Journal of Equine Veterinary Science 104 (2021) 103690

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Research Paper

The Effect of Three Levels of Concentrate and Grain Processing on Feeding Behavior, Nutrient Digestibility, Blood Metabolites and Fecal pH Of Turkmen Horses

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ARTICLE INFO

Article history:
Received 17 April 2021
Received in revised form 7 June 2021
Accepted 7 June 2021
Available online 15 June 2021

Keywords:
Blood metabolites
Digestibility
Feeding behavior
Forage to concentrate ratio
Grain processing

ABSTRACT

The aim of this study was to investigate, the effect of different levels of concentrates and grain processing on feeding behavior, nutrient digestibility, fecal pH and blood metabolites in the horse. Sixteen 5 to 11 years old Turkmen horses with an initial body weight 433±50 kg were used in this experiment based on completely randomized design. Four treatments were studied, in three treatments were used 20, 25 and 30% of concentrate containing processed grains (A20, A25 and A30, respectively), and in one treatment was used 25% of concentrate containing whole grain (B25). The amount of feed intake, chewing and swallowing rate and total intake for forage and concentrate were not affected by experimental treatments ($P>$ .05). By increasing the concentrate level up to 30%, the digestibility coefficients of dry matter, organic matter, crude protein, ash-free neutral detergent fiber, ash-free acid detergent fiber and digestible energy increased. The highest digestibility coefficients were observed in A30 treatment ($P<$ 0.05). The digestibility of organic matter, crude protein, ash-free neutral detergent fiber and digestible energy in A25 treatment significantly increased compared to B25 ($P<$ 0.05). The concentration of total protein, triglycerides, cholesterol and low-density lipoprotein were not affected by experimental treatments ($P>$ 0.05). The concentration of glucose increased with increasing concentrate for treatment A30 ($P<$ 0.05). In conclusion, comparing the two levels of 25% concentrate showed that the use of processed grains compared to unprocessed grains had no effect on feeding behavior, fecal pH and blood parameters. The use of 30% concentrate containing processed grains improved digestion without adversely affecting feeding behavior and fecal pH.

1. Introduction

Today, horse management practices are very different from the past. To increase energy density and provide essential nutrients to high-yielding horses, concentrate is used as a substitute or in combination with forage mixtures [1]. Due to the fact that horses are herbivores (cecal digestors), the use of high levels of concentrate can lead to some metabolic diseases (e.g., colic), so the use of concentrate in the diet has become a challenge [2]. For example, when a small amount of oats is given to a horse, approximately 80% of its starch is digested and absorbed in the small intestine [3]. But when the amount of oats in the horse's diet exceeds 20% of the diet, digestibility in the small intestine is reduced by 58%. The use of concentrate in horse nutrition should be in the lowest proportion of forage and normally the concentrate should be used in less than 50% of the diet and at the desired level of 20 to 30% [4]. In this way, a 450 kg mature horse fed 2% of its body weight per day, of which 1.5 to 3 kg is allocated to concentrate [4]. It has been reported in a study that not processing grains has an adverse effect on starch fermentation in the gastrointestinal tract, which leads to laminitis by increasing lactic acid production in the gastrointestinal

Conflict of interest statement: The authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no financial support for this work that could have influenced its outcome.

Animal welfare/ethical statement: The Guide for the Care and Use of Agricultural Animals in Research and Teaching [10] was followed for housing, feeding, transport, proper and humane care and use of animals, veterinary care, occupational health and safety, program management and procedures. The Committee of Animal Science of Tarbiat Modares University (Iran) approved the experimental protocols.

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https://doi.org/10.1016/j.jevs.2021.103690
0737-0806/

tract [5]. By fermenting large volumes of starch in the large intestine, lactic acid is produced, so increasing the production of this acid leads to a change in the microbial population of this area of the gastrointestinal tract due to a decrease in pH [6]. Decreasing the pH of the large intestine lead to reduces the number of fiber degradation bacteria [7], fiber digestion [8], reduce the production of volatile fatty acids and its absorption [6]. On the other hand, the use of concentrates containing processed ingredients (e.g., cereal grains) that increase the digestibility of starch and protein in the primary part of the gastrointestinal tract can reduce the negative effects of increasing concentrate in the horse diet [9]. Because grain processing improves the effect of small intestinal enzymes on starch granules, so that most of starch are digested and absorbed in the small intestine. Therefore, the use of processed grain due to its effect on pre-cecum digestion can be a desirable solution in the horse management. In this study, our aim was to compare three levels of concentrate containing processed cereal grains (20, 25 and 30%) with unprocessed cereal grain (25%) on feeding behavior, nutrient digestibility, blood parameters and fecal pH.

