments, horses were randomly assigned to each treatment in each period.

The bed of each box was filled with wood chips and the feces collected in the box bed of each horse were cleaned daily, and the entire bed cover was changed weekly. Throughout the project, the horses had free access to water and salt block. To neutralize exercise effect on digestibility trait, horses' exercise was equalized [62,63]. In this way, the training schedule of all 6 horses throughout the week consisted of one day of rest, one day of lunge for 15 minutes of trotting, and the remaining five days the horses walked for 10 minutes, trotted for 20 minutes, and slow trotting for 10 minutes by the rider for a total of 40 minutes in the mange.

In the Azmoun riding club, the horses' daily feeding program included 3 meals of hay, the first meal at 5:30 am, 1:30 pm, and 8:30 pm. To prevent respiratory issues and to reduce the dust from dry forage, in all meals, hay was first completely wet and then given to the horses. All horses were given 4 kg of hay in the morning and evening meals and 2.5 kg of hay in the noon meal (10.5 kg in total).

The composition of the concentrate consumed to feed the horses had minor changes throughout the study. For this reason, at the end of each period, samples were taken from the consumed concentrate (approximate analysis data is given in Table 1). The concentrates were completely pelleted and nutty, and 1 kg was provided to the horses 1 hour prior to the midday hay meal, at 12 to 12:30.

Table 1. Approximate analysis of feed samples at the end of three periods.

Nutrients	%CP	%ADF	%EE	%OM
End of the first period	13.01	33.07	1.498	92.2
End of the second period	12.58	32.78	1.524	92.92
End of the third period	12.50	32.77	1.828	94.92

Horses were randomly assigned to each of the experimental treatments in every period (each treatment 6 repetitions, 3 periods). The design was carried out in the form of a rotating design in 3 periods, and each treatment was repeated twice in each period, and at the end of the study, all 6 horses had fed experimental treatments. The experimental treatments included C) Omega-3-free supplement (control treatment), P) flaxseed calcium fat powder (Persialin fat powder, Kimiya Danesh Alvand Co., Qom, Iran), and R) flaxseed oil (Persianlin flaxseed oil, Kimiya Danesh Alvand Co., Qom, Iran). The nutritional value and chemical composition of the treatments are given in Table 2. The experimental treatments were weighed using a digital scale (1 mg accuracy), 100 grams of fat powder and 100 grams of oil were weighed in a special container (containing a spray) and used throughout the survey. Flaxseed oil treatment was sprayed on 1 kg of concentrate and then given to the horses. In addition, the fat powder treatment was completely combined with 1 kg of concentrate and given to the horses 1 hour before consuming hay. In previous research, a 3-week period was considered to investigate the effects of fat supplements on horses' nutrition, and it has been argued that this period is sufficient for changes in metabolism as a result of dietary changes [21,25,26,32] and this study was also carried out in a period of three weeks. The treatments containing oil and fat powder were fed for 14 days in each period, and after that, blood and feces samples were obtained at the end of the experimental period. 4 days were allocated for the adaptation process (25 grams on the first day, 50 grams on the second day, 75 grams on the third day, and 100 grams on the fourth day). The horses received 100 grams of experimental treatments from the fourth day for 10 days. At the end of each experimental period, 7 days were considered as experimental free-course to make prepared to initiate the next period, when the horses did not receive the oil or fat powder treatments, and their daily ration only included 11.5 kg of hay plus 1 kg of concentrate.

Table 2. Chemical composition of fat supplements.

Item	Oil	
	Persialin Fat Powder	Persialin Flaxseed oil

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 March 2023 doi:10.20944/preprints202303.0386.v1

Fatty Acids, %		
C16:0	10	8
C18:0	7	5
C18:1 ω-9	21	22
C18:2 ω-6	18	12
C18:3 ω-3	42	50
ω-6/ω-3	0.42	0.24
Crude Fat	85	100
PUFA	60	62

In each experimental period, the concentrate fed in all treatments was the same, and they were kept in the club's feed warehouse. Horses were kept in individual boxes with mangers. To evaluate the palatability index (Delobel et al. method [4]), during feeding on the last 4 days of each period, the duration of consumption of 1 kg of concentrate was recorded in experimental treatments.

This study evaluates the digestibility of acid detergent insoluble fiber (ADF), crude protein (CP), ether extract (EE), organic matter (OM), and acid insoluble ash (AIA) in feed and feces samples. To measure ADF, Van Soest method [64] and the Fibertec 1010 device was used. To measure protein and crude fat Kjeldahl and Soxhlet devices were used respectively, and AIA and organic matter of the samples, was measured based on AOAC method [65]. Due to the lack of standard protocols for collecting partial feces samples, the method of collecting total feces throughout the last 2 days of each period was selected and performed based on the method suggested by Martin-Rosset et al. [66]. To achieve this purpose, the beds of individual horse boxes were filled with wood shavings, and in the last 2 days of each period, 24 hours before sampling, the feces in the bed were not cleaned. After the first meal of feeding at 7-8 a.m. from a total of 100 to 200 grams of feces collected from different parts of the box bed, samples were taken. After sampling on the first day, the bed of the boxes was cleaned. On the second day, sampling was done in the same way.

At the end of each period samples of consuming concentrate were taken (500 grams), and a sample of alfalfa was taken at the beginning of the study to represent the alfalfa consumed throughout the entire period. To measure the dry matter percentage at the end of the study, the feed and feces samples were placed in an oven with a temperature of 65°C for 72 hours. Within 2 days after drying, the ration and feces samples were completely mixed and ground with a 1 mm sieve, and AIA was used as an internal marker to calculate the amount the nutrients digestibility. Several studies in horses have shown that the digestibility coefficients obtained by AIA marker are statistically similar to those obtained with total collection [67–70].

To evaluate blood metabolites (glucose, albumin, total protein, triglyceride, cholesterol, and blood urea nitrogen), at the beginning of each experimental period before applying experimental treatments (as a covariate factor) and at the end of each period (main record), 2.5 hours after feeding hay at 8 a.m. blood samples, from the jugular vein through vacuum tubes containing EDTA, K3 anticoagulant, were obtained. Obtained blood samples were centrifuged at 3,000 × g for 15 min at 4°C to separate plasma. The plasma was poured into 3 ml-microtubes and preserved at -20°C till biochemical analysis. The plasma samples were analyzed to determine concentrations of glucose (GOD-PAP), albumin (Bromocresol Green method), total protein (TP) (Biuret with sample blank), and blood urea N (BUN) (Berthelot) utilizing commercial kits following the manufacturer’s instructions (Pars Azmoon Co.). The ELISA device (Hiperion, MPR4+, Microplate Reader, Germany) of the Physiology Laboratory of the Department of Animal Sciences, University of Tehran was used to evaluate glucose, albumin, total protein, and BUN, cholesterol, and triglyceride were measured using the optical absorption spectrophotometer of the Nutrition Laboratory of the Animal Science Department.

