Veterinary and Animal Science 27 (2025) 100417

Contents lists available at ScienceDirect

Veterinary and Animal Science

journal homepage: www.elsevier.com/locate/vas

Productive performance, rumen parameters, carcass quality, antioxidant profile and methane emission in lambs supplemented with triticale hay

G.A. Izadi [a], Y. Rouzbehan [a,*], J. Rezaei [a], M.J. Abarghuei [b]

[a] Department of Animal Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, P.O. Box 14115-336, Tehran, Iran
[b] Animal Science Research Department, Fars Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Shiraz, Iran

ARTICLE INFO

Keywords:
Triticale hay
Grey Shirazi lamb
Digestibility
Growth performance
Ruminal variables
Carcass characteristics

ABSTRACT

This study aims to measure the effects of different dietary concentrations of triticale hay (TH) on productive performance, carcass characteristics, microbial protein synthesis (MPS), ruminal and blood variables, and antioxidant power in 40 fattening male Gray Shirazi lambs (BW of 33.2 ± 1.1 kg) over 81 days in a completely randomized design (10 animals/diet). Four levels of TH (0.00, 13.30, 26.50, and 40.00 g/100 g dry matter; DM) were included in the diet, instead of alfalfa hay + barley straw. Dietary inclusion of TH decreased DM and nutrient intakes, but increased phenolic and flavonoid intakes, and digestibility of DM and nutrients (L, $P < 0.01$). Feeding TH did not affect daily gain, feed conversion ratio, carcass weight, dressing percentage, and *Longissimus* muscle area, decreased (L, $P < 0.05$) fat-tail, total carcass fat, and carcass stearic acid but increased (L, $P < 0.05$) lean-to-total carcass fat ratio, carcass oleic acid, polyunsaturated fatty acids (FA), and antioxidant power. Dietary TH increased ruminal MPS and cellulolytic bacteria but decreased ammonia-N, protozoa, and *in vitro* methane production (L, $P < 0.01$). Nitrogen retention and rumen short-chain FA were not affected by TH addition. Increasing dietary TH levels lowered (L, $P < 0.01$) blood cholesterol, triglyceride, and urea-N, but did not affect glucose, total protein, albumin, globulin, nitrate, and nitrite. Overall, TH can be included, up to 40 % of DM in the diet of fattening lambs, without effects on performance, to improve carcass quality, FA composition, and antioxidant capacity and reduce ruminal methane production.

1. Introduction

Nutrients in forage, including energy, protein, vitamins, and minerals, are the foundation for all rationing systems in optimizing productivity (NASEM, 2016). Conventional forages used in the diet of ruminants are alfalfa hay (AH), corn silage, and grass, which are the most water-demanding crops (Dhiman & Satter, 1997; McKenzie & Wood, 2011). Altered weather patterns and other factors (*i.e.,* dam and reservoir construction, 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 hybrid triticale (x *Triticosecale* Wittmack), that need less water than conventional forages and can be grown and harvested as hay and display desirable traits from both parent species (wheat and rye) (Glamoclija et al., 2018).

Triticale is a winter-grown crop, which has a higher dry matter (DM) concentration than barley or oats and can resist harsh environmental conditions such as cold, drought, and soil salinity (Coblentz et al., 2022) making it suitable for production in marginal areas (*i.e.,* arid, saline, or soils with extreme pH and heavy metal toxicity) (Eudes, 2015). 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 & Badry, 2020). According to the results presented by Keles et al. (2016), DM and digestible DM yields obtained per hectare improved with the progression of the triticale growth stage, so that they were greater in the milky and dough stages than in the booting and flowering stages.

Triticale hay is a common forage that can be used in the ration of ruminants in the form of silage or hay. Santana et al. (2019) reported

Abbreviations: AH, alfalfa hay; ADFom, ash-free acid detergent fibre; BW, body weight; CH_4, methane; CP, crude protein; DM, dry matter; FA, fatty acids; FCR, feed conversion ratio; ME, metabolisable energy; NDFom, ash-free neutral detergent fibre; NH_3-N, ammonia-N; OM, organic matter; PD, purine derivatives; SCFA, short chain fatty acids; TAC, total antioxidant capacity; TH, triticale hay; TMR, total mixed ration; TP, total phenolics.

* Corresponding author at: Department of Animal Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, P.O. Box14115-336, Iran.
E-mail address: rozbeh_y@modares.ac.ir (Y. Rouzbehan).

https://doi.org/10.1016/j.vas.2024.100417

Available online 18 December 2024

that the DM intake (DMI) in dairy cows fed on TH showed no differences from those offered AH. Additionally, Ashkvari et al. (2024) proposed that triticale, which is cultivated using less water than corn forage and alfalfa, is a potential alternative crop and can increase milk productivity and milk fat and decrease methane release in dairy cows. Moreover, Guamán-Rivera et al. (2023) reported that the DM intake of triticale (cv. Titania) and hooded barley (cv. Mochona) in sheep was similar, though, triticale had a slightly greater nutritive value than hooded barley. Furthermore, Keles et al. (2016) reported that the feeding value of TH for fattening lambs was intermediate (DMI of 850 g/d along with ADG of 110 g/d), *i.e.,* lower than the feeding value of wheat and oats, but higher than that of barley or rye. They stated that the awns in triticale harvested between ear emergence and the milk stage reduced its potential as a forage. Akbağ (2022), also, noted that barley and triticale forages have adequate metabolizable energy (ME) and crude protein (CP) levels to meet the nutritional requirements of adult goats of 50 kg body weight (BW) in early lactation. The variation in the animal's performance when fed triticale forage was possibly due to a combined effect of dissimilarities in its nutritive value and morphological structure, which is in turn affected by cultivar, moisture levels soil type, and harvesting stage (Keles et al., 2016).

On the other hand, Giorgio et al. (2019) reported that some triticale varieties contain considerable amounts of phenolics, which these compounds may improve performance, efficiency, estimated microbial protein synthesis (MPS), N retention, and antioxidant capacity in ruminants (Ahmed et al., 2024). Phenolics, also, decrease ruminal protozoa and methanogen populations leading to a reduction of enteric methane (CH_4) emissions (Newbold et al., 2015). Therefore, it was hypothesized that lambs fed TH diets would improve their performance increase TAC of the blood, and reduce ruminal CH_4 emissions. Hence, the objective of the current study was to assess the effect of dietary inclusion of different levels of TH, substituted for alfalfa hay (AH) + barley straw, on *in vivo* diet digestibility, performance, carcass quality, rumen and blood variables, blood antioxidant capability, EMPS, N balance, and *in vitro* ruminal methane production in fattening male Gray Shirazi lambs.

2. Materials and methods

The study was done at the Agricultural and Natural Resources Research and Education Center of Fars (Shiraz City, Fars province, Iran). The FASS (2010) guidelines were followed as to the care and use of the experimental lambs. All experimental protocols (proposal 9,930,092, 001, 2020) were approved by the Tarbiat Modares University Committee of Animal Science.

2.1. Plant preparation and chemical analysis

Triticale was sown in the autumn and winter of 2020 at the field (30° 01′ 56″ N, 53° 08′ 32″ E) in Fars Agricultural Training Center, Ali Abad Kamin, Pasargad, Fars, Iran, and harvested in the spring at the milky-dough stage, due to higher DM and digestible DM yields than those in the boot and flowering stages. The mean yearly temperature and rainfall from 2020 to 2021 were 16.6 °C and 260 mm, respectively. Moreover, the mean temperature and rainfall during the growing period are shown in Table 1. After harvesting the fodder, five samples were taken from different locations in the field to determine fresh forage DM. Harvested biomass was air-dried for 5 days to reach approximately 95 % DM and then baled as hay.

For chemical analysis (Table 2), samples of the feedstuffs, including TH, AH, and barley straw, were dried to a constant weight to obtain DM and then milled through a 1-mm sieve (Felini et al., 2024). Nitrogen concentrations of the fodders were recorded using AOAC (2012) procedure 990.03, adapted for an automatic distiller Kjeldahl (Kjeltec Analyzer, Foss Analytics, Hillerød, Denmark), and CP derived as $N \times 6.25$. Method 942.05 of AOAC (2012) was followed to determine ash content, 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. The forage ether extract (EE) concentration was determined using the AOAC (2012) technique 2003.05, which involved the exhaustive extraction of 3 g of sample in a Soxhlet apparatus using petroleum ether (boiling range 40 to 60 °C) as the extractant. The ash-free neutral detergent fiber (NDFom) concentrations of the fodders were measured without sodium sulfite and expressed exclusive residual ash according to Van Soest et al. (1991). Ash-free acid detergent fiber (ADFom) was determined gravimetrically as the residue remaining after extraction with an acid detergent solution (Van Soest et al., 1991). The

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

Month	Average 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

Table 2
Average chemical analysis (g/100 g DM or as stated), *in vitro* organic matter digestibility, and metabolisable energy of alfalfa, barley straw, and triticale ($n = 5$).

Item	Alfalfa	Barley straw	Triticale	SEM	P-value
	Fresh	Dried	Fresh		
Dry matter, g/100 g fresh weight	33.31[c]	94.82[a]	36.32[b]	0.56	<0.01
Chemical analysis	hay	Dried	hay		
Crude protein	13.90[a]	3.03[c]	8.33[b]	0.33	<0.01
Ash	7.34[b]	9.05[a]	7.00[b]	0.15	<0.01
Ash-free neutral detergent fiber	49.34[c]	73.08[a]	58.12[b]	0.33	<0.01
Ash-free acid detergent fiber	36.70[b]	47.24[a]	37.52[b]	0.40	<0.01
Lignin(sa)[1]	7.54[b]	10.83[a]	6.66[b]	0.28	<0.01
Ether extract	2.21[b]	1.24[c]	3.32[a]	0.09	<0.01
Non-fiber carbohydrates[2]	27.31[a]	13.63[c]	23.32[b]	0.34	<0.01
Starch	2.57[b]	1.65[c]	12.88[a]	0.25	<0.01
Water-soluble carbohydrates	4.11[b]	1.23[c]	8.00[a]	0.27	<0.01
Total phenolic compounds	0.58[b]	0.03[c]	0.88[a]	0.02	<0.01
Total tannin[3]	0.33[b]	0.01[c]	0.63[a]	0.19	<0.01
Flavonoids[4]	0.83[b]	0.01[c]	1.08[a]	0.03	<0.01
Nitrate	0.20[a]	0.02[c]	0.12[b]	0.02	<0.01
IVOMD[5],%	61.15[b]	40.03[c]	64.80[a]	0.67	<0.01
ME[6], MJ/kg DM	8.73[b]	5.91[c]	9.13[a]	0.11	<0.01

[1] Lignin(sa) = lignin determined by solubilisation of cellulose with 72 % sulphuric acid solution;
[2] Non-fiber carbohydrates = 100 – (% CP +% Ash +% EE +% NDF);
[3] Expressed as tannic acid equivalent;
[4] Expressed as quercetin equivalent;
[5] IVOMD = *in vitro* organic matter digestibility (%) calculated as 14.88 + (0.889 × mL gas production after 24 h) + (0.45 × CP) + (0.0651 × ash);
[6] ME = metabolisable energy (MJ/kg DM) calculated as 2.20 + (0.136 × mL gas production after 24 h) + (0.057 × CP) + (0.0029 × CP^2) (Menke et al., 1979).

samples were analyzed for lignin(sa) according to AOAC (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. Once weighed, crucibles were placed in a muffle for 4 h at 490 °C to incinerate the residue. Ash content was then subtracted from the initial weight to calculate the lignin(sa) content. The water-soluble carbohydrate (WSC) of the samples was extracted with distilled water (0.2 g sample in 200 mL water) and its concentration was determined using the anthrone reaction assay, where the absorbance of the extract was measured by a photometer (Hall et al., 1999). Total phenolic compounds (TP) were measured using the Folin-Ciocalteau reagent method. The sample (200 mg) was dissolved in mixed acetone and water (10 mL; 70:30 vol./vol.) using an ultrasonic bath for 20 min. The contents were centrifuged at 3000 × *g* for 10 min at 4 °C and the supernatant was kept on ice until analysis. Non-tannin phenolics (NTP) were determined by 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 (3000 × *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 (TT) were calculated as the difference between TP and NTP. Tannic acid (Merck GmbH, Darmstadt, Germany) was used as the standard to express the data. The total flavonoid content was determined by the colorimetric method according to Quettier-Deleu et al. (2000). The amount of 0.1 mL of diluted sample was mixed with 0.02 mL of 5 % (w/v) aluminium chloride methanolic solution. After 30 min of incubation at laboratory temperature, the absorbance of the reaction mixture was measured at 405 nm using a microplate reader (Epoch, USA). The results were expressed as the equivalent of quercetin. The colorimetric method of Singh (1988), based on diazotization, was used to determine nitrate content. Briefly, 100 mg of the sample was mixed with 50 mL of acetic acid (2 %), shaken for 20 min, and filtered through filter paper. Then, the nitrate concentration was measured colorimetrically at 540 nm. Atomic absorption spectrometry was used according to AOAC (2012) method 968.08 to obtain the Ca level, and the phosphorus level was obtained using colorimetry according to AOAC (2012) method 965.17.

