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Replacing the forage portion of the ration with triticale hay improves the performance of Holstein dairy cows

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ABSTRACT

The aim of the present study was to assess the effect of replacing the forage portion (alfalfa, corn silage, and barley straw) in the diet of lactating Holstein cows with triticale hay (TH, × *Triticosecale* L.) on DMI, digestibility, ruminal fermentation variables, estimated microbial-N synthesis (EMNS), and milk production and composition. Eight Holstein cows were used in a replicated Latin square design (two 4 × 4 squares) with four 28-d periods and 4 treatments, including a TH-free diet (control), and diets replacing 33%, 66%, and 100% of the forage portion with TH. Cows were fed ad libitum with 10% carryover during the experimental periods. Intakes and in vivo digestibilities of dry matter, organic matter, crude protein, and ash-free neutral detergent fiber, rumen pH, ammonia-N, total and individual short-chain fatty acids, protozoa, and bacteria populations were evaluated using specific methods. Moreover, in vitro total gas and methane release and in vivo urinary purine derivatives, EMNS, milk production, and composition were measured. The results showed that TH diets lowered DMI, compared with the control. Apparent digestibilities of DM, OM, CP, and NDF increased with dietary TH inclusion. The addition of TH instead of the diet forage portion increased in vivo rumen pH; acetic, propionic, valeric, and isovaleric acids concentrations; cellulolytic bacteria number; and in vitro gas production. In vivo rumen ammonia-N, short-chain fatty acids, butyric acid, in vivo and in vitro total protozoa and *Entodiniinae* numbers, and in vitro methane production decreased with increasing dietary levels of TH instead of the forage portion. The dietary addition of TH did not affect milk yield, protein, and lactose, but increased fat-corrected milk, milk fat, fat-corrected milk:DMI ratio, and milk yield:DMI ratio. Milk urea N decreased, but urinary purine derivatives excretion and EMNS increased with increasing levels of TH in the diet. For variables with significant changes, except for isovaleric acid, there was a linear response of animals to increasing levels of TH in the diet. Results suggest that TH, which is grown with less water compared with alfalfa, corn forage, and straw, is a potential alternative to those forages by increasing milk production efficiency, milk fat, and decreasing methane emission.
Key words: dairy cattle, triticale hay, rumen variables, milk production, milk composition

INTRODUCTION

Nutrients in forage, including energy, protein, vitamins, and minerals, are the foundation for all rationing systems to optimize productivity (NASEM, 2016). The main conventional forages used in the diet of lactating cows are alfalfa hay (**AH**), corn silage, and grass, which are the most water-demanding crops (Dhiman and Satter, 1997; McKenzie and Wood, 2011). Altered weather patterns and other factors (i.e., constructing dams and reservoirs, intensive agriculture, deforestation, and war conflict) causing drought may limit the availability of water for irrigation, leading to producers' need for alternative crops, such as triticale, that do not use as much water as conventional forages. The winter-crop triticale (× *Triticosecale* L.) can be grown and harvested as hay and displays desirable traits from each of its parent species (Glamoclija et al., 2018). Triticale is a modern cereal grain, a hybrid of wheat and rye, which inherited the ability of the rye to survive the high frosts, but has a higher protein value than rye, and higher grain yields and increased tolerance to diseases were inherited from wheat (Glamoclija et al., 2018). Triticale forage is grown as a winter-to-spring crop utilizing less water than alfalfa (Santana et al., 2019). The DM yield of fall-sown triticale ranges between 7.5 and 16.3 t/ha (Bilgili et al., 2009; Keles et al., 2016; Salama and Badry, 2020). Triticale hay (**TH**) is characterized by the high concentrations of CP (82–212 g/kg DM), ME (8.2–12.0 MJ/kg

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DM), NDF (469–581 g/kg DM), and NFC (159–256 g/kg DM), as well as low ADL concentration (36–70 g/kg DM), along with high DM digestibility (684–926 g/kg) and palatability, compared with more common winter forage crops (Keles et al., 2016). However, delaying the harvest to the hard-dough stage can increase yield and reduce feeding costs without affecting nutritional value (Delogu et al., 2002; Lestingi et al., 2007; O'Keefe et al., 2022). Starch accumulation during the dough stages of the kernel may corroborate with the dilution effect of CP, sugar, and fiber concentrations, resulting in a general enhancement of feeding value from the milk-to-dough stages (Delogu et al., 2002; Lestingi et al., 2007; O'Keefe et al., 2022). Nitrate concentration in TH decreased at maturity compared with its concentration at the boot stage (Gulmezoglu et al., 2010). However, Coblentz et al. (2018) noted that harvesting at the boot stage rather than the soft-dough stage might improve some nutritional characteristics, particularly as a protein forage source, in the climate conditions of central Wisconsin.

Several investigations have been carried out regarding the feeding value of triticale silage in dairy cattle. For example, Khorasani et al. (1993) recorded a decline in DMI but no effect on milk production when replacing 50% of dietary DM as AH with triticale silage, and similar findings were observed by Vatandoost et al. (2007) when 14% of dietary DM as corn silage was replaced with triticale silage. In these trials, the triticale had been dough-stage harvested and fed to cows producing up to 30 kg/d of milk. Other work that replaced 10% (on a DM basis) of corn silage with triticale silage (boot-stage harvest) for cows producing more than 40 kg/d of milk did not affect DMI but lowered milk production and N use efficiency (Harper et al., 2017).

There has been limited work using TH to replace AH in the dairy cow ration, but Santana et al. (2019) replaced AH with boot-stage harvested TH and found minimal influence on dairy cow lactation performance or N utilization.

