Elevated CO₂ affects plant nitrogen and water-soluble carbohydrates but not *in-vitro* metabolisable energy

J.F. Panozzo^{1*}, C.K. Walker¹, P. Maharjan¹, D.L. Partington², and C.J. Korte¹

¹Department of Jobs, Precincts and Regions, Agriculture Victoria, Private Bag 260, Horsham, Victoria 3400, Australia

²Department of Jobs, Precincts and Regions, Agriculture Victoria Napier Road Hamilton, Victoria 3400, Australia

* Corresponding author. Dr Joe Panozzo, Department of Jobs, Precincts and Regions, Agriculture Victoria, Private Bag 260, Horsham, Victoria 3400, Australia

Email address joe.panozzo@ecodev.vic.gov.au

Abstract:

The effects of elevated concentrations of atmospheric CO₂ (e[CO₂]) on the nutritive value of wheat vegetative-matter and grain as a feed-stock for ruminants were investigated in a study undertaken at the Australian Grains Free Air CO₂ Enrichment (AGFACE) facility. The study included using two commercial wheat cultivars (Janz and Yitpi) and two genetic selections from a Seri/Babex population (SB003 and SB062) which had previously been characterised for low and high water-soluble carbohydrate accumulation efficiency. The trial was grown under ambient (~390 μmol mol⁻¹) and elevated (~550 μmol mol⁻¹) CO₂ conditions and plants harvested at tillering, anthesis and physiological maturity. Composition analyses to determine the nutritive value for ruminant-feed were undertaken on stems, leaves and grain. Plant and grain nitrogen were reduced in the e[CO₂] treatments and as expected the water-soluble carbohydrates increased. All genotypes responded to e[CO₂] with the effects of altered composition evident within 60 days of sowing. Determinants of ruminant-feed quality such as neutral- and acid detergent fibre and estimated *in-vitro* metabolisable energy were not significantly affected. The reduced plant and grain N will impact on the nutritive

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/JAC.12359](https://doi.org/10.1111/JAC.12359)

This article is protected by copyright. All rights reserved

value and supplementation may be required. The impact of $e[CO_2]$ on chemical composition of wheat plants may be greater if the predicted climate change is associated with concomitant abiotic stress such as high ambient temperature or low soil moisture.

Highlights:

Elevated carbon dioxide reduced plant and grain nitrogen
Water soluble carbohydrates increased under elevated CO_2
All cultivars are affected by $e[CO_2]$ but the magnitude of the response varies
Estimated metabolisable energy was not affected

Keywords:

AGFACE
Elevated carbon dioxide
Wheat
Ruminant nutrition
Nitrogen
Water soluble carbohydrates

1. Introduction

Climate change and climate variability, arising from increased levels of atmospheric carbon dioxide will impact on future agricultural productivity and subsequently affect food production for humans and animals. With atmospheric carbon dioxide concentrations predicted to increase to $550 \mu\text{mol } CO_2 \text{ mol}^{-1}$ by the middle of the 21st century (Carter, Jones et al. 2007; IPCC 2013), there is a substantial focus on assessing the potential impact of CO_2 -mediated climate change on agricultural production. It is also recognised that elevated concentrations of carbon dioxide $e[CO_2]$ has a positive, 'fertilisation' effect on plant growth under favourable soil moisture and optimum temperature conditions resulting in increased biomass. However, associated with climate change are more frequent periods of higher ambient temperatures which accelerates plant development but contributes to a decrease in post-anthesis growing-degree days. These effects may alter both composition of the plant and/or of the grain (Panozzo, Eagles et al. 2001). Furthermore, variability

in climatic conditions such as water availability, temperature and carbon dioxide will interactively affect dry matter production and grain composition (Dellar, Topp et al. 2018).

Within the major broad-acre crops, most of the research on the effects of $e[CO_2]$ has focused on wheat and the impact on plant growth (Kimball 2016; Poorter 1993; Seneweera and Norton 2011), grain yield, (Fitzgerald, Tausz et al. 2016; Kimball 2016; Taub, Miller et al. 2008), plant- macro and micro nutrients (Dietterich, Zanobetti et al. 2015; Manderscheid, Bender et al. 1995) and end-use quality traits (Fernando, Panozzo et al. 2014a; Högy, Brunnbauer et al. 2013; Högy and Fangmeier 2008; Panozzo, Walker et al. 2014). Furthermore, research on the impact of $e[CO_2]$ on biomass-quality and its nutritional value for ruminant nutrition has been reported in a number of studies including Akin, Kimball et al. (1995), Ehleringer, Cerling et al. (2002) and Taweel, Tas et al. (2005). These studies have concentrated on pasture grasses and there has been limited studies on the effects on wheat-vegetative matter and its nutritional value if used as a feed source for ruminants (Porteous, Hill et al. 2009).

