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Nutritional value of barley, triticale and oat grain varieties based on *in vitro* gas production and fermentation parameters, and Cornell Net Carbohydrate and Protein System

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Abstract This research was conducted to evaluate the nutritional value of different varieties of barley (*Turkmen*, *Reyhan* and *Gorgan*), triticale (*Juvanilo*, *Rondo* and *Massa*) and oat (*Wild* and *Canadian*) grains. The chemical composition of the samples was determined using the standard methods of AOAC. Gas production (GP) test was performed to estimate the *in vitro* fermentation parameters. *In vitro* digestibility was determined by the batch culture procedure. The carbohydrate fractions of the Cornell Net Carbohydrate and Protein System (CNCPS) were also measured. Crude protein (CP) content was more in triticale and barley than oat grains (P<0.01). Triticale grains had higher contents of neutral detergent fiber (NDF), acid detergent fiber (ADF), and lower lignin compared to barley and oat grains (P<0.01). The amounts of GP potential and fermentation parameters were higher in triticale and barley than oat grains (P<0.01). Partitioning factor (PF, P<0.01), microbial biomass (MB, P<0.05) and efficiency of microbial biomass (EMB P<0.01) were more in different varieties of triticale and barley than oat varieties. According to CNCPS, carbohydrate portions and total carbohydrate content in triticale and barley grains were higher than in oat grains (P<0.01). The highest and lowest contents of none- fiber carbohydrates (NFC), starch, and soluble sugars were observed in triticale and oat varieties, respectively. Totally, triticale and barley grains had a better nutritional value than oat grains. Triticale grains were as good as barley grains in most aspects, while being superior in some traits.

Keywords: *in vitro* digestibility, ruminal fermentation parameters, CNCPS model, grain variety

Introduction

Cereal grains i.e., maize, barley, wheat, triticale, rye, oats and sorghum, are important components of the ruminant diets. The aim of feeding grains to ruminants is their high starch content enabling high energy density in diets (Aboagye et al., 2021).

Barley, one of the most important cereals in livestock feeding, can play a major role in rumen microbial biomass production and supply of maintenance requirements, milk yield and reproduction (Church, 1991; Giraldo et al., 2019). Triticale is rapidly expanding in several production systems. Its ability to produce higher biomass and grain yield compared with other cereals over a wide range of soil and climatic conditions has enhanced its adaption in many countries (Hinojosa et al., 2002; Szempliński et al., 2021). Oat is ranked sixth in the world among cereals. It is widely used as animal feed throughout the world. Oat is well adapted to specific environmental conditions (soil types and low fertility) and various types can easily adapt to different climatic conditions (Hoffmann, 1995; Libera et al., 2021).

Nutrient content of cereal grain varies due to some factors such as variety and environment. Grains differ in their starch content and effective rumen degradability (ERD). The digestibility and utilization of starch is instrumental for the nutritive value of grains in ruminants (Humer and Zebeli, 2017).

Accurate estimate of the nutritional value of cereal grains is an important step in meeting animal nutritional requirements (Lee et al., 2016). *In-vitro*, *in vivo* and *in situ* methods are used to evaluate the nutritional value of the feedstuffs.

Some *in vitro* methods like Tilly and Terrey (1963) and the innovative method of Menke et al. (1979) using ruminal fluid, as well as other none ruminal fluid methods using enzymes have provided the possibility to evaluate the ruminal fermentation of samples. Using these methods, the potential digestibility process of the feed can be estimated (Valentin et al., 1999). One of the reliable *in vitro* methods for determining the feedstuff nutritional value is Cornell Net Carbohydrate and Protein System (CNCPS). This system is capable of predicting the nutrient requirements of the animal, feedstuff utilization rate, and excretion rate of nutrients in production conditions, considering the basic principles like rumen performance, microbial growth, feedstuff digestion, passage rate, and animal physiology (Tylutki et al., 2008).

In ruminant nutrition, ruminal fermentability of cereal grains is an important index in identifying their nutritional value. Such that it can affect the place of starch digestion in gastrointestinal tract and providing microbial protein and has a significant effect on rumen environment. These effects can significantly affect feed consumption in livestock production through changing the pH, production of volatile fatty acids (VFAs), and cellulose degradation in the rumen (Zamani-Amirabad et al., 2015).

The aim of this study was to compare the nutritional value of different varieties of barley, triticale and oat grains using chemical analysis, gas production (GP) test, *in vitro* digestibility trial and CNCPS model.

Materials and methods

Sample preparation

Varieties of triticale (*Juvanilo*, *Rondo* and *Massa*), barley (*Torkman*, *Reyhan* and *Gorgan*) and oat (*Wild* and *Canadian*) grains were harvested from the Agriculture and Natural Resources Center of Gonbad Kavous, Golestan Province, Iran. Then the samples were milled - to pass 1 mm sieve and evaluated using the following procedures.