Experimental design and data analysis

Data was analysis by SAS statistical software [71] (version 9.1, 2000, SAS Inst. Inc., Cary, NC) and GLM procedure. The averages compared by LSMeans and the probability of less than or equal to 0.05 ($P \leq 0.05$) were considered a significant difference. All the data were first analyzed with the complete statistical model and if the covariance factor was meaningless, it was removed from the equation.

Results

Palatability

The results related to the comparison of the palatability average among the experimental treatments are shown in Table 3. Delobel et al.'s [4] method was used to measure palatability. Based on this method, at the end of each period, 4 observations were recorded on the feeding of each horse with experimental treatments on 4 consecutive days.

Table 3. Average palatability of experimental treatments.

	Control	Fat powder	Flaxseed oil	SEM	P value
Palatability	5.45^{b}	7.33^{a}	5.29^{b}	0.31	0.0014

^{a,b}Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$). Experimental treatments were: No fat supplement (C), Persialin fat powder (P), Persialin flaxseed oil (R).

Digestibility of insoluble fiber in acid detergent (ADF), crude protein (CP), ether extract (EE), and organic matter (OM)

The results related to the comparison of the average of ADF, crude protein, ether extract, and organic matter digestibility among experimental treatments are shown in Table 4.

Table 4. Average of nutrients digestibility (%).

Items	Control	Fat powder	Flaxseed oil	SEM	P value
ADF	59.22	65.35	61.75	5.79	0.47
Crude protein	84.14	86.39	84.38	2.22	0.49
Crude fat	64.88	66.53	61.55	7.94	0.63
Organic matter	74.84	78.11	75.25	3.42	0.51

^{a,b}Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$). Experimental treatments were: No fat supplement (C), Persialin fat powder (P), Persialin flaxseed oil (R).

Blood metabolites

Blood serum glucose concentration

Glucose concentration in all horses was in the normal range (75-115mg/dl) reported in previous study [99]. The results related to the comparison of the average concentration of blood serum glucose among experimental treatments are shown in Table 5. The effect of the experimental treatment in the model was insignificant, and blood serum glucose was not affected by the experimental treatments.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 March 2023 doi:10.20944/preprints202303.0386.v1

Table 5. Effect of experimental treatments on blood serum glucose (mg/dL).

Major factor effect					
	1	2	3	SEM	P value
Treatment effect	88.62	81.34	96.16	7.26	0.19
Period effect	94.57	84.45	87.10	7.26	0.36
Treatment/Period interaction					
	Control	Fat powder	Flaxseed oil	SEM	P value
First period	97.22	80.15	106.34	11.98	0.16
Second period	77.97	94.04	81.34	11.98	0.37
Third period	90.67	69.84	100.79	11.98	0.10

[a,b]Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$). Experimental treatments were: No fat supplement (C), Persialin fat powder (P), Persialin flaxseed oil (R).

Blood serum total protein concentration

The effect of experimental treatment in the used model was insignificant. No significant difference was observed between the experimental treatments, and the treatments did not affect blood serum total protein (Table 6).

Table 6. Effect of experimental treatments on blood serum total protein (g/dL).

Major factor effect					
	1	2	3	SEM	P value
Treatment effect	7.09	7.12	7.99	0.43	0.17
Period effect	7.72	7.03	7.45	0.43	0.31
Treatment/Period interaction					
	Control	Fat powder	Flaxseed oil	SEM	P value
First period	7.64	7.16	8.36	0.77	0.34
Second period	6.63	7.18	7.28	0.77	0.52
Third period	7.00	7.01	8.33	0.68	0.20

[a,b]Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$). Experimental treatments were: No fat supplement (C), Persialin fat powder (P), Persialin flaxseed oil (R).

Blood serum albumin concentration

The effect of experimental treatment in the used model was significant, and the albumin concentration of the flaxseed oil treatment in the first and third periods was significantly higher than the albumin concentration of the fat powder treatment and the control group (Table 7).

Table 7. Effect of experimental treatments on blood serum albumin (g/dL).

Major factor effect					
	1	2	3	SEM	P value
Treatment effect	2.91^b	2.99^b	3.47^a	0.14	0.02
Period effect	3.30	3.05	3.02	0.14	0.21
Treatment/Period interaction					
	Control	Fat powder	Flaxseed oil	SEM	P value

First period	3.06[b]	2.88[b]	3.96[a]	0.25	0.01
Second period	2.95	3.33	2.88	0.25	0.24
Third period	2.72[b]	2.76[b]	3.58[a]	0.25	0.04

[a,b]Mean values within a row with unlike superscript letters were significantly different (P < 0.05). Experimental treatments were: No fat supplement (C), Persialin fat powder (P), Persialin flaxseed oil (R).

Blood serum triglyceride concentration

The effect of experimental treatment was not significant in the used model. None of the treatments of fat powder or Persialin flaxseed oil had a significant effect on the blood serum triglyceride concentration in any period (Table 8).

Table 8. Effect of experimental treatments on blood serum triglyceride (mg/dL).

Major factor effect					
	1	2	3	SEM	P value
Treatment effect	34.20	17.27	18.26	5.93	0.07
Period effect	27.87	23.83	18.03	6.07	0.29
Treatment/Period interaction					
	Control	Fat powder	Flaxseed oil	SEM	P value
First period	33.14	27.28	23.18	10.36	0.55
Second period	37.67	13.96	19.85	10.36	0.11
Third period	31.79	10.58	11.73	10.36	0.17

[a,b]Mean values within a row with unlike superscript letters were significantly different (P < 0.05). Experimental treatments were: No fat supplement (C), Persialin fat powder (P), Persialin flaxseed oil (R).

Blood serum cholesterol concentration

The effect of experimental treatment was insignificant in the model, but the covariate factor of serum cholesterol was significant in the model. In this experiment, however, no significant difference was observed between the treatments, and the experimental treatments did not affect the blood cholesterol of the horses (Table 9).

Table 9. Effect of experimental treatments on blood serum cholesterol (mg/dL).

Major factor effect					
	1	2	3	SEM	P value
Treatment effect	34.20	68.81	71.13	2.95	0.20
Period effect	27.87	68.69	66.50	3.29	0.50
Treatment/Period interaction					
	Control	Fat powder	Flaxseed oil	SEM	P value
First period	68.21	73.38	68.09	5.21	0.47
Second period	65.42	68.12	72.54	5.26	0.37
Third period	61.80	64.94	72.75	5.57	0.23

[a,b]Mean values within a row with unlike superscript letters were significantly different (P < 0.05). Experimental treatments were: No fat supplement (C), Persialin fat powder (P), Persialin flaxseed oil (R).

Blood urea nitrogen concentration (BUN)

The effect of experimental treatment was insignificant in the used model. No significant difference was observed between the averages of experimental treatments, and the level of blood urea nitrogen (BUN) was not affected by any treatment (Table 10).

Table 10. Effect of experimental treatments on blood serum BUN (mg/dL).

