Fecal volatile fatty acids and blood metabolites in the Turkmen horse associated with type and source of cereal grains

Ehsan Direkvandi\textsuperscript{a} and Rohallah Kamyab Kalantari\textsuperscript{b}

\textsuperscript{a}Department of Animal Science, Faculty of Animal and Food Science, Khuzestan Ramin Agriculture and Natural Resources University, Mollasani, Iran; \textsuperscript{b}Department of Animal Science, Faculty of Agriculture, Guilan University, Rasht, Iran

\section*{ABSTRACT}
In this experiment, corn – oat, barley – wheat and barley – oats were used as a source of starch in 1, 2 and 3 rations respectively. The ratio of concentrate to forage in all treatments were 35:65. The results showed the concentration of total protein and cholesterol were not affected by the type and source of cereal grains ($P > 0.05$). Serum glucose and triglyceride concentration significantly increased in corn – oats treatment ($P < 0.05$). Also, in this experiment total volatile fatty acids (VFA), acetate (A), propionate (P) and A/P concentrations were not affected by the type of cereal grains ($P > 0.05$), but valerate and butyrate concentrations significantly increased in barley – wheat treatment ($P < 0.05$). On the other hand, concentrations of isobutyrate and isovalerate significantly increased in corn – oats treatment ($P < 0.05$). Fecal pH, were not significantly different between corn – oats and barley – oats treatments ($P > 0.05$), although the lowest pH was observed in barley – wheat treatment (6.02). According to the results, treatment 1 (corn – oats) improved fecal VFA and blood metabolites in the comparison of the other treatments.

1. Introduction
Horses are grazing animal. They are eating and foraging between 12.5 ± 2.5 h per 24 h (Ellis 2010). Forage is the main source of energy, while the horses grazing in the pasture. Because of different structure and function of the digestive tract in the horse than other monogastric animals (Frape 2004), fermentation of plants cell walls in the large intestine leading to reduce the use of forage nutrients (Karlsson et al. 2000) and increased gastrointestinal diseases (i.e. colic and laminitis) is a result of a change in the hindgut microflora (Bailey et al. 2000) and increased gastrointestinal diseases (i.e. colic and laminitis) is a result of a change in the hindgut microflora (Bailey et al. 2000) and increased gastrointestinal diseases (i.e. colic and laminitis) is a result of a change in the hindgut microflora (Bailey et al. 2000) and increased gastrointestinal diseases (i.e. colic and laminitis) is a result of a change in the hindgut microflora (Bailey et al. 2000) and increased gastrointestinal diseases (i.e. colic and laminitis) is a result of a change in the hindgut microflora (Bailey et al. 2000) and increased gastrointestinal diseases (i.e. colic and laminitis) is a result of a change in the hindgut microflora (Bailey et al. 2000) and increased gastrointestinal diseases (i.e. colic and laminitis) is a result of a change in the hindgut microflora (Bailey et al. 2000). The digestion of NDF and ADF decreased when forage of ration was replaced with barley, as a result, cellulose degrading bacteria in the large intestine decreases (Drogoul et al. 2001; Julliand et al. 2001). In another study, when 60% or more of ration forage was replaced with oat, digestibility of cellulose and ADF was reduced (Drogoul et al. 2001), so the production of VFA falls down too (Kohnke et al. 1999). The digestion of NDF and ADF decreased when forage of ration was replaced with barely, as a result, cellulose degrading bacteria in the large intestine decreases (Drogoul et al. 2001; Julliand et al. 2001). In another study, when 60% or more of ration forage was replaced with oat, digestibility of cellulose and ADF was reduced. In this situation, Kohnke et al. (1999) reported that absorption of VFA was reduced. These changes in the large intestine leading to reduce the use of forage nutrients (Karlsson et al. 2000) and increased gastrointestinal diseases (i.e. colic and laminitis) is a result of a change in the hindgut microflora (Bailey et al. 2004). There are different management in the horse industry and also different uses of the horse in human life and increase the use of cereal grains in the horse diet. In this study, the object of...
is to research the effects of types and sources of cereal grains on fecal VFA and blood metabolites in Turkmen horses.

2. Materials and methods

2.1. Animals and diets

Three types of diet with different sources of starch were used in the experiment. They contained corn-oats (CO) as ration No. 1, barley-wheat (BW) as ration No. 2 and barley-oats (BO) as ration No. 3. The other part of these rations consisted of forage and wheat straw, which was mixed in a ratio of 2 to 1 as a forage part. The rations were formulated according to NRC (2007) that they are shown in Table 1. Also, the chemical compositions are shown in Table 2. The diet was based on 35% concentrate and 65% forage.