2.2. Animals, experimental diets, and study period

This experiment was conducted with 4 trial diets (with different TH concentrations) in the form of total mixed ration (TMR) and 10 replications (lamb as an experimental unit) per diet (*i.e.,* one lamb in each pen). Forty male Grey Shirazi lambs with an average BW of 33.2 ± 1.1 kg and 6.5 months of age were used in this study. The Grey Shirazi sheep is a native breed of the Fars province, Iran. It is a moderate fat-tail in conformation that could be classified as a medium breed and has an average BW of 45 to 55 kg for ewe and 70 to 80 kg for ram (Karimi et al., 2022a). This breed is originally a range sheep, well adapted for utilizing low-quality feed under adverse environmental conditions in Iran, and does not have a high growth rate (Makarechian et al., 1978).

The treatments were 1- diet without TH (control), 2- diet containing 13.30 g TH/100 g DM, 3- diet containing 26.50 g TH/100 g DM, and 4- diet containing 40.00 g TH/100 g DM. The TH was used instead of AH + barley straw. The diets were adjusted based on the lambs' nutritional requirements (NRC, 2007), with the forage-to-concentrate ratio being 40:60 % of DM (Table 3). The experimental animals were given a 14-day adjustment period to acclimatize to the experimental environment and diets before starting the 81-day fattening period. Before the start of the experiment, the lambs were washed, and an anti-parasite drug and enterotoxaemia vaccine were administered.

Table 3
Ingredients and chemical composition (g/100 g DM or as stated) of the diets (n = 5) containing different triticale hay (TH) levels fed to fattening lambs.

	TH level in diet (g/100 g DM)			
Item	0.00	13.30	26.50	40.00
Feed ingredients				
Alfalfa hay	24.90	16.60	8.40	0.00
Barley straw	15.10	10.10	5.10	0.00
Triticale hay	0.00	13.30	26.50	40.00
Soybean meal (44 % CP)	7.55	7.55	7.55	7.55
Canola meal	1.89	2.74	3.58	4.47
Barley grain	21.90	21.90	21.90	21.90
Corn grain	14.60	14.60	14.60	14.60
Wheat bran	12.30	11.37	10.49	9.50
Salt	0.33	0.33	0.33	0.33
Oyster shell	0.00	0.24	0.47	0.71
Di-calcium phosphate	0.49	0.33	0.14	0.00
Mineral and vitamin premix[1]	0.28	0.28	0.28	0.28
Sodium bicarbonate	0.66	0.66	0.66	0.66
Chemical composition[2]				
Dry matter, g/100 g (as-fed)	92.25	92.14	92.10	92.07
Crude protein	13.38	13.31	13.24	13.17
Ash-free neutral detergent fiber	34.57	34.88	35.20	35.44
Ash-free acid detergent fiber	20.46	20.54	20.59	20.65
Lignin(as)[3]	4.70	4.23	3.72	3.23
Ether extract	3.51	3.42	3.33	3.25
Non-fiber carbohydrates	39.70	39.64	39.57	39.59
Starch	27.27	28.82	30.00	31.16
Ash	8.84	8.75	8.66	8.55
Calcium	0.98	0.97	0.96	0.96
Phosphorus	0.47	0.47	0.46	0.46
Calcium/Phosphorus	2.09	2.06	2.09	2.09
Total phenolic compounds	0.30	0.38	0.44	0.55
Total tannin[4]	0.16	0.24	0.29	0.35
Flavonoids[5]	0.30	0.37	0.45	0.52
Nitrate	0.08	0.07	0.07	0.06
ME[6], MJ/kg DM	10.76	10.81	10.87	10.92
Diet price, Toman[7]/kg DM	10,018	9855	9674	9513
Diet price, US $/kg DM	0.143	0.141	0.138	0.136

[1] Premix contained (per kg): Ca, 120 g; P, 30 g; Na, 55 g; Mg, 20 g; Zn, 3 g; Fe, 3 g; Mn, 2 g; Cu, 280 mg; Co, 100 mg; Se, 1 mg; K, 215 mg; I, 100 mg; vitamin E, 100 mg; vitamin A, 500,000 IU; vitamin D3, 100,000 IU; antioxidant, 400 mg; carrier, up to 1000 g

[2] The composition was determined via chemical methods;

[3] Lignin(sa) = lignin determined by solubilisation of cellulose with 72 % sulphuric acid solution;

[4] Expressed as tannic acid equivalent;

[5] Expressed as quercetin equivalents;

[6] ME = metabolisable energy calculated as 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);

[7] Toman = Iranian currency.

2.3. Intake, growth performance, and in vivo digestibility

The experimental lambs were housed individually in the concrete floor pens (1.5 *m* × 1.5 m) with bedding of wood chips. They were fed *ad libitum* with balanced diets, as TMR, at 08:00 h and 16:00 h daily to allow for 5 % orts with clean water always available. The weight of daily TMR distributed to each animal was recorded. Moreover, the corresponding orts were collected and weighed daily before the morning feed, and the diets were adjusted every week accordingly. Dry matter intake (DMI) was determined by subtracting the daily DM weight of ort from the TMR distributed. Moreover, the daily representative samples of the TMR and orts were collected immediately after mixing based on the quartering method. The samples were preserved for later chemical analysis to calculate daily nutrient intake.

Lambs were weighed, using a digital scale, every 20 days before the morning feed and average daily gain (ADG) was calculated using a linear regression model of BW over time. The feed conversion ratio (FCR) was estimated as daily DMI (g) divided by ADG (g).

Whole tract apparent digestibility amounts of DM, organic matter (OM), CP, NDFom, and EE were determined by the total feces collection technique (Galyean, 2010). On day 71, randomly 6 lambs per dietary treatment were housed in individual metabolism crates (0.6 *m* × 1.3 m), equipped with separate containers for collecting 24-h urine and feces. The crates were provided with a tray for total feces collection and underneath this tray another tray to roll into a separate bucket for total urine collection. The digestibility trial lasted for 7 continuous days, 2 days for adaptation, and then 5 days for sampling (*i.e.,* days 71 to 77). During this period, feed offered, orts and feces of each lamb were weighed each day using a digit analytical balance, and 10 % representative samples were kept. At the end of the trial, the obtained samples were pooled, and thoroughly mixed for each lamb per diet. The samples were analyzed chemically, as mentioned above, and the following equation was used to estimate the digestibility of nutrients:

Digestibility of dietary nutrient (%) = [(g daily intake of the nutrient – g daily nutrient excreted in the feces) / g daily intake of the nutrient] × 100

2.4. EMPS and N retained

During the period of measuring digestibility (*i.e.,* from day 71 to day 77), 24-h urine was also collected from animals in metabolism crates (6 replications per treatment), as mentioned for feces. The total urine produced daily was collected in plastic vessels containing 100 mL of sulphuric acid solution (10 %, vol/vol), to ensure the final pH was below 3.0. To prevent the precipitation of purine derivatives (PD) in urine during storage, 10 % of the daily amount was diluted fivefold with distilled water and stored at −20 °C to estimate PD and N. After thawing and filtration, total PD, including allantoin, uric acid, and xanthine + hypoxanthine, was determined using the photometry methods of Chen and Gomes (1992). Daily excreted urinary PD levels were utilized in estimating exogenous purines absorbed, and the EMPS was obtained as per Chen and Gomes's (1992) equations.

For estimation of the N balance in the experimental sheep, the urinary, fecal, diet, and ort N concentrations were measured and the data were used to obtain the daily N retention as daily N intake (g) – daily N excreted in urine and feces (g).

2.5. In vivo ruminal fermentation characteristics and in vitro methane release

To evaluate rumen fermentation, samples of rumen fluid were collected from all animals per treatment via esophageal tube on days 35 and 70, before morning feed distribution (0 h) and 3 h afterwards. An electric vacuum pump with a maximum continuous pressure of 68.9 kPa was utilized to extract rumen liquor. The pH value of the fluid was read using a pH meter (model Sartorius PT-10, Gottingen, Germany). Five milliliters of samples were treated with 1 mL of HCl (0.2 *N*) solution and frozen for ammonia-N (NH_3—N) analysis following the phenol-hypochlorite photometric method described by Galyean (2010). For analysis of ruminal short-chain fatty acids (FA) (SCFA), 5 mL of strained rumen fluid was mixed with 0.15 mL of 50 % sulphuric acid solution and stored at −20 °C. After defrosting in the laboratory, the ruminal liquid was centrifuged (10,000 × *g* for 10 min; 4 °C). Then, an aliquot of supernatant (1 mL) was mixed with 25 % meta-phosphoric acid solution (0.2 mL), including 2 g of 2-ethylbutyric acid per L (as internal standard), and injected to a UNICAM gas chromatograph (SB Analytical, Cambridge, UK), as per the method of Galyean (2010). A capillary column with dimensions of 10 *m* × 0.535 mm ×1 μm (Agilent J & W HP-FFAP, 19095F-121; Agilent Technologies, Santa Clara, CA) was used in the analysis, with the injection volume of 0.5 μL and helium as carrier gas.

Rumen protozoa were quantified according to Dehority (2003), where a 5-mL aliquot of the rumen contents was preserved at 4 °C in combination with 5 mL of 50% formalin solution. Then, protozoa were counted by a light microscope and hemocytometer (Improved Neubauer, Hawksley, UK). Five milliliters samples of rumen fluid were used to determine the population of cellulolytic bacteria using the Hungate tubes based on the most probable number procedure (Bryant, 1972; Dehority, 2003)

To estimate methane release, *in vitro* gas production test was performed using a batch system, in the 100-mL glass syringes (Menke et al., 1979). According to Demeyer et al. (1988) method, 24-h gas production was recorded then 4 mL of NaOH (10 *M*) solution was injected into each glass syringe to absorb the produced CO_2, and the leftover gas was considered to be approximately equivalent to CH_4 (naturally, it also contains minor amount of hydrogen). Moreover, the syringe content samples were prepared for *in vitro* protozoa assay according to Dehority (2003), as mentioned above. The *in vitro* study was conducted with 4 dietary treatments, 3 replications per treatment, two syringes (samples) per replication, and 2 batches, in different weeks, per treatment.