Characterizing ruminal variables is basic to understanding the feed metabolism efficiency and utilization in lactating cows (Van Soest, 1994). In this study, dairy cow performance and in vivo ruminal pH, ammonia-N, short-chain fatty acid (**SCFA**) profiles; protozoa and cellulolytic bacteria populations; estimated microbial-N synthesis (**EMNS**), and in vitro methane (CH_4) were evaluated when TH replaced other dietary forages. The hypothesis was that dietary TH substitution would enhance milk yield and improve rumen variables in lactating cows.

MATERIALS AND METHODS

Compliance

The *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (FASS, 2010) was followed for all aspects of this study. The Tarbiat Modares University Animal Science Committee (proposal 9830092001, 2019) approved the experimental protocol.

Crop Preparation

Triticale seeds (× *Triticosecale* Wittmack) were obtained from the Fars Agricultural and Natural Resources Research and Education Centre, **AREEO**), Shiraz, Iran. Triticale was sown on November 7, 2020, at a seed rate of 200 kg/ha as a winter crop after corn and harvested after 205 d at the milky-dough stage. The crop was grown at 1,865 m above sea level. at latitude and longitude of 30° 03′ N and 53° 13′ E, respectively. During the growing period, the mean annual temperature and rainfall from 2020 to 2021 were 16.6°C and 260 mm, respectively (Table 1). The soil on the site is classified as clayey (650 g/kg clay, 300 g/kg silt, and 100 g/kg sand), with a second-level classification as calcic xerosols in the Food and Agriculture Organization of the United Nations taxonomy. The crop was irrigated using a sprinkler system, with the first irrigation applied after sowing at a rate of 500 m^3/ha. Due to the decrease in precipitation and to increase the yield of the crop, 4 stages of irrigation were used on April 4, April 19, May 5, and May 20, 2021, respectively. Water applied at each stage was 480 m^3/ha and the cumulative precipitation was 10 mm. The mean total volumes of water applied were 2,430 m^3/ha. Fertilizer (46–0-0) was applied as 100 kg of urea/ha (46 kg of N/ha).

The corn (*Zea mays*) crop had been harvested in the summer of 2020 from the field where triticale was grown subsequently. Corn seeds (hybrid SC 704) were obtained from AREEO (as above) and harvested at 83 d at the milk stage. The mean total volume of water applied to corn was 6,000 m^3/ha. The corn forage was chopped and placed in a trench silo at a volume of 250 t and left to ensile. Laboratory analyses were used to determine the nutrients levels of forages (Table 2).

Analytical Procedures

Sampling was done from different points of each forage mass during the experimental period. The forage samples (AH, corn silage, barley straw, and TH) were dried for 48 h at 55°C in a forced-air oven (Memmert GmbH,

Table 1. Monthly temperature, precipitation, and relative humidity during the growing season of triticale

Month	Temperature, °C			Precipitation, mm	Relative humidity, %
	Minimum	Maximum	Mean		
October 2020	4.7	26.2	15.5	0	20
November 2020	3.5	17.8	10.7	2.2	54
December 2020	−0.7	13.1	6.2	2.0	66
January 2021	−1.3	11.8	5.3	4.1	56
February 2021	0.2	15.4	7.8	1.1	50
March 2021	5.6	22.6	14.1	0.9	35
April 2021	10.2	26.6	18.4	0	30
May 2021	11.3	30.5	20.9	0.2	28

Schwabach, Germany) to determine DM and then milled for analysis. Nitrogen levels were measured using AOAC International (2012) method 990.03, adapted for an automatic distiller Kjeldahl (Kjeltec Auto 1030 Analyzer, Tecator, Foss Analytics, Hillerød, Denmark), and CP was calculated as N × 6.25. Method 942.05 of AOAC International (2012) was used for ash-content determination, by using 2 g of the sample, which was incinerated in a muffle furnace (LMF4, Carbolite, Bamford, Sheffield, UK) maintained at 600°C for 5 h. Method 2003.05 of AOAC International (2012) was followed for ether extract (**EE**) determination, which involved exhaustive extraction of 3 g of sample in a Soxhlet apparatus using petroleum ether (boiling range 40–60°C) as the extractant. Neutral detergent fiber was measured without sodium sulfite and expressed exclusive of residual ash according to Van Soest et al. (1991). Ash-free ADF was determined gravimetrically as the residue remaining after extraction with an acid-detergent solution (AOAC International, 2012; method 973.18). The samples were analyzed for ADL according to AOAC International (2012) method 973.18. Briefly, the ADF residue was suspended within the crucible in 40 mL of sulfuric acid (72%) for 3 h. Residues were then washed with boiling water and dried overnight at 65°C. Once weighed, crucibles were placed in a muffle furnace for 4 h at 490°C to incinerate the residue. Ash content was then subtracted from the initial weight to calculate the ADL content. Calcium concentration in the samples was determined with AOAC International (2012) method 968.08 using an atomic absorption spectrophotometer (AA-6200, Shimadzu, Japan), and phosphorus was determined by the colorimetric procedure (method 965.17; Genova, Essex, UK). Water-soluble carbohydrate levels were measured using the anthrone reaction

Table 2. Average chemical composition (mean ± SD),[1] secondary metabolites (g/100 g of DM or as stated), and ME (MJ/kg of DM) of alfalfa, corn silage, barley straw, and triticale