Major factor effect					
	1	2	3	SEM	P value
Treatment effect	34.96	32.26	31.53	1.22	0.09
Period effect	32.84	31.23	34.68	1.22	0.09
Treatment/Period interaction					
	Control	Fat powder	Flaxseed oil	SEM	P value
First period	34.66	29.72	34.14	2.08	0.17
Second period	32.70	32.76	28.23	2.08	0.20
Third period	37.51	34.31	32.23	2.08	0.14

[a,b]Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$). Experimental treatments were: No fat supplement (C), Persialin fat powder (P), Persialin flaxseed oil (R).

Discussion

Several studies evaluated the acceptability of oil enriched diets [21,72,73] and compared the palatability of various fats and oils [18]. Previous research investigated the effect of consumption of different sources of oil including soybean oil [26], corn oil [74], and even animal fats [32,75] on the palatability of the diet and they had no negative effect on the feed intake. In studies on horses, there are very few reports on the effects of flaxseed oil consumption on palatability. The research results of some researchers showed the negative effect of flaxseed oil, as a source of omega-3, on the palatability of consumed food [44,76,77]. In this study, there was no significant difference between the average of the flaxseed oil treatment and the control group, but the fat powder treatment significantly reduced the palatability. The reduction in feed intake may be due to the calcium salts of fatty acids leading to unpleasant taste, and sometimes a decrease in feed intake has been reported in other animals such as cattle. Delobel et al. [4] reported an insignificant difference in the palatability of the control and the oil treatment group testing the effect of flaxseed oil supplementation on the feed intake.

After realizing the effect of horse sports activities on the digestibility of the diet's dry matter, the speed of material passing through the intestines, and the fermentation capacity of the microbes at the end of the digestive tract, the researchers conducted various experiments to investigate the effects of horse sports activities such as jumping, endurance and running on nutrients' digestion and absorption. They conducted surveys for the digestibility of various fat supplements and checked varying levels of their consumption and their effects on the digestibility of other nutrients such as crude fiber, crude protein, etc [4,6,78]. Results of numerous researches indicated that the addition of fat supplements to the diet had no effect on the apparent digestibility of cell wall contents [79,80], neutral detergent fibre [14,30,79–82] or acid-detergent fibre [79]. However, other researchers reported an increase in apparent digestibility [4,6,83–87] after the feeding diets containing fat. Improving digestibility in horses leads to a reduction in the risk of metabolic diseases such as colic [8]. The usage of oil in horses' ration creates a coating on the surface of the forage, which can increase the speed of passage of materials throughout the digestive system, and as a result, the components of the feed and nutrients are exposed less time for the digestion, and the digestion is incomplete and can cause the difference between the 3 experimental treatments. In this study the effect of the experimental treatments (fat powder and flaxseed oil) was insignificant in any of the investigated digestive traits with each other and comparing the control group. The results of experiments conducted by Meyer et

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 March 2023 doi:10.20944/preprints202303.0386.v1

al. [88] and Jansen et al. [89] indicated that the use of fat sources in horses' ration decreased protein digestibility. Based on the results obtained by Markey and Kline [78] horses consuming the control treatment had greater digestibility of DM, OM, total dietary fiber, and GE than did those fed the supplemental fat treatments (10% hydrogenated fat; or 10% soybean oil). According to Meyer et al. [88] consuming high amounts of fat in the diet may reduce the apparent digestibility in the small intestine due to the higher endogenous nitrogen flow, which is related to the stimulation of digestive secretions. Delobel et al. [4] reported that the consumption of 80 grams of flaxseed oil significantly increased the digestibility of dry matter (DM), crude fiber (CF), neutral detergent-insoluble fiber (NDF), and crude fat compared to the control treatment and disagree with the results of the current study. However, there was no significant difference between the control group and the oil treatment group for the digestibility of crude protein (CP) and acid detergent-insoluble fiber (ADF), and it is consistent with the results of the present experiment. Rich et al. [80] reported that ponies consuming a diet containing 10% blended fat had decreased NDF digestibility compared to the control or those consuming 10% corn oil. In another study ponies consuming diets containing 7.5 or 15% peanut oil had increased NDF digestibility compared to ponies consuming the control diet or those consuming corn oil, animal fat, or blended fat at the same levels, and ponies consuming any one of the fat sources had higher ADF digestibility than the control [80]. Meyer et al. [88] observed that increasing the daily fat intake from 0.1 to 1 to 2 grams per kilogram of body weight leads to an increase in pre-ileum fat digestibility. According to previous studies, adding fat supplementation have an insignificant effect on the digestibility of the fibrous part of the feed [6,14,32,79,80,85]. Other studies also reported a decrease in the digestibility of crude fiber, ADF, and NDF [89–91]. In another study the addition of 10% animal fat to the diet of exercising horses increased NDF digestibility by 12 to 13 percentage units compared to the control [87].

Lindberg and Karlsson [92] found that adding fat to horses' diets increased apparent ADF digestibility and did not affect total fiber digestibility. In horses, the part of the digestive tract where fermentation occurs is located after the small intestine, which probably explains the difference in digestibility between ruminants and horses [4]. According to Karlsson et al. [93] replacing fermentable carbohydrates with fat reduced some of the negative effects of starch on fiber digestion, because research has shown that reducing the amount of starch in the diet improves fiber digestion.

The insignificant difference in ADF digestibility of the diet between the 3 treatments of Persialin flaxseed oil, Persialin fat powder, and the control group is an acceptable result because it shows that the consumption of 100 grams of fat powder or Persialin flaxseed oil in the diet does not reduce the digestibility and it is possible to use both supplements. One of the objectives of this study was to investigate the effects of fat powder comparing flaxseed oil on creating the coating on the forage surface and the effect on the digestibility of diet's nutrients. The results showed no decrease in ADF digestibility between the two experimental treatments compared to the control group. On the other hand, we assumed that the consumption of flaxseed oil, following the increase in the speed of passage of nutrients through the digestive system, will reduce the digestibility of nutrients compared to the two treatments of fat powder and the control group, and maybe lead to a significant difference in digestibility between the control and fat powder treatments with the flaxseed oil treatment, and the flaxseed oil treatment have lower digestibility than the other two treatments. Another assumption was that following the increase in the passage speed of nutrients throughout the digestive tract, a large part of the undigested starch and flaxseed oil reach the caecum of horses, and by creating an acidic environment in the cecum, causing digestive disorders, and the increase in the passage rate reduces the digestibility of CP, EE, and OM. However, since the digestibility of any of the nutrients does not decrease after consuming flaxseed oil, it can be concluded that a ration with high energy level and omega-3 (fat powder and flaxseed oil) can be consumed in the horse's diet without any problem.

In this study the digestibility of DM and OM were not influenced ($P > 0.05$) by experimental treatments. These results are similar to findings of other researchers [32,80]. Previous studies indicated that adding fat to the horses' diet increased fat digestion [6,14,28,30,79–81,85,86] and the results of this study were not in line with the previous study results. Bush et al.[6] conducted a survey to evaluate the effects of corn oil (5,10,15%) in horses' diet on CP digestibility and concluded that in contrast to previous work [80,87], there was no effect of fat supplementation on CP digestibility. Delobel et al.[4] showed that the inclusion of flaxseed oil in the concentrate improved the EE digestibility up to 83.7% as opposed to 57.3% for the control ration.