While much of the grain produced globally is destined for human consumption, approximately 25% of production is used for stockfeed and in both grain markets, the value is often based on its grain composition which determines the nutritive value and processing functionality. In addition, some long-photoperiod C_3 plants including cereal crops are often grazed by ruminants early in plant-growth and prior to the reproductive phase of plant development. After grazing, the crop is allowed to recover and develop through to grain filling and the grain is harvested. For these farming practices, the composition of the cereal plant at the early growth-stage is associated with the concentration of plant nitrogen and water-soluble carbohydrate (WSC) which will determine the nutritional value and affect the palatability of the vegetative matter and voluntary-intake, ultimately impacting on live-weight gain. Additional farm management practices include allowing the ruminants to graze the straw and grain residues after harvest to reduce field biomass and volunteer plants after post-harvest rain.

In-vitro ruminant-feed quality is defined by a series of analytical assays that determine both the positive and deleterious components of nutrition. These include plant tissue and grain nitrogen often reported as crude protein (%Nx6.25), structural carbohydrates measured separately as neutral- (NDF) and acid detergent fibre (ADF), non-structural water-soluble carbohydrates (WSC) including sugars and fructans, total inorganic material (% Ash) and *in-vitro* dry matter digestibility (IVDMD). Based on these parameters, it is possible to derive an estimate of *in-vitro* metabolisable energy (ME) which is used to quantify the value of the feed ration to maximise production either as live-weight gain or milk output in ruminants. The use of forage-based feeding systems presents

challenges for animal nutritionists and agronomists as the composition of the pasture can be highly variable affected by soil nutrition, soil moisture or a range of abiotic factors. Therefore, pasture-based feeds are often supplemented with more expensive grain-based diets. However, this option is often reliant on sourcing highest quality grain but at the least-cost. Under a climate change scenario, this option will become more difficult.

Forage-quality can be negatively impacted under e[CO₂] conditions resulting in increased plant carbohydrate reserves and biomass which contributes to a dilution in both plant tissue-nitrogen and grain nitrogen irrespective of the nitrogen-availability in the soil (Conroy 1992). Reduced nitrogen content in pastures has been shown to decrease digestibility and conversion of ingested matter retarding ruminant live-weight gain. In addition, as ruminant digestibility is affected by microbial activity within the rumen, an altered balance in the concentration of nitrogen-containing compounds and carbohydrate (structural and non-structural) will impact on the rate of digestion (Van Soest, Robertson et al. 1991).

A positive aspect of e[CO₂] is an increase in non-structural carbohydrates which can be quantified as insoluble starch, fructans and sugars including glucose, fructose and sucrose. The concentration of sugars in plant tissue vary diurnally and are at their highest concentration during the day when advantageously most herbivores graze. The concentration of the mono- and disaccharide sugars will impact the rate of grazing and palatability, although this is difficult to quantify (Tava, Berardo et al. 1995).

Furthermore, increased rates of photosynthesis due to e[CO₂] have been reported to increase the synthesis of carbon-based structural carbohydrates such as lignin. The reported change in lignin in C₃ grasses is small (Newman et al. 2003) or inconsistent (Poorter, Van Berkel et al. 1997), although these effects may be species-dependent (Hartley, Jones et al. 2000). For forage analysis, lignin is not usually measured directly but determined as NDF (lignin, hemicellulose and cellulose) and as ADF (cellulose and lignin). These determinants are measured as they may impede digestibility. Contrary to these reported effects, (Akin, Kimball et al. 1995) found that e[CO₂] did not alter *in-vitro* digestibility although changes in ADF and NDF were noted which were dependent on the growth stage of the plant.