Item	Alfalfa	Corn silage	Barley straw	Triticale hay
Chemical composition				
DM, g/100 g fresh weight	33.8 ± 0.2	23.3 ± 0.6	94.4 ± 0.8	36.8 ± 0.2
CP	13.7 ± 0.4	6.77 ± 0.1	3.13 ± 0.5	8.16 ± 0.2
Ash	7.49 ± 0.7	6.76 ± 0.4	10.2 ± 0.2	7.14 ± 0.1
NDF	51.1 ± 1.2	53.1 ± 0.5	74.6 ± 0.7	59.3 ± 1.0
ADF	36.8 ± 0.4	29.4 ± 0.6	47.8 ± 0.9	37.8 ± 0.4
ADL	7.53 ± 0.4	4.91 ± 0.1	11.1 ± 0.3	6.31 ± 0.2
EE	3.15 ± 0.2	2.49 ± 0.1	2.29 ± 0.4	4.04 ± 0.4
NFC[2]	24.6 ± 2.0	30.9 ± 0.6	9.79 ± 1.1	21.4 ± 0.7
Starch	5.44 ± 0.1	18.9 ± 0.7	0.86 ± 0.1	13.9 ± 0.7
WSC[3]	4.45 ± 0.2	10.3 ± 0.5	1.40 ± 0.1	8.01 ± 0.8
Secondary metabolites[4]				
Total phenolic compounds	0.66 ± 0.1	0.49 ± 0.0	0.04 ± 0.0	0.82 ± 0.1
Total tannin	0.42 ± 0.1	0.31 ± 0.0	0.02 ± 0.0	0.59 ± 0.1
Flavonoids,[5] mg/kg of DM	840 ± 20	410 ± 17	10 ± 1.7	1,010 ± 23
Nitrate	0.159 ± 0.01	0.050 ± 0.01	0.034 ± 0.0	0.123 ± 0.01
ME[6]	8.87 ± 0.2	8.91 ± 0.2	5.75 ± 0.4	9.68 ± 0.1

[1]Determined from the laboratory analysis.

[2]Non-fiber carbohydrates = 100 − (% CP + % ash + % ether extract + % NDF).

[3]WSC = water-soluble carbohydrate.

[4]Expressed as tannic acid equivalents.

[5]Expressed as quercetin equivalents.

[6]ME (MJ/kg of DM) = 2.20 + 0.136 × gas production + 0.057 × CP + 0.0029 × CP^2; estimated using gas-production technique as described by Menke et al. (1979).

assay, and the absorbance of the extract was measured by a spectrophotometer (MAFF, 1986). Total phenolics were measured using the Folin–Ciocalteau method (Makkar, 2000). The sample (200 mg) was dissolved in acetone:water (10 mL; 70:30 vol./vol.) in an ultrasonic bath for 20 min. The contents were centrifuged at 3,000 × *g* for 10 min at 4°C and the supernatant was kept on ice until analysis. Nontannin phenolics were determined using absorption to insoluble polyvinylpyrrolidone. The insoluble polyvinylpyrrolidone (100 mg) was weighed into test tubes. Distilled water (1 mL) and then 1 mL of tannin-containing extract were added and the mixture was vortexed. The tube was kept at 4°C for 15 min, vortexed again, and centrifuged (3,000 × *g*) for 10 min at 4°C and the supernatant was collected. Phenolic content in the supernatant was measured by the Folin–Ciocalteau reaction and this result was accepted as the nontannin phenolics (Makkar, 2000). Total tannins were calculated as the difference between total phenolics and nontannin phenolics. Tannic acid (Merck GmbH, Darmstadt, Germany) was used as the standard to express the data. Total flavonoid was measured by the method of Quettier-Deleu et al. (2000) and expressed as quercetin equivalent, in milligrams per gram of DM. The colorimetric method of Singh (1988) was used to determine nitrate content. Briefly, 100 mg of a DM sample was gently mixed with 50 mL of acetic acid (2%) for 20 min and filtered through filter paper; nitrate concentration was measured colorimetrically at 540 nm.

Animals and Treatments

The animal study was a balanced replicated Latin square (two 4 × 4 squares) with four 28-d periods. A total of 8 Holstein cows, averaging 120 ± 20 DIM (in their third lactation), at 623 ± 33 kg BW, and 29.8 ± 0.9 kg of milk/d were selected. They were housed individually in tiestalls with 24 h access to water. The diets in the form of TMR (Tables 2 and 3) were fed ad libitum (allowing for 10% orts). The 4 diets were (1) control, ration without TH, (2) 33% TH in the forage portion, (3) 66% TH in the forage portion, and (4) 100% TH in the forage portion. Each period lasted 28 d (21 d for adaptation + 7 d for sampling and collection of data). Diets were matched for CP and NE_L (NRC, 2001) and fed twice daily (0600 and 1800 h).

Feed Intake and Nutrient Digestibility

Daily feed intake per animal was obtained from the daily-distributed TMR weight minus the daily ort weight. Whole tract apparent digestibility of DM and nutrients was measured using acid-insoluble ash as a marker (McGeough et al., 2010). Fecal grab samples were obtained from each cow daily on the last 7 d of each period of the trial. Samples of feed offered, orts, and feces from each cow were taken daily in the final week and kept frozen at −20°C for subsequent analysis. They were analyzed for DM, CP, NDF, ADF, EE, and ash after drying at 60°C and milling to pass through a 1-mm sieve (Wiley mill; Thomas Scientific, Gloucester, NJ). Digestibility coefficients were then calculated.