The analysis of blood metabolites in horses is a useful measure to evaluate the health and well-being, nutritional requirements, and appropriateness for selecting the type of activity [94]. The concentration of blood metabolites is also an efficient tool to show feed efficiency, because it indicates the nutritional conditions of animals [95,96]. Most of the results pertain to the analysis of blood metabolites in this study were in the range of the results of the sources specific to horses [97,98], which indicated optimal health conditions in them.

Consuming more daily feed and higher digestibility of consumed feed can increase blood glucose concentration [100]. The consumption of fat supplemented diet (up to 12%) enhances skeletal muscle stores in horses by as much as 51% [25,26,32]. Glycogen provides a significant energy substrate for skeletal muscles through exercise activity, with the amount of glycogen utilization increasing exponentially as exercise intensity increases [34,35]. Therefore, a fat-added diet may improve exercise performance through insuring that sufficient glycogen stores are provided and are used to accommodate the elevated glycogen demands occurring during exercise [21].

There were 2 hypotheses about serum glucose concentration:

1) The consumption of a high omega-3 fat supplement increases insulin sensitivity after feeding omega-3 in horses, and after consuming food blood glucose returns to normal level (close to fasting blood glucose) faster, following that blood glucose decreases compared to the control group.

2) On the other hand, we expected that due to the body use of fat as a source of energy at rest, the glucose absorbed from the food will not be absorbed by the cells and will remain in the bloodstream, causing an increase in blood glucose in the experimental groups compared to the control group.

Similar to the study by Hucko et al. [101] the experimental groups, fed omega-3 supplements for 4 weeks, had higher blood glucose than the control group. The results of the study by Harkins et al. [21] indicated that following the consumption of fat in the diet of racing horses, the consumed glucose is stored in the form of glycogen in the muscle tissue, and the blood plasma glucose level increases in the resting case. However, contrary to these two hypotheses, no significant difference was observed between the treatments of fat powder and flaxseed oil on blood glucose, and the treatments of fat powder and flaxseed oil did not show significant increase in blood glucose compared to the control group. O'Connor et al. [17] indicated that plasma glucose concentrations were not different between groups (fed corn oil and fish oil as fat supplements) during exercise but were lower (treatment × time interaction; $P < 0.01$) for the fish oil group during recovery. Hodge et al. [102] reported that both mare and foal plasma glucose and insulin concentrations were not affected by experimental treatments including fish oil and fish oil plus soybean oil as fat supplements.

According to the survey conducted by Long et al. [103], the concentration of albumin has an inverse relationship with the acute phase proteins (APP), and following the increase in serum albumin the concentration of acute phase proteins decreases. Therefore, it can be concluded that the treatment of Persialin flaxseed oil may have reduced the inflammatory response in the first and third periods in horses by increasing the concentration of serum albumin. But in the second period, no significant difference was observed between any of the experimental treatments.

Following the consumption of fat supplements such as fish oil, many studies reported a significant increase in blood serum triglycerides in horses after exercise. This increase following the consumption of fat supplements can be attributed to 2 processes:

1) Increasing the activity of lipoprotein lipase in the oxidation of fatty acids [104,105]

2) Activation of the negative regulation effect (in the direction of reduction) on triglyceride synthesis enzymes by fatty acids [106]

However, throughout this study, no significant difference was observed between the groups consuming fat supplements because the blood samples were taken from the horses at rest and were not examined before and after daily exercise. For this reason, the insignificant difference between the blood triglyceride concentration of horses in the experimental groups, consuming fat supplements, and the control group is a correct result. Hucko et al. [101] also did not observe any significant difference between the control group and the fat supplement-consuming group following the consumption of omega-3 supplements in sports horses.

In another study conducted by Monteverde et al. [107] 70 ml of the fat supplement was used and blood samples were taken from horses before and after exercise, the triglyceride level increased significantly after the horses' sports activities, and also the consumption of this supplement improved the adaptation of the horse's metabolism to the sports activities by increasing the capacity of consuming fatty acids through the horses' muscle tissues. The results of Sembratowicz et al. [108] showed that when flaxseed oil was used in the diet of horses instead of soybean oil, the blood triglyceride concentration and the Atherogenic part of LDL cholesterol reduced, and the percentage of HDL-TC increased. Omega-3 found in unsaturated fatty acids can reduce blood plasma triglyceride concentration by preventing their synthesis in the intestinal wall and liver [109]. Piccione et al. [110] reported a significant decrease in triglycerides and NEFAs concentration in experimental groups compared with control groups during both training period and after the simulated events following omega-3 fatty acids supplementation. Decreased serum triglycerides are the most consistently reported result of fish oil supplementation in horses [111].

Hucko et al. [101] did not observe a significant difference in blood cholesterol levels between the control and the treated group following the daily consumption of 150 gr of omega-3 supplement obtained from Oenothera oil, which is consistent with the results of this study. In horses, LDL constitutes up to 15% of blood lipoproteins [112]. LDL contains 42% cholesterol, while high-density lipoproteins contain 21% to 36% cholesterol. LDL is produced by liver lipase of low-density lipoproteins and is responsible for transporting cholesterol to peripheral tissue [113]. O'Connor et al. [17] noted that serum cholesterol concentrations were lower in horses receiving fish oil (treatment effect; $P < 0.05$), but serum triglycerides were not affected by treatment.

Conclusion

Based on the results of this research, both fat powder and Persialin flaxseed oil supplements can be used up to 10% of horse concentrate as an energy source as well as a source of omega-3 unsaturated fatty acid. The lack of reduction in digestibility of nutrients after adding these supplements also shows that these two products are not superior to each other. It can be concluded that the consumption of 100 gr of Persialin flaxseed oil or fat powder in the diet of horses will lead to beneficial impacts such as increasing the level of the immune index and reducing inflammation.

Acknowledgments: The authors are grateful to the staff of the Azmoun riding club for their assistance in this experiment. This work was financially supported mainly by University of Tehran. M. D.-B. designed the study. E.-P ran farm experiment, H. G. M.-F. and E. P. and V. M.-Z. conducted the research. H. G. M.-F. and V. M.-Z. analyzed the data and wrote the main part of the manuscript. M. D.-B. assisted in manuscript preparation. H. G. M.-F. and V. M.-Z. and M. D.-B. prepared the revised final manuscript. All authors interpreted the findings and critically reviewed the manuscript. All authors read and approved the final manuscript.
The authors declare that no conflicts of interest exist.