Chemical analysis

Chemical composition of the samples including dry matter (DM), ash, ether extract (EE), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) was determined according to the standard methods of AOAC (2005).

Animals and diet

Three rumen fistulated Dallagh sheep (45 ± 2.5 kg) were used for *in situ* and *in vitro* trials. The animals were fed a total mixed ration (TMR) according to the standard of the experiments on maintenance level (Table 1; NRC, 2001) twice daily in equal meals at 08:00 h and 17:00 h and had free access to drinking water.

Table 1. Ingredients and chemical composition of diet

Ingredient	g/kg of total diet DM
Alfalfa hay	200
Corn silage	500
Barley grain	120
Wheat barn	175
Mineral–vitamin premix[1]	5
Chemical composition (**DM basis**)	
Metabolizable energy (MJ/kg)	2.48
Crude protein (%)	10.90
Calcium (%)	0.73
Phosphorus (%)	0.35

[1]Mineral–vitamin premix contained per Kg DM: Ca, 170 g; P, 60 g; Mg, 50 g; Fe, 3 g; Cu, 2 g; Mn, 4 g; Zn, 6 g; Co, 0.1 g; I, 0.25 g; Se, 0.03 g; NaCl, 250 g; vitamin A, 300000 IU; vitamin D3, 60000 IU; vitamin E, 0.5 g

Gas production test

Gas production was measured according to the standard method (Menke et al., 1979). Rumen fluid was collected through rumen fistula before the morning feeding and transferred to the laboratory immediately. Artificial saliva and the filtered rumen fluid were poured into the special balloon at a ratio of 2:1 (volume of artificial saliva to rumen fluid). Then, CO_2 gas was injected into the balloon and kept in a 39°C water bath. Finally, 30 mL of the mixture was poured into a glass vials containing 200 mg of the sample. The vials were sealed with rubber caps covered with aluminum foil, and put in a 39°C water bath immediately. During this step, the glass vials were shaken at definite intervals. The volume of GP was calculated cumulatively at the time intervals of 2, 4, 6, 8, 12, 24, 36, 48, 72, and 96 h after the incubation. The GP parameters were estimated according to following equation by SAS software (Ørskov and MacDonald, 1979):

$$y = b\,(1 - e^{-ct})$$

where, ‘y’ is gas produced at time t (mL/g of DM), ‘b’ is GP from the fermentable insoluble fraction (mL), ‘e’ is Euler's number, ‘c’ is GP rate for b fraction (mL/ h), and ‘t’ is the incubation time (h).

Metabolizable energy (ME) organic matter digestibility (OMD), and short chain fatty acids (SCFAs) of the samples were estimated using Getachew et al. (1998) and Menke and Steingass (1988) equations:

$$ME = 2.20 + 0.136\ GP + 0.057\ CP + 0.0029\ CF$$

$$NE = -0.36 + 0.1491\ GP + 0.0054\ CP + 0.0139\ EE - 0.0054XA$$

$$OMD = 14.88 + 0.889\ GP + 0.45\ CP + 0.0651\ XA$$

$$SCFAs = 0.0222\ GP - 0.00425$$

where, ME is metabolizable energy (MJ/kg of DM), GP is net gas production after 24 h incubation (mL/200 mg of DM), CP is crude protein (% of DM), CF is crude fiber (% of DM), EE is ether extract (% of DM), OMD is organic matter digestibility (%), SCFAs is short chain fatty acids (mmol/200 mg DM), and XA is Ash amount (% of DM).

In vitro digestibility

Digestibility determination of the samples was done using the batch culture method (Theodorou et al., 1994). The samples were first grinded to 1 mm and then dried. Ruminal fluid was collected by fistula before the morning feeding. Artificial saliva was prepared according to Menke et al. (1979). A portion (500 mg) of each sample was poured in each glass vial and 50 mL of a combination of artificial saliva and ruminal fluid with a ratio of 2 to 1 was added to each vial. Then, CO_2 gas was blown into the glass vial for 10 seconds, and the vials were then sealed by a rubber cap and aluminum cover. The vials were then put in a warm water bath at 39 °C. After 24 hours, the vials were removed from the water bath and transferred to the ice container. The vial contents were filtered through a filter paper and the indigested contents were separated from the liquid phase and dried at 60°C for 48 h; the apparent digestibility of the sample was then calculated. The pH of the liquid phase of samples was measured.

Rumen fermentation parameters

The ammonia nitrogen (NH_3-N) content of the sample was determined using the phenol-hypochlorite method (Broderick and Kang, 1980). A spectrophotometer device at 630 nm wave length was used to read the light absorption. Production of the microbial biomass was calculated using the following equation (Makkar, 2010).

$$MB = GP \times (PF - 2.2)$$

where, MB is microbial biomass (mg/g of DM), GP is net gas production after 24 h incubation (mL/g of DM), and PF is partitioning factor (mg/mL); PF is the mg of digested real organic matter (OM) to mL of GP. Microbial biomass efficiency (EMB) was calculated as MB divided by the fermentable real OM at the end of incubation time (24 h).