In Vivo Rumen Fermentation Variables and In Vitro Methane Production

For each period, rumen fluid was taken by an esophageal tube on d 28, at 3 h after the a.m. feed. An electric vacuum pump (Gast model 0823-v13q-g608nex, Septic Solutions Inc., Dieterich, IL) with 68.9 kPa of maximum continuous pressure was used to obtain rumen liquor. During sampling, the head of the animal was restrained, and ruminal fluid was collected by passing the tubing using an oral speculum down the esophagus into the rumen. The tubing was gently pushed through the rumen mat to collect ruminal contents. Approximately 180 to 200 cm of the stomach tube was inside the cow with the remainder being outside of the cow, thus providing the flexibility to move the tube and extract ruminal fluid. Approximately 100 mL of initially sampled ruminal fluid was discarded due to possible saliva contamination, and then the pH of the remainder was recorded with a pH meter (Sartorius PT-10, Gottingen, Germany). Samples of 5 mL each were added to 1 mL 0.2 *N* HCl and frozen for ammonia-N analysis by the method of Galyean (2010). In addition, 1-mL rumen fluid samples were taken for SCFA analysis according to the method of Galyean (2010). Rumen protozoa were enumerated according to the method of Dehority (2003). The cellulolytic bacteria population was quantified by using 5-mL samples of rumen fluid according to the method of Bryant (1972) and the most-probable-number procedure of Dehority (2003).

In vitro gas production was measured after 24 h incubation using a batch system following the method of Demeyer et al. (1988). Gas production was recorded after 24 h, then 2 mL of 10 *M* NaOH was added to each syringe, and the remaining gas was recorded as CH_4. Samples were taken from each syringe to allow the in vitro enumeration of protozoa (Dehority, 2003).

Milk Yield and Milk Composition

Cows were milked twice daily (at 0700 h and 1900 h) by an automatic milking system (Lely Astronaut A4, Maassluis, the Netherlands), and milk production per

Table 3. Ingredient and chemical composition (g/100 g of DM or as stated) of the diets containing different TH levels fed to lactating Holstein cows

	Level of TH in diet (g/100 g of forage DM)			
Item	0	33	66	100
Alfalfa hay	22.9	15.4	7.7	0.0
Corn silage	17.7	11.9	5.9	0.0
Barley straw	4.8	3.2	1.6	0.0
Triticale hay	0	15.1	30.2	45.4
Soybean meal	16.9	16.9	16.9	16.9
Canola meal	1.2	2.7	4.4	6.1
Barley	14.7	14.9	15.1	14.9
Corn	8.4	8.4	8.4	8.4
Wheat bran	8.4	6.7	4.8	3.4
Fat powder	1.8	1.6	1.5	1.3
Vitamin supplement[1]	0.52	0.52	0.52	0.52
Mineral supplement[1]	0.20	0.20	0.20	0.20
Salt	0.29	0.29	0.29	0.29
Dicalcium phosphate	0.27	0.20	0.13	0.02
Sodium bicarbonate	1.2	1.2	1.2	1.2
$CaCO_3$	0.17	0.39	0.56	0.76
Bentonite	0.47	0.47	0.47	0.47
Chemical composition[2]				
DM	63.0	63.3	63.0	62.8
OM	92.7	92.7	92.3	92.3
CP	16.3	16.2	16.2	16.2
NDF	34.4	34.7	34.9	35.3
ADF	19.9	20.2	20.5	20.9
ADL	3.87	3.80	3.58	3.42
Forage ash-free NDF	24.7	25.5	26.1	26.9
EE	3.90	3.90	3.90	3.90
NFC[3]	38.1	37.9	37.3	36.9
Starch	25.8	26.7	27.2	28.2
Calcium	0.70	0.70	0.70	0.70
Phosphorus	0.40	0.40	0.40	0.40
RDP, % CP	69.9	70.4	70.9	71.4
Secondary metabolites				
Total phenolic compounds	0.43	0.45	0.52	0.65
Total tannin	0.26	0.27	0.33	0.43
Flavonoids,[4] mg/kg DM	299	321	362	420
Nitrate	0.130	0.121	0.117	0.112
NE_L,[5] Mcal/kg of DM	1.59	1.59	1.58	1.57
Price[6] (Toman/d)	112,477	110,379	105,333	98,828
Price ($/d)	3.21	3.15	3.01	2.82
Price (Toman/kg of DM)	5,378	5,253	5,039	4,726

[1]Sepahandaneh, Isfahan, Iran.

[2]Calculated from each feed composition, which were analyzed.

[3]Nonfiber carbohydrates = 100 − (% CP + % ash + % EE + % NDF).

[4]Expressed as quercetin equivalents.

[5]Predicted values from NRC (2001) model.

[6]Prices are for total feed per day. USD$1 is equivalent to 35,040 Tomans (Iranian currency).

cow was recorded in graduated jars (Agri and SD Co., Frankfurt, Germany). Before milking began, milk samples were taken by hand to check for mastitis. In the final 7 d of each study period, milk samples were collected from each cow and each milking. Potassium dichromate was added to preserve the samples and they were maintained at 4°C before analysis of protein, fat, lactose, and SNF using a Milko Scan 133B (Foss Electric, Hillerød, Denmark). Fat-corrected milk (4%) was computed as FCM = 0.4 × milk yield (kg/d) + 15 × milk fat (kg/d; NRC, 2001). Feed-conversion efficiencies were obtained by dividing milk yield or FCM by DMI.

Urinary PD and EMNS

Urine samples were obtained by manual stimulation from each cow on d 23 to 28 of each experimental period. Samples were acidified using 0.072 *N* H_2SO_4 at 4 times the sample volume and frozen until analysis. After thawing and filtration, the samples were analyzed for

Table 4. Effect of substituting TH for alfalfa, corn silage, and barley straw on nutrient intake and apparent digestibility in lactating Holstein cows (n = 8)