Twelve adult Turkmen horses (2 stallions and 10 non-pregnant mares) with an average age of 6 ± 1.8 years were used. Before the experiment, the horses were treated against both external and internal parasites. The horses’ average weights were estimated (425 ± 44 kg). They were placed in individual stalls with a straw and sawdust bedding. The adaptation period lasted 21 days and sampling period lasted 7 days. During the experiment, water and salt/mineral licks were freely available at all times. In this research, feeding of concentrate and forage were performed at hours 07, 13, 19 and 01 for concentrate and hours 07:30, 13:30, 19:30 and 01:30 for forage. Horse’s weights were calculated according to Hall (1971), as follows:

\[
\text{Body weight (kg)} = \left(\text{Chest width (cm)}\right)^2 \times \left(\text{Body length (cm)}\right)/11880.
\]

2.2. Fecal fluid samples collection and analysis

Fresh fecal compressed after excretion and pH of fecal fluid was measured by using a portable pH metre (Muller 2012). For the analysis of VFA, 1 mL of fecal fluid was added to 0.25 mL of an acid solution containing 200 mL/L of orthophosphoric acid and 20 mmol/L 2-ethyl-butyric acid and was stored at −20°C until analysis. The concentration of VFA was measured by Gas Chromatography and acid ethyl butyrate was used as an internal standard (Stewart and Duncan 1985).

| Ingredients         | CO   | BW   | BO   |
|---------------------|------|------|------|
| Alfalfa hay         | 429  | 429  | 429  |
| Wheat straw         | 221  | 221  | 221  |
| Micronized barley   | –    | 187  | 199.5|
| Micronized Wheat    | –    | 82.5 | –    |
| Extruded corn       | 192  | –    | –    |
| Oats                | 77.5 | –    | 70   |
| Roasted soybeans    | 63   | 63   | 63   |
| Vitamin and mineral supplements | 7    | 7    | 7    |
| Vegetable oil mixture | 10.5 | 10.5 | 10.5 |

| Chemical composition (Forage and Concentrate) | CO | BW | BO | SEM | P-value |
|-----------------------------------------------|----|----|----|-----|---------|
| Dry matter                                    | 955| 951| 948| 45.0| 0.604   |
| Organic matter                                | 879| 873| 869| 33.3| 0.183   |
| Crude protein                                 | 117| 113| 114| 24.5| 0.484   |
| Ether extract                                  | 38 | 39 | 41 | 11.8| 0.841   |
| Ash                                           | 80 | 78 | 81 | 1.50| 0.983   |
| NDForm                                        | 464| 475| 485| 32.9| 0.118   |
| ADFom                                         | 315| 307| 313| 23.2| 0.384   |
| Lignin                                        | 72.5 | 72.6 | 73.3 | 11.8 | 0.740 |
| Hemicellulose                                 | 157 | 161 | 169 | 7.49 | 0.527   |
| Digestible energy (MJ/kg DM)                  | 10.9| 10.3| 10.1| 1.29| 0.492   |

CO = corn-oats; BW = barley-wheat; BO = barley-oats; SEM = standard error of means; NDForm = ash-free neutral detergent fibre; ADFom = ash-free acid detergent fibre.

2.3. Blood sampling and analysis

In day 27 day of experiment period, blood was collected 8 times during 8 h. Blood was sampled at 06:30, 07:30, 08:30, 09:30, 10:30, 11:30, 12:30 and 13:30, respectively. Approximately 10 mL of blood sample was collected from jugular veins using tubes containing an anticoagulant. The blood samples were allowed to clot for 24 h at 4°C and centrifuged (700 x g for 20 min at 4°C), and the serum was separated and frozen at −20°C until measuring biochemical parameters. Glucose, triglycerides, total protein, albumin, creatinine, blood urea nitrogen (BUN), total bilirubin, total cholesterol, low-density lipoprotein (LDL), high-density lipoproteins (HDL) and very-low-density lipoprotein (VLDL) were determined by using enzymatic methods and spectrophotometer (Jenway, Genova, UK). In this experiment, ZiestChem’s (Tehran, Iran) kits were used.