Carbohydrate parameters based on Cornell Net Carbohydrate- Protein System (CNCPS)

The parameters of carbohydrate portions were determined by CNCPS and calculated by Tylutki et al. (2008) method (Table 2). The methods for determination of DM, CP, ash, EE, NDF, and ADF were explained in the preceding section. Neutral detergent insoluble nitrogen (NDIN), sugars, Klason lignin, and starch were determined by Licitra et al. (1996), Rong et al. (1996), Cousins (1976), and polarimetry (Parvane, 2007) methods, respectively.

Statistical analysis

The data were analyzed for a completely randomized design (CRD). The statistical model was as below:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where, Y_{ij} is the value of each observation, μ is total mean, T_i is treatment effect, and e_{ij} is the random residual error.

Data processing was done using the SAS (9.1) software and GLM procedure. Significant differences between means of treatments were assessed by the least significant difference (LSD), and the differences among treatments were declared significant at P<0.05.

Table 2. Equations for calculating available and degradable portions of feed carbohydrates in the rumen

Components	Definition	Tylutki et al. (2008) equations[1]
CHO	Total carbohydrate	100-CP-EE-Ash
C	Unavailable NDF	NDF×ADL×2.4/100
B3	Available NDF	NDF-C
NFC	Non fiber carbohydrates	CHO-NDF
A1	Volatile fatty acids	Acetic acid + Propionic acid + Butyric acid
A2	Lactic acid	-
A3	Organic acids	-
A4	Sugars	-
B1	Starch	-
B2	Soluble fibers	NFC-A1-A2-A3-A4-B1

[1]CP; crude protein, EE; ether extract, NDF; neutral detergent fiber, ADL; acid detergent lignin

Results and discussion

Chemical composition

Chemical composition in different varieties of barley, triticale, and oat grains (Table 3) was different (P< 0.05). The DM content in oat was higher than in barley, but less than triticale. Ash and OM contents in oat grains were higher and lower than those in barley and triticale grains, respectively. The highest level of ash was observed in wild oat (4.17% DM) and the highest level of OM was seen in *Massa* variety of triticale (98.07% DM). Barley grains contained more CP than triticale and oat grains. The CP content was higher in *Torkman* and *Reyhan* varieties of barley, as well as *Massa* variety of triticale than other varieties of the studied cereals (16.01, 15.31, and 15.31% DM, respectively). Oat grains contained more EE and cell wall components, NDF, and ADF than barley and triticale grains. The highest levels of EE (7.03% DM), NDF (41% DM), ADF (17% DM), and lignin (7.7% DM) were observed in *Wild* oat.

Chemical composition of feed is affected by many factors such as the growth stage, species and variety, climate conditions, soil fertility, and the management from planting to harvest (Ammar et al., 2008; NRC, 2001). Parand et al. (2018) reported DM, ash, EE, NDF, and ADF contents of barley grains as 89.4, 2.4, 1.7, 21.5, and 5.4% DM, respectively. Also, NRC (2001) estimated DM, EE, NDF, ADF, and lignin contents of barley grains as 91, 2.2, 20.8, 7.2, and 2.9% DM, respectively. In an experiment on different varieties of triticale grains by Gholami et al. (2012), mean contents of DM, ash, and starch for the studied varieties were reported as 93.23, 1.71, and 58.6% DM, respectively. In the study by Parand et al. (2018), chemical composition of triticale grains, including DM, ash, EE, CP, NDF, and ADF was reported as 88.2, 2.4, 1.2, 10.3, 11.8, and 3.3% DM, respectively. Heger and Eggum (1991) estimated the sugar content of winter type triticale grains as 4.3 to 7.6% DM that was similar to the present study. Aman (1987) estimated the ash and EE contents of oat grains as 3.00 and 5.7% DM. Biel et al. (2014) indicated DM, ash, CP, EE, and CF content of oat grains to be 90.25, 2.11, 10.49, 5.84, and 10.45% DM, respectively. In the study by Pan et al. (2021), CP content of oat grains was 8.3% DM. Inconsistency in published data could be due to the genetic, environmental and climatic, as well as varietal differences and possibly methodology (Gholami et al., 2012).