Abbreviations

PUFA (Polyunsaturated fatty acids), ALA (Alpha-linolenic acid), TC (Total cholesterol), HDL (High-density lipoprotein), DM (Dry matter), CP (Crude protein), GE (Gross Energy), CF (crude fiber), NDF (Neutral detergent-insoluble fiber), ADF (Acid detergent fiber), EE (Ether extract), OM (Organic matter), AIA (Acid-insoluble ash), TP (Total protein), BUN (Blood urea nitrogen), APP (Acute phase proteins), LDL (Low-density lipoprotein), NEFA (Non-esterified fatty acids)

References

1. Warren, L. K. (2004). The skinny on feeding fat to horses. In Proc. Horse Breeders and Owners Conference (pp. 127-136).

2. Piccione, G., Arfuso, F., Fazio, F., Bazzano, M., & Giannetto, C. (2014). Serum lipid modification related to exercise and polyunsaturated fatty acid supplementation in jumpers and thoroughbred horses. Journal of Equine Veterinary Science, 34(10), 1181-1187.
3. Cichorska, B., Komosa, M., Nogowski, L., Maćkowiak, P., & Józefiak, D. ACCEPTED AUTHOR VERSION OF THE MANUSCRIPT: Significance of nutrient digestibility in horse nutrition–a review.
4. Delobel, A., Fabry, C., Schoonheere, N., Istasse, L., & Hornick, J. L. (2008). Linseed oil supplementation in diet for horses: Effects on palatability and digestibility. Livestock Science, 116[1–3], 15-21.
5. Davidson, N., & Harris, P. (2007). Nutrition and welfare. In The welfare of horses (pp. 45-76). Springer, Dordrecht.
6. Bush, J. A., Freeman, D. E., Kline, K. H., Merchen, N. R., & Fahey Jr, G. C. (2001). Dietary fat supplementation effects on in vitro nutrient disappearance and in vivo nutrient intake and total tract digestibility by horses. Journal of Animal Science, 79(1), 232-239.
7. Cohen, N., Gibbs, P., & Woods, A. (1999). Dietary and other management factors associated with equine colic. J. Am. Vet. Med. Assoc, 45, 96-98.
8. Potter, G. D., Arnold, F. F., Householder, D. D., Hansen, D. H., & Brown, K. M. (1992). Digestion of starch in the small or large intestine of the equine. Pferdeheilkunde, 1(4), 107-111.
9. Harking, J. D., Morris, G. S., Tulley, R. T., Nelson, A. G., & Kamerling, S. G. (1992). Effect of added dietary fat on racing performance in Thoroughbred horses. Journal of Equine Veterinary Science, 12(2), 123-129.
10. Williams, F., & Ashby, K. (1992). Horse related injuries. Hospital, 1989(93).
11. Clarke, L. L., Roberts, M. C., & Argenzio, R. A. (1990). Feeding and digestive problems in horses: physiologic responses to a concentrated meal. Veterinary Clinics of North America: Equine Practice, 6(2), 433-450.
12. Greet, T. R. C., & Rossdale, P. D. (1987). The digestive system. In Veterinary Notes for Horse Owners (pp. 5-24). Stanley Paul and Co. Ltd., London, UK.
13. Sprouse, R. F., Garner, H. E., & GREEN, E. M. (1987). Plasma endotoxin levels in horses subjected to carbohydrate induced laminitis. Equine Veterinary Journal, 19(1), 25-28.
14. Kane, E., Baker, J. P., & Bull, L. S. (1979). Utilization of a corn oil supplemented diet by the pony. Journal of Animal Science, 48(6), 1379-1384.
15. Saastamoinen, M., & Särkijärvi, S. (2020). Effect of linseed (Linum usitatissimum) groats-based mixed feed supplements on diet nutrient digestibility and blood parameters of horses. Animals, 10(2), 272.
16. Bazzano, M., Rizzo, M., Arfuso, F., Giannetto, C., Fazio, F., & Piccione, G. (2015). Increase in erythrocyte osmotic resistance following polyunsaturated fatty acids (PUFA) supplementation in show jumper horses. Livestock Science, 181, 236-241.
17. O'Connor, C. I., Lawrence, L. M., St. Lawrence, A. C., Janicki, K. M., Warren, L. K., & Hayes, S. (2004). The effect of dietary fish oil supplementation on exercising horses. Journal of animal science, 82(10), 2978-2984.
18. Holland, J. L., Kronfeld, D. S., Rich, G. A., Kline, K. A., Fontenot, J. P., Meacham, T. N., & Harris, P. A. (1998). Acceptance of fat and lecithin containing diets by horses. Applied Animal Behaviour Science, 56(2-4), 91-96.
19. Hintz, H. F., & Cymbaluk, N. F. (1994). Nutrition of the horse. Annual review of nutrition, 14, 243-267.
20. Kronfeld, D. S., Ferrante, P. L., & Grandjean, D. (1994). Optimal nutrition for athletic performance, with emphasis on fat adaptation in dogs and horses. The Journal of nutrition, 124(suppl_12), 2745S-2753S.
21. Harkins, J. D., & Kamerling, S. G. (1992). Effects of induced alkalosis on performance in Thoroughbreds during a 1,600-m race. Equine Veterinary Journal, 24(2), 94-98.
22. Kouba, J. M., Burns, T. A., & Webel, S. K. (2019). Effect of dietary supplementation with long-chain n-3 fatty acids during late gestation and early lactation on mare and foal plasma fatty acid composition, milk fatty acid composition, and mare reproductive variables. Animal reproduction science, 203, 33-44.
23. Pagan, J. D., Geor, R. J., Harris, P. A., Hoekstra, K., Gardner, S., Hudson, C., & Prince, A. (2002). Effects of fat adaptation on glucose kinetics and substrate oxidation during low-intensity exercise. Equine Veterinary Journal, 34(S34), 33-38.
24. Duren, S. E., Pagan, J. D., HARRIS, P. A., & CRANDELL, K. G. (1999). Time of feeding and fat supplementation affect plasma concentrations of insulin and metabolites during exercise. Equine Veterinary Journal, 31(S30), 479-484.
25. Oldham, S. L., Potter, G. D., Evans, J. W., Smith, S. B., Taylor, T. S., & Barnes, S. W. (1989). Storage and mobilization of muscle glycogen in racehorses fed a control and high-fat diet. In Proc. 11th Equine Nutr. Physiol. Symp (pp. 57-62).
26. Hambleton, P. L., Slade, L. M., Hamar, D. W., Kienholz, E. W., & Lewis, L. D. (1980). Dietary fat and exercise conditioning effect on metabolic parameters in the horse. Journal of Animal Science, 51(6), 1330-1339.
27. Kronfeld, D. S. (1996). Dietary fat affects heat production and other variables of equine performance, under hot and humid conditions. Equine veterinary journal, 28(S22), 24-34.
28. Scott, B. D., Potter, G. D., Evans, J. W., Reagor, J. C., Webb, G. W., & Webb, S. P. (1989). Growth and feed utilization by yearling horses fed added dietary fat. Journal of Equine Veterinary Science, 9(4), 210-214.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 March 2023 doi:10.20944/preprints202303.0386.v1