	Level of TH in diet (g/100 g of forage DM)					*P*-value		
Item	0	33	66	100	SEM	T[1]	Linear	Quadratic
Intake, kg/d or as stated								
DM	23.76	23.04	22.18	20.73	0.32	<0.01	<0.01	0.263
OM	22.04	21.37	20.48	19.12	0.29	<0.01	<0.01	0.245
CP	3.86	3.76	3.62	3.37	0.06	<0.01	<0.01	0.223
EE	0.95	0.86	0.86	0.78	0.03	<0.01	<0.01	0.627
NFC[2]	9.49	8.68	8.46	8.38	0.22	<0.01	<0.01	0.112
Starch	5.88	5.83	5.77	5.73	0.09	0.321	0.106	0.959
NDF	8.21	7.99	7.75	7.34	0.11	<0.01	<0.01	0.392
ADL	0.92	0.88	0.79	0.71	0.02	<0.01	<0.01	0.207
Flavonoids, g/d	6.89	7.38	7.98	8.69	0.12	<0.01	<0.01	0.342
Nitrate, g/d	30.9	27.9	25.9	23.2	0.37	<0.01	<0.01	0.714
Apparent digestibility, %								
DM	63.78	64.46	64.67	65.21	0.21	<0.01	<0.01	0.747
OM	67.31	67.66	68.33	69.89	0.58	<0.01	<0.01	0.300
CP	67.85	68.18	70.32	72.22	0.44	<0.01	<0.01	0.089
NFC	76.52	76.55	76.79	77.02	0.25	<0.01	0.134	0.699
NDF	52.66	54.25	56.30	58.68	0.53	<0.01	<0.01	0.614

[1]T = the main effect of the treatment.

[2]Nonfiber carbohydrates = 100 − (% CP + % ash + % EE + % NDF).

creatinine, to allow the estimation of daily urine volume as (BW × 29)/urinary creatinine concentration (mg/L), according to Valadares et al. (1999). Total purine derivative (**PD**), allantoin, and uric acid were determined by the methods of Chen and Gomes (1992). Daily excreted urinary PD levels were used in the estimation of exogenous purines absorbed and ruminal EMNS calculated according to Chen and Gomes (1992).

Statistical Analysis

In this study, a total of 8 cows were used in 2 Latin squares. Data were analyzed in a 4 × 4 replicated Latin square design, using the MIXED procedure of SAS (version 9.1, SAS Institute Inc., Cary, NC), considering the treatment as a fixed effect and the cow and period as random effects. The statistical model was

$$Y_{ijk(l)} = \mu + R_j + C_{ik} + T_l + e_{ijk(l)}, \quad (1)$$

where $Y_{ijk(l)}$ is a dependent variable, μ is the overall mean, R_j is the effect of row (period), C_{ik} is the effect of column (animal) within replicate (square), T_l is the effect of treatment (diet), and $e_{ijk(l)}$ is the residual (random error). The period and square with treatment interactions were not significant, so they were not further reported. Contrast statement was used to determine the linear and quadratic cow response to increasing concentrations of the extract in the diet. Differences between treatments were declared significant at $P \leq 0.05$ using the Tukey correction for multiple comparisons.

RESULTS

Feed Intake and Nutrient Digestibility

Except for starch, replacing the forage portion of the diet with TH had a significant effect ($P < 0.01$) on the feed intake (Table 4), where daily intakes of DM, OM, CP, EE, NDF of OM, and ADL ($P < 0.01$) linearly decreased as TH levels increased in the diet. Substitution levels of 66% and 100% of TH instead of the forage portion of the diet caused a considerable reduction in feed intake of lactating cows compared with the control. The increased dietary TH rate had no effect on starch intake ($P > 0.05$), but flavonoid intake increased linearly ($P < 0.01$), and the maximum intake of flavonoids was at the level of 100% substitution of the dietary forage portion with TH.

Except for starch, a positive effect ($P < 0.01$) on diet digestibility was observed with the inclusion of increasing levels of TH in the diet of animals, so that the digestibility coefficients of DM, OM, CP, and NDF of OM increased linearly ($P < 0.01$) with dietary TH addition. The quadratic effect of the treatment on feed intake and diet digestibility in the cows was not significant and the maximum digestibility was observed when 100% of forage portion was replaced with TH.

Rumen Fermentation Variables and In Vitro Methane Production

According to Table 5, replacing the forage portion of the diet with different levels of TH had a significant effect ($P < 0.01$) on rumen fermentation variables, except

for the acetic acid:propionic acid ratio and individual protozoa other than *Entodiniinae*. The animal response to the treatments was linear, so that in vivo pH; acetic, propionic, valeric, and isovaleric acid concentrations; cellulolytic bacteria ($P < 0.01$); and in vitro gas production ($P = 0.012$) increased linearly with the addition of increasing rates of TH in the diet. The increasing levels of TH led to a linear decrease in the in vivo ammonia concentration, total SCFA, butyric acid, total protozoa, *Entodiniinae* ($P < 0.01$), and in vitro CH_4 production, total protozoa, and *Entodiniinae* ($P < 0.01$). There were no linear or quadratic effects on the acetic acid:propionic acid ratio and other individual protozoa families ($P > 0.05$). Except for isovaleric acid, there was no significant quadratic effect ($P > 0.05$) of TH feeding on rumen fermentation variables in the cows. All 3 levels of the forage substitution caused a decrease in rumen ammonia and an increase in pH compared with the control group, but considerable differences in ruminal protozoa, SCFA, cellulolytic bacteria, protozoa, and methane, compared with the control, were observed at 66% and 100% replacement levels.

Milk Yield and Milk Composition

The effects of substituting TH for other forages in the diet on milk yield, milk composition, and feed efficiency are shown in Table 6. There was no significant main effect of the treatment on milk yield, protein, lactose, and BW change ($P > 0.05$), but other milk variables were affected ($P < 0.01$) by feeding TH. In this respect, MUN decreased linearly ($P < 0.01$) with increasing dietary TH rates, but FCM, milk fat, milk yield-to-DMI ratio, and FCM-to-DMI ratio ($P < 0.01$) increased linearly, and no significant quadratic effect was observed regarding these variables. There were no significant linear or quadratic differences ($P > 0.05$) in milk yield, protein, lactose, and BW change among the experimental groups.