2.6. Blood metabolites

Two blood samples were taken from all the lambs on days 35 and 70, the first before the morning feed and the second sampling 3 h later. Approximately 10 mL of blood were taken via the jugular vein into the evacuated tubes, without any anticoagulant. After centrifugation (at 1500 × *g*; 15 min), the obtained separated serum was used to measure the concentrations of triglyceride, cholesterol, glucose, total protein, albumin, and urea-N by Pars Azmun Diagnostics kits (Tehran, Iran) and photometric methods. An average value from the 2 blood samples was reported. The total antioxidant capacity (TAC) of the blood samples was assessed using the ferric reducing antioxidant power (FRAP) method (Benzie & Strain, 1996), where the ferric-tripyridyltriazine complex reduces to ferrous form due to antioxidants. The intensity of the developed blue color was read photometrically at a wavelength of 593 nm and the ferrous sulphate solution was used as a standard to prepare calibration curves and calculations.

2.7. Carcass components characteristics and harvested carcass data

At the end of the fattening period, the experimental lambs were killed by the method of Farid (1991). The lamb hot carcass weights (HCW) were recorded then the carcasses were cooled (4 °C; 24 h) to allow the measurements of cold carcass weight (CCW). The cold dressing percentage (CDP) of the experimental sheep was calculated as the ratio of CCW to live BW. The obtained carcasses were cut between the 12th and 13th ribs and the width, depth, and area of the *Longissimus lumborum* muscle were measured according to Esenbuga et al. (2009). The depth of fat was measured at the 12th rib. Fat-tail, subcutaneous fat (SCF), intermuscular fat (IMF), lean, and bones were carefully separated and weighed (Farid, 1991). The TAC values from the *M. longissimus thoracis* (loin) muscle samples were obtained following the FRAP assay, as mentioned above (Benzie & Strain, 1996). Also, the extraction of intramuscular FA from subcutaneous fat of the loin was conducted by the method of Vasta et al. (2009). Duplicate 1 g sub-samples were used to determine the intramuscular FA via gas chromatography (Vasta et al., 2009), and quantitative analysis was completed using the FA methyl ester (FAME) method (Supelco, Bellefonte, PA, USA).

2.8. Statistical analyses

Data on digestibility, growth, carcass characteristics, offal, PD, microbial protein, and N retention were analyzed statistically as a completely randomized design using the MIXED procedure of SAS (SAS 9.1., SAS Institute) with the following model including dietary treatment as a fixed effect:

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

In the mentioned model, Y_{ijk} is dependent observation, μ is the

general mean, T_i is the treatment effect, and e_{ij} is residual error. The lambs' initial weights were applied as a covariance factor in the model but were insignificant and removed. Data obtained at different times, including feed intake, rumen and blood variables, were analyzed as repeated measurements with dietary treatment, time, and their interactions as fixed effects. In the statistical models, the animal was considered random.

The *in vitro* data were analyzed as repeated measures utilizing the model described below:

$$Y_{ijkl} = \mu + T_i + D_j + H_k + (TD)_{ij} + (TH)_{ik} + (DH)_{jk} + (TDH)_{ijk} + E_{ijkl}$$

where Y_{ijkl} is the dependent observation, μ is the total mean, T_i denotes the treatment effect, D_j is the sampling effect, H_k is the run effect, $(TD)_{ij}$ is the interaction between treatment and sampling, $(TH)_{ik}$ is the interaction between treatment and run, $(DH)_{jk}$ is the interaction between sampling and run, $(TDH)_{ijk}$ is the interaction between treatment and sampling and run, and E_{ijkl} is the residual error.

Additionally, a polynomial contrast was used to obtain the *P*-values of the linear or quadratic effects of feeding TH on measured variables. Statistical significance was defined as $P < 0.05$.

3. Results

3.1. Chemical composition of the experimental forages

The chemical compositions of TH, AH, and barley straw are listed in Table 2. The contents of CP, lignin(sa), non-fiber carbohydrates (NFC), and nitrate were significantly lower ($P < 0.01$) in TH (8.33, 6.66, 23.32, and 0.12 g/100 g DM, respectively) than in AH (13.90, 7.54, 27.31, and 0.20 g/100 g DM, respectively). The contents of NDFom, EE, starch, WSC, and IVOMD were higher ($P < 0.01$) in TH than in AH (58.12 vs. 49.34, 3.32 vs. 2.21, 12.88 vs. 2.57, 8.00 vs. 4.11, and 64.80 vs. 61.15 g/100 g, for TH *vs*. AH, respectively). Moreover, TH contained greater ($P < 0.01$) concentrations of TP, TT, and flavonoids (0.88, 0.63, and 1.08 g/100 g DM, respectively) compared to those in AH (0.58, 0.33, and 0.83 g/100 g DM, respectively).

3.2. Intake, in vivo digestibility and performance

According to Table 4, the main effect of feeding TH on feed intake was significant ($P < 0.05$), so daily DMI was lower in the animals fed with the 26.50 and 40.00 g TH-containing diets (1450 and 1430 g/d, respectively), compared to those fed the diets containing 0.00 and 13.30 g TH (1620 and 1547 g/d, respectively). The polynomial contrast showed that the intakes of DM, OM, CP, NDFom, NFC, starch, and EE decreased linearly (L, $P < 0.01$) as TH levels increased in the diet so that

Table 4
Effect of triticale hay (TH) level in the diet on feed intake ($n = 10$), digestibility ($n = 6$), and growth performance ($n = 10$) of fattening lambs.

	TH level in diet (g/100 g DM)							*P*-value[8]
Item	0.00	13.30	26.50	40.00	SEM[7]	Trt	Lin	Quad
Intake, g/d								
Dry matter	1620^{a}	1547^{a}	1450^{b}	1430^{b}	41.90	0.01	< 0.01	0.88
Organic matter	1452^{a}	1405^{a}	1301^{b}	1292^{b}	33.30	< 0.01	< 0.01	0.08
Crude protein	209^{a}	200^{a}	183^{b}	179^{b}	10.11	< 0.01	< 0.01	0.51
Ash-free NDF[1]	535^{a}	534^{a}	507^{b}	492^{b}	19.84	0.01	< 0.01	0.03
Non-fiber carbohydrates	654^{a}	619^{a}	562^{b}	572^{b}	13.62	< 0.01	< 0.01	0.90
Starch	404^{a}	401^{a}	369^{b}	360^{b}	11.23	0.01	< 0.01	0.31
Ether extract	54.32^{a}	52.30^{ab}	49.40^{bc}	48.72^{c}	1.78	0.02	< 0.01	0.88
Lignin(sa)[2]	76.28^{a}	64.76^{b}	53.43^{c}	45.58^{d}	2.03	< 0.01	< 0.01	0.97
TP[3]	4.85^{d}	5.90^{c}	6.44^{b}	7.89^{a}	0.21	< 0.01	< 0.01	0.93
TT[4]	2.52^{d}	3.74^{c}	4.28^{b}	5.02^{a}	0.10	< 0.01	< 0.01	0.90
Flavonoids	0.49^{d}	0.58^{c}	0.66^{b}	0.76^{a}	0.02	< 0.01	< 0.01	0.89
Apparent digestibility,%								
Dry matter	62.32^{c}	64.64^{bc}	67.06^{ab}	69.60^{a}	1.58	0.02	< 0.01	0.90
Organic matter	64.40^{c}	67.10^{bc}	69.89^{ab}	72.10^{a}	1.46	0.02	< 0.01	0.67
Crude protein	70.12^{b}	72.91^{b}	78.14^{a}	81.54^{a}	1.88	0.01	< 0.01	0.41
Ash-free NDF	58.92^{c}	61.57^{bc}	64.44^{ab}	67.46^{a}	1.56	0.01	< 0.01	0.84
Non-fiber carbohydrates	64.41^{b}	67.92^{b}	71.79^{a}	71.82^{a}	2.03	0.02	< 0.01	0.20
Total digested nutrient, g/d								
Dry matter	1009	1000	972	976	26.73	0.89	0.84	0.80
Organic matter	935	943	909	932	23.44	0.81	0.83	0.75
Crude protein	147	146	142	146	8.76	0.99	0.99	0.99
Ash-free NDF	315	329	327	332	10.12	0.69	0.70	0.70
Non-fiber carbohydrates	421	420	403	410	10.25	0.60	0.67	0.63
Growth performance								
Initial body weight, kg	33.77	33.30	33.11	32.71	1.04	0.70	0.75	0.88
Final body weight, kg	53.21	51.04	50.12	50.42	1.21	0.13	0.16	0.37
ADG[5], g/d	239	218	210	218	8.45	0.14	0.15	0.11
Feed conversion ratio	6.78	7.10	6.90	6.56	0.29	0.50	0.45	0.12
Feeding costs/d, Toman[6]	16229^{a}	15249^{a}	14030^{b}	13600^{b}	366	< 0.01	< 0.01	0.33
Feeding costs/d, US $	0.232^{a}	0.218^{a}	0.201^{b}	0.194^{b}	0.01	< 0.01	< 0.01	0.33

[1] NDF = neutral detergent fiber;
[2] Lignin(sa) = lignin determined by solubilization of cellulose with 72 % sulphuric acid solution;
[3] TP = total phenolic compounds;
[4] TT = total tannin;
[5] ADG = average daily gain; [5]Toman = Iranian currency;
[6] Toman = Iranian currency;
[7] SEM = standard error of the means;
[8] Trt = main effect of treatment; Lin = linear effect; Quad = quadratic effect. In each row, different letters indicate significant differences among treatments ($P < 0.05$).

the intakes were lower in lambs receiving the 26.50 and 40.00 g TH diets than those animals fed with the 0.00 and 13.30 g TH diets. However, the intakes of plant secondary metabolites, *i.e.,* TP, TT, and flavonoid, increased linearly (4.85 to 7.89, 2.52 to 5.02, and 0.49 to 0.76 g/d, respectively) with increasing dietary rates of TH (L, $P < 0.01$).

Feeding TH had a significant effect ($P < 0.05$) on the *in vivo* diet digestibility such that the linear increases (L, $P < 0.01$) were observed in the digestibility coefficients of DM, OM, CP, NDF, and NFC as dietary TH levels increased. The DM digestibility was the highest (67.06 and 69.60 %) in 26.50 and 40.00 g TH-containing groups, intermediate (64.64 %) in 13.30 g TH diet and the lowest (62.32 %) in the TH-free diet. A similar trend was observed for the digestibility of the nutrients.

The final BW of the lambs was 50.12 to 53.21 kg, which was not statistically different among the groups. Moreover, the animals' growth performance during the fattening period revealed no differences in terms of ADG (218 to 239 g/d) and FCR (6.56 to 7.10) values between the TH-fed and control lambs. It is noteworthy that daily feeding costs decreased considerably (0.232 to 0.194 US $) with increasing levels of TH in the animal diet.