Table 3. Chemical composition of barley, triticale and oat grain varieties (% DM)

Treatments	Chemical composition (DM%)							
	Dry matter	Ash	Organic matter	Crude protein	Ether extract	Neutral detergent fiber	Acid detergent fiber	lignin
Triticale *Juvanilo*	94.00^{a}	2.19^{c}	97.80^{ab}	14.00^{b}	1.47^{f}	23.00^{e}	6.00^{d}	2.75^{e}
Triticale *Rondo*	92.78^{cd}	2.38c	96.42^{c}	13.56^{b}	1.48^{f}	24.00^{de}	5.00^{d}	2.75^{e}
Triticale *Massa*	92.93^{bc}	1.92^{c}	98.07^{a}	15.31^{a}	1.43^{f}	25.00^{e}	6.00^{d}	2.50^{e}
Barley *Torkman*	91.43^{f}	3.31^{b}	96.67^{bc}	16.01^{a}	2.95^{e}	29.00^{c}	6.00^{d}	3.25^{d}
Barley *Reyhan*	91.69^{ef}	3.38^{b}	96.60^{c}	15.31^{a}	3.42^{c}	30.33^{bc}	8.00^{c}	3.37^{cd}
Barley *Gorgon*	93.59^{ab}	3.19^{b}	96.80^{bc}	14.09^{b}	3.25^{d}	29.00^{c}	9.00^{bc}	3.75^{c}
Oat *Canadian*	92.72^{cd}	3.10^{b}	96.88^{bc}	13.56^{b}	5.90^{b}	31.00^{b}	10.50^{c}	5.00^{b}
Oat *Wild*	92.23^{de}	4.17^{a}	95.81^{c}	10.94^{c}	7.03^{a}	41.00^{a}	17.00^{a}	7.75^{a}
SEM	0.232	0.173	0.386	0.327	0.050	0.514	0.550	0.137
P- Value	<0.0001	<0.0001	0.0166	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Orthogonal contrasts								
Triticale Vs barley	0.3136	0.0001	0.2034	0.0597	<0.0001	<0.0001	0.0103	0.0034
Triticale Vs oat	0.0036	0.03916	0.0230	0.0002	<0.0001	<0.0001	<0.0001	<0.0001
Barley VS oat	0.0123	<0.0001	0.0043	0.0014	<0.0001	<0.0001	<0.0001	<0.0001

SEM: standard error of the mean

a,b: Within column, means with common superscript(s) are not different (P>0.05).

Gas production parameters

The GP data (through 96 h incubation) is presented in Figure 1. The *Rondo* and *Massa* varieties of triticale and *Reyhan* variety of barley produced more gas at different incubation times. In contrast, *Wild* oat and *Canadian* oat produced less gas. Orthogonal contrast and mean comparison of GP and estimated parameters of different triticale, barley, and oat grain varieties are shown in Table 4. According to orthogonal contrasts, GP potential and estimated parameters including OMD, ME, NE, and SCFAs in triticale and barley grains were higher than those in oat grains (P<0.01). Based the mean comparisons, the highest values for these traits (P<0.01) were observed in *Rondo* variety of triticale (354.08 mL, 59.59%, 9.00 Mcal/kg, 5.44 MJ/kg, 1.01 mmol/200 mg of DM, respectively), although there was no significant difference with that of *Massa* variety of triticale (348.11 mL, 57.79%, 8.72 Mcal/kg, 5.23 MJ/kg, 1.059 mmol/ 200 mg of DM) and *Reyhan* barley (350.02 mL, 57.62%, 8.72 Mcal/kg, 5.23 MJ/kg, 1.054 mmol/200mg of DM).

The amount of *in vitro* GP is a good measure of digestibility, final fermentation, and production of microbial protein from initial substances in the rumen. Gases (CH_4 and CO_2), VFAs, and MB are the end products of ruminal digestion. *In vitro* GP results from microbial activity (directly) and neutralization of VFAs by bicarbonate in the culture media (indirectly) (Bayat Kouhsar et al., 2021). The amount of GP is dependent on chemical composition of the feedstuff. So, factors such as species harvesting time, and maturity stage of plant affect the amount of produced gas (Sabzekar, 2014). The majority of GP results from carbohydrates fermentation. Although the amount of carbohydrate plays the main role in the rate of GP, the type of carbohydrate is also of special importance. There is a negative correlation between NDF, as well as ADF and the rate and volume of produced gas (Haddi et al., 2003).

In the present study, the higher proportion of cell wall in wild oat varieties led to limitation in fermentation process and consequently, GP was decreased. A reduction in ADF and NDF contents leads to the better access of rumen microorganisms to soluble carbohydrates, and more effective fermentation and greater GP (Sommart et al., 2000). With the increase in starch content, GP from fermentation process would increase. In the present study, triticale and barley grains contained more starch than oat grains, resulting in more GP. Also, there is an interaction between the carbohydrate and nitrogen sources on GP kinetics. Through higher carbohydrate and nitrogen availability, improvement in fermentation parameters and GP would be expected (Babayi et al., *2016*). Molecular structure of starch granules is one of the factors affecting the fermentation rate and GP of grains. But, along with this factor, the difference in protein structure seems to be the important reasons for the difference in GP parameters amongst various species of cereals. Protein matrix and its interaction with starch granules can affect ruminal degradation of grains. In this study, the reason for less GP in oat grains could be related to the protein in the endosperm, which prevents bacterial attachment and microbial fermentation in the rumen (Krieg et al., 2017). Similar to the present research, in the study by Gulsen et al. (2002), *in vitro* GP of oat grains was less than that of barley and triticale grains. The volume of GP is reflective of feedstuff fermentation and SCFAs production. In the present study, similar to the higher potential of GP in triticale and barley grains, the values of the estimated parameters, including the OMD, ME, NE, and SCFAs were higher than in oat grains. Zamani-Amirabad et al. (2015) reported similar results through comparing nutritional value of commercial varieties of barley. Increased GP, means more OM degradation and consequently greater digestibility and higher fatty acid production. Thereafter, more ME would be provided for the animal.