Urinary PD and EMNS

The main effect of the treatment on the amounts of urinary PD and EMNS was significant ($P < 0.01$) and the biggest difference with the control was observed at the substitution levels of 33% and 66% of TH instead of the forage portion. Animal response to treatment was linear for these variables. Allantoin ($P = 0.013$) and uric acid ($P = 0.012$) excreted in daily urine increased linearly with the level of TH added in the diet. Moreover, total absorbed and excreted PD and EMNS increased linearly ($P < 0.01$) with increasing dietary rates of TH. However, the quadratic effect of treatment on these traits was insignificant.

Table 5. Effect of substituting TH for alfalfa hay, corn silage, and barley straw on the in vivo and in vitro ruminal variables in lactating Holstein cows (n = 8)

	Level of TH in diet (% of forage DM)					*P*-value		
Item	0	33	66	100	SEM	T[1]	Linear	Quadratic
In vivo experiment								
Ruminal pH	6. 03	6.23	6.27	6.31	0.06	<0.01	<0.01	0.162
Ammonia-N, mg/dL	22.11	19.90	17.00	14.40	0.36	<0.01	<0.01	0.584
Total SCFA, mmol/L	71.4	71.0	69.5	69.1	0.29	<0.01	<0.01	0.972
SCFA, mol/100 mol								
Acetic acid	62.9	63.2	63.7	64.3	0.11	<0.01	<0.01	0.189
Propionic acid	26.3	26.5	26.7	27.0	0.07	<0.01	<0.01	0.483
Butyric acid	10.1	9.4	8.4	7.4	0.08	<0.01	<0.01	0.080
Isovaleric acid	0.34	0.38	0.55	0.66	0.01	<0.01	<0.01	<0.01
Valeric acid	0.37	0.43	0.60	0.69	0.01	<0.01	<0.01	0.164
Acetic:propionic	2.39	2.38	2.39	2.38	0.01	0.957	0.628	0.889
Total protozoa, log_{10}/g of digesta	6.14	6.13	6.09	6.07	0.01	<0.01	<0.01	0.152
Entodiniinae	5.91	5.88	5.84	5.77	0.01	<0.01	<0.01	0.119
Diplodiniinae	5.33	5.33	5.33	5.34	0.003	0.527	0.178	0.606
Dasytrichidae	5.13	5.15	5.13	5.14	0.01	0.781	0.943	0.847
Isotrichidae	5.21	5.22	5.21	5.19	0.04	0.477	0.216	0.339
Ophryoscolex	4.79	4.84	4.70	4.77	0.09	0.747	0.632	0.927
Cellulolytic bacteria, log_{10}/g of digesta	7.07	7.27	8.53	9.73	0.34	<0.01	<0.01	0.176
In vitro experiment								
Total protozoa, log_{10}/g of digesta	6.03	6.01	6.00	6.00	0.01	<0.01	<0.01	0.133
Entodiniinae	5.78	5.72	5.68	5.63	0.02	<0.01	<0.01	0.727
Gas production, mL/g of DM	201	206	212	214	2.73	0.034	0.012	0.233
Methane, mL/g of DM	44.79	43.66	41.68	39.94	0.36	<0.01	<0.01	0.408

[1]T = the main effect of the treatment.

DISCUSSION

Triticale Yield

The triticale was harvested at the milky-dough stage of the kernel with a DM yield of 14.2 t/ha, which was higher than the boot-stage harvested triticale yield of 5.7 t/ha reported by Santana et al. (2019). The DM yields of fall-sown triticale ranged between 7.5 and 16.3 t/ha (Bilgili et al., 2009; Keles et al., 2016; Salama and Badry, 2020).

Feed Intake and Nutrient Digestibility

The lower feed intake of the lactating cows with the dietary addition of TH may be connected to the presence of rough awns reducing palatability and this would explain the lower DMI observed (Smith et al., 2018). In our trial, TH was moisturized in an attempt to alleviate this problem but the use of new triticale varieties that are awnless or have much-reduced awns is recommended (Smith et al., 2018). As expected, the lower DMI with the increased dietary TH concentration led to lower OM, CP, NDF, and NFC intakes. However, Santana et al. (2019) noted that DMI was unaffected in cows fed TH in comparison with an AH-based diet. Differences between our results and those of Santana et al. (2019) relate to plant growth stage at harvest; in this study the triticale was at the milky-dough stage (CP = 81, NDF = 60, ADL = 6.3, and NFC = 214 g/kg of DM) whereas the latter was at the boot stage (CP = 18.5, NDF = 53.0, ADL = 4.5, and NFC = 315 g/kg of DM). This discrepancy may also be related to the basal diet type and ingredients. Table 3 shows that nitrate levels in the TH diets were lower than what is considered toxic (i.e., <10 g/kg of DM; Aiello, 1998), which indicates that TH can be a valuable ruminant forage.

The linear increases in DM, OM, CP, and NDF digestibilities in cows receiving TH may be related to the lower intake of TH rations (Table 4). This is because when ruminants consume less feed, the passage rate of digesta in the alimentary canal is slower and digestibility increases as a result of higher retention time. Moreover, increasing TH levels in the diet decreased daily ADL intake, improving digestibility (Van Soest, 1994; Table 4). In the other study, Santana et al. (2019) reported no difference in OM and CP digestibility in dairy cows given TH or AH diets when their feed intakes were similar. However, they recorded a tendency for NDF digestibility to increase linearly (P = 0.07) when TH diets replaced AH diets, suggesting a faster passage rate but lower fiber digestibility for legumes compared with grasses (Kuoppala et al., 2009).