29. Snyder, J. L., Meacham, T. N., Fontenot, J. P., & Webb Jr, K. E. (1981). Heat production in pony geldings fed fat supplemented diets. In Proc. 7th Equine Nutr. Physiol. Soc. pp (pp. 144-148).
30. Kane, E., & Baker, J. P. (1977). Utilization of an corn oil supplemented diet by the horse. In Proc. 5th Equine Nutr. Physiol. Soc (p. 41).
31. Nogradi, N., Couetil, L. L., Messick, J., Stochelski, M. A., & Burgess, J. R. (2015). Omega-3 fatty acid supplementation provides an additional benefit to a low-dust diet in the management of horses with chronic lower airway inflammatory disease. Journal of Veterinary Internal Medicine, 29(1), 299-306.
32. Meyers, M. C., Potter, G. D., Evans, J. W., Greene, L. W., & Crouse, S. F. (1989). Physiologic and metabolic response of exercising horses to added dietary fat. Journal of Equine Veterinary Science, 9(4), 218-223.
33. Scott, B. D., Potter, G. D., Greene, L. W., Hargis, E. S., & Anderson, J. G. (1992). Efficacy of a fat-supplemented diet on muscle glycogen concentrations in exercising Thoroughbred horses maintained in varying body conditions. Journal of Equine Veterinary Science, 12(2), 109-113.
34. Nimmo, M. A., & Snow, D. H. (1983). Changes in muscle glycogen, lactate and pyruvate concentrations in the Thoroughbred horse following maximal exercise. Equine Exercise Physiology, 3, 237-244.
35. Pagan, J. D., Essen-Gustavsson, B., Lindholm, A., & Thornton, J. (1987). The effect of dietary energy source on exercise performance in Standardbred horses.
36. Newsholme, LeechAR: Biochemistry For The Medical Sciences, John Wiley and Cons, Chichester, England, pp339- 373,198,3.
37. Hess, T., & Ross-Jones, T. (2014). Omega-3 fatty acid supplementation in horses. Revista Brasileira de Zootecnia, 43, 677-683.
38. Hall, J. A., Van Saun, R. J., & Wander, R. C. (2004). Dietary (n-3) fatty acids from Menhaden fish oil alter plasma fatty acids and leukotriene B synthesis in healthy horses. Journal of veterinary internal medicine, 18(6), 871-879.
39. Ross-Jones, T., Hess, T., Rexford, J., Ahrens, N., Engle, T., & Hansen, D. K. (2014). Effects of omega-3 long chain polyunsaturated fatty acid supplementation on equine synovial fluid fatty acid composition and prostaglandin E2. Journal of Equine Veterinary Science, 34(6), 779-783.
40. King, S. S., Maiero, A., Marlo, T., Roser, J. F., Webel, S. K., & Jones, K. L. (2009). The effect of omega-3 fatty acid supplementation on cortisol and prolactin concentrations in response to common stressors in horses. Journal of Equine Veterinary Science, 29(5), 421-422.
41. Manhart, D. R., Scott, B. D., Gibbs, P. G., Coverdale, J. A., Eller, E. M., Honnas, C. M., & Hood, D. M. (2009). Markers of inflammation in arthritic horses fed omega-3 fatty acids. The Professional Animal Scientist, 25(2), 155-160.
42. Proudman, S. M., Cleland, L. G., & James, M. J. (2008). Dietary omega-3 fats for treatment of inflammatory joint disease: efficacy and utility. Rheumatic Disease Clinics of North America, 34(2), 469-479.
43. James, M. J., Gibson, R. A., & Cleland, L. G. (2000). Dietary polyunsaturated fatty acids and inflammatory mediator production. The American journal of clinical nutrition, 71(1), 343s-348s.
44. Morris, D. D. (1991). Endotoxemia in horses: a review of cellular and humoral mediators involved in its pathogenesis. Journal of Veterinary Internal Medicine, 5(3), 167-181.
45. Morris, D. D., Henry, M. M., Moore, J. N., & Fischer, K. (1989). Effect of dietary linolenic acid on endotoxin-induced thromboxane and prostacyclin production by equine peritoneal macrophages. Circulatory shock, 29(4), 311-318.
46. Mélo, S. K. M., Diniz, A. I. A., de Lira, V. L., de Oliveira Muniz, S. K., Da Silva, G. R., Manso, H. D. C., & Manso Filho, H. C. (2016). Antioxidant and haematological biomarkers in different groups of horses supplemented with polyunsaturated oil and vitamin E. Journal of animal physiology and animal nutrition, 100(5), 852-859.
47. Hansen, R. A., Waldron, M. K., Allen, K. G. D., Hannah, S. S., & Harris, M. (2004, May). Long chain n-3 PUFA improve biochemical parameters associated with canine osteoarthritis. In Proceedings of the American Oil Chemists Society Meeting, Cincinnati, OH.
48. Lau, C. S., Morley, K. D., & Belch, J. J. F. (1993). Effects Of Fish Oil Supplementation On Non-Steroidal Anti—Inflammatory Drug Requirement In Patients With Mild Rheumatoid Arthritis—A Double-Blind Placebo Controlled Study. Rheumatology, 32(11), 982-989.
49. Kremer, J. M., Lawrence, D. A., Jubiz, W., Digiacomo, R., Rynes, R., Bartholomew, L. E., & Sherman, M. (1990). Dietary fish oil and olive oil supplementation in patients with rheumatoid arthritis clinical and immunologic effects. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology, 33(6), 810-820.
50. Pilar, B., Güllich, A., Oliveira, P., Ströher, D., Piccoli, J., & Manfredini, V. (2017). Protective role of flaxseed oil and flaxseed lignan secoisolariciresinol diglucoside against oxidative stress in rats with metabolic syndrome. Journal of food science, 82(12), 3029-3036.
51. Ali, Z. Y., El-Yamany, M., Tawfeeq, M., Elhariry, M. Y., & Ahmad, H. H. (2017). Flaxseed oil effectively reduces the risk of development of atherosclerosis in rats fed on high cholesterol diet. Annual Research & Review in Biology, 1-17.

