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Interrelationships of winter wheat varieties on rumen fermentation rate, forage biomass production, and grain yield dynamics under the grazed out by steers.

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ABSTRACT

Little information is available comparing wheat forage varieties, rumen fermentation and biomass production for different wheat (*Triticum aestivum* L.) cultivars. A combination of grazing and *in vitro* experiments was conducted at Texas A & M (**TAM**) AgriLife Research and Extension Center, Vernon, TX from 2003 to 2004. Our objective was to determine the effects of wheat varieties (**WV**) and forage allowances (**FA**) on *in vitro* rumen fermentation rate, forage biomass production, time of sampling, and grain yields under grazing by steers. The 2003 experiment consisted of 14 commercial wheat cultivars (a part of the UVT collection) and was a part of the TAM Wheat Breeding Program statewide evaluation test. Hereford steers (*Bos taurus* L.; 220 to 233 kg) were grazed from November 2003 to May 2004. Grain yield was measured in May 2004. Across WV, total forage protein and soluble protein content were declined from February to March 2004. Total forage protein contents were higher for HG-9, Thunderbolt and Maton Rye than for Lockett, Cutter, Jagalene and Triticale in March. *In vitro* dry matter (**DM**) digestibility (**IVDMD**) was greater (P<0.01) for Maton Rye than for other WV. Forage DM production varied between FA (P<001), grazing (P<0.001), and WV (P<0.001), with no FA x WV and FA x WV x grazing interactions. Total forage DM production was greater (P<0.001) for Gagalene, Longhoern, TAM 111, TAM Bar 501, Lockett, Marton rye, and Jagalene than for HG-9, Triticale 5019, and Triticale 6331 WV in high-FA during grazing, but DM production in un-grazed plot was higher for Triticale 5019, TAM Bar 501 and Jagalene than for HG-9 and Ogallala varieties during the end of March. Across **FA**, forage DM production (kg DM/ha) in high-FA was higher (P<0.001) DM production than low-FA. Potential *in vitro* rumen DM disappearance rate (a+b; %) was not differences among WV and time of sampling date, but instantly solubilized rate (a) in the rumen was higher (P<0.05) for Maton rye and Cutter in February and Triticale, Maton rye and Jagalene in March 17 than for other WV, which lead to differentiation of high soluble carbohydrate among forage WV. Similar to rumen DM disappearance rate, instantly NDF solubilized rate (a: %) in the rumen were higher (P<0.05) for Triticale, Maton rye and Jagalene in March 17 than for other WV, suggests that higher rate of instantly solubilized of DM, rate of disappearance rate, and instantly solubilized NDF in the rumen may lead to initiating trigger factor for frothy bloat and need to further study. When steers grazing on high-FA, grain yields were greater (P<0.01) for 2174, Lockett, Thurnderbolt, TAM 111, and HG-9 than for low-FA, with Gagalene and Cutter being produced higher (P<0.01) grain yield than other WV. The pull of date (**POD**) response to grain yield depends upon the time of date with no or variable responses of grain yield with increasing time of date up to March 08. When POD after March 08 or thereafter, grain yield progressively decreased with time. Therefore, beneficial effects of POD for dual purpose winter wheat occur in the range from February 19 to March 08.

Key words: Wheat varieties, Forage allowances, Disappearance rate, Gas production, Grain yield

Abbreviations: ADF, neutral detergent fiber; FA, forage allowance; DM, dry matter; DMDR, dry matter disappearance rate; IVDMD, in vitro dry matter digestibility; NDF, neutral detergent fiber; NLIN, Non-linear regression; NPN, non-protein nitrogen; WV, wheat variety; TAM, Texas A & M; POD, pull of date;

1. Introduction

Winter wheat (*Triticum aestivum* L.) is the major source of high-quality winter forage for grazing cattle and annually sown on about 10 million ha (Christiansen et al. 1989) in the southern Great Plains of the USA. Production of winter wheat for dual-purpose use (forage and grain) is a complicated process involving the interaction of livestock production with wheat grain production. Relatively few studies to determine the consequences of grazing on wheat grain yield have been conducted (Christiansen et al., 1989; Redmon et al., 1996). Holliday (1956) summarized several studies and found that grazing reduced grain yield while others reported that grain yield was greater on plots that had been mechanically clipped or grazed. Redmon et al. (1995) also reported that under some circumstances fall-winter grazing could increase the grain yield of tall varieties. An evaluation of these research findings would lead one to conclude that if grazing is properly managed, fall-winter grazing will not reduce grain yield. Profitability of wheat production systems in the region depends on income derived from both cattle and grain production. Wheat cultivar development has been directed toward grain production (Carver et al. 1991, Winter and Thompson 1990), while wheat pasture grazing research has focused on the impacts of intensity and timing of defoliation on subsequent grain yields (Winter and Thompson 1990, Winter and Musick 1991, Christiansen et al. 1989).

Selection of wheat varieties (**WV**) is one of the most important management decisions for dual-purpose production. High concentrations of crude protein and soluble nitrogen fractions in wheat forage are often associated with frothy bloat conditions in cattle. The death loss of stocker cattle grazing wheat due to bloat ranges 2 to 3% in the southern

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Great Plains each year. Beef cattle research on wheat pasture has focused on bloat problems (Horn and Frost 1982; Min et al., 2005) and the evaluation supplementation and management strategies (Horn et al.1981, Mader and Horn 1986, Min et al., 2006; 2007). Supplementation strategies to increase stocking density have also been evaluated by Cravey et al. (1993) and Horn et al. (1995). Noticeably lacking are effects of WV selection on forage allowance, grazing intensity, and seasonal patterns of biomass production associated with ruminal fermentation and grain yield. Our objectives were to characterize and elucidate the relationships between forage biomass production dynamics, *in vitro* rumen fermentation, and grain yields under stocker cattle graze-out management for 14 wheat cultivars differing in forage production potential.