In Vivo Rumen Fermentation Variables and In Vitro Protozoa and Methane Production

Dietary TH showed no effect on rumen pH biologically; pH values were between 6.03 and 6.31 (Table 5), which is considered to be within the range (i.e., 5.7–7.0) of normal rumen microbial activity (Dehority, 2003).

In all experimental animals, ruminal ammonia-N (14.4 to 22.1 mg/dL rumen liquor) was well more than the minimum level needed for optimum microbial growth (5.0 mg/dL; Sinclair et al., 1993). Decreasing rumen ammonia levels with higher dietary TH levels can partly be explained by higher rumen EMNS (Table 6), that is, the incorporation of more ammonia into the microbial

Table 6. Effect of substituting TH for alfalfa, corn silage, and barley straw on DMI, milk production, milk composition, MUN, and FCM in lactating Holstein cows (n = 8)

	Level of TH in diet (g/100 g of forage DM)					*P*-value		
Item	0	33	66	100	SEM	T[1]	Linear	Quadratic
Milk yield, kg/d	28.33	28.62	28.64	28.00	0.62	0.894	0.728	0.455
FCM[2]	24.83	25.74	26.41	26.39	0.28	<0.01	<0.01	0.109
Milk fat, g/kg	31.80	33.26	34.83	35.98	0.75	<0.01	<0.01	0.837
Milk fat, kg/d	0.90	0.95	0.99	1.01	0.02	<0.01	<0.01	0.289
Milk protein, g/kg	30.84	30.20	30.91	31.11	0.49	0.592	0.487	0.398
Milk protein, kg/d	0.87	0.86	0.88	0.87	0.02	0.918	0.793	0.883
Lactose, g/kg	49.60	48.91	49.56	49.60	0.58	0.806	0.852	0.585
Lactose, kg/d	1.41	1.40	1.42	1.39	0.03	0.946	0.787	0.711
Milk yield/DMI	1.19	1.25	1.29	1.36	0.03	<0.01	<0.01	0.883
FCM/DMI	1.05	1.12	1.19	1.28	0.02	<0.01	<0.01	0.694
Price (Toman[3]/kg of milk)	3,988	3,860	3,698	3,537	89.3	<0.01	<0.01	0.852
Price ($/kg of milk)	0.114	0.110	0.106	0.101	0.002	<0.01	<0.01	0.852
Urea N, mg/dL	12.49	12.17	11.94	11.39	0.26	0.027	<0.01	0.679
BW change, g/d	471	450	428	443	28.57	0.806	0.414	0.541

[1]T = the main effect of the treatment.

[2]FCM (40 g of fat/kg) = 0.4 × milk yield (kg/d) + 15 × milk fat (kg/d).

[3]Toman = Iranian currency.

mass. Lower rumen ammonia levels also relate to lower protozoa numbers, especially *Entodiniinae* spp. (Table 5) because these are the primary species involved in bacterial lysis (Belanche et al., 2012). It may also be an effect of the lower daily CP intake of cows receiving increasing levels of TH.

The SCFA are endpoints of microbial fermentation and are the main provider of energy in ruminants (Dehority, 2003), so their lower production is disadvantageous for the animal. A major effect of TH addition in the diet was a decreased ruminal concentration of SCFA (Table 5). The lower daily OM intake of the cows (Table 4), resulting in lower daily OM fermentation, may explain the lowering of SCFA as TH in the diet increased (Dehority, 2003). Moreover, the reduction of ruminal SCFA in cows fed TH diets may partly be a consequence of the reduced protozoa numbers (Newbold et al., 2015). This reduction is because protozoa activities are linked to total SCFA concentrations; they engulf starch granules, store them as glycogen, and later ferment them to produce SCFA (Newbold et al., 2015). The increased ruminal acetic acid concentrations with the increasing dietary TH rates are related to the higher cellulolytic bacteria numbers (Table 5) because their OM fermentation endpoint is acetic acid (Newbold et al., 2015). Paula et al. (2016) also reported that phenolics (Table 3) lowered *Entodinium* numbers, which favored acetic acid production, similar to the result seen in this research. Higher propionic acid levels with TH diets may relate to a decrease in CH_4 production in vitro because this competes for hydrogen ion acceptors and negatively correlates with propionic acid levels (Newbold et al., 2015). Protozoa also produce butyric acid from fermenting carbohydrates (Williams and Coleman, 1992; Babayemi et al., 2004), which may explain the lower butyric acid levels in cows fed increasing concentrations of TH in the diet.

The lower *Entodiniinae* numbers and, consequently, lower total protozoa numbers in TH-fed cows, compared with control animals, may relate to their higher polyphenol intake, particularly flavonoids (Oskoueian et al., 2013). However, there is no consistency in the phenolics' effect on protozoa among research, due to differences in diet type, animals, sampling methods, and plant metabolite type and concentration (Patra and Saxena, 2011), and differences in the adaptability of protozoa (Wallace et al., 2002). The TH addition did not affect *Diplodiniinae*, *Isotrichidae*, and *Ophrioscolecinae* numbers, implying that different protozoa species respond differently to diets according to their needs and available substrates (Dehority, 2003).

The increased ruminal cellulolytic bacteria numbers in the cows fed the increasing TH concentrations may be a consequence of higher valeric and isovaleric acid levels because these acids act as growth factors for these bacteria (Liu et al., 2014). Additionally, reduced *Entodiniinae* protozoa numbers (bacterial predators; Dehority, 2003) could lead to the greater populations of cellulolytic bacteria. In this study, decreasing CH_4 production with the increase in dietary concentration of TH was mostly related to the decreased protozoa numbers, because rumen protozoa, particularly the *Entodiniinae* subfamily, are associated with methanogens and subsequent CH_4 production (Kamra, 2005). The TH-fed cows also had increased ruminal concentrations of propionic acid, the production of which reduces hydrogen availability (Patra et al., 2017). The increased consumption of flavonoid in cows fed TH diets led to lower CH_4 production, which suggests that these molecules may be able to act as bioactive regulators in ruminants (Kim et al., 2015). Ruminant CH_4 emissions account for approximately 16% of global CH_4 (Knapp et al., 2014) so feeds such as TH may help to improve the environment.

