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 winter wheat forage varieties, rumen fermentation and biomass production for different wheat \textit{(Triticum aestivum L.)} cultivars. A combination of grazing and \textit{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 \textit{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 \textit{(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. \textit{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<0.01), 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 Jagalene, 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 \textit{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 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 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, Thunderbolt, TAM 111, and HG-9 than for low-FA, with Galagala 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 \textit{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 \textit{(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
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 Gravey 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° 57' N, 99° 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 (TEX), 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. Plant size was 5 by 15 ft and 10 lb S/acre. 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.

2.3. 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. Forage biomass production 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° 57' N, 99° 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.

2.4. 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 CO₂ gas and held at 39°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 CO₂ gas. All syringes were lubricated with dose syringe oil (Jupiter Vet products; Harrisburg, PA) 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°C in ziplock bags within 30 min after harvesting and remained frozen until analysis. Forage samples for DM was dried at 60°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 ANKOM220/220 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA) according to the methodology supplied by the company, which is based on the
method 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 Driskov 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 < 0.01), 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 Jagalene, 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 February (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 gas production rate (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, Stormondell, TAM 111, and HG-9 than for low-FA, with Jagalene 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, IVMD 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 observations of Malinosski et al. (2005), who reported that wheat forages in vegetative stage (January to March) had higher CP content, soluble protein and IVMD 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 sugars by rumen microflora into ruminal fluid promotes the formation of a polysaccharide biofilm layer and gases 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 IVMD 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 build up 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 IVMD.

In addition to temperature, light intensity and plant phenolic compounds (e.g. condensed tannins) may also play a role in determining IVMD 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, 1996). 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 IVMD, the amount of phenol content in selected winter WV were compared among different varieties (Figure 4). The relationship between plant phenol content and WV were similar in Lockette, HG-9, and Cutter, but IVMD 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 (Salawa et al., 1997) and precipitated with soluble protein (Martin and Martin, 1982). The presence of Caillandra 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 the 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

| Varieties | Total Protein (% DM) | Proportion of protein fractions (%) | IVDMD at 8 h (%) |
|-----------|----------------------|------------------------------------|-----------------|
|           |                      | Insoluble | Soluble | Non-protein nitrogen |                      |
| February 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^{a}              |
| 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^{a}           | 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                  |
Table 2. Dry matter (DM) production (forage mass; kg DM/ha) during steers grazing winter wheat varieties

| Wheat variety/ time | February 19 H-FA L-FA | March 09 H-FA L-FA | March 17 H-FA L-FA | March 23 H-FA L-FA | 50% heading H-FA 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               | 292189               | 280          | 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    | 0.12  | 0.001 |
| 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    | 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 Varieties | DM Disappearance Rate | Rumen Gas Production |
|-----------------|------------------------|-----------------------|
|                 | Instantly Solubilized Disappearance Rate | Potential Disappearance Rate | Rate of Gas Production | Potential Gas Production |
|                 | a (%) | c (%/h) | a + b (%) | c (ml/h) | b (ml/6h) |
| February 19     |       |         |          |         |         |
| Triticale       | 27.2<sup>ab</sup> | 27.6 | 82.8 | 3.1 | 39.2<sup>b</sup> |
| Lockett         | 23.7<sup>ab</sup> | 26.0 | 77.7 | 3.7 | 43.8<sup>a</sup> |
| HG-9            | 23.6<sup>ab</sup> | 26.1 | 77.9 | 4.7 | 52.1<sup>a</sup> |
| Thunderbolt     | 28.0<sup>ab</sup> | 27.0 | 82.2 | 4.3 | 47.0<sup>a</sup> |
| Maton rye       | 34.7<sup>a</sup> | 30.4 | 82.3 | 3.5 | 42.3<sup>a</sup> |
| Cutter          | 33.2<sup>a</sup> | 29.1 | 80.2 | 3.5 | 41.6<sup>a</sup> |
| Jagalene        | 30.9<sup>ab</sup> | 28.3 | 77.7 | 4.1 | 44.5<sup>a</sup> |
| SEM             | 4.51 | 2.48 | 6.81 | 0.99 | 5.90 |
| March 08        |       |         |          |         |         |
| Triticale       | 34.1 | 28.2 | 75.2 | 6.3 | 71.5<sup>b</sup> |
| Lockett         | 32.5 | 27.9 | 84.8 | 7.9 | 92.2<sup>a</sup> |
| HG-9            | 29.7 | 27.6 | 82.8 | 7.1 | 82.8<sup>ab</sup> |
| Thunderbolt     | 30.6 | 27.5 | 83.0 | 6.4 | 74.3<sup>b</sup> |
| Maton rye       | 35.2 | 31.3 | 82.7 | 7.4 | 83.1<sup>ab</sup> |
| Cutter          | 29.8 | 27.7 | 83.1 | 6.8 | 77.1<sup>ab</sup> |
| Jagalene        | 28.7 | 27.2 | 81.5 | 7.2 | 79.8<sup>ab</sup> |
| SEM             | 3.91 | 2.15 | 5.90 | 0.99 | 5.90 |
| March 17        |       |         |          |         |         |
| Triticale       | 34.5<sup>a</sup> | 28.6<sup>a</sup> | 86.9 | 7.0 | 84.6<sup>ab</sup> |
| Lockett         | 24.0<sup>b</sup> | 26.2<sup>b</sup> | 76.9 | 8.3 | 97.8<sup>a</sup> |
| HG-9            | 23.6<sup>b</sup> | 23.5<sup>b</sup> | 80.3 | 7.9 | 92.5<sup>c</sup> |
| Thunderbolt     | 27.6<sup>ab</sup> | 26.7<sup>ab</sup> | 79.9 | 7.0 | 79.9<sup>ab</sup> |
| Maton rye       | 36.6<sup>a</sup> | 30.9<sup>a</sup> | 83.5 | 8.1 | 91.3<sup>ab</sup> |
| Cutter          | 27.2<sup>ab</sup> | 26.1<sup>ab</sup> | 79.4 | 6.9 | 76.6<sup>b</sup> |
| Jagalene        | 34.7<sup>a</sup> | 29.0<sup>a</sup> | 81.8 | 7.9 | 89.8<sup>ab</sup> |
| SEM             | 3.91 | 2.15 | 5.89 | 1.00 | 5.90 |