3.3. Carcass components characteristics and harvested carcass data

The main effect, as well as linear and quadratic effects, of the dietary TH levels were not significant regarding HCW, CCW, and CDP of the lambs (Table 5), where their values were 25.55 to 27.39 kg, 24.95 to 26.96 kg, and 49.57 to 51.31 %, respectively. Fat-tail, total carcass fat (TCF), and SCF + IMF weights decreased linearly (L, $P < 0.01$) and the lean-to-TCF ratio increased linearly (L, $P < 0.01$) with increasing levels of dietary TH, but carcass offal parts were not affected. The lambs receiving 26.50 and 40.00 g TH-containing diets had the lower fat-tail and TCF and the higher lean-to-TCF ratio (3.45 to 3.58 kg, 6.01 to 6.63 kg, and 2.02 to 2.23, respectively), compared to 0.00 and 13.30 g TH-fed groups (4.23 to 4.31 kg, 7.45 to 7.67 kg, and 1.71 to 1.76, respectively). In terms of the SCF + IMF weight, the value detected for the lambs receiving a 40.00 g TH diet (2.57 kg) was lower than the other animals. The carcass TAC value enhanced linearly (L, $P = 0.01$) with increasing dietary TH concentrations, so that the carcass TAC of the lambs fed the diets without or containing 40.00 g of TH was 0.19 and 0.22 mmol Fe^{3+}/100 g, respectively.

This study highlighted differences in carcass FA profiles among the experimental groups (Table 5). The lambs receiving the increasing dietary TH levels had significantly higher unsaturated FA and lower saturated FA percentages in their carcass fat (L, $P < 0.05$). In this regard, including 40.00 g TH per 100 g diet DM, instead of AH + straw, resulted in the maximum proportion of carcass unsaturated FA (*i.e.,* 54.80 vs. 57.10 g/100 g of total FA in 0.00 and 40.00 g TH groups, respectively).

Table 5
Effect of triticale hay (TH) in the diet on harvested carcass, carcass components, total antioxidant capacity (as the units stated), and carcass fatty acid profile (g/100 g of total fatty acids) in fattening lambs ($n = 10$).

	TH level in diet (g/100 g DM)					*P*-value[12]		
Item	0.00	13.30	26.50	40.00	SEM[11]	Trt	Lin	Quad
Harvested data								
HCW[1], kg	27.39	25.83	25.55	25.75	0.73	0.24	0.24	0.36
CCW[2], kg	26.96	25.42	24.95	25.27	0.69	0.21	0.21	0.31
CDP[3],%	50.72	51.31	49.81	49.57	1.03	0.30	0.35	0.44
LL[4] area, cm^2	12.16	11.78	12.90	12.81	0.46	0.30	0.32	0.60
Fat thickness, mm	4.02	3.09	3.41	3.53	0.32	0.31	0.70	0.31
Carcass components								
Lean, kg	13.44	13.12	13.38	13.14	0.34	0.81	0.81	0.89
Bone, kg	4.68	4.16	4.63	4.49	0.10	0.81	0.83	0.08
SCF[5]+IMF[6], kg	3.36^{a}	3.22^{a}	3.05^{a}	2.57^{b}	0.10	< 0.01	< 0.01	0.12
Fat-tail, kg	4.31^{a}	4.23^{a}	3.58^{b}	3.45^{b}	0.21	0.02	0.02	0.89
Total carcass fat[7], kg	7.67^{a}	7.45^{a}	6.63^{b}	6.01^{b}	0.30	0.02	0.02	0.62
Lean/bone	2.86	3.14	2.89	2.92	0.12	0.45	0.82	0.55
Lean/total carcass fat	1.76^{b}	1.71^{b}	2.02^{a}	2.23^{a}	0.10	< 0.01	0.02	0.25
TAC[8], mmol Fe^{2+}/100 g	0.19^{b}	0.20^{b}	0.20^{ab}	0.22^{a}	0.01	0.02	0.01	0.38
Saturated fatty acids	45.26^{a}	44.57^{ab}	44.02^{bc}	42.90^{c}	0.31	< 0.01	< 0.01	0.84
C14:0	3.09	3.01	2.77	2.67	0.22	0.15	0.17	0.96
C16:0	26.53	26.20	26.00	26.09	0.30	0.67	0.68	0.78
C17:0	0.77	0.76	0.75	0.83	0.05	0.35	0.51	0.37
C18:0	14.92^{a}	14.60^{a}	14.50^{a}	13.31^{b}	0.20	< 0.01	< 0.01	0.48
Unsaturated fatty acids	54.70^{c}	55.43^{bc}	55.98^{ab}	57.10^{a}	0.38	< 0.01	< 0.01	0.66
C16:1	2.78	2.91	2.95	2.91	0.23	0.71	0.71	0.56
C17:1	0.69	0.53	0.55	0.59	0.07	0.20	0.47	0.22
C18:1	45.81^{c}	46.30^{bc}	46.50^{ab}	47.29^{a}	0.31	0.01	0.01	0.67
C18:2 *cis* (n-6)	4.32^{c}	4.48^{bc}	4.68^{ab}	4.94^{a}	0.06	< 0.01	< 0.01	0.40
C18:3 *cis* (n-3)	0.16^{b}	0.17^{ab}	0.17^{ab}	0.19^{a}	0.01	0.02	0.03	0.30
C20:4 (n-6)	0.82^{b}	0.90^{ab}	0.98^{a}	1.00^{a}	0.04	< 0.01	< 0.01	0.39
DTA[9], C22:4 *cis* (n-6)	0.03^{b}	0.04^{ab}	0.04^{ab}	0.05^{a}	0.003	0.03	0.04	0.84
DPA[10], C22:5 (n-3)	0.09^{c}	0.10^{bc}	0.11^{ab}	0.13^{a}	0.01	< 0.01	< 0.01	0.64

[1] HCW = hot carcass weight;
[2] CCW = cold carcass weight;
[3] CDP = cold dressing percentage;
[4] LL = longissimus lumborum;
[5] SCF = subcutaneous fat;
[6] IMF = inter-muscular fat;
[7] Total carcass fat = fat-tail + SCF + IMF;
[8] TAC = total antioxidant capacity;
[9] DTA = Docosatetraenoic acid;
[10] DPA, Docosapentaenoic acid;
[11] SEM = standard error of the means;
[12] Trt = main effect of treatment; Lin = linear effect; Quad = quadratic effect. Within a row, different letters indicate significant differences among treatments ($P < 0.05$).

The minimum carcass fat and the maximum values of TAC and unsaturated FA were observed in lambs fed the diet containing 40.00 g TH (*i.e.,* total replacement of the TMR forage portion with TH).

3.4. EMPS and N retained

According to Table 6, there was a linear incremental response in the contents of urinary allantoin (L, $P = 0.046$), xanthine + hypoxanthine (L, $P = 0.019$), excreted total PD (L, $P < 0.01$), absorbed total PD (L, $P < 0.01$), and daily EMPS (L, $P < 0.01$) with increasing dietary TH rates. Statistical comparison showed that total PD excreted (14.86 to 15.41 mmol/d), PD absorbed (16.94 to 17.57 mmol/d), and EMPS (77.00 to 79.86 g/d) were greater in the lambs fed with all TH-containing diets compared to those values (13.37, 15.13 mmol/d and 68.72 g/d, respectively) observed in the control group ($P < 0.05$).

Observations during days 71 to 77 revealed that the addition of enhancing TH levels in the diet caused a linear decrease in daily N consumption (L, $P = 0.02$) and fecal N excretion (L, $P = 0.03$). In this regard, daily N intake in the 26.50 and 40.00 g TH groups (29.88 and 29.52 g/d, respectively) was lower than the control animals (35.14 g/d). On the other hand, daily fecal N excretion in 26.50 and 40.00 g TH groups (7.14 and 6.64 g/d, respectively) was lower than the 0.00 and 13.30 g TH treatments (10.51 and 9.09 g/d, respectively). However, daily-retained N (10.26 to 11.41 g/d) was not affected by feeding different TH concentrations.

3.5. In vivo ruminal fermentation characteristics and in vitro methane release

Increasing dietary levels of TH had no significant effect on *in vivo* rumen pH (6.41 to 6.58) and total SCFA (66.57 to 68.47 mmol/L) concentration (Table 7). The individual SCFA proportions were also not influenced by the diets. The ruminal NH_3–N concentration, total protozoa number, *Entodiniinae* and *Diplodiniinae* populations, and cellulolytic bacteria were affected by feeding the TH levels in diets ($P < 0.05$). Enhancing dietary levels of TH led to a linear enhancement of *in vivo* ruminal cellulolytic bacteria (L, $P < 0.01$), with the maximum value (8.03 Log_{10}/mL) in 40.00 g TH group, intermediate values (7.37 to 7.53 Log_{10}/mL) in 13.30 and 26.50 g TH groups, and minimum value (6.47 Log_{10}/mL) in the control. However, a linear decrease (L, $P = 0.03$) was detected in the *in vivo* ruminal NH_3–N concentration as the level of TH increased in the diet (the maximum and minimum NH_3–N concentrations of 14.57 and 11.46 mg/dL in the control and 40.00 g TH-fed lambs, respectively). Moreover, the *in vitro* total protozoa (L, $P < 0.01$), *Entodiniinae* (L, $P < 0.01$), *Diplodiniinae* (L, $P = 0.02$), and CH_4 production (L, $P < 0.01$) lowered linearly with the inclusion of higher TH levels in the diet so that the minimum values were detected in the 40.00 g TH treatment.

3.6. Blood serum variables

Table 8 shows that the diets containing different TH concentrations did not affect the blood serum glucose, total protein, albumin, globulin, albumin-to-globulin ratio, nitrate, and nitrite (58.13 to 59.44 mg/dL, 6.09 to 6.24, 3.12 to 3.27, 3.57 to 3.62 g/dL, 0.88 to 0.92, 6.58 to 6.70, and 0.13 to 0.14 μg/mL, respectively). The other blood variables were affected by the treatment, and the serum cholesterol (L, $P < 0.01$), triglyceride (L, $P < 0.01$), and urea-N (L, $P < 0.01$) concentrations decreased linearly, but TAC (L, $P < 0.01$) increased linearly with the increased dietary TH levels. The lowest cholesterol (59.39 mg/dl), triglyceride (19.64 mg/dl), and urea-N (9.28 mg/dL) concentrations and the highest TAC level (0.28 mmol Fe^{2+}/l) were observed in lambs fed on the 40.00 g TH-containing diet. The values for the control group were 62.67, 22.00, 12.23 mg/dL, and 0.23 mmol Fe^{2+}/l, respectively.

4. Discussion

4.1. Yield and chemical composition of TH and alfalfa

In this study, the yield of triticale harvested at the milky-dough stage was 14.2 t DM/ha, *i.e.,* a higher yield than that reported (*i.e.,* 5.7 t/ha) by Santana et al. (2019) when the crop was harvested at the earlier boot stage. The DM yields of autumn-sown triticale cultivars range from 5.7 to 16.3 t/ha (Bilgili et al., 2009; Keles et al., 2016; Salama & Badry, 2020). Coblentz et al. (2018) recorded that the mean DM yield of triticale at the boot stage (3.80 t DM/ha) was less than the yield of the soft dough stage (12.64 t DM/ha). This wide range could be due to differences in cultivar, growth stage, and location (Liebert et al., 2023).

Ruminant performance is a function of intake, availability, nutrient digestibility, and metabolic efficiency (Cherney & Mertens, 1998). Harvest time management is the most critical factor affecting forage quality and yield (Cherney et al., 2020). Other studies reveal how harvest stage, cultivar, and growing season affect triticale forage chemistry (Coblentz et al., 2018; Manni et al., 2021). Triticale forage DM varies

Table 6
Effect of triticale hay (TH) level in the diet on urinary purine derivatives, estimated microbial protein synthesis, and N retention of fattening lambs, measured from day 71 to day 77 of the experimental period ($n = 6$).