A positive correlation has been suggested between the calculated ME and NE in GP test and the availability and digestibility of feed components (Van Soest, 1994). Similar to the present study, Salabi et al. (2010) reported that *in vitro* digestibility of triticale grains was significantly higher than that of barley grains.

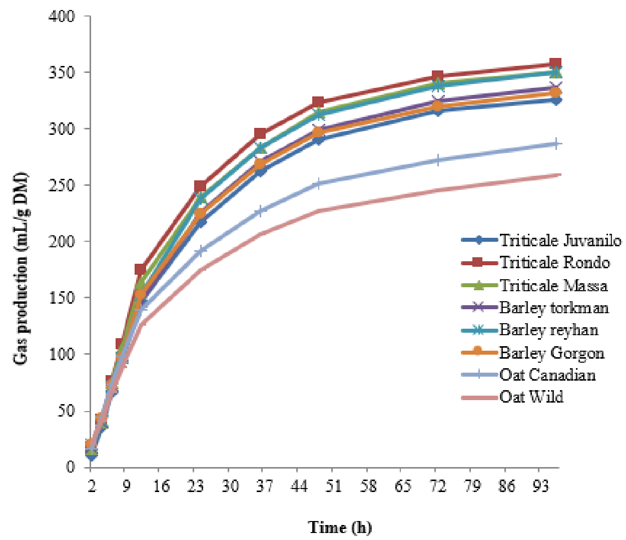

Figure 1. Gas production trend of barley, triticale and oat grain varieties.

Table 4. Gas production parameters and estimated parameters of barley, triticale and oat grain varieties

Treatments	**Gas production parameters**		**Estimated parameters**			
	Gas production potential (mL)	Gas production rate (mL/h)	Organic matter digestibility (%)	Metabolizable energy (Mcal/kg)	Net energy (MJ/kg)	Short chain fatty acids (mmol/200 mg DM)
Triticale *Juvanilo*	324.56^{c}	0.050^{b}	53.23^{c}	8.00^{c}	4.71^{c}	0.959^{c}
Triticale *Rondo*	354.08^{a}	0.055^{a}	59.59^{a}	9.00^{a}	5.44^{a}	1.101^{a}
Triticale *Massa*	348.11^{ab}	0.052^{ab}	57.79^{ab}	8.72^{ab}	5.23^{ab}	1.059^{ab}
Barley *Torkman*	336.16^{bc}	0.048^{b}	55.16^{bc}	8.32^{bc}	4.94^{bc}	0.999^{bc}
Barley *Reyhan*	350.02^{ab}	0.049^{b}	57.62^{ab}	8.72^{ab}	5.23^{ab}	1.054^{ab}
Barley *Gorgon*	330.23^{c}	0.050^{b}	54.85^{bc}	8.28^{bc}	4.91^{bc}	0.994^{bc}
Oat *Canadian*	280.32^{d}	0.051^{ab}	48.12^{d}	7.29^{d}	4.17^{d}	0.846^{d}
Oat *Wild*	253.30^{e}	0.050^{b}	44.59^{e}	6.75^{e}	3.78^{e}	0.770^{e}
SEM	5.510	0.001	1.050	0.165	0.120	0.023
P- Value	<0.0001	0.0632	<00001	<0.0001	<0.0001	<0.0001
Orthogonal contrast						
Triticale Vs barley	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Triticale Vs oat	<0.0001	0.3943	<0.0001	<0.0001	<0.0001	<0.0001
Barley VS oat	<0.0001	0.3943	<0.0001	<0.0001	<0.0001	<0.0001

SEM: standard error of the mean

a,b: Within column, means with common superscript(s) are not different (P>0.05).

In vitro digestibility and rumen fermentation parameters

Orthogonal contrasts and mean comparisons of dry matter digestibility (DMD), OMD, NH_3-N, culture media pH and estimated parameters of triticale, barley, and oat grains are shown in Table 5. DMD and OMD in triticale - grains were higher than in barley grains (P<0.05), and in barley grains more than oat grains (P<0.01). The highest contents of DMD and OMD were observed in *Rondo* and *Massa* varieties of triticale (80.50 and 83.56%, 80.00 and 83.68%, respectively) and the lowest ones were observed in *Wild* oat (46.50 and 51.50%, respectively).