2.4. Feed analyses

Before the chemical analysis, feed samples were oven-dried at 55°C for 48 h and then were passed through a 1 mm sieve (Wiley mill, Swedesboro, USA). Dry matter, ash, organic matter, crude protein and ether extract were analyzed according to the methods of AOAC (1999). Then, ash-free neutral detergent fibre (NDForm) and ash-free acid detergent fibre (ADFom) were analyzed using the method proposed by Van Soest et al. (1991). Lignin was determined by solubilization of cellulose with a sulfuric acid. The amount of cellulose (ADF-ADL) and hemi-cellulose (NDF-ADF) was calculated by different methods. Digestible energy of the diet was calculated as follows (NRC 2007):

\[
\text{DE (Mcal/kg DM)} = 2.118 + (0.01218 \times \text{CP}) - (0.00937 \times \text{ADF}) - (0.00383 \times (\text{NDF} - \text{ADF})) + (0.04718 \times \text{EE}) + (0.02035 \times \text{NSC}) - (0.0263 \times \text{ASH}).
\]

2.5. Statistical analysis

The data obtained from VFA was analyzed as a randomized complete balanced design using General Linear Models (GLM) procedure on SAS software (SAS 2008), which is based on the following statistical model:

\[
Y_{ij} = \mu + T_i + e_{ij}
\]
Where $Y_{ij}$ is observation (VFA), $\mu$ is the general mean, $T_i$ is the effect of type’s cereal grains and $e_{ij}$ is the standard error of term. Also, data of blood metabolite were analyzed as repeated measurements using the MIXED procedures of SAS (2008), based on the statistical model:

$$Y_{ijk} = \mu + T_i + H_j + (TH)_{ij} + e_{ijk}$$

where $Y_{ijk}$ is observation (blood metabolite), $\mu$ is the general mean, $T_i$ is the effect of feeding frequency, $H_j$ is the effect of sampling hours, $(TH)_{ij}$ is interactions between the effect of feeding frequency and sampling hours and $e_{ijk}$ is the standard error of term. All data were analyzed to determine linear (Lin) and quadratic (Q) responses to feeding frequency using orthogonal contrasts of SAS.

### 3. Results

#### 3.1. Serum biochemical parameters

The results of the effect of mixture sources of cereal grains on blood metabolites are shown in Table 3 and Figure 1. As can be seen, serum glucose and triglyceride concentration significantly increased in treatment CO ($P < 0.05$). A peak serum glucose and triglyceride concentration was observed in CO treatment (99.5 and 25.44 mg/dL at 07:30 and 08:30, respectively), whereas the lowest concentration of glucose and triglyceride was observed in the BW treatment (88.7 and 25.44 mg/dL at 09:30 and 07:30, respectively) (Figure 1).

Serum total bilirubin concentration significantly increased in treatment BW (1.54 mg/dL) ($P < 0.05$). However, between treatment CO and BO total bilirubin was not affected by experimental treatments. Although, the highest concentrations of total protein, BUN, total cholesterol and LDL were observed in BW treatment (6.41 g/dL, 23.0, 152 and 78.8 mg/dL, respectively). But, the concentration of total protein, BUN, total cholesterol and LDL were not affected by mixture sources of cereal grains ($P > 0.05$). The highest concentrations albumin, creatinine and HDL were observed in treatment BO (3.65 g/dL, 1.41 and 67.2 mg/dL, respectively). However, these parameters and VLDL was not affected by mixture sources of cereal grains ($P > 0.05$).

#### 3.2. Fecal volatile fatty acids

The results of the effect of mixture sources of cereal grains on fecal VFA and pH are shown in Table 4. As can be seen, in the experiment fecal pH there were no significant differences between treatments CO and BO ($P > 0.05$), although the lowest pH was observed in the treatment BW (6.02) ($P < 0.05$). The highest amount of total VFA, acetate and propionate were observed in treatment CO (respectively 95.3, 58.9 and 17.9 mmol/L), however, total VFA, acetate, propionate and A/P concentrations were not affected by mixture source of cereal grains ($P > 0.05$). Valerate and butyrate concentrations significantly increased in treatment BW ($P < 0.05$) (respectively 1.53 and 13.6 mmol/L). On the other hand, concentrations of isobutyrate and isovalerate significantly increased in treatment CO respectively with average 2.33 and 4.93 mmol/L, and the lowest amount of isobutyrate and isovalerate was observed in treatment BO respectively with average 1.83 and 3.96 mmol/L ($P < 0.05$). Also, the results showed that the concentration of acetate, propionate, butyrate, valerate and A/P was intermediate for BO treatment.