2. Materials and Methods

2.1. Experimental Design

A combination of WV, FA, grazing intensity, and *in vitro* experiments was conducted under the grazing at Texas A & M Agricultural Research and Extension Center, Vernon, TX from fall 2003 through spring 2004. Grain yield was measured in 2004. The experimental was a 2 x 2 factorial, using two different forage allowances (high vs. low) and grazing intensity (grazed vs. un-grazed) during grazing period by stocker cattle (*Bos taurus* L.) to measure forage nutrient contents, forage biomass production, in vitro ruminal fermentation, and grain yield. Consequently, in vitro ruminal gas production was conducted at the same time of forage biomass measured. Research was conducted on continuously cropped wheat forage comprised of six 4.0 ha paddocks in West Walker farm, Vernon, Texas (33^o 57$'$ N, 99^o 26$'$ W). Grazing commenced on 10 November 2003 through April 2004. Wheat forage protein dynamics, related with forage allowance (**FA**) and time of date are presented.

2.2. Pasture management

The evaluated wheat entries are grouped into a Uniform Wheat Variety Trial (UVT), consisting of released cultivars, and the Texas Elite Trial (TXE), which consists of advanced experimental breeding lines developed by the TAM Wheat Breeding Program. At the Smith-Walker Research Unit (Fig. 1), experiments were planted in October 2003 on Rotan clay loam. The 14 commercial wheat cultivars (a part of the UVT collection) and was a part of the TAM Wheat Breeding Program statewide evaluation test. Pastures were fertilized with 60 lb N/ac 20 lb P2O5/acre, 20 lb K2O/acre, and 10 lb S/acre. Wheat entries were planted in a tilled seedbed with a precision planter (Wintersteiger, Salt Lake City, UT) at a seeding rate of 23 seeds/sq ft. Plot size was 5 by 15 ft with 3 replicated each wheat variety. The experimental plots were a part of a larger grazing study at this location. Wheat was continuously grazed by cattle, commencing on February, Initial cattle weights averaged 216 kg/head and the stocking density was 2.3 acre/animal based on historical stocking densities in the region.

2.3. Measurements of biomass production, and *in vitro* dry matter digestibility, and grain yield of wheat forage

Wheat forage protein dynamics, related with forage allowance and plant stage of growth, are presented. Forage biomass production was conducted with 14 varieties, but in vitro ruminal fermentation and grain yield experiment were conducted with 7 and 9 varieties, respectively. Wheat varieties forage samples were harvested at Feb. 19, Mar 08, and Mar 17, 2004. End of experiment, wheat grains were harvested by combine to measure grain yield in each varieties. Data presented least squares means for each varieties in in vitro rumen incubated with mixed rumen microorganisms. Rumen fluid obtained from fresh wheat forage diet to rumen fistulated steer used in the in vitro experiment. Freshly minced forage (5 g) was incubated with rumen fluid in the rumen during 6 h.

2.4. *In vitro* gas production

Duplicated in vitro ruminal gas production was measured as plunger displacement (cc) at 0, 1, 2, 3, 4, 5, and 6 h incubation periods (Paisley and Horn 1998; Min et al., 2005). Flask stoppers were equipped with rubber tubing connected to 60 ml syringes (Tyco Health Care Ltd., Mansfield, MA). Ruminal gas production was determined from an in vitro rumen incubation procedure in which 5 g of minced fresh wheat forage was placed in 250 ml volumetric flasks containing 20 ml of ruminal fluid, 30 ml of artificial saliva, pH 6.8 which was saturated with CO_2 gas and held at 39^{o}C (Min et al., 2005). Rumen fluid was collected from two cannulated steers fed Bermuda grass hay, mixed and strained through four layers of cheesecloth and flushed with CO_2 gas. All syringes were lubricated with dose syringe oil (Jupiter Vet products; Harrisburg, PN) to assure consistent plunger resistance and movement.

2.5. Chemical Analysis.

Total crude protein, soluble and insoluble protein concentrations were measured in wheat forage samples (5.0 g/fresh weight) harvested at ground level once per month during February to April in growing season. At each harvest date, forage samples were collected from different parts of the plots to ensure that plant material collected on the previous date was not harvested again. Samples were frozen at -20^{o}C in ziplock bags within 30 min after harvesting and remained frozen until analysis. Forage samples for DM was dried at 60^{o}C for 48 h. Total CP, soluble protein, insoluble protein and non-protein nitrogen (NPN) from fresh forage samples were determined by the Kjeldahl digestion procedure (AOAC, 1990; Min et al., 2005). Concentrations of NDF and ADF were sequentially determined using an ANKOM200/220 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA) according to the methodology supplied by the company, which is based on the methods described by Van Soest et al., (1991). Methane gas was determined from 6 h in vitro incubation gas samples in an open-circuit respiration calorimetric system (Puchala et al., 2005; Sable Systems; Henderson, NV).

2.6. Statistical Analysis

Data were analyzed as a repeated measures analysis with Proc Mixed procedures of SAS (SAS, 1987). Data are presented as least square mean values, together with the SEM. Variables in this experiment included plant chemical composition, forage biomass production, in vitro ruminal gas production, and grain yield. The model included WV, FA, grazing intensity, and associated interactions. In vitro gas production rate was calculated using the exponential equation of ∅rskov and McDonald (1979).

$$Y = a + b(1-e^{-ct})$$

Where Y was defined as gas production in time *t*; a, b, and c being constants of the exponential equation where a = the gas production at time 0, b = the proportion gas production during time (*t*), and c = the rate of gas production of the 'b' fraction. The constants b and c for each treatment were calculated with the method described by Min et al. (2000) using the Non-Linear Regression (NLIN) procedure from SAS (1987). The response of b and c to forage allowance and stage of growth, fertilizer treatment and associated interactions were analyzed using the Proc Mixed procedure of SAS.

3. Results

3.1. Forage quality, biomass production and *in vitro* dry matter digestibility (IVDMD) of winter wheat forages

The chemical composition and IVDMD were varied between forage varieties (Table 1). Across forage cultivars, total protein and soluble protein content were declined from February to March 2004 (Table 1, Figure 3). Total forage protein contents were higher for HG-9, Thunderbolt and Maton Rye than for Lockett, Cutter, Jagalene and Triticale in March. Soluble protein content was also similar trend with total protein. *In vitro* dry matter digestibility (IVDMD) was higher ($P< 0.01$) for Maton Rye (69, 71, and 70 %) than for other forage varieties on February 19, March 6 and March 17, respectively.