The DMD content in *Torkman* variety of barley (76.50%) was not different from *Massa* variety of triticale and OMD content (33.80%) did not differ from *Rondo* and *Massa* varieties of triticale.

The differences between digestibility coefficients of different varieties of grain can largely be related to the internal structure of starch granules and their amylose content, the type and strength of the protein matrix and other differences in the texture of the grains (caused by the environmental and genetic differences) (Li et al., 2001). For example, triticale starch has larger granules than wheat and maize (Bressler et al., 2012). Larger granules contain more amylose and are hydrolyzed faster than smaller granules (Bressler et al., 2012). Our data on DMD, OMD, and pH are consistent with those of Georgiades and Hadjipanayiotou (1985) in barley (75.5%, 77.2%, and 6.6) and triticale (76.9%, 78.4%, and 6.4). Assefa and Ledin (2001) reported the average *in vitro* OMD of three oat varieties as 554 g/kg DM, and Doran et al. (2007) reported the *in vivo* OMD of this grain as 626 g/kg DM). The difference in the digestibility amongst varieties might be due to the differences in the chemical composition and ratio of grains (Santhosh Reddy et al., 2021). For example, the lower amount of lignin, NDF, and ADF in triticale and barley varieties is an important reason for their higher *in vitro* digestibility. Lignin acts as an indigestible part and a limiting factor of the activity of microbial enzymes on cell wall polysaccharides (Filya, 2004). Nadeau (2007) also observed that the *in vitro* digestibility of triticale and barley grains was higher than that of oat grains.

In the present study, concentration of NH_3-N in *Juvanilo* and *Massa* varieties of triticale (4.28 and 3.69 mg/dL) was higher than the other varieties (P<0.01). Protein degradation leads to the production of NH_3-N, amino acids, and peptides in the rumen (Firkins et al., 2007). The concentration of NH_3-N is an indicator of conversion efficiency of dietary nitrogen into bacterial nitrogen. This parameter is one of the important components in estimating DM consumption and digestibility (Alaei et al., 2022). The pH value of culture media in different varieties of triticale grains was lower than barley and oat varieties (P<0.01). Amongst the treatments, the highest pH value of the culture media was related to *Wild* oat (6.64). The rumen fluid pH is a balance of the concentration of major VFAs in the rumen (acetate, propionate, butyrate, and lactate), ammonia, buffer and saliva. Rumen pH is an important indicator for rumen microbial ecosystem status in ruminants.

In the present study, the pH of rumen samples (6.36 to 6.64) was within the normal physiological range of 6.5-6.7 (Bayatkouhsar et al., 2022). Higher pH is indicative of less VFA production and lower digestibility (Getachew et al., 1998). In this study, in oat which has lower digestibility than other grains, the pH of culture media was higher.

The amount of PF in triticale and barley grains was higher than in oat grains (P<0.01). The highest amount was observed in *Rondo* triticale and *Torkman* barley varieties (3.26 and 3.27 mg/mL, respectively); however, their difference with *Massa* (3.14 mg/mL) and *Juvanilo* triticale (3.08 mg/mL) was not significant. GP after 24 h incubation was higher in oat grains than triticale and barley grains (P<0.01). The highest amount was observed in *Wild* oat (431.86 mL). MB production and EMB in triticale and barley grains were higher than those in oat grains (P<0.01). The highest value for MB and EMB were observed in *Rondo* and *Massa* triticale and *Torkman* barley varieties, respectively (132.96 mg/g DM and 0.326, 123.23 mg/ g DM and 0.326) but there was no difference between *Juvanilo* triticale and other varieties in this respect (108.75 mg/ g DM, and 0.283). Microbial protein synthesis as the source and efficiency of microbial protein production is of great of much importance in ruminant nutrition. Factors such as CP and carbohydrate contents, affect the amount of microbial protein (Blummel and Orskov, 1993).

The PF, an indicative of feed quality, is defined as a ratio of true degraded substrate (in mg) to the volume of produced gas through the incubation time (24 or 48 h). Blummel et al. (1997) reported PF in common feeds to be between 2.74 and 4.65 mg/mL. In the present study, PF of grains was in standard range of 2.46- 3.27 mg/mL. In other words, PF indicates the fact that how much of the rumen degraded OM has been consumed in the production of VFAs or microbial biomass (Alaei et al., 2022). In the present study, the PF values in triticale and barley grains were larger than in oat grains. In oat and triticale grains, GP after 24 h incubation was reduced and conversely, MB production and EMB were increased compared to those in oat, as shown by the PF values.