	TH level in diet (g/100 g DM)					*P*-value[6]		
Item	0.00	13.30	26.50	40.00	SEM[5]	Trt	Lin	Quad
UPD[1], mmol/d								
Allantoin	9.10^{b}	10.19^{a}	10.56^{a}	10.64^{a}	0.32	0.03	0.05	0.69
$X + H$[2]	0.87^{b}	0.97^{a}	1.00^{a}	1.01^{a}	0.04	0.04	0.02	0.08
Uric acid	3.39	3.70	3.75	3.75	0.17	0.32	0.19	0.25
TPD[3] excreted	13.37^{b}	14.86^{a}	15.31^{a}	15.41^{a}	0.42	0.02	< 0.01	0.14
TPD absorbed	15.13^{b}	16.94^{a}	17.46^{a}	17.57^{a}	0.51	0.02	< 0.01	0.14
EMPS[4], g/d	68.72^{b}	77.00^{a}	79.33^{a}	79.86^{a}	2.30	0.03	< 0.01	0.14
N balance, g/d								
N intake	35.14^{a}	33.54^{ab}	29.88^{b}	29.52^{b}	1.25	0.02	0.02	0.77
Fecal N	10.51^{a}	9.09^{a}	7.14^{b}	6.64^{b}	0.78	< 0.01	0.03	0.97
Urinary N	13.84	13.04	12.48	12.56	1.77	0.80	0.83	0.72
N retained	10.79	11.41	10.26	10.32	1.83	0.89	0.87	0.86

[1] UPD = urinary purine derivatives;
[2] $X + H$ = xanthine and hypoxanthine;
[3] TPD = total purine derivatives;
[4] EMPS = estimated microbial protein synthesis;
[5] SEM = standard error of the means;
[6] Trt = main effect of treatment; Lin = linear effect; Quad = quadratic effect. In each row, different letters indicate significant differences among treatments ($P < 0.05$).

Table 7
Effect of triticale hay (TH) level in the diet on the *in vivo* ruminal variables of fattening lambs ($n = 10$) and *in vitro* methane emission.

	TH level in diet (g/100 g DM)					*P*-value[3]		
Item	0.00	13.30	26.50	40.00	SEM[2]	Trt	Lin	Quad
In vivo experiment								
pH	6.44	6.44	6.58	6.41	0.13	0.54	0.89	0.35
Ammonia-N, mg/dL	14.57^{a}	13.38^{ab}	12.61^{bc}	11.46^{c}	0.70	0.03	0.03	0.99
Total SCFA[1], mmol/L	68.47	68.35	67.62	66.57	1.98	0.76	0.34	0.75
Individual SCFA, mol/100 mol								
Acetic acid (A)	71.86	72.29	72.52	72.72	1.62	0.59	0.20	0.81
Propionic acid (P)	17.26	17.28	17.30	17.30	1.62	0.99	0.96	0.98
Butyric acid	8.69	8.22	7.97	7.73	0.48	0.32	0.09	0.75
Isobutyric acid	1.22	1.23	1.26	1.30	0.10	0.89	0.48	0.92
Valeric acid	0.54	0.53	0.53	0.52	0.06	0.99	0.81	0.97
Isovaleric acid	0.43	0.45	0.42	0.43	0.07	0.99	0.95	0.95
A:P	4.16	4.19	4.19	4.20	0.18	0.99	0.81	0.94
Total protozoa, Log_{10}/mL	5.23^{a}	5.20^{ab}	5.18^{bc}	5.15^{c}	0.01	0.01	0.02	0.34
Entodiniinae	4.91^{a}	4.88^{b}	4.84^{c}	4.82^{c}	0.03	< 0.01	0.02	0.31
Diplodiniinae	4.56^{a}	4.53^{ab}	4.52^{ab}	4.46^{b}	0.03	0.04	0.02	0.54
Dasytrichidae	4.16	4.14	4.16	4.16	0.06	0.97	0.92	0.84
Isotrichidae	4.47	4.41	4.44	4.40	0.05	0.34	0.22	0.88
Ophryoscolex	3.85	3.84	3.86	3.85	0.04	0.99	0.99	0.99
Cellulolytic bacteria, Log_{10}/mL	6.47^{c}	7.37^{b}	7.53^{b}	8.03^{a}	0.31	< 0.01	< 0.01	0.99
In vitro experiment								
Total protozoa, Log_{10}/mL	5.12^{a}	5.08^{b}	5.07^{bc}	5.05^{c}	0.01	< 0.01	< 0.01	0.33
Entodiniinae	4.83^{a}	4.79^{b}	4.76^{c}	4.74^{d}	0.01	< 0.01	< 0.01	0.27
Diplodiniinae	4.39^{a}	4.37^{b}	4.35^{bc}	4.33^{c}	0.01	< 0.01	< 0.01	0.16
Gas production, mL/g DM	177^{c}	197^{b}	213^{a}	220^{a}	5.19	0.02	<0.01	0.25
Methane,% of total gas	18.09^{a}	14.52^{b}	11.86^{bc}	10.62^{c}	0.99	<0.01	<0.01	0.67
Methane, mL/g DM	32.00^{a}	28.67^{ab}	25.33^{bc}	23.33^{c}	0.98	< 0.01	< 0.01	0.55

[1] SCFA = short chain fatty acid;
[2] SEM: standard error of the means;
[3] Trt = main effect of treatment; Lin = linear effect; Quad = quadratic effect. In each row, different letters indicate significant differences among treatments ($P < 0.05$). For the *in vivo* trial, the average of repeated sampling of the rumen liquor was collected from 10 animals per treatment on days 35 and 70, before the morning feed distribution (0 h) and 3 h after that. No significant interaction between times of sampling was observed, therefore, an average was reported.

Table 8
Effect of triticale hay (TH) level in the diet on blood metabolites and total antioxidant capacity in fattening lambs ($n = 10$).

	TH level in diet (g/100 g DM)					*P*-value[3]		
Item	0.00	13.30	26.50	40.00	SEM[2]	Trt	Lin	Quad
Glucose, mg/dL	58.13	59.10	59.38	59.44	0.76	0.65	0.66	0.85
Cholesterol, mg/dL	62.67^{a}	61.22^{ab}	60.02^{b}	59.39^{b}	0.39	< 0.01	< 0.01	0.31
Triglyceride, mg/dL	22.00^{a}	21.06^{b}	20.30^{bc}	19.64^{c}	0.28	< 0.01	< 0.01	0.76
Urea-N, mg/dL	12.23^{a}	10.64^{b}	9.76^{bc}	9.28^{c}	0.28	< 0.01	< 0.01	0.10
Total protein, g/dL	6.24	6.19	6.24	6.09	0.13	0.45	0.47	0.71
Albumin, g/dL	3.24	3.27	3.22	3.12	0.04	0.09	0.09	0.22
Globulin, g/dL	3.57	3.62	3.59	3.58	0.09	0.72	0.87	0.72
Albumin/Globulin	0.92	0.89	0.88	0.88	0.04	0.50	0.52	0.77
Nitrate, µg/mL	6.70	6.70	6.65	6.58	0.07	0.53	0.52	0.61
Nitrite, µg/mL	0.14	0.13	0.13	0.13	0.004	0.30	0.30	0.43
TAC[1], mmol Fe^{2+}/L	0.23^{c}	0.24^{b}	0.27^{a}	0.28^{a}	0.003	< 0.01	< 0.01	0.19

[1] TAC = total antioxidant capacity;
[2] SEM = standard error of the means;
[3] Trt = main effect of treatment; Lin = linear effect; Quad = quadratic effect. In each row, different letters indicate significant differences among treatments ($P < 0.05$). The average of repeated sampling of the blood collected from 10 animals per treatment on days 35 and 70, before the morning feed distribution (0 h) and 3 h after that. No significant interaction between times of sampling was observed, therefore, an average was reported.

between 35.0 and 41.6 g/100 g fresh-weight at the early-dough stage (maturity stage 3) which is optimum for making silage and between 32.9 and 38.0 g/100 g fresh-weight at the soft-dough stage (maturity stage 4) (De Zutter et al., 2023) and DM accumulation increases rapidly with relatively higher temperatures (Delogu et al., 2002). In this study, the growing season weather data heralded a drier and warmer March-May compared to other months, which may account for higher DM levels (36.8 g/100 g fresh-weight; Table 2) than those reported by Coblentz et al. (2018; *i.e.,* 34.0 g/100 g fresh-weight) and Fazaeli et al. (2011; *i.e.,* 31.0 g/100 g fresh-weight).

The CP concentration of TH in this experiment was higher than the 8.0 g/100 g DM reported by Karimi et al. (2022b) but lower than that of Fazaeli et al. (2011) and Santana et al. (2019) reports (10.23 and 18.50 g/100 g DM, respectively). The lower CP concentration in TH was associated with later growth stages (Keles et al., 2016). In the present work, despite the lower CP and higher NDFom concentrations of TH than AH, the OM digestibility of TH was relatively higher probably due to its lower lignin(sa) and higher starch, WSC, and plant secondary metabolites, as these compounds affect the digestibility coefficient and feed consumption (Van Soest, 1994; Wu, 2018). On the other hand, although the CP concentration of TH (8.33 g/100 g DM) was lower than AH (13.9 g/100 g DM), but it was higher than the minimum suggested level (> 8 g/100 g DM) supporting sufficient ruminal ammonia and microbial growth (Norton, 1998), and it probably does not hurt the forage digestion. The results suggest that these cultivars are valuable ruminant feed sources (Coblentz et al., 2018). In another study by Keles

et al. (2016), the nutritive levels of triticale forage, at the final maturity stage, were 6.0, 2.1, 8.2, 58.1, 35.1, 7.0, 68.4, 66 48.9, 25.6 g/100 g, and 8.2 MJ/kg DM for ash, EE, CP, NDF, ADF, lignin(sa), *in vitro* true DM digestibility, *in vitro* NDF digestibility, NFC, and ME, respectively.

The TP concentration in TH (< 1 g/100 g DM) was well below the levels that have an adverse effect on nutrient digestibility (Oliveira et al., 2010). Ruminants are especially susceptible to nitrate toxicity because the rumen microbiota can reduce nitrate to ammonia, with nitrite being an intermediary fermentation product that is 10 times more toxic than nitrate (Aiello, 1998). This study showed that nitrate concentration in the TH diets was below the toxic level for un-acclimated animals (*i.e.,* < 1.00 g/100 g on a DM basis; Aiello, 1998).

4.2. Intakes, in vivo digestibility, and animal performance

The lower DMI in lambs fed the increasing levels of dietary TH may, in part, be associated with the plant's rough awns that can reduce diet palatability and acceptability (Smith et al., 2018). Therefore, it is recommended that growers consider planting reduced awn or awnless varieties, as noted by Smith et al. (2018), or moistening the feed. The lower OM, CP, NDFom, NFC, starch, and EE intake with increasing dietary TH levels were parallel to the reduction in daily DMI. Keles et al. (2016) reported that the feeding value of TH for fattening lambs, harvested at the same growth stage as this trial, was intermediate (DMI of 850 g/d along with ADG of 110 g/d), *i.e.,* lower than the feeding value of wheat and oats, but higher than that of barley or rye. They stated that the awns in triticale harvested between ear emergence to the milk stage reduced its potential as a forage. Santana et al. (2019) reported that the DMI in dairy cows fed on TH showed no difference from those offered AH. Differences between this study's results and those of Santana et al. (2019) could be related to the growth stage at harvest. They compared the quality of TH harvested at the milky-dough stage (CP of 8.1, NDF of 60, lignin of 6.3, and NFC of 21.4 g/100 g DM) to that harvested at the boot stage (CP of 18.5, NDF of 53.0, lignin of 4.5, and NFC of 31.5 g/100 g DM). In terms of ensiled fodder, McCartney and Vaage (1994) noted a reduced performance in heifers fed on triticale silage compared to an ensiled barley or oat diet and this was primarily related to poor palatability because of its coarse texture.