Across FA, forage DM production (kg DM/ha) in high-FA was higher ($P < 0.001$) than low-FA with or without animals grazing. Forage DM production varied between FA (P < 001), grazing ($P < 0.001$), and WV ($P < 0.001$) (Table 2), with no FA x WV or FA x WV x grazing interactions. Total biomass forage production was greater ($P < 0.001$) for Gagalene, Longhoern, TAM 111, TAM Bar 501, Lockett, Marton rye, and Jagalene than for HG-9, Triticale 5019, and Triticale 6331 varieties in high-FA during grazing (Fig. 2b), but dry matter production in un-grazed plot was higher for Triticale 5019, TAM Bar 501 and Jagalene than for HG-9 and Ogallala varieties during the end of March.

3.2. *In vitro* experiment

The *in vitro* dry matter (**DM**) disappearance rate (**DMDR**) and cumulative *in vitro* gas production incubated with rumen fluid in steer are shown in Table 3. Potential DMDR (a+b; %) was not differences among WV and time of date, but instantly solubilized rate (a) in the rumen was higher ($P < 0.05$) for Maton rye and Cutter in February and Triticale, Maton rye and Jagalene in March 17 than for other WV, which lead to differentiation of high soluble carbohydrate among forage WV. The estimated rate of DMDR (c; %/h) in March 17 was greater ($P < 0.05$) for Triticale, Maton rye and Jagalene than for other WV. *In vitro* rate of gas production (c; ml/h) exhibited no response to wheat forage varieties, but potential gas production rate (a+b; ml/6h) was lower ($P < 0.01$) for Triticale than for other WV in February 19. Potential gas production in March 17 was lower ($P < 0.01$) for Cutter than for other WV (Table 3).

The *in vitro* neutral detergent fiber (**NDF**) disappearance rates incubated with rumen fluid in steers are shown in Table 4. Similar to DMDR, potential NDF disappearance rate (b; %) was not differences among WV and time of date, but instantly NDF solubilized rate (a: %) in the rumen were higher ($P < 0.05$) for Triticale, Maton rye and Jagalene in March 17 than for other WV, suggests that higher rate of instantly solubilized of dry matter, rate of disappearance rate, and instantly solubilized NDF in the rumen may lead to initiating trigger factor for frothy bloat and need to further study.

In vitro methane gas productions are shown in Table 5. *In vitro* ruminal methane gas production was greater ($P < 0.05$) for Maton rye than for other WV (Table 5), suggesting that selected wheat varieties may affect rumen methane gas production and may use for future methane gas mitigation strategies.

3.3. Forage allowance (FA) and grain yield

In the grazing experiment, high-FA to steers grazing WV had greater ($P < 0.001$) for average grain yield (28.2 vs. 25.5 BU/ac DM) than for low-FA, respectively. When steers grazing on high-FA, grain yields were greater ($P < 0.01$) for 2174, Lockett, Thurnderbolt, TAM 111, and HG-9 than for low-FA, with Gagalene and Cutter being produced higher ($P < 0.01$) grain yield than other WV. Grain yield exhibited FA x WV (rep) interactions ($P < 0.02$), resulting from increasing grain yield with high-FA, and decreasing or remaining relatively unchanged grain yield with low-FA. The pull of date (**POD**) response to grain yield depends upon the time of date with no or variable responses of grain yield with increasing time of date up to March 08. When POD after March 08 or thereafter, grain yield progressively decreased with time, as shown in Fig. 5. Therefore, beneficial effects of POD for dual purpose winter wheat occur in the range from February 19 to March 08. There were no FA x POD, wheat varieties x POD, FA x WV x POD interactions (Table 6).

4. Discussion

The most significant finding in this study was that high-FA and optimum POD (before March 8) increased forage biomass production and grain yield without detrimental effects of winter wheat forage and grain production. Collectively, this suggests that FA and POD had a profound effect on dual purpose winter wheat grazing management. *In vitro* ruminal methane gas production was greater for Maton rye than for other WV, suggesting that selected WV may affect rumen methane gas production and may use for future methane gas mitigation strategies.

Crude protein contents in winter wheat forages were higher for HG-9 (28.1), Thunderbolt (28.1) and Maton Rye (27.8% DM) than for Lockett (21.6), Cutter (24.5), Jagalene (23.4) and Triticale (25.6 % DM) in March 17. Soluble protein content was also similar trend with total protein among WV. Consequently, IVDMD was greater for Maton Rye (69, 71, and 70 %) than for other forage varieties on February 19, March 6 and March 17, respectively. These findings agree with the date of Min et al. (2005), who reported that wheat forages in vegetative stage (January to March) had higher CP content, soluble protein and IVDMD values. High-quality wheat forage contains high level of crude protein (18 % DM) and comprises a high proportion of soluble protein (53%) and *in vitro* DMD (94-95%; Min et al., 2005).

Horn et al., (1977, 1999) reported that bloat promoting wheat pastures had greater concentration of CP (35 vs. 25% CP) and soluble protein (62 vs. 45%) compared with non-bloat promoting pastures. Subsequent work reported that wheat forage from bloat-provocative pastures contained less DM (22 vs. 28%) and markedly lower NDF (35 vs. 45%) content (Horn et al., 1977). Soluble proteins and soluble carbohydrate (Lorenzo et al., 2015) are potential precursors of frothy bloat in cattle grazing on wheat pasture (Bartley *et al*. 1975). Highly succulent wheat forage is subjected to a rapid fermentation in the rumen, with release of ammonia, volatile fatty acids, and fermentative gases at a much higher rate than normal (Min et al. 2005, 2006). The rapid release of soluble nutrients by rumen microflora into ruminal fluid promotes the formation of a polysaccharide biofilm layer and gasses trapped in the polysaccharide biofilm layer, causing distention of the rumen, impairing the eructation mechanism and interfering with respiration. (Cheng et al., 1998; Howarth *et al*. 1986; Majak *et al*. 2008). Our present study suggests that an instantly high solubilized (fast initial solubilized) wheat forages in the rumen in Maton rye and Cutter in February or Triticale, Maton rye and Jagalene in March may lead to promoting bloat in steers grazing winter wheat. Our study shown that the estimated rate of disappearance rate (c; %/h) in March 17 was greater for Triticale, Maton rye and Jagalene than for other WV. *In vitro* rate of gas production (c; ml/h) exhibited no response to wheat forage varieties, but potential gas production rate (a+b; ml/6h) was lower for Triticale than for other WV, indicating that estimated *in vitro* gas production data itself may not enough to estimate the bloat potential.