There is now strong evidence of the implications of increased e[CO₂] associated with climate-change and its impact on future agricultural production and food security. While the effects of CO₂ on pastures are well documented, compositional changes in wheat vegetative-matter particularly related to ruminant feed quality is lacking. In this study, we investigated the effects of e[CO₂] on the

composition and *in-vitro* metabolisable energy value of wheat vegetative-matter throughout plant development and in the resulting grain, particularly if used as a feed source for ruminants.

2. Materials and Methods

2.1 Site description and field experiment

The four wheat cultivars (*Triticum aestivum* L.) used in this study were Janz, Yitpi, SB003 and SB062. Janz and Yitpi are Australian commercially released cultivars while SB003 and SB062 are recombinant inbred lines derived from a Seri/Babax population described by Olivares-Villegas, Reynolds et al. (2007). Dreccer, van Herwaarden et al. (2009) characterised several genotypes derived from the Seri/Babax population of which, SB003 was identified as low WSC-accumulation and high-tillering capacity, whereas SB062 had a high WSC-accumulation potential and low-tillering capacity.

The field experiment was conducted at the Australian Grains Free-Air CO₂ Enrichment (AGFACE) site at Horsham (36° 45'07"S; 142° 06'52" E) Victoria, Australia as previously described by (Mollah, Norton et al. 2009) and Mollah, Partington et al. (2011). The field trial was designed as a randomised complete-block with two CO₂ treatments; ambient concentrations a[CO₂] (~390 μmol mol⁻¹ daylight concentration) and elevated concentration e[CO₂] (~550 μmol mol⁻¹). Sensors within the ring adjusted the flow of CO₂ throughout the growing season to compensate for variation in wind speed and wind direction. The field trial consisted of 8 rings, each 16 m in diameter. Four rings were allocated for each CO₂ treatment. Within each ring the four genotypes were sown in 4 m plots, 1.4 m wide consisting of 5 drill rows with a 0.27 m row space. The soil nitrogen measurements (NO₃-N) were taken at sowing and after harvest. In 2011 the readings were 180 kg.ha⁻¹ at sowing and 97 kg.ha⁻¹ at harvest. In 2012, the readings were 112 kg.ha⁻¹ at sowing and 38 kg.ha⁻¹ at harvest.

Within a few days after sowing, injection of CO₂ began into the ring-space of the e[CO₂] treatment and continued until near harvest. Plant development was recorded using the Zadoks decimal scale (DC) (Zadoks, Chang et al. 1974). Ambient temperatures and rainfall were recorded throughout the growth stage until physiological maturity and were similar in both years (Panozzo, Walker et al. 2014). The trial was irrigated at prior to tillering (~DC29) and prior to anthesis (~DC60) and mid-grain filling (~DC70). Irrigation was carried out via an overhead spray system with the equivalent of 20mm of rain applied at each watering stage. Rainfall during the growing season was similar in both years with 218 mm recorded in 2011 and 180 mm in 2012.

157

158 2.2 Plant sampling and analyses

159 Plant tissue of each genotype was hand-harvested from quadrant samples at tillering (DC31), and
160 anthesis (DC65). Vegetative samples at DC31 were partitioned in to stems, and leaves. While at
161 DC65 samples were portioned in to stems, leaves and spikes. All plant samples were then dried at
162 40°C for 72 hours in a high-velocity air oven prior to analysing for nutritive composition. At
163 physiological maturity (DC90), whole plants (without roots) were hand-harvested and the grain
164 threshed from the spikes. Both the grain, and plant material consisting predominantly of stems,
165 were retained for nutritive composition analysis.