The linear increases in DM, OM, CP, and NDF digestibilities (11.10, 11.28, 15.00, and 13.50%, respectively) in lambs receiving the enhancing dietary rates of TH may partly be related to the lower intake of the TH-containing rations, as mentioned above. This is because when ruminants consume less daily feed, the passage rate of digesta in the alimentary canal is slower and digestibility increases due to the higher retention time, which would improve microbial and enzymatic breakdown and attack (Van Soest, 1994; Wu, 2018). Moreover, increasing TH levels in the diet decreased the diet lignin(sa) concentration (from 4.70 to 3.23 g/100 g DM in 0.00 and 40.00 g TH diets, respectively) and daily lignin(sa) intake (from 76.28 to 45.58 g/d in 0.00 and 40.00 g TH groups, respectively), which results in the digestibility improvement (Van Soest, 1994). This means that there was a reduction in the digestion inhibitory agent in the digestive system.

In addition, the higher daily intake of the plant secondary metabolites (TP and flavonoids) in the TH-fed groups reduced the population of the rumen protozoa, as bacterial predators, which led to an increase in cellulolytic bacterial numbers (Newbold et al., 2015) and improvement of diet digestibility. It should also be noted that the reduction of protozoa that have fibrolytic activity (Newbold et al., 2015) could potentially lead to reduced diet digestibility. However, the similarity in SCFA concentrations among all the experimental groups in this study indicates that the reduction in these protozoa is not affecting total fibrolytic activity (*i.e.,* the overall results of decreasing protozoa population and increasing cellulolytic bacteria numbers have led to improving the digestibility). On the other hand, it is also expected that high levels of phenolic compounds (> 50 g/kg DM per day) could disrupt the digestibility of nutrients, particularly CP, indicating that the effects of these compounds on digestibility may differ depending on their level in the diet (Da Silva Aguiar et al., 2023). However, it is possible, that high tannin addition formed complexes with the protein that allowed the protection of proteins against degradation in the rumen, and highly digested in the post-rumen (abomasum) as described by Da Silva Aguiar et al. (2023).

Finally, it is important to note that total digested nutrients (g/d) were similar among treatments, which is due to the simultaneous increase in diet digestibility (%) and decrease in daily nutrient intake (g/d) with increasing TH levels in the diet.

Under normal conditions when lambs are not stressed, changes in BW are based on variations in feed intake and digestibility (Nasri et al., 2011). In this experiment, there were no significant differences in the ADG and FCR of the experimental lambs, despite a linear decrease in daily DMI of the TH-fed animals. This could be attributable to the enhanced nutrient digestibility (as discussed above) of the TH-containing rations. Considering the intake and digestibility data, it appears that the total amount of absorbable nutrients supplied to the animals in the different experimental groups was the same so the total digested nutrients (g/d) were similar among the groups. Results suggest that TH can form the forage portion of the diet of fattening lambs, replacing AH + straw, with no adverse effect on the performance of animals. Therefore, the use of TH, which can be cultivated more easily, even under suboptimal field conditions, may represent a promising strategy to safeguard livestock operations in at-risk areas, especially considering the challenges posed by climate change (Keles et al., 2016; Buonaiuto et al., 2021).

4.3. Carcass characteristics and non-carcass components

All the lambs reached the expected harvest weight and did not have any health problems. No significant differences among all the lambs regarding carcass weights were related to the similar FCR of all the experimental groups, *i.e.*, optimal DM conversion into BW gain Olfaz et al. (2005) (see above).

The HCW is a key datum in fattening lambs and together with fat content influences meat price. Tables 5 and 6 illustrate that more of the DM consumed by the control lambs has been converted into fat rather than meat (*i.e.,* feeding TH produces a better carcass quality). This improved carcass quality in TH-fed lambs may be financially beneficial to meat producers. Likewise, there was no difference in CDP among the lambs because CDP and CCW are positively related (Van De Voorde & Verbeke, 1979). On the other hand, the lack of differences in carcass cut weights (loin, hand, brisket, and neck) among lambs receiving increasing amounts of TH in the diet was due to the positive correlation between carcass traits and BW (Fabrega et al., 2011; Hailu et al., 2011).

The significantly lower SCF + IMF and fat-tail, numerically lower back-fat thickness, and the higher lean-to-TCF ratio in the TH-fed animals were in line with their significantly lower blood triglyceride and cholesterol levels. Moreover, dietary polyphenols could decrease adipogenesis and fat accumulation (Kim et al., 2014; Trindade et al., 2019) and promote lipolytic activity *in vivo* (Kim et al., 2014). In another study, Zhong et al. (2009) reported that polyphenols decrease intramuscular fat and improve meat quality in goats. Also, some researchers noted that the improved weights of the loin and muscles with the inclusion of the plant secondary metabolites (tannins) in the diet might be attributable to the formation of tannin-protein complex reducing ruminal proteolysis and providing the animal body with greater bypass protein (Fernandes et al., 2021). Consumers generally prefer a leaner carcass (Esenbuga et al., 2009) and, in this study, the data on body fat reflect the better carcass quality in TH-fed lambs, *i.e.,* energy intake and metabolism in sheep receiving TH shifted towards a lower fat, leaner carcass.

Ashkvari et al. (2024) recorded phenolic levels in TH (0.82 g/100 g of DM) and these chemicals have been shown to increase carcass antioxidant activity as mentioned by Scerra et al. (2022), who fed a high percentage of phenolic-containing almond hulls to fattening lambs and

reported improved meat oxidative stability and no effect on growth performance. These findings can explain the increased TAC in the carcasses of TH-fed lambs in our work and this could lengthen carcass shelf life by reducing lipid oxidation (Scerra et al., 2022). However, the extent of the effects of phenolics depends on their biological activity, which is associated with their chemical nature and not just their concentrations in the diet (Rodríguez et al., 2014). Therefore, the source of tannins, types and their concentration in the diet need to be considered.

Phenolics and tannins have selective activity on the rumen bacteria (particularly *Fusocillus* spp. and *Clostridium proteoclasticum*), reducing biohydrogenation and enhancing unsaturated FA, including conjugated linoleic acid, levels in produced meat. The addition of these chemicals to the diet can change the meat FA profile towards a more health-promoting FA profile (Kamel et al., 2018). Accordingly, the higher carcass unsaturated FA content in TH-fed lambs in this study could be related to their higher daily phenolic and flavonoid intakes and their controlling effect on rumen biohydrogenation. Partial prevention of the ruminal unsaturated FA biohydrogenation by polyphenolic compounds could have resulted in a further effective transfer of diet polyunsaturated FA to the body tissues (Vasta et al., 2019). The improvement in meat quality in terms of FA profile could significantly contribute to the provision of essential FA in the human diet (Nguyen et al., 2018). Similarly, other lamb studies have recorded increases in 18:2 n-6 and 18:3 n-3 levels (Vasta et al., 2009; Kamel et al., 2018). On the other hand, different studies have examined the effect of tannins on meat FA deposition with various results being recorded due to differences in diet type, tannin chemistry and concentration, and ruminant species (Besharati et al., 2022).

4.4. EMPS and N retained

Based on Chen and Gomes (1992), PD levels in urine reflect the amount of microbial protein in the duodenum. The linear increase of EMPS with the higher dietary TH content in this trial may relate to the lower ruminal protozoa counts, especially *Entodiniinae*, in TH-fed lambs. This is because these protozoa are responsible for bacterial predation, so reducing their population leads to higher ruminal bacterial growth (Belanche et al., 2012). In addition, the improved EMPS may have resulted from more OM and CP degradation (Wu, 2018) and improved synchrony of CP and ME in the rumen of TH-fed lambs (Kaur et al., 2010).

Lambs fed on the TH-free diet had a higher N intake, but their retained N was not significantly different from that of lambs fed TH, which was due to the higher fecal N (*i.e.,* lower CP digestibility) in lambs fed on the TH-free diet. This means that the control diet resulted in a lower N utilization efficiency compared to the TH diets (30.7 vs. 34.4 g N retained/100 g N intake, respectively). This unchanged N retention among the lambs was in line with the similar ADGs of the experimental groups.

4.5. In vivo ruminal fermentation characteristics and in vitro methane release

The rumen pH values (between 6.41 and 6.58) of the experimental animals were within the range (*i.e.,* 5.7 to 7.0) of normal rumen microbial activity (Dehority, 2003). Rumen pHs in this study's lambs were comparable to those observed by Ashkvari et al. (2024) in TH-fed Holstein lactating cows. All groups of lambs had ruminal NH_3—N concentrations (11.46 to 14.57 mg/dL liquor) above the minimum level (5 mg/dL liquor) necessary for ideal microbial growth (Sinclair et al., 1993). The linearly decreased rumen ammonia in lambs fed with higher levels of TH relates to their greater EMPS, *i.e.,* the more NH_3—N absorption by the rumen microbiota leads to better growth. Another reason may be the decreased protozoa, especially *Entodiniinae* spp., counts in the rumen of the TH-fed lambs compared to the control, as these protozoa are the major types with bacterial lysis function (Belanche et al., 2012). In addition, the effect of lower daily CP consumption of TH-fed sheep should be considered in the rumen ammonia reduction.

One of the final products of rumen microbial fermentation is SCFA, which is an important source of energy supply in ruminants, so diets improving their ruminal levels will lead to better animal performance (Wu, 2018). As mentioned above, although lambs fed TH-containing diets had a decreased OM intake, their total and individual ruminal SCFA contents were not affected. These results could be justified by the higher OM digestibility (Wu, 2018) in TH-fed lambs, *i.e.,* all lambs obtained similar digestible OM intake. It seems that the TH-free diet was utilized less efficiently (*i.e.,* the lower digestibility of OM) than the TH diets, but lambs fed on the TH-free diet compensated for this lower efficiency by increasing their daily OM intake, leaving ruminal SCFA unchanged.

The lower ruminal *Entodiniinae, Diplodiniinae*, and total protozoa count in TH-fed lambs, compared to the control, may be attributable to their higher TP and flavonoid intake as noted by Oskoueian et al. (2013) and de Paula et al. (2016). Ashkvari et al. (2024) also reported that the higher dietary flavonoid intake in dairy cows receiving TH resulted in lower protozoa numbers, similar to the result seen in this research. However, it should be noted that due to differences in ration condition, livestock type, and the nature and concentration of plant metabolites (Patra & Saxena, 2011), as well as protozoa adaptability (Wallace et al., 2002), the effect of phenolics on protozoa is not the same among the different studies. The feeding of TH showed no effect on ruminal *Diplodiniinae, Isotrichidae*, and *Ophrioscolecinae* indicating various protozoa families respond differently based on their nutritional needs and available substrates (Dehority, 2003).

The enhanced population of the cellulolytic bacteria counts in the rumens of TH-receiving lambs, compared to the control animals, could be attributed to the lower protozoa numbers as bacterial predators (Dehority, 2003). Ashkvari et al. (2024) observed similar findings when TH replaced the dietary forage portion of TMR in lactating Holstein cows.

The linear decline in the *in vitro* ruminal methane release with the increasing dietary TH levels was in line with decreased ruminal protozoa numbers, due to the presence of TP and flavonoids, since protozoa provide hydrogen ions and are closely related to methanogen groups (Kamra, 2005). The lower methane production in the higher flavonoid-containing diets indicates that this secondary metabolite acts as a bioactive regulator in ruminant animals, as noted by Kim et al. (2015). The decreased *in vitro* CH_4 production was in agreement with the result observed by Ashkvari et al. (2024) in TH-fed lactating Holstein cows. Based on these findings, proportional inclusion of TH, instead of AH + straw, in the diets of ruminants may be a useful way to decline methane emissions.