In vitro rumen gas production has been positively correlated with plant protein fractions and IVDMD when incubated with mixed rumen microorganisms (Min et al., 2005). However, the concentration of soluble protein and sugars in wheat leaves may also important for bloat precursor and both components are vary, depending on genotypic and environmental conditions (Lorenzo et al., 2015; Tognetti et al., 1990). Exposure of grasses to low temperature induces a steady buildup of both soluble protein and sugar components, while reversion to non-chilling conditions determines a very rapid decline in their concentration (Lorenzo et al., 2015; Tognetti et al., 1990). Considerable variation in the capacity to accumulate sugars and proteins exists among wheat cultivars: cultivars which undergo deeper cold-acclimation with winter hardy cultivars are able to accumulate substantially higher amounts of compatible solutes in their cells compared with less hardy cultivars (Lorenzo et al., 2015; Tognetti et al., 1990). Because of the transient nature of solute accumulation under cold conditions, the ratio between rapidly fermentable non-structural carbohydrates and proteins, and structural components of grass cells may vary with temperature, and thus wheat pastures might present a variable bloat risk while maintaining a constantly high IVDMD.

In addition to temperature, light intensity and plant phenolic compounds (e.g. condensed tannins) may also play a role in determining IVDMD and bloat risk. Previous research at our laboratory (Malinosski et al., 2015) suggests that wheat forages under high solar radiation conditions has greater level of total phenolic than under low solar radiation as well as different WV. A reduction in light intensity has been associated with reduced forage quality (increase lignin content) in some evergreen species (Blair et al., 1983). Interest in plant phenolic compounds has increased among nutritionists, physiologists, and plant breeders because of their antioxidant and protein-precipitating properties and their role in forage quality, plant defenses, and animal health (Min et al., 2003). The level of tannins in plants can vary among species and genotype (Roberts et al., 1993; Springer et al., 2002) and can change due to biotic stress (herbivory and disease; Richard et al., 2000) and environmental factors (light, nutrients, water, and temperature; Barry and Forss, 1983; Hemming and Lindroth, 1999). Forages with moderate condensed tannin concentrations between 2 and 4 percent have been proposed as suitable for bloat prevention (Min et al., 2003; 2006) and would reduce the cost of intervention practices for bloat prevention. Protein foams formed in the rumen are collapsed by condensed tannins in a dose dependent process (Tanner et al., 1995). Moderate tannin levels offer additional benefits due to the precipitating reaction between tannins and soluble proteins in the rumen fluid. To further understand the effect of phenol content on IVDMD, the amount of phenol content in selected winter WV were compared among forage varieties (Figure 4). The relationship between plant phenol content and WV were similar in Lockette, HG-9, and Cutter, but IVDMD was increased with decreasing phenol content in Jagalene (MacKown et al., 2008), suggesting that phenol content in the wheat forages may have responses of digestibility in the rumen of steers and wheat forage bloat. Plant phenols have found inhibitory effects on the fiber digestibility in the rumen. In sheep fed grass hay with addition of quebracho tannin, *in sacco* digestibility was decreased (Salawu et al., 1997) and precipitated with soluble protein (Martin and Martin, 1982). The presence of *Calliandra* tannins in the diet (2-3% DM) reduced the population of fiber degrading bacteria (McSweeney et al., 2011). The decreased digestibility was associated with phenol compounds probably resulted from the formation of complexes between phenol and soluble protein, and decreased ruminal bacterial activities (Min et al., 2002, 2003). In addition to light intensity, phenol concentration in WV may also play a role in determining IVDMD and bloat risk. Future experiments should focus on the remobilization of cool- and solar radiation-induced soluble protein, sugar, and phenol concentrations, and their association with rumen gas production in animals.

There are a number of important implications of the dual-purpose winter wheat between fall-winter forage production and grain yield for producers and researchers. The optimal POD for dual-purpose wheat depends primarily upon the relative value of fall-winter forage and wheat grain yield. A careful evaluation of several research findings would lead one to conclude that if grazing is properly managed, fall-winter grazing will not reduce grain yield (Christiansen et al., 1989; Redmon et al., 1995). Proper management implies sufficient fertility, grazing initiation delayed until after the root system is well developed, low to moderate stocking density, and grazing termination prior to the development of the first hollow stem. Grazing POD necessary to prevent grain yield reduction of semidwarf cultivars also appear to be much earlier than for taller wheat cultivars (Redmon et al., 1995). The reason for the difference in grazing tolerance is not clear; however, research suggests that semidwarf cultivars require maximum leaf area at anthesis for maximum grain yield. Tall wheat cultivars are not affected in the same manner, and decreased leaf area to later grazing does not reduce grain yield of taller wheat cultivars to the same extent as for the semidwarf cultivars. In the current grazing experiment, the POD response to grain yield depends upon the time of date with no or variable responses of grain yield with increasing time of date up to March 08. When POD after March 08 or thereafter, grain yield progressively decreased with time. Therefore, beneficial effects of POD for dual purpose winter wheat occur in the range from February 19 to March 08.

5. Conclusion

It can be concluded that high-FA and optimum POD (before March 8) increased forage biomass production and grain yield without detrimental effects of winter wheat forage and grain production. Collectively, this suggests that FA and POD had a profound effect on dual purpose winter wheat grazing management. In addition, selected wheat cultivars have the potential to reduce frothy bloat in stocker cattle while producing wheat grain.

Conflict of interest statement

We declare that there was no conflict of interest in carrying out this work.

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Figure 1. The effect of winter wheat varieties on rumen fermentation rate, forage biomass production, and grain yield dynamics under the grazed out by steers during grazing season in 2003-2004, Vernon, TX.