2. Materials and Methods

The experiment was performed at Tarbiat Modares University (Tehran, Iran) with cooperation from the Zoljanah Equestrian Club.

2.1. Experimental Design

The experiment was performed based on randomized complete balanced design with three treatments and four replicates in each treatment. The experiment consisted of 28 days, of which 21 days were considered to be adaptation and 7 days for data collection. During the adaptation period, the horses were exercised by lunging twice a day (morning and evening for 20 minutes). Behavioral observations, feed intake and fecal sampling were performed on days 22 to 28. Blood plasma sampling was performed on day 27 of the experiment. The Guide for the Care and Use of Agricultural Animals in Research and Teaching [10] was followed for housing, feeding, transport, proper and humane care and use of animals, veterinary care, occupational health and safety, program management and procedures. The Committee of Animal Science of Tarbiat Modares University (Iran) approved the experimental protocols.

2.2. Animals, Diets and Experimental Treatments

Sixteen 5 to 11 years old Turkmen horses (15 mares and 1 gelding) with an initial body weight 433±50 kg were used in this experiment. All horses were examined by a veterinarian and then treated against internal and external parasites. They were housed in an individual stalls of dimensions 4m × 5m with a sawdust bedding. During the experiment, water and salt licks were freely available at all times. The experimental diet consisted of two parts: forage and concentrate, which were provided separately for each experimental treatment. The forage used in this experiment contains: alfalfa hay and wheat straw, which was fed in a ratio of 2 to 1, respectively. Two concentrates were used in this experiment, concentrate A, which contained processed grains and was fed at three levels 20, 25 and 30% (A20, A25 and A30, respectively), and concentrate B, which contains unprocessed grains and was fed at the level of 25% (B25). The ingredients used in these two concentrates were the same and the only difference was the type of grain processing. The ingredients used in the diets were formulated according to NRC (2007) [11]. The ingredients and chemical composition of the diets is presented in Table 1. The ration was divided into four parts and fed at 07:00 hours, 13:00 hours, 19:00 hours and 01:00 hour recommended by Direkvandi et al. (2021) [12].

Table 1
Ingredients and chemical composition used in the experimental treatments.

Ingredients (g/kg DM)	Diets[a]			
	B25	A20	A25	A30
Alfalfa hay	500	533	500	467
Wheat straw	250	267	250	233
Micronized wheat	-	11.5	23.7	36.4
Micronized barley and Steam flake	-	23.1	35.8	47.9
Extruded corn	-	23.1	34.6	47.9
Steam flake Oats	-	11.5	24.2	36.4
Wheat grain	23.7	-	-	-
Barley grain	35.8	-	-	-
Corn grain	34.6	-	-	-
Oats grain	24.2	-	-	-
Roasted soybeans	17.3	17.3	17.3	17.3
Rice Bran	17.3	17.3	17.3	17.3
Beet pulp	17.3	17.3	17.3	17.3
Premix feed[b]	62.4	62.4	62.4	62.4
Vegetable oil mixture[c]	10.4	10.4	10.4	10.4
Vitamin and mineral supplements	6.93	6.93	6.93	6.93
Chemical composition (g/kg DM)				
Dry matter	935	944	945	947
Organic matter	824	816	823	831
Crude protein	116	115	116	118
Ether extract	36.2	35.3	37.5	39.8
NDFom	498	516	496	476
ADFom	333	348	331	314
DE[d] (MJ/kg DM)	8.99	8.97	9.01	9.29

Abbreviations: ADFom, ash-free acid detergent fiber; DE, digestible energy; NDFom, ash-free neutral detergent fiber.
[a] B25, 25% of concentrate containing whole grains; A20, 20% of concentrate containing processed grains; A25, 25% of concentrate containing processed grains; A30, 30% of concentrate containing processed grains.
[b] A mixture of soybean, canola, and sunflower meal.
[c] A mixture of soybean, sunflower, and canola oil.
[d] Calculated according to the NRC (2007) [11].