166 Oven-dried plant material was ground using a Foss-Tecator Cyclotec impeller mill (Foss ANA
167 Denmark) fitted with a 0.5mm screen. The standard suite of forage-nutritive quality-traits were
168 undertaken which included %nitrogen (%N), total water-soluble carbohydrates (%WSC), neutral
169 detergent fibre (%NDF), acid detergent fibre (%ADF), ash (%Ash) and metabolisable energy (ME)
170 calculated from *in-vitro* dry-matter digestibility (IVDMD). All assays were determined by near
171 infrared reflectance spectroscopy (NIRS) using a Foss XDS Rapid Content Analyser (Foss ANA
172 Denmark) using established spectroscopic techniques (Lundberg, Hoffman et al. 2004; Muir, Ward et
173 al. 2014). The collected NIR spectra were corrected for scatter with standard normal variance (SNV)
174 and detrending. Mathematical treatment included a second-order derivative with gap and smooth
175 sizes of five and five data points, respectively. Algorithms were developed using modified partial
176 least squares (mPLS) algorithm and cross-validation technique. WinISI software V 4.6.8.14739
177 (Infrasoft International LLC, Hilleroed, Denmark) was used for all data processing. The NIR algorithms
178 used to determine these traits were those used routinely in this laboratory for ruminant nutrition
179 projects and commercial applications and have been developed using accumulated spectral
180 reference data for more than 5 years. The number of data points for each trait are listed in Table 1.
181 The NIRS algorithms were developed using the standard reference methods as follows: plant and
182 grain N was determined by Dumas combustion (CCD-Method-02-03 2003), NDF and ADF (AFIA 2014)
183 based on the method of Van Soest, Robertson et al. (1991), WSC using the anthrone method (AFIA
184 2014), ash by combustion at 650 °C. The estimated metabolisable energy for cereal vegetative
185 matter was derived from an algorithm based on *in-vitro* dry organic matter digestibility which was
186 determined by pepsin cellulase digestion. Briefly, samples were digested with acidified pepsin for
187 24h followed by digestion with heat-stable α -amylase and a third digestion with cellulase for 24h.
188 The digested solution was filtered through sintered crucibles. The residue was dried to determine
189 the dry matter digestibility. The values were adjusted to *in-vitro* dry matter digestibility (IVDMD)

using a linear regression based on forage standards on known *in-vivo* digestibility determined through animal feeding trials. Subsequently, ME were calculated using the following equations: ME (MJ/kg DM) = $0.203 \times (6.83 + 0.847 \times \% \text{IVDMD}) - 3.001$ (AFIA 2014); and for grains samples, ME (MJ/kg DM) = $0.858 + 0.138 \times (2.11 + (0.961 \times \% \text{IVDMD})) + 2.72 \times \% \text{Fat}$ (AFIA 2014).

Approximately 10% of the samples tested by NIRS were randomly selected and cross-validated against the reference methods described above. Table 1 details the calibration statistics for each NIR-estimated trait.

2.3 Biometrics

Statistical analyses were carried out based on a split-plot design nested within each year. The fixed factors were year, [CO₂] and genotype. The main factor within each year was [CO₂] with plots being split into subplots to examine the differences between genotypes. Data for plant composition at each sampling point and grain were analysed using GenStat® (16th Edition) (Payne, Welham et al. 2013) as described by Panozzo, Walker et al. (2014).

3. Results

3.1 Overall treatment effects

Elevated CO₂ had a significant effect on both plant N and WSC in all plant components and at all stages of plant growth (Table 2). In most growth stages, cultivars responded differently under e[CO₂] except at DC31 where there was a significant response ($P < 0.05$) to increased %WSC and a concomitant decrease in %N was measured in the stem tissue. In latter stages of plant growth, no effect of e[CO₂] and genotypes were observed as was also the case for the three-way interaction of years, CO₂ and genotypes. For other components of forage quality such as *in-vitro* ME and estimates of structural carbohydrates (NDF and ADF), e[CO₂] did not significantly alter the concentration within the vegetative matter (Table 2).

3.2 Nitrogen

The impact of e[CO₂] on plant N was manifested early in plant development and exhibited by significantly lower %N in both the leaf and stem tissue in the DC31 sampling ($P < 0.05$) in all four

cultivars in 2011 and similarly in 2012 except for SB003. The mean decrease, in %N between elevated and ambient CO₂ treatments at DC31 was between 0.3-0.6%. As plants developed from DC31 to DC65, plant N decreased by approximately 3% in both the stems and leaves irrespective of CO₂ treatment levels. Significantly lower %N was also measured in leaf tissue at the DC65 for the e[CO₂] treatment compared to the a[CO₂] ($P<0.05$). Conversely, there were no significant differences in %N of the stems or spikes at DC65 in the e[CO₂] treatment, however in the leaf tissue, e[CO₂] contributed to significantly lower %N. At completion of grain-filling (DC90), there was a significant effect ($P<0.05$) due to e[CO₂] with a reduction in N of between 1-1.8% (Figure 1).