Table 1. Chemical composition and in vitro dry matter digestibility (IVDMD) of winter wheat forage

Wheat Varities[1]	Total Protein (% DM)	Proportion of protein fractions (%) Insoluble	Soluble	Non-protein nitrogen	IVDMD at 8 h (%)
Febrary 19					
Triticale	22.6$^{b\ (0.08)}$	42.6^{a}	52.4^{b}	5.0	57.9^{b}
Lockett	22.9^{ab}	42.5^{a}	53.2^{b}	4.3	52.4^{c}
HG-9	23.0^{ab}	31.7^{b}	63.7^{a}	4.6	52.3^{c}
Thunderbolt	23.5^{ab}	30.2^{b}	65.1^{a}	4.7	57.6^{b}
Maton rye	27.3^{a}	38.1^{ab}	56.7^{ab}	5.2	68.5^{a}
Cutter	24.3^{ab}	35.5^{ab}	59.8^{ab}	4.7	62.1^{ab}
Jagalene	25.1^{ab}	34.6^{ab}	60.9^{a}	4.5	58.6^{b}
March 6					
Triticale	28.1^{a}	41.7^{b}	56.3^{ab}	2.0	63.9^{b}
Lockett	24.0$^{b\ (0.08)}$	46.1^{a}	52.1^{b}	1.8	61.3^{b}
HG-9	28.5^{a}	38.3^{b}	60.1^{ab}	1.6	65.8^{b}
Thunderbolt	28.5^{a}	33.6^{b}	64.2^{a}	2.2	61.7^{b}
Maton rye	27.2^{ab}	41.5^{b}	56.5^{ab}	2.0	71.0^{a}
Cutter	24.9^{ab}	42.6^{ab}	55.8^{ab}	1.7	62.6^{b}
Jagalene	27.1^{ab}	44.7^{ab}	53.6$^{b(0.08)}$	1.7	60.9^{b}
March 17					
Triticale	25.6$^{b\ (0.07)}$	36.8^{b}	61.3^{a}	1.9	63.1^{a}
Lockett	21.6^{b}	55.5^{a}	42.0^{b}	2.5	56.8^{b}
HG-9	28.1^{a}	40.9^{ab}	56.8^{ab}	2.2	56.7^{b}
Thunderbolt	28.1^{a}	35.2^{b}	62.7^{a}	2.1	59.3^{b}
Maton rye	27.8^{ab}	41.0^{ab}	56.9^{ab}	2.1	70.3^{a}
Cutter	24.5$^{b\ (0.06)}$	48.3^{a}	49.3^{ab}	2.5	55.0^{a}
Jagalene	23.4$^{b\ (0.06)}$	51.8^{a}	45.7^{b}	2.5	63.0^{a}
SEM	1.71	3.67	3.68	0.36	2.22
Average					
Triticale	21.7^{b}	42.6^{a}	53.3^{b}	4.1	58.3^{b}
Lockett	21.7^{b}	44.9^{a}	51.4^{b}	3.7	53.5^{c}
HG-9	23.6^{ab}	36.5^{b}	59.7^{a}	3.7	54.0^{c}
Thunderbolt	24.5^{a}	32.5^{b}	63.5^{a}	3.9	55.3^{bc}
Maton rye	24.5^{a}	42.9^{a}	53.2^{b}	3.9	62.7^{a}
Cutter	23.4^{ab}	40.0^{a}	56.3^{ab}	3.7	56.3^{bc}
Jagalene	23.1^{ab}	41.7^{a}	54.3^{ab}	3.9	57.3^{bc}
SEM	0.80	1.90	1.90	0.16	1.53

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Table 2. Dry matter (DM) production (forage mass; kg DM/ha) during steers grazing winter wheat varieties

Wheat variety/ time	February 19 H-FA	February 19 L-FA	March 09 H-FA	March 09 L-FA	March 17 H-FA	March 17 L-FA	March 23 H-FA	March 23 L-FA	50% heading H-FA	50% heading L-FA	Never grazed
1 Lockett											
Un-grazed	193	93	224	112	242	146	314	199	1226	1094	1956
Grazed	-	-	257	68	213	99	297	255	-	-	-
2 2174											
Un-grazed	99	24	141	84	183	102	305	187	1234	882	1894
Grazed	-	-	103	52	152	85	275	182	-	-	-
3 Thunderbolt											
Un-grazed	157	51	133	85	144	83	352	293	1111	989	1301
Grazed	-	-	170	54	149	49	224	208	-	-	-
4 Jagalene											
Un-grazed	154	63	215	111	203	113	412	322	1013	972	1763
Grazed	-	-	186	76	194	100	314	157	-	-	-
5 Cutter											
Un-grazed	75	49	175	114	184	103	284	238	1298	1285	1765
Grazed	-	-	126	59	155	89	271	161	-	-	-
6 Longhorn											
Un-grazed	75	32	152	79	160	124	284	229	1361	1030	1854
Grazed	-	-	135	52	174	92	314	195	-	-	-
7 HG-9											
Un-grazed	54	45	142	87	148	78	259	157	1366	1374	1508
Grazed	-	-	83	42	120	54	182	102	-	-	-
8 Ogallala											
Un-grazed	155	66	190	129	212	126	267	233	1221	1256	1847
Grazed	-	-	176	57	179	96	263	123	-	-	-
9 TAM 111											
Un-grazed	107	44	257	124	185	106	322	229	1251	827	1638
Grazed	-	-	154	55	147	76	310	161	-	-	-
10 Sturdy 2K											
Un-grazed	108	41	165	89	167	118	313	229	950	869	1599
Grazed	-	-	113	56	129	84	246	229	-	-	-
11 Triticale 5019											
Un-grazed	177	123	248	255	251	215	437	216	1535	1501	2297
Grazed	-	-	320	114	158	89	199	190	-	-	-
12 Triticale 6331											
Un-grazed	165	140	244	203	247	164	322	221	1158	1162	3654
Grazed	-	-	158	96	170	97	204	157	-	-	-
13 TAM BAR 501											
Un-grazed	186	54	146	109	178	96	416	289	1209	865	1771
Grazed	-	-	129	58	204	78	327	212	-	-	-
14 Maton Rye											
Un-grazed	179	178	215	161	292	189	280	195	-	-	1820
Grazed	-	-	169	80	184	104	292	149	-	-	-
P values											
FA	0.001		0.001		0.001		0.001		0.06		-
Grazing (G)	-		0.001		0.001		0.001		-		-
FA x G	**ND**		**0.05**		**NS**		**NS**		**-**		**-**
Wheat varieties (WV)	**NS**		**0.001**		**0.001**		**NS**		**0.12**		**0.001**
WV x FA	NS		NS		NS		NS		NS		-
WV x FA x G	NS		NS		NS		NS		-		-

H-FA=High forage allowance (FA). L-FA= low forage allowance. G=grazing

Table 3. *In vitro* Experiment: Effect of wheat varieties upon the in vitro rates of disappearance of dry matter (DM) suspended in ANCOM filter bags and in vitro rumen gas production from wheat forages during in vitro incubation with rumen fluid.