2.3. Collection Procedures

During the experiment period for estimating the feed intake, feed offered and residue were recorded daily before morning feeding, and feed and residue samples were stored at -20°C for subsequent chemical analysis. Chewing and swallowing rates in horses were observed and recorded by two observers who also recorded the duration of alfalfa hay and concentrate intake per meal at all meals for each horse. Therefore, in each meal, after presenting the feed individually (concentrate and forage), the rate of chewing and swallowing was observed for at least 5 minutes [13]. The number of chewing and swallowing per kg of dry matter (DM) was calculated for each horse. Also, the total feed intake time for each horse was measured during feeding.

To measure digestibility of nutrients and fecal pH, fresh fecal grab samples were collected from the rectums of all horses three times per day post-feeding on days 22 to 28 of the sampling period, and grab was mixed with 20 mL of distilled water, the pH of the fecal fluid was measured using a portable pH meter [14]. Fecal samples were transferred to aluminum pans and dried at 50 °C in a forced-air oven for 48 hours, then ground to pass through a 1-mm Wiley mill screen, and a single composite sample was prepared for each horse by mixing equal amounts (on a dry matter basis) from the three samples. Feed, residue and fecal samples were analyzed for DM, organic matter (OM), ether extract (EE), ash-free neutral detergent fiber (NDFom) and ash-free acid detergent fiber (ADFom) and crude protein (CP). In this experiment chromium oxide (Cr_2O_3) was used as an indigestible external marker to determine the digestibility of nutrients. The marker was mixed with concentrate and given to horses during the experiment and digestibility of nutrients was calculated according to Church (1993) [15].

On the 27th day of the experiment period, blood was collected at 3 time points. The venesection was performed at 6:30 (before first feed), 8:30 and 10:30, respectively. Approximately 10 mL of blood sample was collected from the jugular vein using tubes containing anticoagulant (Becton Dickinson, Rutherford, NJ, USA). The blood samples were centrifuged (3,000 × g for 15 minutes at 4°C) and the plasma was separated and frozen at -20°C until measuring biochemical parameters. Biochemical parameters including glucose, triglycerides, total protein, cholesterol and low-density lipoprotein (LDL) were determined using enzymatic methods and by spectrophotometer (Jenway, Genova, UK) and using kits of the Pars Azmun Company (Tehran, Iran). The analyses were performed in the animal nutrition laboratory of the Faculty of Agriculture, Tarbiat Modares University.

2.4. Chemical and Statistical Analyses

Feed and fecal DM, ash (number 924.05), CP (N × 6.25; number 984.13) and EE (number 954.02), ADFom (method 973.18) were analyzed according to the methods of AOAC (1990) [16]. Furthermore, NDFom was analyzed using the method of presented by Van Soest et al. (1991) [17] and Cr_2O_3 was determined as described by Kozloski et al. (1993) [18]. Digestible energy (DE) of the diet was calculated according to NRC (2007) [11].

The data obtained from assessing nutrient digestibility and feeding behavior of horses were analyzed as a randomized complete design using General Linear Models (GLM) procedure in SAS software [19], which is based on the statistical model: $Y_{ij} = \mu + T_i + e_{ij}$. Where Y_{ij} is observation (nutrient digestibility, feeding behavior), μ is the general mean, T_i is the effect of experimental treatment and e_{ij} is the standard error term. Blood metabolite and fecal pH data were analyzed as repeated measurements using the MIXED procedures of SAS, based on the statistical model: $Y_{ijk} = \mu + T_i + H_j + (TH)_{ij} + e_{ijk}$. Where Y_{ijk} is observation (blood metabolite and fecal pH), μ is the general mean, T_i is the effect of experimental treatment, H_j is effect of sampling hours, $(TH)_{ij}$ is interactions between effect of experimental treatment and sampling hours and e_{ijk} is the standard error of term. Means were compared by the Duncan multiple comparison tests at $P < .05$.