Carbohydrate parameters based on Cornell Net Carbohydrate- Protein System (CNCPS)

Orthogonal contrasts and mean comparison of carbohydrate parameters based on CNCPS (Table 6) revealed significant differences amongst grain varieties (P<0.05). There was more carbohydrate in triticale and barley grains than in oat grains, but no difference was observed between oat and barley grains. Total carbohydrates in *Rondo*, *Juvanilo*, and *Massa* varieties of triticale were higher than in other varieties (82.57, 82.33, 81.33% DM, respectively). *Wild* oat, *Canadian* oat, as well as *Torkman* and *Reyhan* varieties of barley presented the lowest amount of carbohydrates (77.85, 77.43, 77.72, and 77.88% DM, respectively). Samiee Zafarghandi et al. (2010) reported the levels of total carbohydrates and non-fiber carbohydrates in barley grains to be 82.32 and 63.04%, respectively. Corresponding values in oat grains were 77.77 and 46.76% DM, respectively (Niu et al., 2007). Our data are in line with these values.

We measured higher values for unavailable NDF (the B3 portion) in *Wild* oat (7.62% DM) than in other varieties, with lowest level recorded in *Massa*, *Juvanilo*, and *Rondo* varieties of triticale (1.50, 1.52, and 1.58% DM, respectively). The C portion or unavailable ADF resists against digestion and hence is of little importance in producing energy (Mertens, 1997). Niu et al. (2007) reported unavailable NDF content of oat to be 4.20% DM. According to Samiee Zafarghandi et al. (2010) and Lanzas et al. (2007), unavailable NDF content of barley was estimated as: 1.00 and 5.8% DM, respectively. The highest level of available NDF was recorded in *Wild* oat (33.37% DM), and the lowest one in *Juvanilo* variety of triticale (21.47% DM). Lanzas et al. (2007) and Samiee Zafarghandi et al. (2010) reported the B3 content in ground barley as 18.6 and 18.28% DM, respectively.

Table 5. Dry matter and organic matter *in vitro* digestibility, and fermentation parameters of barley, triticale and oat grain varieties

Treatments	Dry matter digestibility (%)	Organic matter digestibility (%)	Ammonia nitrogen (mg/dL)	pH	Partitioning factor (mg/mL)	Gas yield (mL)	Microbial biomass (mg/g DM)	Efficiency of microbial biomass
Triticale *Juvanilo*	75.50^{cd}	78.01^{bc}	4.28^{a}	6.37^{d}	3.08^{abc}	328.87^{cd}	108.75ab	0.283^{abc}
Triticale *Rondo*	80.50^{a}	83.56^{a}	3.50^{b}	6.40^{d}	3.26^{a}	309.43^{d}	132.96a	0.326^{a}
Triticale *Massa*	80.00^{ab}	83.68^{a}	3.69^{ab}	6.36^{d}	3.14^{ab}	326.41^{cd}	123.23a	0.300^{ab}
Barley *Torkman*	76.50^{bc}	80.33^{ab}	3.20^{b}	6.49^{c}	3.27^{a}	310.13^{d}	127.57a	0.326^{a}
Barley *Reyhan*	69.00^{e}	74.12^{c}	2.99^{b}	6.47^{c}	2.98^{bc}	348.02^{bc}	94.05bc	0.261^{bc}
Barley *Gorgon*	72.00^{de}	76.24^{bc}	3.27^{b}	6.49^{c}	2.99^{bc}	343.39^{bc}	97.60bc	0.263^{bc}
Oat *Canadian*	63.50^{f}	68.00^{d}	3.03^{b}	6.58^{b}	2.90^{c}	357.83^{b}	79.95c	0.240^{c}
Oat *Wild*	46.50^{g}	51.50^{e}	2.05^{c}	6.64^{a}	2.46^{d}	431.86^{a}	26.06d	0.105^{d}
SEM	1.303	1.548	0.263	0.015	0.077	7.587	8.638	0.018
P- Value	<0.0001	<0.0001	0.0004	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Orthogonal contrast								
Triticale Vs barley	0.0011	0.0117	0.0111	<0.0001	0.3115	0.1601	0.1054	0.3028
Triticale Vs oat	<0.0001	<0.0001	0.0069	<0.0001	<0.0005	<0.0001	0.0001	0.0002
Barley VS oat	<0.0001	<0.0001	<0.0001	1.0000	0.0001	<0.0001	<0.0001	<0.0001

SEM: standard error of the mean

a,b: Within column, means with common superscript(s) are not different (P>0.05).