Wheat	DM Disappearance rate			Rumen gas production	
Varities[1]	Instantly Solubilized a (%)	Rate of[2] Disappearance c (%/h)	Potential Disappearance rate[2] a + b (%)	Rate of gas production c (ml/h)	Potential gas production b (ml/6h)
Febrary 19					
Triticale	27.2^{ab}	27.6	82.8	3.1	39.2^{b}
Lockett	23.7$^{b0.08}$	26.0	77.7	3.7	43.8^{a}
HG-9	23.6$^{b0.08}$	26.1	77.9	4.7	52.1^{a}
Thunderbolt	28.0^{ab}	27.0	82.2	4.3	47.0^{a}
Maton rye	34.7^{a}	30.4	82.3	3.5	42.3^{a}
Cutter	33.2^{a}	29.1	80.2	3.5	41.6^{a}
Jagalene	30.9^{ab}	28.3	77.7	4.1	44.5^{a}
SEM	4.51	2.48	6.81	0.99	5.90
March 08					
Triticale	34.1	28.2	75.2	6.3	71.5^{b}
Lockett	32.5	27.9	84.8	7.9	92.2^{a}
HG-9	29.7	27.6	82.8	7.1	82.8^{b}
Thunderbolt	30.6	27.5	83.0	6.4	74.3^{b}
Maton rye	35.2	31.3	82.7	7.4	85.1^{ab}
Cutter	29.8	27.7	83.1	6.8	77.1^{ab}
Jagalene	28.7	27.2	81.5	7.2	79.8^{ab}
SEM	3.91	2.15	5.90	0.99	5.90
March 17					
Triticale	34.5^{a}	28.6$^{a\ (0.1)}$ 86.9	7.0	84.6^{ab}	
Lockett	24.0^{b}	26.2^{ab}	76.9	8.3	97.8^{a}
HG-9	23.6^{b}	23.5^{b}	80.3	7.9	92.5^{a}
Thunderbolt	27.6^{ab}	26.7^{ab}	79.9	7.0	79.9^{ab}
Maton rye	36.6^{a}	30.9^{a}	83.5	8.1	91.3^{ab}
Cutter	27.2^{ab}	26.1^{ab}	79.4	6.9	76.6^{b}
Jagalene	34.7^{a}	29.0$^{a\ (0.07)}$	81.8	7.9	89.8^{ab}
SEM	3.91	2.15	5.89	1.00	5.90

[1]Rumen fluid obtained from fresh wheat forage diet to rumen fistulated steer used in the in vitro experiment. Fresh minced forage in the filter bag incubated with rumen fluid in the rumen. [2]Disapperance rate was estimated by measuring the loss of plant constitutes from ANCOM filter bags, incubated with rumen fluid from steers.

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Table 4. *In vitro* Experiment: Effect of wheat varieties upon the in vitro rates of disappearance of neutral detergent fiber (NDF) from fresh wheat forages suspended in ANCOM filter bags during in vitro incubation with rumen fluid.

Wheat Varities[1]	Instantly Solubilized a (%)	Rate of [2] Disapperance c (%/h)	Potential Disappearance rate[2] a + b (%)
Febrary 19			
Triticale	23.7	23.9[ab]	86.8
Lockett	22.7	20.3[b]	98.5
HG-9	23.3	20.2[b]	99.2
Thunderbolt	24.2	25.3[ab]	86.3
Maton rye	31.1	27.3[a]	93.8
Cutter	29.8	26.5[ab]	91.8
Jagalene	27.4	25.7[ab]	89.0
SEM	4.37	2.34	10.06
March 08			
Triticale	29.6	26.0	102.9
Lockett	27.8	25.5	88.4
HG-9	25.3	25.4	86.2
Thunderbolt	25.9	25.0	86.3
Maton rye	30.9	26.9	92.9
Cutter	25.4	25.2	85.6
Jagalene	24.4	24.9	84.9
SEM	3.79	2.02	8.71
March 17			
Triticale	30.6[a]	26.6[a]	93.0
Lockett	23.0[b]	22.7[b (0.1)]	88.0
HG-9	22.1[bc]	20.8[b]	92.4
Thunderbolt	25.3[ab]	25.0[ab]	85.5
Maton rye	34.5[a]	27.3[a]	95.0
Cutter	25.3[ab]	25.1[ab]	85.3
Jagalene	32.3[a]	26.6[a]	93.0
SEM	3.79	2.02	8.72

[1]Rumen fluid obtained from fresh wheat forage diet to rumen fistulated steer used in the in vitro experiment. Fresh minced forage in the filter bag incubated with rumen fluid in the rumen.
[2]Disapperance rate was estimated by measuring the loss of plant constitutes from ACOM filter bags, incubated with rumen fluid from steers.

Table 5. Effect of wheat varieties upon the *in vitro* rumen methane gas production incubated with rumen fluid.

Wheat varities[1]	Methane gas production (ml/h)[2]
Triticale	3.5[b] (0.06)
Lockett	5.3[ab]
HG-9	3.1[b]
Thunderbolt	2.2[b]
Maton rye	6.4[a]
Cutter	3.8[b] (0.09)
Jagalene	4.4[ab]
SEM	1.09

[1]Wheat varieties forage samples were harvested at Feb. 03, Feb. 19, Mar 08, and Mar 17, 2004. Data presented least squares means for each varieties in in vitro rumen incubated with mixed rumen microorganisms.

[2]Rumen fluid obtained from fresh wheat forage diet to rumen fistulated steer used in the in vitro experiment. Freshly minced forage (5 g) was incubated with rumen fluid in the rumen during 6 h.

[a,b]Means within a column not followed by the same letter differ ($P < 0.05$).

SEM = Standard error of the mean.