3. Results and Discussion

3.1. Feeding Behavior

Feed consumption behaviors are influenced by feed type (forage or concentrate), forage and concentrate type and its processing and even horses used in experimental treatments (body size, animal species, physiological state) [20]. In present study, the amount of concentrate intake, chewing and swallowing rate and total intake time increased with increasing concentrate level from 20 to 30% (from 1.73 to 2.60 kg). However, none of these parameters were affected by the experimental treatments ($P > 0.05$) (Table 2). In agreement with the results of the present study, it has been reported that with increasing the level of concentrate in the diet and chewing rate, the duration of concentrate intake increases [21].

The rate of chewing per kg of DM and the duration of concentrate intake in B25 treatment numerically increased compared to A25 treatment. It seems that the use of whole grain in B25 treatment compared to its peer treatment (A25) has caused this difference. Because whole grains require more force to break than processed grains, resulting in more jaw movements. Processing can cause changes in size, density and texture of feed. In this regard, it has been reported that the duration of feed intake and chewing activity depends on the type and physical form of feed [22,23]. However, similar to our results it has been reported that grain processing had no effect on eating time [24].

Table 2
Effect of different level of concentrate to forage on feeding behavior.

Item	Diets[a]				SEM	*P* Value
	B25	A20	A25	A30		
Concentrate						
Intake (g/min)	141	134	146	157	18.3	0.339
No. of chews/kg DM	643	529	561	576	63.7	0.642
No. of swallowing/kg DM	11.0	10.8	11.0	12.4	1.18	0.257
Total intake time (min)	15.8	14.9	15.2	16.3	1.15	0.069
Forage						
Intake (g/min)	43.1	45.0	44.4	41.7	8.72	0.345
No. of chews/kg DM	2521	2609	2495	2518	133	0.277
No. of swallowing/kg DM	37.5	36.4	39.4	40.8	2.45	0.119
Total intake time (min)	156	162	154	151	9.46	0.406

Abbreviations: SEM, standard error of the means.
[a] B25, 25% of concentrate containing whole grains; A20, 20% of concentrate containing processed grains; A25, 25% of concentrate containing processed grains; A30, 30% of concentrate containing processed grains.

Table 3
Effect of different level of concentrate and processing on nutrient digestibility (g/kg DM).

Item	Diets[a]				SEM	*P* Value
	B25	A20	A25	A30		
Dry matter	512^{bc}	498^{c}	531^{b}	609^{a}	7.22	0.001
Organic matter	506^{c}	512^{c}	547^{b}	607^{a}	9.55	0.001
Crude protein	527^{c}	523^{c}	545^{b}	580^{a}	7.83	0.001
NDFom	358^{c}	357^{c}	373^{b}	408^{a}	5.76	0.001
ADFom	307^{b}	259^{c}	274^{bc}	336^{a}	9.86	0.001
DE (MJ/kg DM)	7.79^{c}	7.80^{c}	7.96^{b}	8.23^{a}	0.02	0.001

Abbreviations: ADFom, ash-free acid detergent fiber; DE, digestible energy; NDFom, ash-free neutral detergent fiber; SEM, standard error of the means.
[a, b, bc, c] Means in the same row with different superscript letters are different ($P < .05$).
[a] B25, 25% of concentrate containing whole grains; A20, 20% of concentrate containing processed grains; A25, 25% of concentrate containing processed grains; A30, 30% of concentrate containing processed grains.

Forage intake, total intake time as well as chewing and swallowing rate were not affected by experimental treatments ($P > .05$) (Table 2). In this study, horses consumed 6.93, 6.50 and 6.10 kg of forage in treatments containing 80, 75 and 70% of forage, respectively, which is why the intake time had a decreasing trend. Because with the increase of NDF, ADF and lignin, the duration of feed consumption increases [25]. Also, the amount of physical effective fiber stimulates chewing and saliva secretion. Therefore, the highest chewing rate was observed in A20 treatment as a result of higher amounts of cell wall fibers in this treatment. Since all horses received the same forage, the observed numerical differences are only related to the amount of forage fed.