4.6. Blood metabolites

The dietary supply and utilization of nutrients and the physiological status of the animal determine the concentration of blood metabolites (Radostits et al., 2007). The findings of the current study show that the blood levels of glucose, urea-N, total protein, and cholesterol were within the normal ranges (50 to 80 mg, 8 to 20 mg, 6 to 7.9 g, and 43 to 103 mg/dL, respectively) previously reported for sheep (Radostits et al., 2007). The serum triglyceride level of all experimental lambs (19.6 to 22.0 mg/dL) was within the range recorded in the blood of fat-tailed Iranian sheep (18 to 50.9 mg/dL mentioned by Mojabi, 2011), but higher than the range presented for other breeds (0 to 14 mg/dL; Radostits et al., 2007).

The reduction of blood cholesterol and triglyceride in TH-fed lambs, compared to the TH-free group, shows that energy uptake and metabolism were different among the animals and these results agree with the differences in carcass fat content. In some cases, polyphenols reduce blood cholesterol due to factors such as changes in lipoprotein metabolism, declined cholesterol absorption by binding bile acids and

preventing pancreatic cholesterol esterase, or increased excretion of bile acids (Kim et al., 2014). There were no differences in blood albumin concentrations among the lambs (*i.e.,* 3.12 to 3.27 g/dL) which is an indication of normal liver function. The similar blood albumin-to-globulin ratio among the animals shows that there were no adverse health effects of feeding TH, which might have influenced performance (Radostits et al., 2007). All lambs had blood nitrate and nitrite concentrations below the levels (10 and 0.5 μg/mL, respectively) defined as excessive (Aiello, 1998) implying that TH is a great potential ruminant forage (Radostits et al., 2007).

The linearly increased TAC in the blood serum of lambs with increasing dietary TH levels could be explained by their higher TP (as strong antioxidants) intake. These compounds have antioxidant ability based on several mechanisms, which include the scavenging of free radicals (Zheng et al., 2009) and singlet O_2 quenching (Mukai et al., 2005). Similar to the carcass TAC, this result indicates that the addition of TH in lambs' diets could be a natural approach to improving antioxidant status and achieving better lamb health.

5. Conclusion

The results show that triticale hay can be successfully included, up to 40.00 g/100 g DM, in the TMR of fattening Gray Shirazi lambs, as a total replacement for alfalfa hay + barley straw, with no adverse effects on performance or carcass weight, and would also decrease body fat and enhance concentrations of polyunsaturated fatty acids and antioxidant activity in the carcass, as well as reduction of methane release and daily feeding cost.

CRediT authorship contribution statement

G.A. Izadi: Writing – original draft. **Y. Rouzbehan:** Writing – review & editing. **J. Rezaei:** Supervision. **M.J. Abarghuei:** Project administration.

Declaration of competing interest

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

Acknowledgements

The authors would like to thank Mr. Gary Easton for correcting the manuscript in English.

Ethical statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes [and feed legislation, if appropriate.

References

Ahmed, M. G., Elwakeel, E. A., El-Zarkouny, S. Z., & Al-Sagheer, A. A. (2024). Environmental impact of phytobiotic additives on greenhouse gas emission reduction, rumen fermentation manipulation, and performance in ruminants. *Environmental Science and Pollution Research, 31*, 37943–37962.

Aiello, S. E. (1998). *The merck veterinary* (8th ed.). Philadelphia, PA, USA: Merck and Co. Inc., National Publishing Inc.

Akbağ, H. I. (2022). Effects of growth stage on nutritional value of barley and triticale forages for goats. *Tropical Grasslands - Forrajes Tropicales, 10*(2), 116–123. https://doi.org/10.17138/tgft(10)116-123

AOAC. (2012). *Official methods of analysis of aoac international* (19th ed.). Washington, DC, USA: Association of Official Analytical Chemists.

Ashkvari, A., Rouzbehan, Y., Rezaei, J., & Boostani, A. (2024). Replacing the forage portion of the ration with triticale hay improves the performance of Holstein dairy cows. *Journal of Dairy Science, 107*, 3531–3542.

Belanche, A., de la Fuente, G., Moorby, J. M., & Newbold, C. J. (2012). Bacterial protein degradation by different rumen protozoal groups. *Journal of Animal Science, 90*, 4495–4504.

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of blood (FRAP) as a measure of antioxidant power: The FRAP assay. *Analytical Biochemistry, 239*, 70–76.

Besharati, M., Maggiolino, A., Palangi, V., Kaya, A., Jabbar, M., Eseceli, H., De Palo, P., & Lorenzo, J. M. (2022). Tannin in ruminant nutrition: Review. *Molecules., 27*, 8273.

Bilgili, U., Cifci, E. A., Hanoglu, H., Yagdi, K., & Acikgoz, E. (2009). Yield and quality of triticale forage. *Journal of Food, Agriculture and Environment, 7*, 556–560.

Bryant, M. P. (1972). Commentary on the Hungate technique for culture of anaerobic bacteria. *American Journal of Clinical Nutrition, 25*, 1324–1328.

Buonaiuto, G., Palmonari, A., Ghiaccio, F., Visentin, G., Cavallini, D., Campidonico, L., Formigoni, A., & Mammi, L. M. E. (2021). Effects of complete replacement of corn flour with sorghum flour in dairy cows fed Parmigiano Reggiano dry hay-based ration. *Italian Journal of Animal Science, 20*, 826–833.

Chen, X. B., & Gomes, M. J. (1992). *Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives—An overview of technical details*. Bucksburn, Aberdeen, UK: Rowett Research Institute.

Cherney, D. J. R., & Mertens, D. R. (1998). Modelling grass utilization by dairy cattle. In J. H. Cherney, & D. J. R. Cherney (Eds.), *Grass for dairy cattle* (p. 416). Wallingford, UK: CABI International.

7th Edn., Vol. 2, eds Cherney, J. H., Kallenbach, R. L., & Picasso Risso, V. D. (2020). Systems for temperate humid areas. In KJ Moore, M Collins, CJ Nelson, & DD. Redfearn (Eds.), *Forages: The science of grassland agriculture* (pp. 369–386). Hoboken, NJ, USA: Wiley & Sons, 7th Edn., Vol. 2, eds.

Coblentz, W. K., Akins, M. S., Kalscheur, K. F., Brink, G. E., & Cavadini, J. S. (2018). Effects of growth stage and growing degree day accumulations on triticale forages: 1) Dry matter yield, nutritive value, and *in vitro* dry matter disappearance. *Journal of Dairy Science, 101*, 8965–8985.

Coblentz, W. K., Ottman, M. J., & Kieke, B. A. (2022). Effects of harvest date and growth stage on triticale forages in the southwest United States: Agronomic characteristics, nutritive value, energy density, and *in vitro* disappearance of dry matter and fiber. *Journal of Animal Science, 100*, 1–16.

Da Silva Aguiar, F., Bezerra, L. R., Cordão, M. A., Cavalcante, I. T. R., de Oliveira, J. P. F., do Nascimento, R. R., de Souza, B. B., Oliveira, R. L., Pereira, E. S., & Filho, J. M. P. (2023). Effects of increasing levels of total tannins on intake, digestibility, and balance of nitrogen, water, and energy in hair lambs. *Animals, 13*, 2497 [PubMed].

de Paula, E. M., Samensari, R. B., Machado, E., Pereira, L. M., Maia, F. J., Yoshimura, E. H., Franzolin, R., Faciola, A. P., & Zeoula, L. (2016). Effects of phenolic compounds on ruminal protozoa population, ruminal fermentation, and digestion in water buffaloes. *Livestock Science, 185*, 136–141.

De Zutter, A., Landschoot, S., Vermeir, P., Van Waes, C., Muylle, H., Rolďan-Ruiz, I., Douidah, L., Boever, J., & Haesaert, G. (2023). Variation in potential feeding value of triticale forage among plant fraction, maturity stage, growing season and genotype. *Heliyon., 9*, e12760.

Dehority, B. A. (2003). *Rumen microbiology* (1st ed.). Nottingham, UK: Nottingham Univ Press.

Delogu, G., Faccini, N., Faccioli, P., Reggiani, F., Lendini, M., Berardo, N., & Odoardi, M. (2002). Dry matter yield and quality evaluation at two phenological stages of forage triticale grown in the Po Valley and Sardinia. *Italy Field Crops Research, 74*, 207–215.

Demeyer, D., De Meulemeester, M., De Graeve, K., & Gupta, B. W. (1988). Effect of fungal treatment on nutritive value of straw. *Meded Fac Landbouwwet Rijksuniv Gent, 53*, 1811–1819.

Dhiman, T. R., & Satter, L. D. (1997). Yield response of dairy cows fed different proportions of alfalfa silage and corn silage. *Journal of Dairy Science, 80*, 2069–2082.

Esenbuga, N., Macit, M., Karaoglu, M., Aksakal, V., Aksu, M. I., Yoruk, M. A., & Gul, M. (2009). Effect of breed on fattening performance, slaughter and meat quality characteristics of Awassi and Morkaraman lambs. *Livestock Science, 123*, 255–260.

Eudes, F. (2015). *Triticale*. Cham. Switzerland: Springer International Publishing.

Fabrega, E., Gispert, M., Tibau, J., Hortos, M., & Oliver, M. A. (2011). Font l Furnols M. Effect of housing system, slaughter weight and slaughter strategy on carcass and meat quality, sex organ development and androstenone and skatole levels in Duroc finished entire male pigs. *Meat Science, 89*, 434–439.

Farid, A. (1991). Carcass physical and chemical composition of three fat-tailed breeds of sheep. *Meat Science, 29*, 109–120.

FASS. (2010). *Guide for the care and use of agricultural animals in research and teaching* (3rd ed.). Champaign, IL, USA: The Federation of Animal Science Societies.

Fazaeli, H., Hajilari, D., Yazdani, A., Zeareahdaran, S., & Mohajer, M. (2011). Comparing of different levels of corn silage substituted with triticale silage in the diet of male growing Zel lambs. *Iranian Journal of Applied Animal Science, 13*(2), 277–285.

Felini, R., Cavallini, D., Buonaiuto, G., & Bordin, T. (2024). Assessing the impact of thermoregulatory mineral supplementation on thermal comfort in lactating dairy cows. *Vet. Anim. Sci., 24*, Article 100363.

Fernandes, J., Pereira Filho, J., Menezes, D., Caldas, A. C., Cavalcante, I., Oliveira, J., Oliveira, R., Júnior, J. S., Cézar, M., & Bezerra, L. (2021). Carcass and meat quality in lambs receiving natural tannins from *Mimosa tenuiflora* hay. *Small Ruminant Research : The Journal of the International Goat Association, 198*, Article 106362.

Galyean, M. L. (2010). *Laboratory procedures in animal nutrition research*. Lubbock, TX, USA: Department of Animal and Food Sciences, Texas Tech University.

Giorgio, D., Di Trana, A., Di Napoli, M. A., Sepe, L., Cecchini, S., Rossi, R., & Claps, S. (2019). Comparison of cheeses from goats fed 7 forages based on a new health index. *Journal of Dairy Science, 102*, 6790–6801.

Glamoclija, N., Starcevic, M., Ciric, J., Sefer, D., Glisic, M., Baltic, M., & Glamoclija, D. (2018). The importance of triticale in animal nutrition. *Veterinary Journal of the Republic Srpska (Бања Лука-Banja Luka), 18*, 73–94.