Table 6. Forage allowance and grain yield

Item	Grain yield (BU/ac DM)		
	Low-FA	High-FA	Mean
1 Jagalene	33.6^{A}	32.4^{A}	33.2^{A}
2 Cutter	32.4^{A}	33.0^{A}	32.9^{A}
3 2174	26.4^{bB}	31.2^{a}	29.3^{B}
4 Lockett	26.0^{bB}	$29.4^{a\ (P = 0.1)B}$	27.5^{B}
5 Ogallala	25.5^{B}	28.2^{B}	26.9^{BC}
6 Thunderbolt	23.3^{bB}	29.1^{aB}	26.1^{BC}
7 S2K	25.0^{B}	24.0^{C}	24.6^{C}
8 TAM 111	21.8^{bB}	26.5^{aAB}	23.7^{C}
9 Longhorn	21.1^{B}	23.8^{C}	22.6^{C}
10 HG 9	19.5^{bB}	$23.1^{a\ (P = 0.1)C}$	22.1^{C}
Mean	25.5^{b}	28.1^{a}	26.9
SEM	0.48	0.52	1.17

ANOVA	*P*-value
Forage allowance (FA)	0.001
Forage allowance (Rep)	0.001
Wheat varieties (WV)	0.001
Pull off day (POD)	0.001
FA x WV	NS
FA (Rep) x WV	0.02
FA x POD	NS
WV x POD	NS
FA x WV x POD	NS

[a b] Means in a row with different letters differ ($P < 0.05$).

[AB] Means within a colum not followed by the same letter differ ($P < 0.05$).

FA = forage allowance.

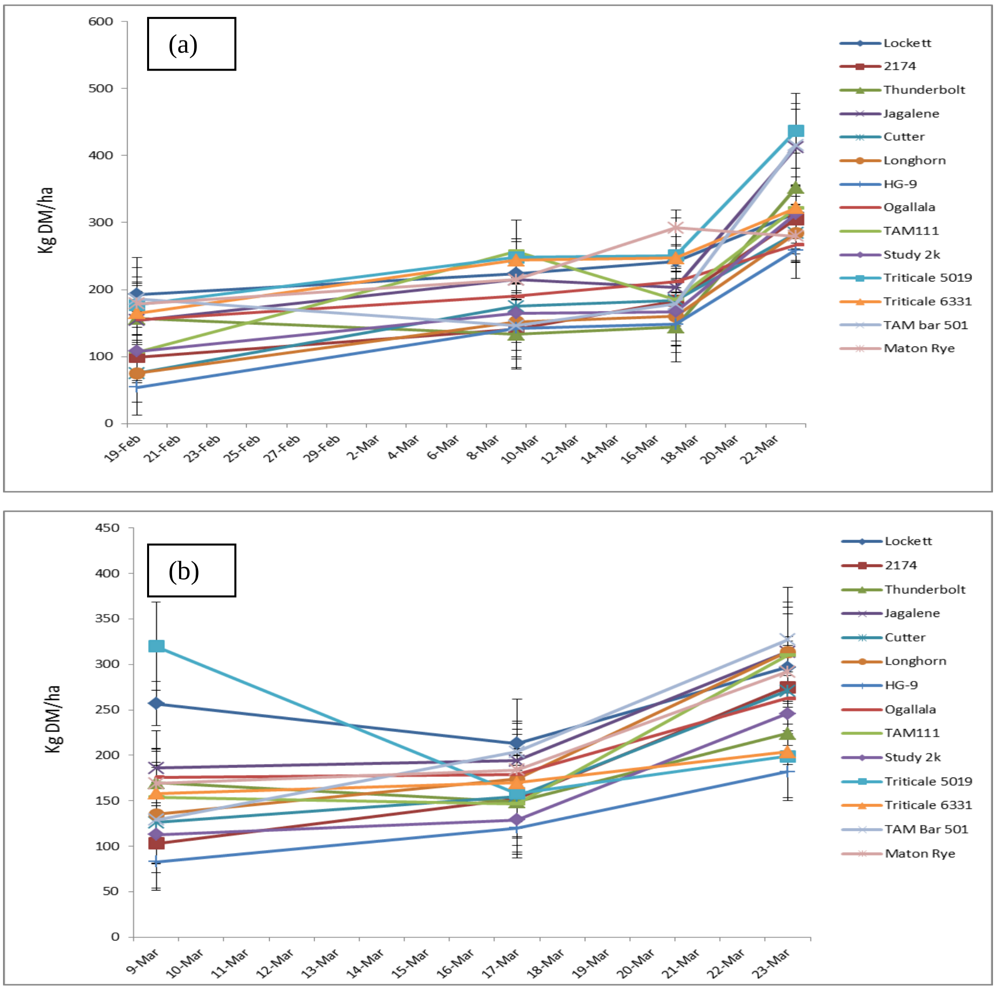

Figure 2. Wheat forage dry matter (DM) production (kg DM/ha) in un-grazed (a) and grazed out (b) during steers grazing winter wheat varieties.