3.2. Digestibility of Nutrients and Fecal pH

By increasing the concentrate level up to 30%, the digestibility coefficients of DM, OM, CP, NDFom, ADFom and DE increased. The highest digestibility coefficients were observed in A30 treatment ($P < .05$) (Table 3). As can be seen from Table 3, no significant difference was observed between A20 and B25 treatments (except for ADFom). Also, comparison of two treatments A25 and B25 showed that the digestibility of OM, CP, NDFom and DE in A25 treatment significantly increased compared to B25 ($P < 0.05$). The increase in digestibility in the present study is probably due to the decrease in forage levels, because naturally with increasing levels of concentrate in the diet digestibility increases due to the decrease in the amount of cell wall fibers. As can be seen in Table 1, with increasing concentrate, the amount of NDFom and ADFom decreased by 40 and 34 g/kg, respectively. According to our results, the digestibility of DM, OM, CP and energy improved with increas-

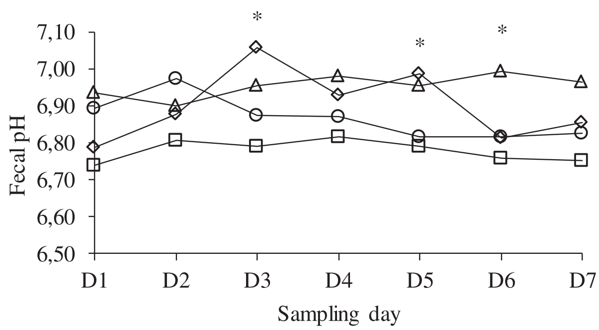

Fig. 1. Effect of different level of concentrate and processing on fecal pH. ◇ (B25), 25% of concentrate containing whole grains; △ (A20), 20% of concentrate containing processed grains; ○ (A25), 25% of concentrate containing processed grains; □ (A30), 30% of concentrate containing processed grains. * Indicates a differ significantly ($P<$ .05) (treatment effect).

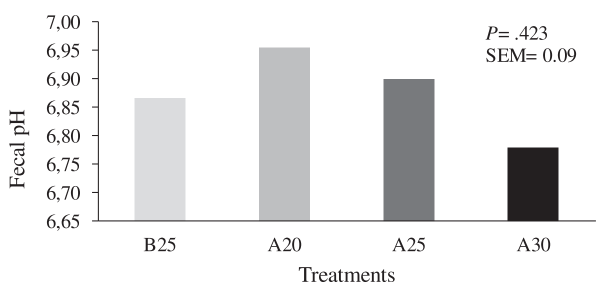

Fig. 2. Effect of different level of concentrate and processing on mean fecal pH (day 1-7). B25, 25% of concentrate containing whole grains; A20, 20% of concentrate containing processed grains; A25, 25% of concentrate containing processed grains; A30, 30% of concentrate containing processed grains. Data were analysed as repeated measurement and this figure we used only the main effect of treatment. Effects are considered significantly different if $P<$ .05. SEM, standard error of the means.

ing concentrate to forage ratio [26]. Contrary to the results of the present study, in an experiment with increasing the level of concentrate from 35 to 50%, digestibility was reduced, which is due to the high level of concentrate in the diet (50%) and its adverse effect on large intestinal microbes and the possibility of subacute acidosis [21]. In the experiment of Karlsson et al. (2000) [26], digestibility of cell wall fibers improved by increasing the concentration up to 20%, however, by increasing the level of concentrate to more than 20% the digestibility of cell wall fibers decreased, due to the negative effect of starch fermentation in the large intestinal on fiber fermentation.

Mechanical processing increases the pre-cecal digestibility of corn starch by more than 15% [27]. Processed grains and starches are digested more efficiently in the gastrointestinal tract than whole and unprocessed grains [11,28], which was consistent with our results. Researchers have reported that grain processing increases the digestibility of cereal starches such as corn, oats, barley and wheat by more than 96% [28]. Therefore, in our study, in A20 treatment, despite the use of less grains, digestibility was almost similar to B25 treatment. Several studies have also reported increased pre-cecal digestibility as a result of micronization [3], extrusion [29] and flocculation [30].