Guamán-Rivera, S., Albanell, E., Ajenjo, O., Casals, R., Elhadi, A., Salama, A. A., & Caja, G. (2023). Performances and nutritional values of a new hooded barley (cv. Mochona) and a high yield triticale (cv. Titania) as hay or silage for sheep under Mediterranean conditions. *Animal Feed Science and Technology, 305*, Article 115784.

Hailu, A., Melaku, S., & Tamir, B. A. (2011). Tassew A. Body weight and carcass characteristics of Washera sheep fed urea treated rice straw supplemented with graded levels of concentrate mix. *Livestock Research for Rural Development, 23*, 8.

Hall, M. B., Hoover, W. H., Jennings, J. P., & Webster, T. K. M. (1999). A method for partitioning neutral detergent-soluble carbohydrates. *Journal of the science of food and agriculture, 79*, 2079–2086.

Kamel, H. E. M., Al-Dobaib, S. N., Salem, A. Z. M., López, S., & Alaba, P. A. (2018). Influence of dietary supplementation with sunflower oil and quebracho tannins on growth performance and meat fatty acid profile of Awassi lambs. *Animal feed science and technology, 235*, 97–104.

Kamra, D. N. (2005). Rumen microbial ecosystem. *Current science, 89*, 124–135.

Karimi, A., Abarghuei, M. J., & Boostani, A. (2022a). Growth performance and carcass traits of purebred and crossbred fattening lambs from Ghezel ram with Grey Shirazi ewe. *Tropical Animal Health and Production, 54*, 26.

Karimi, A., Abarghuei, M. J., & Zarei, M. (2022b). Using Triticale (*Triticosecale*), Vetch (*Vicia Sativa*) and Triticale Vetch mixture silages in feeding of Grey Shirazi fattening male lambs. *Animal Science Journal = Nihon Chikusan Gakkaiho, 133*, 45–58.

Kaur, R., Garcia, S. C., Fulkerson, W. J., & Barchia, I. M. (2010). Utilisation of forage rape (Brassica napus) and Persian clover (*Trifolium resupinatum*) diets by sheep: Effects on whole tract digestibility and rumen parameters. *Animal Production Science, 50*, 59–67.

Keles, G., Ates, S., Coskun, B., Alatas, M. S., & Isik, S. (2016). Forage yields and feeding value of small grain winter cereals for lambs. *Journal of the Science of Food and Agriculture, 96*, 4168–4177.

Kim, H., Bartley, G. E., Arvik, T., Lipson, R., Nah, S. Y., Seo, K., & Yokoyama, W. (2014). Dietary supplementation of chardonnay grape seed flour reduces plasma cholesterol concentration, hepatic steatosis, and abdominal fat content in high-fat diet-induced obese hamsters. *Journal of Agricultural and Food Chemistry, 62*(8), 1919–1925.

Kim, E. T., Guan, L., Le, S. J., Lee, S. M., Lee, S. S., Lee, I. I . D.., Lee, S. K., & Lee, S. S. (2015). Effects of flavonoid-rich plant extracts on *in vitro* ruminal methanogenesis, microbial populations and fermentation characteristics. *Asian-Australasian Journal of Animal Sciences, 28*, 530–537.

Liebert, J., Cherney, J. H., Ketterings, Q. M., Mirsky, S. B., Pelzer, C. J., & Ryan, M. R. (2023). Winter cereal species, cultivar, and harvest timing and harvest timing affect trade-offs between forage quality and yield. *Frontiers in Sustainable Food Systems, 7*, Article 1067506.

Makarechian, M., Farid, A., & Goshtasbpour-Parsi, B. (1978). A study of sheep breed differences in utilizing feed high in wheat straw. *Iranian Journal of Agricultural Research, 6*(2), 107–115.

Makkar, H. P. S. (2000). *Quantification of tannins in tree foliage. a laboratory manual for the fao/iaea co-ordinated research project on use of nuclear and related techniques to develop simple tannin assays for predicting and improving the safety and efficiency of feeding ruminants on tanniniferous tree foliage. joint fao/iaea of nuclear techniques in food and agriculture. animal production and health sub-programme*. Vienna, Austria: FAO/IAEA Working Document, IAEA.

Manni, K., Lötjönen, T., & Huuskonen, A. (2021). Comparing spring triticale varieties to barley and wheat varieties when harvested as whole crop. *Agriculture and Food Science, 30*, 24–35.

McCartney, D. H., & Vaage, A. S. (1994). Comparative yield and feeding value of barley, oat and triticale silage. *Canadian Journal of Animal Science, 74*, 91–96.

McKenzie, R.H., Wood, S.A., 2011. Crop water use and requirements. Accessed Apr. 30, 2023. https://www.agriculture.alberta.ca.

Menke, K. H., Raab, L., Salewski, A., Steingass, H., Fritz, D., & Schneider, W. (1979). The estimation of the digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor *in vitro*. *Journal of Agricultural Research, 93*, 217–222.

Mojabi, A. (2011). *Veterinary clinical biochemistry* (2nd ed.). Tehran, Iran: Noorbakhsh Publ.

Mukai, K., Nagai, S., & Ohara, F. (2005). Kinetic study of the quenching reaction of singlet oxygen by tea catechins in ethanol solution. Free Radic. *Biology and Medicine, 39*, 752–761.

NASEM. (2016). *Nutrient requirements of beef cattle, 8th rev*. Washington, DC, USA: The National Academies Press.

Nasri, S., Ben Salem, H., Vasta, V., Abidi, S., Makkar, H. P. S., & Priolo, A. (2011). Effect of increasing levels of Quillaja saponaria on digestion, growth and meat quality of Barbarine lamb. *Animal Feed Science and Technology, 164*, 71–78.

Newbold, C. J., de la Fuente, G., Belanche, A., Ramos-Morales, E., & McEwan, N. R. (2015). The role of ciliate protozoa in the rumen. *Frontiers in Microbiology, 6*, 1–14.

Nguyen, Q. V., Le, V. H., Nguyen, D. V., Malau-Aduli, B. S., Nichols, P. D., & Malau-Aduli, A. E. O. (2018). Supplementing grazing dairy ewes with plant-derived oil and rumen-protected EPA plus DHA pellets enhances health-beneficial n-3 long-chain polyunsaturated fatty acids in sheep milk. *European Journal of Lipid Science and Technology, 20*, 256.

Norton, B. W. (1998). The nutritive value of tree legumes. Editors. In R. C. Gutteridge, & H. M. Shelton (Eds.), *Forage tree legumes in tropical agriculture. tropical grassland society of australia inc.*. QLD, Australia: St Lucia, Brisbane.

NRC. (2007). *Nutrient requirements of small ruminants: Sheep, goats, cervids, and new world camelids*. Washington, DC, USA: National Academy Press.

Olfaz, M., Ocak, N., Erener, G., Cam, M. A., & Garipoglu, A. V. (2005). Growth, carcass and meat characteristics of Karayaka growing rams fed sugar beet pulp, partially substituting for grass hay as forage. *Meat Science, 70*, 7–14.

Oliveira, R. A., Narciso, C. D., Bisinotto, R. S., Perdomo, M. C., Ballou, M. A., Dreher, M., & Santos, J. E. P. (2010). Effects of feeding polyphenols from pomegranate extract on health, growth, nutrient digestion, and immunocompetence of calves. *Journal of Dairy Science, 93*, 4280–4291.

Oskoueian, E., Abdullah, N., & Oskoueian, A. (2013). Effects of flavonoids on rumen fermentation activity, methane production, and microbial population. *Biomedical Research International*, Article 349129.

Patra, A. K., & Saxena, J. (2011). Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *Journal of the Science of Food and Agriculture, 91*, 24–37.

Quettier-Deleu, C., Gressier, B., Vasseur, J., Dine, T., Brunet, C., Luyckx, M., & Trotin, F. (2000). Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. *Journal of Ethnopharmacology, 72*, 35–42.

Radostits, O. M., Gay, C. C., Blood, D. C., & Hinchliff, K. W. (2007). *Veterinary medicine. a text book of the diseases of cattle, sheep, goats and horses* (10th ed.). London, UK: W.B. Saunders Ltd.

Rodríguez, R., De La Fuente, G., Gomez, S., & Fondevila, M. (2014). Biological effect of tannins from different vegetal origin on microbial and fermentation traits *in vitro*. *Animal Production Science, 54*(8), 1039–1046.

Salama, H. S. A., & Badry, H. H. (2020). Forage and grain yields of dual-purpose triticale as influenced by the integrated use of *Azotobacter chroococcum* and mineral nitrogen fertilizer. *Italian Journal of Agronomy, 16*, 1719.

Santana, O. I., Olmos-Colmenero, J. J., & Wattiaux, M. A. (2019). Replacing alfalfa hay with triticale hay has minimal effects on lactation performance and nitrogen utilization of dairy cows in a semi-arid region of Mexico. *Journal of Dairy Science, 102*, 8546–8558.

Scerra, M., Bognanno, M., Foti, F., Caparra, P., Cilione, C., Mangano, F., Natalello, A., & Chies, L. (2022). Influence of almond hulls in lamb diets on animal performance and meat quality. *Meat Science, 192*, Article 108903.

Sinclair, L. A., Garnsworth, P. C., Newbold, J. R., & Buttery, P. J. (1993). Effect of synchronizing the rate of dietary energy and nitrogen release on rumen fermentation and microbial protein synthesis in sheep. *Journal of Agricultural Science, 120*, 251–263.

Singh, J. P. (1988). A rapid method for determination of nitrate in soil and plant extracts. *Plant and soil, 110*, 137–139.

Smith, S. R., Benson, B., & Thomason, W. (2018). Growing small grains for forage in Virginia. *Virginia cooperative extension, virginia tech*. Available at: Https://www.pubs.ext.vt.edu.

Trindade, P., Soares, E., Monteiro, E., Resende, Â., Nunes, N. M., Mello, V., da Costa, D. F., & Daleprane, J. (2019). Antiadipogenic effects of acaí on high fat diet-fed mice and 3t3-l1 adipocytes: A potential mechanism of action. *Current Developments in Nutrition, 3*(Suppl. 1). nzz031.OR34-04-19.

Van De Voorde, G., & Verbeke, R. (1979). La conformation et les caractéristiques d'abattage de taureaux. *Revue De L'Agriculture., 32*, 117–120.

Van Soest, P. J. (1994). *Nutritional ecology of the ruminant*. Ithaca, NY, USA: Cornell University Press.

Van Soest, P. J., Robertson, J., & Lewis, B. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of dairy science, 74*(10), 3583–3597.

Vasta, V., Priolo, A., Scerra, M., Hallett, K. G., Wood, J. D., & Doran, O. (2009). Δ9 desaturase protein expression and fatty acid composition of longissimus dorsi muscle in lambs fed herbage or concentrate with or without tannins. *Meat Science, 82*, 357–364.

Wallace, R. J., McEwan, N. R., McIntosh, F. M., Teferedegne, B., & Newbold, C. J. (2002). Natural products as manipulators of rumen fermentation. *Asian-Australasian Journal of Animal Sciences, 15*, 1458–1468.

Wu, G. (2018). Principles of animal nutrition. *LLC*.

Zheng, G., Xu, L., Wu, P., Xie, H., Jiang, Y., Chen, F., & Wei, X. (2009). Polyphenols from longan seeds and their radical-scavenging activity. *Food Chemistry, 116*, 433–436.

Zhong, R. Z., Tan, C. Y., Han, X. F., Tang, S. X., Tan, Z. L., & Zeng, B. (2009). Effect of dietary tea catechins supplementation in goats on the quality of meat kept under refrigeration. *Small Ruminant Research: The Journal of the International Goat Association, 87*(1–3), 122–125.