Milk Yield and Composition

A linear decrease in feed intake was recorded with the addition of TH to the diet of the cows, but this made no difference to milk yields. This would suggest that cows could compensate for reduced DMI via improved digestibility and feed efficiency and from BW reserves. The BW of TH-fed cows in this study remained unchanged, leading to the conclusion that improved feed efficiency was the reason for unaffected milk yields. These results lend adequate support to TH use in dairy cow rations. However, there are inconsistencies in studies involving TH in cow diets. Santana et al. (2019) recorded milk production declining linearly (from 37.6 to 36.9 kg/d) when TH replaced AH completely, but there was no effect on DMI. Harper et al. (2017) showed that boot-stage harvested ensiled triticale would support milk yields higher than 41 kg/d when it replaced 10% of DM instead of corn silage. This substitution did not affect DMI but it did reduce milk yield and efficiency by small but significant amounts (1.5 kg of milk/d and 0.06 kg milk yield/kg DMI). It was suggested that the replacement of starch (in corn silage) for digestible fiber (in triticale silage) resulted in a lower energy supply. In the current study, starch levels were kept the same among all the diets. Moreover, the lower in vitro CH_4 production from TH diets (Table 5) indicates higher energy efficiency along with more EMNS (Table 7), leading to improvements in milk efficiency.

Milk fat concentration presented a linear response to increasing TH in the diet, up to +13% when TH made up 100% of the forage (Table 6). The higher milk fat percentage in cows fed TH diets may be related to the higher digestibility of NDF (Table 4) resulting in higher production of the main milk fat precursor (i.e., acetic

Table 7. Effect of substituting TH for alfalfa, corn silage, and barley straw on PD concentrations in the urine and EMNS (g/d) in lactating Holstein cows (n = 8)

	Level of TH in diet (% of forage DM)					*P*-value		
Item	0	33	66	100	SEM	T[1]	Linear	Quadratic
Urinary excretion, mmol/d								
Allantoin	312	326	329	332	5.28	0.037	0.013	0.322
Uric acid	34.8	36.3	37.5	38.1	0.93	0.034	0.012	0.669
Total PD excreted	347	362	366	370	5.68	0.050	<0.01	0.323
Total PD absorbed	350	368	373	378	6.69	0.029	<0.01	0.323
EMNS, g/d	255	268	271	274	4.74	<0.01	<0.01	0.323

[1]T = the main effect of the treatment.

acid; Table 5; Van Soest, 1994). Higher milk fat levels were associated with higher rumen pH in cows fed TH diets. In comparison, Harper et al. (2017) recorded that the substitution of corn silage with triticale silage (at 10% dietary DM) did not affect milk fat level or yield. Milk protein levels in this study were similar among the animals, indicating that EMNS and different dietary CP intakes of the experimental cows did not limit milk production (Krause et al., 2002). However, Santana et al. (2019) observed that milk protein decreased linearly at a rate of 2 g/d per percentage of TH in the DM leading to a decline of 2.5% when TH completely replaced AH. Harper et al. (2017) recorded a lowering of milk protein yield (5.5%) and lactose (6.5%) when triticale silage was substituted for corn silage at a rate of 10% of the DM.

Milk urea N is a part of milk N that is used to assess the status of protein and energy in dairy cattle (Moore and Varga, 1996). In this work, the MUN concentration was significantly lower in TH-fed cows, but the range of MUN concentration in all cows (i.e., 12.5–11.4 mg/dL) was within normal range (11–14 mg/dL) recorded by Moore and Varga (1996), which indicates that dietary TH did not affect the cows' protein and energy balance. Santana et al. (2019) observed similar ranges of MUN when TH completely replaced AH in the ration (12.3 vs. 12.5 mg/dL for AH and TH diets, respectively).

Urinary PD and EMNS

In the present experiment, cows fed TH had higher EMNS than the control group. This relates to lower rumen protozoa numbers, particularly *Entodinium* sp. (Table 5), because these are involved in the breakdown of most rumen bacteria. The consequence of this is that the microbial-N flow to the small intestine is increased, leading to an increase in the amino acid levels being absorbed (Belanche et al., 2012). The different EMNS levels were not reflected in milk protein levels, indicating that differing dietary CP intakes did not limit milk production (Krause et al., 2002).

Finally, in view of the economic impact on producers, results show that, it is 11% more expensive to produce a kilogram of milk using conventional feeding practices (i.e., alfalfa, corn silage, and barley straw) compared with a TH-based diet (Tables 3 and 6).

CONCLUSIONS

Results suggest that TH can form the forage portion of the diet of lactating Holstein cows, replacing alfalfa, corn silage, and barley straw, with no adverse effect on cow performance. Dietary inclusion of TH reduced feed consumption but positively affected milk production efficiency and milk fat, and also reduced in vitro CH_4 production. This forage can be an excellent choice for dairy farms where the cultivation of alfalfa and corn silage is difficult due to arid conditions and water shortages.

NOTES

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Abbreviations used: AH = alfalfa hay; AREEO = Agricultural Research, Education and Extension Organization; EE = Ether extract; EMNS = estimated microbial-N synthesis; PD = purine derivative; SCFA = short-chain fatty acid; TH = triticale hay.

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