<sup>1</sup>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. <sup>2</sup>Disappearance rate was estimated by measuring the loss of plant constitutes from ANCOM filter bags, incubated with rumen fluid from steers.
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 Varieties¹ | Instantly Solubilized a (%) | Rate of Disappearance c (%/h) | Potential Disappearance rate a + b (%) |
|------------------|----------------------------|-------------------------------|---------------------------------------|
| February 19      |                            |                               |                                       |
| Triticale        | 23.7                       | 23.9ab                        | 86.8                                  |
| Lockett          | 22.7                       | 20.3b                         | 98.5                                  |
| HG-9             | 23.3                       | 20.2b                         | 99.2                                  |
| Thunderbolt      | 24.2                       | 25.3ab                        | 86.3                                  |
| Maton rye        | 31.1                       | 27.3a                         | 93.8                                  |
| Cutter           | 29.8                       | 26.5ab                        | 91.8                                  |
| Jagalene         | 27.4                       | 25.7ab                        | 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        | 30.6a                      | 26.6a                         | 93.0                                  |
| Lockett          | 23.0b                      | 22.7b (0.1)                   | 88.0                                  |
| HG-9             | 22.1bc                     | 20.8b                         | 92.4                                  |
| Thunderbolt      | 25.3ab                     | 25.0ab                        | 85.5                                  |
| Maton rye        | 34.5a                      | 27.3a                         | 95.0                                  |
| Cutter           | 25.3ab                     | 25.1ab                        | 85.3                                  |
| Jagalene         | 32.3a                      | 26.6a                         | 93.0                                  |
| SEM              | 3.79                       | 2.02                          | 8.72                                  |

¹Rumen 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.

²Disappearance 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 varieties | Methane gas production (ml/h)* |
|-----------------|--------------------------------|
| Triticale       | 3.5<sup>b</sup> (0.06)        |
| Lockett         | 5.3<sup>ab</sup>              |
| HG-9            | 3.1<sup>b</sup>               |
| Thunderbolt     | 2.2<sup>b</sup>               |
| Maton rye       | 6.4<sup>a</sup>               |
| Cutter          | 3.8<sup>b</sup> (0.09)        |
| Jagalene        | 4.4<sup>ab</sup>              |
| SEM             | 1.09                           |

<sup>1</sup>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.

<sup>2</sup>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.

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

SEM = Standard error of the mean.
| 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^{B}\text{C} |          |
| 7 S2K     | 25.0^{B}              | 24.0^{C} | 24.6^{C} |          |
| 8 TAM 111 | 21.8^{bB}             | 26.5^{aB}\text{AB} | 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\text{b} Means in a row with different letters differ (P < 0.05).

^AB\text{AB} Means within a column 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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