#### 4. Discussion

##### 4.1. Serum biochemical parameters

In our study, a mixture of corn and oats increased the plasma glucose concentrations compared to the other treatments (94.6 mg/dL). Similar with our results, Vervuert and Coenen (2005) was reported that the most of the peak plasma glucose concentration 120.6±23.4, 118.8±14.4 and 109.8±9 mg/dL for unprocessed oats, corn and barley, respectively. Because the coefficient digestibility of corn and oats starch is about 97% and 96% respectively, and the value of corn and oats starch is respectively 97% and 96.7% (Arnold et al. 1981). Also, in ponies feed with oats leads to increased blood glucose compared with the diets containing barley and corn (Vervuert and Coenen 2005). Because oats starch digestibility is more than corn and barley in prececal (Kienzle et al. 1992; Potter et al. 1992; Meyer et al. 1995).

Blood NH$_3$ and urea are influenced by endogenous and exogenous sources (i.e. catabolism of excess dietary AA or

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**Table 3. Effect of mixture sources of cereal grains on blood metabolites.**

| Parameters      | CO       | BW       | BO       | SEM      | L   | Q    | H     | T × H |
|-----------------|----------|----------|----------|----------|-----|------|-------|-------|
| Proteins (g/dL) | 6.37     | 6.41     | 6.35     | 0.16     | 0.71| 0.70 | 0.01  | 0.01  |
| Albumin         | 3.30     | 3.21     | 3.65     | 0.09     | 0.05| 0.22 | 0.01  | 0.14  |
| Metabolites (mg/dL) |        |          |          |          |     |      |       |       |
| Creatinine      | 1.39     | 1.38     | 1.41     | 0.07     | 0.06| 0.12 | 0.01  | 0.03  |
| Glucose         | 94.6$^a$ | 92.4$^b$ | 93.1$^{a,b}$ | 0.61     | 0.05| 0.52 | 0.01  | 0.79  |
| BUN             | 21.7     | 23.0     | 22.1     | 0.72     | 0.11| 0.13 | 0.01  | 0.06  |
| Total bilirubin | 1.43$^a$ | 1.54$^b$ | 1.40$^b$ | 0.04     | 0.02| 0.43 | 0.01  | 0.05  |
| Triglycerides   | 26.2$^a$ | 26.0$^b$ | 26.1$^{a,b}$ | 0.52     | 0.03| 0.55 | 0.01  | 0.43  |
| Total cholesterol | 151     | 152      | 150      | 0.57     | 0.89| 0.06 | 0.01  | 0.01  |
| LDL             | 76.5     | 78.7     | 75.5     | 0.91     | 0.05| 0.35 | 0.01  | 0.30  |
| HDL             | 66.8     | 66.5     | 67.2     | 0.33     | 0.56| 0.08 | 0.01  | 0.09  |
| VLDL            | 8.61     | 8.51     | 7.26     | 0.08     | 0.64| 0.35 | 0.01  | 0.42  |

CO = corn-oats; BW = barley-wheat; BO = barley-oats; SEM = standard error of means; BUN = blood urea nitrogen; LDL = low-density lipoprotein; HDL = high-density lipoprotein; VLDL = very-low-density lipoprotein.

$^1$T: effect of mixture sources of grain; H: effect of sampling hours; L: linear effect of mixture sources of grain; Q: quadratic effect.
In the current experiment, serum total protein and BUN concentration were increased in treatment BW, but serum total protein and BUN concentration were not affected by experimental diets. Given that the level of CP in the diets was almost the same (11.7, 11.3 and 11.4 mg/dL for CO, BW and BO, respectively), BUN was not affected by the mixture sources of cereal grains (Hussein et al. 2004). Ott (2001) reported that as a result of consumption dietary with high CP, BUN increased, however, NH3-N was not affected by dietary with high CP.

Plasma creatinine levels are independent of protein and water intake, urine production rate and exercise. Therefore, since its production rate is constant, high levels of plasma creatinine indicates kidney disease. However, the observed changes in serum creatinine concentrations, it was in the normal range of serum creatinine concentrations in horses. Although the highest concentration of serum creatinine was observed in BO treatment (1.41 mg/dL). The high levels of unconjugated bilirubin indicated that hemoglobin are too much destroyed. Although in this experiment total bilirubin significantly increased in treatment BW (1.54 mg/dL), but this value was in the normal range of total bilirubin mature in horses (0.1–1.9 mg/dL) (Barton & LeRoy 2007).