52. Wang, Y. W., Sunwoo, H., Cherian, G., & Sim, J. S. (2004). Maternal dietary ratio of linoleic acid to alpha-linolenic acid affects the passive immunity of hatching chicks. Poultry Science, 83(12), 2039-2043.
53. Lemieux, I., Lamarche, B., Couillard, C., Pascot, A., Cantin, B., Bergeron, J., ... & Després, J. P. (2001). Total cholesterol/HDL cholesterol ratio vs LDL cholesterol/HDL cholesterol ratio as indices of ischemic heart disease risk in men: the Quebec Cardiovascular Study. Archives of internal medicine, 161(22), 2685-2692.
54. Gehlen, H. (2010). Equine cardiologic diagnostics in practice. Praktische Tierarzt, 91(8), 668-675.
55. Shahidi, F., & Ambigaipalan, P. (2018). Omega-3 polyunsaturated fatty acids and their health benefits. Annual review of food science and technology, 9, 345-381.
56. Schumacher, J., DeGraves, F. J., & Spano, J. S. (1997). Clinical and clinicopathologic effects of large doses of raw linseed oil as compared to mineral oil in healthy horses. Journal of Veterinary Internal Medicine, 11(5), 296-299.
57. O'Neill, W., McKee, S., & Clarke, A. F. (2002). Flaxseed (Linum usitatissimum) supplementation associated with reduced skin test lesional area in horses with Culicoides hypersensitivity. Canadian Journal of Veterinary Research, 66(4), 272.
58. Allman, M. A., Pena, M. M., & Pang, D. (1995). Supplementation with flaxseed oil versus sunflowerseed oil in healthy young men consuming a low fat diet: effects on platelet composition and function. European Journal of Clinical Nutrition, 49(3), 169-178.
59. Casali, R. E., Hale, J. A., LeNarz, L., Faas, F., & Morris, M. D. (1986). Improved graft patency associated with altered platelet function induced by marine fatty acids in dogs. Journal of Surgical Research, 40(1), 6-12.
60. Calder, P. C. (2009). Polyunsaturated fatty acids and inflammatory processes: new twists in an old tale. Biochimie, 91(6), 791-795.
61. Iranian Council of Animal Care (1995). Guide to the Care and Use of Experimental Animals, vol. 1. Isfahan: Isfahan University of Technology.
62. Zeyner, A., Kretschmer, E., Fuchs, R., Kaske, H., & Hoffmann, M. (2003, October). Investigation on influence of exercise intensity on the digestibility of the feed in adult riding horses. In Proc. 7th ESVCN Conference, Hannover (p. 74).
63. Pagan, J. D., Harris, P., Brewster-Barnes, T., Duren, S. E., & Jackson, S. G. (1998). Exercise affects digestibility and rate of passage of all-forage and mixed diets in thoroughbred horses. The Journal of Nutrition, 128(12), 2704S-2707S.
64. Van Soest, P. V., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of dairy science, 74(10), 3583-3597.
65. AOAC Official Method of Analysis. 15th ed. Washington, DC, USA: Association of Official Analytical Chemists; 1990.
66. Martin-Rosset, W., Vermorel, M., Doreau, M., Tisserand, J. L., & Andrieu, J. (1994). The French horse feed evaluation systems and recommended allowances for energy and protein. Livestock Production Science, 40(1), 37-56.
67. Miraglia, N., Bergero, D., Bassano, B., Tarantola, M., & Ladetto, G. (1999). Studies of apparent digestibility in horses and the use of internal markers. Livestock Production Science, 60(1), 21-25.
68. Cuddeford, D., & HUGHES, D. (1990). A comparison between chromium-mordanted hay and acid-insoluble ash to determine apparent digestibility of a chaffed, molassed hay/straw mixture. Equine Veterinary Journal, 22(2), 122-125.
69. Orton, R. K., Hume, I. D., & Leng, R. A. (1985). Effects of exercise and level of dietary protein on digestive function in horses. Equine Veterinary Journal, 17(5), 386-390.
70. Sutton, E. I., Bowland, J. P., & McCarthy, J. F. (1977). Studies with horses comparing 4N-HCl insoluble ash as an index material with total fecal collection in the determination of apparent digestibilities. Canadian Journal of Animal Science, 57(3), 543-549.
71. SAS, S. (2008). STAT Software Release 9.2. Cary, NC, USA: SAS Institute Inc.
72. Zeyner, A., Behnisch, C., & Gropp, J. (2003). Investigations on the palatability and digestibility of fish oil containing diets in adult riding horses. Proc. 7th Congr. Europ. Soc. Vet. Comp. Nutr.(ESVCN). Hannover, 3, 04-75.
73. Harris, P. A., Pagan, J. D., CRANDELL, K. G., & DAVIDSON, N. (1999). Effect of feeding thoroughbred horses a high unsaturated or saturated vegetable oil supplemented diet for 6 months following a 10 month fat acclimation. Equine Veterinary Journal, 31(S30), 468-474.
74. Topliff, D. R., Potter, G. D., Dutson, T. R., Kreider, J. L., & Jessup, G. T. (1983). Diet manipulation and muscle glycogen in the equine. In Proc 8th Equine Nutrition and Physiology Symposium (pp. 224-229).
75. Hintz, H. F., Schryver, H. F., & Stevens, C. E. (1978). Digestion and absorption in the hindgut of nonruminant herbivores. Journal of animal science, 46(6), 1803-1807.
76. Hansen, R. A., Savage, C. J., Reidlinger, K., Traub-Dargatz, J. L., Ogilvie, G. K., Mitchell, D., & Fettman, M. J. (2002). Effects of dietary flaxseed oil supplementation on equine plasma fatty acid concentrations and whole blood platelet aggregation. Journal of Veterinary Internal Medicine, 16(4), 457-463.