The effect of experimental treatments on daily fecal pH changes as well as mean pH is shown in Figs. 1 and 2. Only on days 3, 5 and 6, a significant difference was observed between the treatments ($P<$ .05). In our study, as shown in Fig. 2, although the mean pH had a decreasing trend with increasing concentrate level, but no significant difference was observed between the treatments ($P>$0.05) and the pH was almost in the neutral range. The mean pH was almost similar between treatments A25 and B25 and processing had no significant effect ($P>$ .05). This indicates that most

Table 4
Effect of different level of concentrate and processing on blood metabolites.

Item	Diets[a]				SEM	*P* Value
	B25	A20	A25	A30		
Total Protein (g/dL)	5.59	5.80	5.33	5.69	0.31	0.200
Glucose (mg/dL)	97.3[b]	97.2[b]	97.4[b]	100[a]	1.36	0.001
Triglycerides (mg/dL)	26.2	26.1	26.2	26.4	0.68	0.541
Cholesterol (mg/dL)	141	139	142	143	4.40	0.071
LDL (mg/dL)	82.1	81.6	80.8	84.1	2.49	0.321

Abbreviations: LDL, low-density lipoprotein; SEM, standard error of the means.
[a,b] Means in the same row with different superscript letters are different ($P<$ .05). Data were analysed as repeated measurement and this table we used only the main effect of treatment.
[a] B25, 25% of concentrate containing whole grains; A20, 20% of concentrate containing processed grains; A25, 25% of concentrate containing processed grains; A30, 30% of concentrate containing processed grains.

of starch is digested and absorbed in the small intestine. On the other hand, it seems that the use of levels less than 50% has low effect on the rate of starch passage to the large intestine. Therefore, in our study, no difference was observed as a result of increasing the level up to 30% and also processing. However, large amounts of grains in the diet increases the possibility of it reaching the large intestine and thus reduces its energy importance and pH. This is usually seen in diets with more than 50% concentrate. The energy value of fermented grains is about 75% of the energy value of cereals, which are digested and absorbed in the small intestine [31]. Passage of high amounts of starch from the small intestine to the large intestine increases lactic acid production in this area. Increased lactic acid is associated with a decrease in gastrointestinal pH. It has been reported in a study that stable horses that received high concentrate diets compared to horses in the pasture had a fecal pH of 6.3 compared to 6.9, respectively [32]. Low pH of the large intestine may adversely affect digestibility and gastrointestinal health. Low pH affects the binding of microbes to feed particles, and also reduces the ability of microbes to break down plant cell wall [33].

3.3. Blood metabolites

The effect of experimental treatments on blood metabolites is presented in Table 4. Total protein, triglyceride, cholesterol and LDL concentrations were not affected by experimental treatments ($P>$ .05). The lack of effect of experimental treatments on total protein concentration indicated an improvement in nutritional status and no use of amino acids and domination to obtain energy. Also, the percentage of protein in different treatments was the same and ranged from 11.5 to 11.8% of the diets. The observed changes in triglyceride and cholesterol concentrations were within the normal range of triglyceride and cholesterol concentrations in horses [34]. The highest glucose concentration was observed in treatment A30 ($P<$0.05). The increase in glucose concentration in A30 treatment was due to the high volume of concentrate used in this treatment, and also increase the digestibility in this treatment [35]. Although in a study similar to our results (comparison A25 and B25), processing had no effect on serum glucose concentration [36], but overall mechanical or thermal processing can increase availability of starch granules for enzymatic digestion in the small intestine [36].

4. Conclusions

In conclusion, the use of processed grains in concentrate A compared to concentrate B had led to improvement of the nutrient digestibility. So that in A20 treatment with 5% less concentrate, no difference was observed in digestibility with B25 treatment. Also, the comparison of two treatments A25 and B25 (equal level of concentrate) showed that digestibility increased in treatment A25 compared to B25. The use of 30% concentrate containing processed grains (A30) improved digestion without adversely affecting feeding behavior and fecal pH. The lack of significant effects on fecal pH, blood parameters, and feeding behavior may have been due to the use of levels less than 50% concentrate.

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