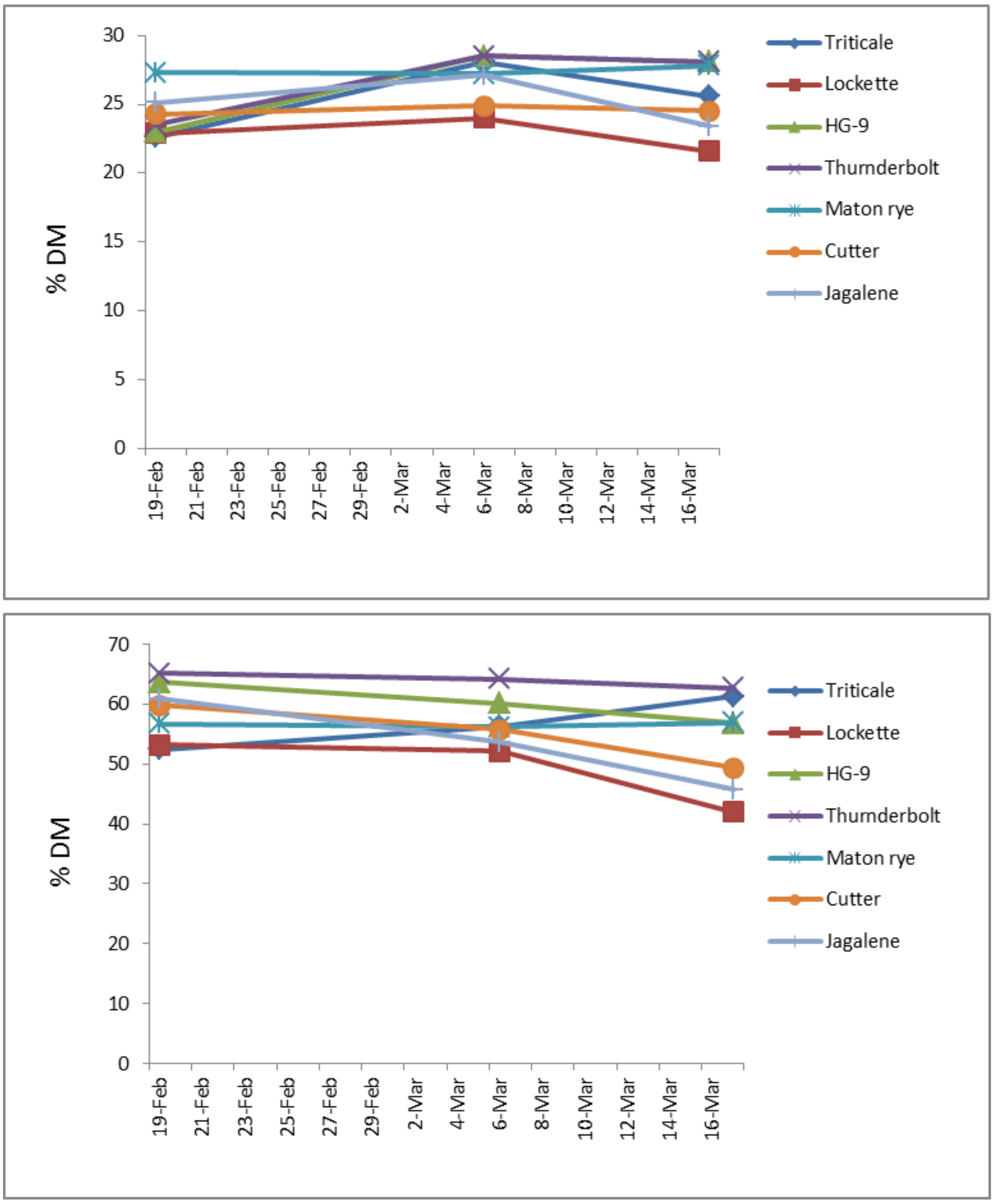

Figure 3. Total protein and soluble protein content of winter wheat forage

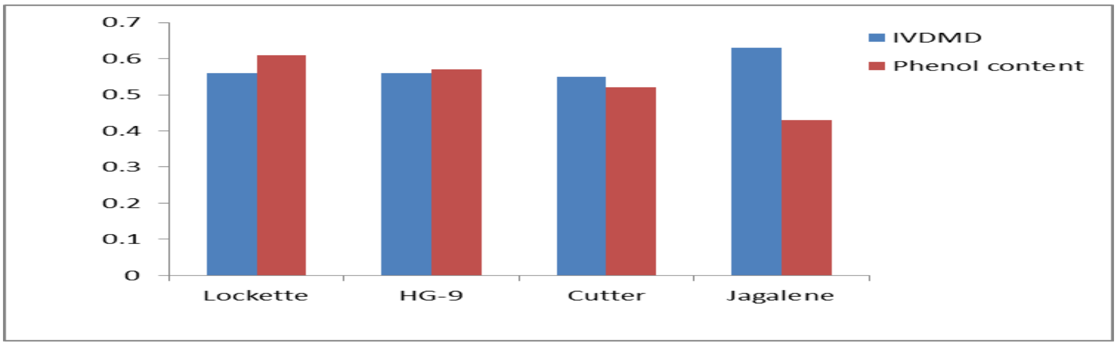

Figure 4. *In vitro* dry matter digestibility (IVDMD, %) and plant phenol content (%) in winter wheat forage. Data of phenol content in winter wheat varieties were obtained from Malinowski et al. (2015) which was the same experimental unit and treated at the same manner.

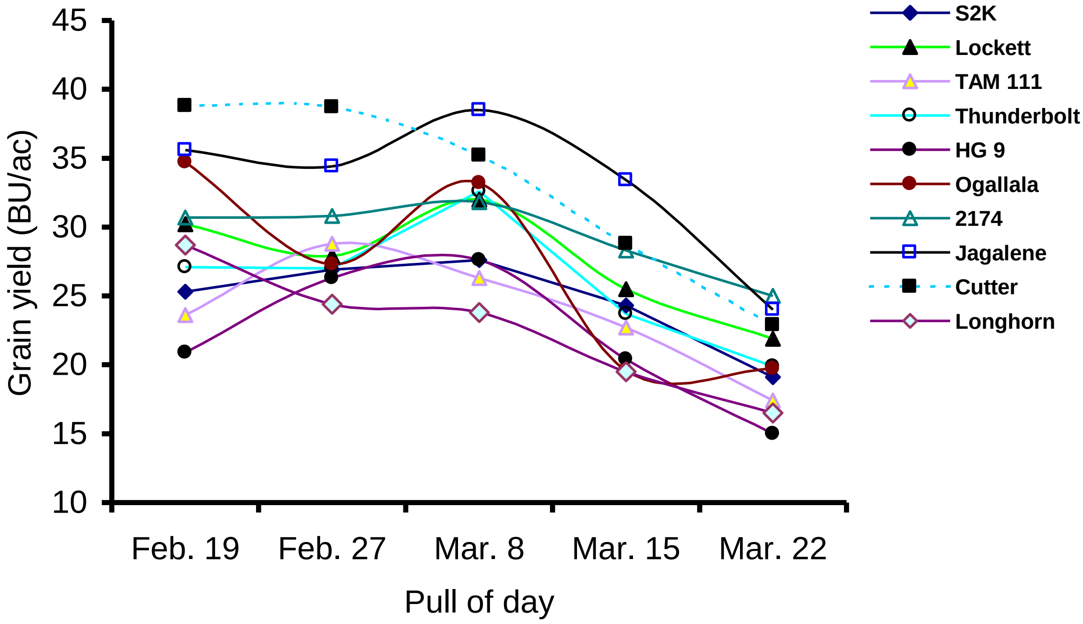

Figure 5. The effect of pull off day (grazed out by steer) on grain yield in winter wheat varieties.

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