The observed changes in serum triglyceride and cholesterol concentrations, it was in the normal range of serum triglyceride and cholesterol concentrations in horses (Radostits et al. 2006). Increasing the level of serum triglyceride concentration greater than 500 mg/dL is indicating of hyperlipidemia (Van Weyenberg et al. 2007). High concentrations of triglycerides in the horses are usually due to metabolic syndromes (equine Cushings disease) or negative energy balance. Therefore, as shown in Figure 1, serum triglyceride concentration decreased after feeding and increased between two meals during energy shortages. But changes in the serum triglyceride concentration have not clinical importance because serum triglyceride concentrations are lower than 500 mg/dL. Watson et al. (1991) reported that LDL makes up 15% of blood lipoprotein in the horse. LDL contains 42% cholesterol while high-density lipoprotein (HDL) contains 21%–36% of cholesterol. LDL is made by hepatic lipase of very low-density lipoprotein (VLDL) and is responsible for cholesterol transfer to peripheral tissues. High serum cholesterol (152 mg/dl) in the treatment of BW leads to an increase in the serum concentration of cholesterol-rich lipoproteins such as LDL (78.7 mg/dl).

### Table 4. Effect of mixture sources of cereal grains on fecal VFA.

| Fecal parameters | CO  | BW  | BO  | SEM | L   | Q   |
|------------------|-----|-----|-----|-----|-----|-----|
| Total VFA mmol/L | 95.3| 95.2| 94.4| 1.47| 0.865| 0.451|
| VFA mmol/L Acetate (A) | 58.9| 55.8| 57.2| 1.19| 0.115| 0.921|
| Propionate (P) | 17.9| 15.9| 16.2| 0.91| 0.015| 0.889|
| Butyrate | 8.37b| 13.6a| 11.2ab| 1.11| 0.005| 0.889|
| Valerate | 1.35b| 1.53a| 1.45b| 0.04| 0.017| 0.758|
| Isobutyrate | 2.33a| 2.18b| 1.83b| 0.09| 0.268| 0.007|
| Isovalerate | 4.93a| 4.34b| 3.96c| 0.11| 0.005| 0.001|
| A/P | 3.29| 3.51| 3.48| 0.19| 0.434| 0.725|
| pH | 6.41a| 6.02b| 6.29ab| 0.05| 0.008| 0.792|

CO = corn-oats; BW = barley-wheat; BO = barley-oats; SEM = standard error of means.

1L: linear effect of mixture sources of grain; Q: quadratic effect of mixture sources of grain.

**Figure 1.** Serum biochemical parameters changes during the 8 h. A = Serum Total Protein; B = Serum glucose; C = Serum triglycerides; D = Serum cholesterol.
4.2. Fecal volatile fatty acids

In our experiment, probably the reduction of pH in BW treatment is being for decreasing of starch digestive ability in the small intestine rather than CO treatment and increasing of the starch crossing into the large intestine (Harper 1979). CO have more digestibility than BW and BO mixture (Direkvandi et al. 2015), in their experiment reported digestibility of dry matter rations containing CO, BW and BO as sources of starch were 509, 473 and 506 g/kg DM, respectively. The barley rolled leads to decreased pH from 6.5 to 6.26 in cecal contents (MacLean et al. 2000; Julliand et al. 2001). Also, Hussein et al. (2004) reported that the use of barley as sources of starch leads to a reduced of pH compared to corn, oats and naked oats. In this experiment, the highest concentration (mmol/L) of acetate and propionate was observed in the treatment CO (Hussein et al. 2004). In the study by Julliand et al. (2001) the diet containing barley leads to decreased acetate and that of increased the propionate concentration. Acetate is the dominant fatty acid in the large intestine rather than CO treatment and increasing of the starch in horses: 2—effects of three hay: grain ratios on digesta passage rate and digestibility in ponies. J Equine Vet Sci. 21:487–491. Ellis AD. 2010. Biological basis of behaviour and feed intake. In: Ellis AD, Longland AC, Coenen M, Miraglia N, editors. The impact of nutrition on the health and welfare of horses. Wageningen: Wageningen Academic Publishers; p. 53–74. EAAP Publication No. 128. Frape D. 2004. Equine nutrition and feeding. 3rd ed. Essex,UK: Blackwell, Oxford Publishing.

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