 doi:10.20944/preprints202303.0386.v1

77. Henry, M. M., Moore, J. N., & Fischer, J. K. (1991). Influence of an omega-3 fatty acid-enriched ration on in vivo responses of horses to endotoxin. American journal of veterinary research, 52(4), 523-527.
78. Markey, A. D., & Kline, K. H. (2006). Effects of dietary fat and yeast culture supplementation on total tract digestibility by horses. The Professional Animal Scientist, 22(3), 261-266.
79. McCann, J. S., Meacham, T. N., & Fontenot, J. P. (1987). Energy utilization and blood traits of ponies fed fat-supplemented diets. Journal of Animal Science, 65(4), 1019-1026.
80. Rich, G. A., Fontenot, J. P., & Meacham, T. N. (1981). Digestibility of animal, vegetable and blended fats by equine. In EQUINE NUTRITION AND PHYSIOLOGY SYMPOSIUM (Vol. 7, p. 30).
81. Davison, K. E., Potter, G. D., Evans, J. W., Greene, L. W., Hargis, P. S., Corn, C. D., & Webb, S. P. (1991). Growth, nutrient utilization, radiographic bone characteristics and postprandial thyroid hormone concentrations in weanling horses fed added dietary fat. Journal of Equine Veterinary Science, 11(2), 119-125.
82. Meyer, H., Gillespie, J. R., & Robinson, N. E. (1987). Nutrition of the equine athlete.
83. Sales, J., & Homolka, P. (2011). A meta-analysis of the effects of supplemental dietary fat on protein and fibre digestibility in the horse. Livestock Science, 136(2-3), 55-63.
84. Jansen, W. L., van Oldruitenborgh-Oosterbaan, M. S., Cone, J. W., De Vries, H. T., Hallebeek, J. M., Hovenier, R., ... & Beynen, A. C. (2007). Studies on the mechanism by which a high intake of soybean oil depresses the apparent digestibility of fibre in horses. Animal feed science and technology, 138(3-4), 298-308.
85. Hughes, S. J., Potter, G. D., Greene, L. W., Odom, T. W., & Murray-Gerzik, M. (1995). Adaptation of Thoroughbred horses in training to a fat supplemented diet. Equine veterinary journal, 27(S18), 349-352.
86. Julen, T. R., Potter, G. D., Greene, L. W., & Stott, G. G. (1995). Adaptation to a fat-supplemented diet by cutting horses. Journal of Equine Veterinary Science, 15(10), 436-440.
87. Webb, S. P., Potter, G. D., & Evans, J. W. (1987). Physiologic and metabolic response of race and cutting horses to added dietary fat. In Proc. 10th Equine Nutr. and Physiol. Symp. p (Vol. 115).
88. Meyer, H., Flothow, C., & Radicke, S. (1997). Preileal digestibility of coconut fat and soybean oil in horses and their influence on metabolites of microbial origin of the proximal digestive tract. Archives of Animal Nutrition, 50(1), 63-74.
89. Jansen, W. L., Geelen, S. N. J., Kuilen, J. V. D., & Beynen, A. C. (2002). Dietary soyabean oil depresses the apparent digestibility of fibre in trotters when substituted for an iso-energetic amount of corn starch or glucose. Equine Veterinary Journal, 34(3), 302-305.
90. Jansen, W. L., Van der Kuilen, J., Geelen, S. N. J., & Beynen, A. C. (2000). The effect of replacing nonstructural carbohydrates with soybean oil on the digestibility of fibre in trotting horses. Equine Veterinary Journal, 32(1), 27-30.
91. Jenkins, T. C., & Palmquist, D. L. (1984). Effect of fatty acids or calcium soaps on rumen and total nutrient digestibility of dairy rations. Journal of dairy science, 67(5), 978-986.
92. Lindberg, J. E., & Karlsson, C. P. (2001). Effect of partial replacement of oats with sugar beet pulp and maize oil on nutrient utilisation in horses. Equine veterinary journal, 33(6), 585-590.
93. Karlsson, C. P., Lindberg, J. E., & Rundgren, M. (2000). Associative effects on total tract digestibility in horses fed different ratios of grass hay and whole oats. Livestock Production Science, 65(1-2), 143-153.
94. Czech, A., Kiesz, M., Kiesz, A., Próchniak, T., Różański, P., & Klimiuk, K. (2019). Influence of type of use, age and gender on haematological and biochemical blood parameters of małopolski horses. Annals of animal science, 19(1), 85-96.
95. Van Weyenberg, S., Buyse, J., & Janssens, G. P. (2007). Digestibility of a complete ration in horses fed once or three times a day and correlation with key blood parameters. The Veterinary Journal, 173(2), 311-316.
96. Pambu-Gollah, R., Cronje, P. B., & Casey, N. H. (2000). An evaluation of the use of blood metabolite concentrations as indicators of nutritional status in free-ranging indigenous goats. South African Journal of Animal Science, 30(2), 115-120.
97. Winnicka, A. Reference Values in Basic Laboratory Analyses in Veterinary Medicine; Warsaw Agricultural University Publishing: Warsaw, Poland, 2015; pp. 97, 108. (In Polish)
98. Krumrych, W. Horse Blood Laboratory Indices-Reference Values and Interpretation; National Veterinary Research Institute: Pulawy, Poland, 2003. (In Polish)
99. Radostits, O. M., Gay, C., Hinchcliff, K. W., & Constable, P. D. (Eds.). (2006). Veterinary Medicine E-Book: A textbook of the diseases of cattle, horses, sheep, pigs and goats. Elsevier Health Sciences.
100. Palumbo, P., Ditlevsen, S., Bertuzzi, A., & De Gaetano, A. (2013). Mathematical modeling of the glucose–insulin system: A review. Mathematical biosciences, 244(2), 69-81.
101. Hučko, B., Mudřík, Z., Mikešová, K., Plachý, V., & Kodeš, A. (2016). Use of Fats in the Diet of Sport Horses Has Its Pros and Cons. Acta Fytotechnica et Zootechnica, 19(1), 3-6.
102. Hodge, L. B., Rude, B. J., Dinh, T. N., & Lemley, C. O. (2017). Effect of ω-3 fatty acid supplementation to gestating and lactating mares: on milk IgG, mare and foal blood concentrations of IgG, insulin and glucose,

placental efficiency, and fatty acid composition of milk and serum from mares and foals. Journal of Equine Veterinary Science, 51, 70-78.
103. Long, A., & Nolen-Walston, R. (2020). Equine inflammatory markers in the twenty-first century: a focus on serum amyloid a. Veterinary Clinics: Equine Practice, 36(1), 147-160.
104. Geelen, S. N., van OLDRUITENBORGH-OOSTERBAAN, M. M. S., & Beynen, A. C. (1999). Dietary fat supplementation and equine plasma lipid metabolism. Equine Veterinary Journal, 31(S30), 475-478.
105. Orme, C. E., Harris, R. C., Marlin, D. J., & Hurley, J. (1997). Metabolic adaptation to a fat-supplemented diet by the thoroughbred horse. British Journal of Nutrition, 78(3), 443-458.
106. Surette, M. E., Whelan, J., Broughton, K. S., & Kinsella, J. E. (1992). Evidence for mechanisms of the hypotriglyceridemic effect of n− 3 polyunsaturated fatty acids. Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism, 1126(2), 199-205.
107. Monteverde, V., Congiu, F., Vazzana, I., Dara, S., Di Pietro, S., & Piccione, G. (2017). Serum lipid profile modification related to polyunsaturated fatty acid supplementation in thoroughbred horses. Journal of Applied Animal Research, 45(1), 615-618.
108. Sembratowicz, I., Zięba, G., Cholewinska, E., & Czech, A. (2020). Effect of Dietary Flaxseed Oil Supplementation on the Redox Status, Haematological and Biochemical Parameters of Horses' Blood. Animals, 10(12), 2244.
109. Xu, J., Rong, S., Gao, H., Chen, C., Yang, W., Deng, Q., ... & Huang, F. (2017). A combination of flaxseed oil and astaxanthin improves hepatic lipid accumulation and reduces oxidative stress in high fat-diet fed rats. Nutrients, 9(3), 271.
110. Piccione, G., Bazzano, M., Bruschetta, D., Giannetto, C., Arfuso, F., & Giudice, E. (2016). Omega-3 fatty acid food enrichment influences some serum acute phase proteins concentration and white blood cell count in athlete horses. Journal of Equine Veterinary Science, 39, 90-96.
111. Hinchcliff KW, Kaneps AJ, Geor RJ. (2004). Equine sports medicine and surgery. Basic and clinical sciences of the equine athlete. 1st ed. Edinburgh, London: Saunders Company.
112. Watson, T. D. G., BURNS, L., Love, S., Packard, C. J., & Shepherd, J. (1991). The isolation, characterisation and quantification of the equine plasma lipoproteins. Equine Veterinary Journal, 23(5), 353-359.
113. Direkvandi, E., Rouzbehan, Y., & Fazaeli, H. (2021). The positive impact of increasing feeding frequency on feed intake, nutrient digestibility, and blood metabolites of Turkmen horses. Journal of Equine Veterinary Science, 98, 103390.