The levels of non-fiber carbohydrates (NFC), starch, and sugar were higher in triticale than barley and oat grains, and higher in barley than oat grains. *Juvanilo*, *Rondo*, and *Massa* triticale varieties recorded the highest concentrations of NFC and starch (59.33 and 58.57% DM for non-fiber carbohydrates, 48.91 and 49.88% DM for starch, respectively), but the *Wild* oat contained the lowest levels (36.85% DM for non-fiber carbohydrates and 30.10% DM for starch, respectively). Overnell-Roy et al. (1998) reported the starch content of barley grains to be in range of 45.9 to 62.8% DM. Khorasani et al. (2000) studying 60 varieties of barley cultivated in different regions, found a range 48.3 to 62.5% DM in starch content. Lanzas et al. (2007) and Samiee Zafarghandi et al. (2010) estimated starch content of barley grains as 52.3 and 51.50% DM, respectively. Gholami et al. (2012) reported starch content of triticale grains as 58.6% in DM. Variation in triticale starch values in this study was less than 53 to 63% in DM reported by Heger and Eggum (1991). Robards et al. (1998) and Niu et al. (2007) reported starch content of oat grains as 46.02 and 42.57% DM, respectively, which is higher than in the present study. In the study by MacArthur and D'appolonia (1979), the amount of sugar in oat grains was reported to be 2.6% which is consistent with the data in the present study. Concentrations of soluble sugars in *Juanilo*, *Rondo*, and *Massa* triticale varieties was higher (6.36, 6.51, and 6.80% DM) than the other grain varieties. The lowest concentration of soluble sugars was observed in *Wild* oat (1.92% DM). Lanzas et al. (2007) and Samiee Zafarghandi et al. (2010) reported the sugar content of barley grains as 2.40 and 2.49% DM, respectively. This is higher than the values in the present study. The level of sugar in different varieties of triticale grains in this experiment was higher than the values reported by Bushuk and Larter (1980), Pena and Bates (1982), and Johnson and Eason (1988). Heger and Eggum (1991) estimated the sugar content of winter triticale grains to be 4.3 to 7.6% DM, which is consistent with the result of the present study.

Triticale varieties contained more soluble fibers compared with barley grains but there were no differences between oat grains versus triticale and barley grains. The concentration of soluble fibers in *Canadian* oat was higher than in other treatments (6.72% DM). Although it did not differ from the values in *Wild* oat, *Juanilo* and *Massa* triticale varieties (4.83, 4.03, and 4.65% DM, respectively). In contrast, the level of soluble fibers in *Torkman* barley variety was lower than other varieties (1.22% DM). No significant difference was observed between *Torkman* barley and *Rondo* triticale, *Reyhan* and *Gorgan* barley varieties (1.22% DM versus 2.18, 2.21, and 3.30% DM). The values for soluble fibers in oat grains are in line with the data of Engstrom et al. (1992). Samiee Zafarghandi et al. (2010) reported the content of soluble fibers in barley grains as 9.50% DM.

Conclusions

Triticale and barley grains recorded higher *in vitro* nutritional values than oat grains. Also, triticale grains were found not to be inferior to barley grains; in fact, triticale out-performed barley in some nutritional characteristics. Considering the climatic conditions of the country and low availability of water resources for cultivation of common agricultural crops, cultivation of triticale is suggested as a proper replacement for barley due to its drought tolerance and adaptability to adverse climatic conditions.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Table 6. Parameters related to carbohydrates in CNCPs model in barley, triticale and oat grain varieties (% DM)

Treatments	Total Carbohydrate	Unavailable neutral detergent fiber	Available neutral detergent fiber	Non fiber carbohydrates	Starch	Soluble Sugar	Soluble fiber
Triticale *Juvanilo*	82.33^{a}	1.52^{e}	21.47^{d}	59.33^{a}	48.91^{a}	6.36^{a}	4.03^{ab}
Triticale *Rondo*	82.57^{a}	1.58^{e}	22.41^{cd}	50.57^{a}	49.88^{a}	6.51^{a}	2.18^{bc}
Triticale *Massa*	81.33^{a}	1.50^{e}	23.50^{c}	56.33^{b}	44.88^{b}	6.80^{a}	4.65^{ab}
Barley *Torkman*	77.72^{c}	2.25^{d}	26.74^{b}	48.72^{cd}	43.54^{bc}	3.95^{b}	1.22^{c}
Barley *Reyhan*	77.88^{c}	2.45^{cd}	27.88^{b}	$47.54d^{e}$	42.11c	3.22^{c}	2.21^{bc}
Barley *Gorgon*	79.47^{b}	2.60^{c}	26.39^{b}	50.47^{c}	44.22^{b}	2.95^{c}	3.30^{bc}
Oat *Canadian*	77.43^{c}	3.72^{b}	27.28^{b}	46.43^{e}	36.82^{d}	2.88^{c}	6.72a
Oat *Wild*	77.85^{c}	7.62^{a}	33.37^{a}	36.85^{f}	30.10^{e}	1.92^{d}	4.83^{ab}
SEM	0.420	0.080	0.517	0.735	0.494	0.175	0.917
P- Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0130
Orthogonal contrast							
Triticale Vs barley	0.1871	<0.0001	0.0007	0.0002	<0.0001	0.0011	0.0109
Triticale Vs oat	0.0002	0.0001	0.0001	<0.0001	<0.0001	<0.0001	0.2062
Barley VS oat	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0683

SEM: standard error of the mean

a,b: Within column, means with common superscript(s) are not different (P>0.05).

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