A similar effect of e[CO₂] was also observed in the second year of this experiment. A significant reduction in %N at DC31 was observed in the leaf tissue of all cultivars and in the stem tissue for Yitpi and Janz. The reduction in %N within the leaf tissue as plants developed from DC31 and DC65 sampling was less in 2012 compared with 2011, with only a 0.1–1.5 reduction in %N observed in 2012 for a[CO₂] and e[CO₂] compared to a 2.6–3.7% reduction in 2011. These differences are most likely due to abiotic effects between the two years. Contrary to 2011, %N in wheat spikes at DC65 in 2012 was significantly lower ($P<0.05$) in the e[CO₂] treatment for all cultivars except Janz. At harvest, a reduction between 1.1 and 1.8% in total N was measured in both years for e[CO₂].

3.3 Water soluble carbohydrates

The concentration of WSC within the plant tissue was found to be inversely correlated to the changes observed for %N at each sampling point. At DC31, the concentration of WSC was greater in stems than in leaves and during plant development, the %WSC further increased at DC65 in the stems. Conversely, %N in the leaf tissue decreased between the DC31 and DC65 growth stages as %N within the spike increased (Figure 2).

As expected, all cultivars responded similarly in changes to WSC throughout plant development and between the two years although the magnitude of the response varied for cultivars and years with a greater response generally observed in 2011 than in 2012. The cultivar SB062 was found to have the highest %WSC within the stems at DC65 of the four cultivars in this study. All cultivars responded positively to e[CO₂] in the early stages of plant development (DC31) resulting in a significantly higher concentration of WSC compared with the a[CO₂] treatment. The relative difference in %WSC between CO₂ treatments varied with growth stages, with the largest differences observed in the stems at DC31 and DC65.

WSC in the leaf tissue generally decreased as plants developed from DC31 to DC65. This decrease was associated with a concomitant increase in %WSC within the spikes. The effects of e[CO₂] within the four genotypes was more consistent in 2012 than in 2011.

3.4 Metabolisable energy

Stems and leaves harvested at DC31 were found to have the highest *in-vitro* ME value compared with plant tissue harvested at latter growth stages. As the wheat plants developed, the *in-vitro* ME value decreased from approximately 11-12 MJ/Kg DM at DC31 to approximately 5-7 MJ/Kg DM in the stems at DC90. In 2011, ME at DC31, in both the a[CO₂] and e[CO₂] treatments was significantly higher in the leaf, than the stem tissue and similarly at DC65 for all cultivars, however, this was not always observed in 2012 particularly at DC31. At DC65, the ME in the leaves was significantly higher than for the stems (Figure 3).

No significant effects of e[CO₂] on *in-vitro* ME was observed in 2011 measured on any component of the plant, however in 2012 the effects of e[CO₂] were more pronounced although not all cultivars responded to e[CO₂]. Where effects of e[CO₂] on *in-vitro* ME were noted, *in-vitro* ME was significantly reduced at higher concentrations of CO₂, but the effects were only observed in Yitpi, SB003 and, SB062 in the leaf tissue at DC65 and stems at DC90. The decrease in *in-vitro* ME at DC65 due to e[CO₂] was 0.9-1.2 MJ/Kg DM and at DC90 the decrease in *in-vitro* ME due to e[CO₂] was 0.7-1.5 MJ/Kg DM ($P<0.05$). The ME values of the grain at DC90 covered a narrow range of 13.2-13.4 MJ/Kg DM for all cultivars and in both years and no differences effects were observed due to e[CO₂].

3.5 Inorganic matter (ash)

The %ash represents the total inorganic material within the plant tissue or grain and comprised of inorganic compounds including silicates and metallic elements. For ruminant-feed analyses, the results are important as the values represent indigestible matter. In this study, no consistent effects were observed in response to e[CO₂] to altered changes in %ash content of either plant tissue throughout the growth stages or in the mature grain (data not shown). The trend however, was for %ash to be reduced in the e[CO₂] treatment particularly in 2012. This is consistent with previous reports that e[CO₂] reduces the concentration of minerals in plants (Erbs, Manderscheid et al. 2010; Högy, Brunnbauer et al. 2013).

3.6 Plant Fibre (Neutral-Detergent Fibre and Acid-Detergent Fibre)

Plant structural carbohydrates consisting predominantly of cellulose, lignin and hemicellulose which were measured as %NDF and %ADF were highest in the stem, rather than the leaf tissue and increased in concentration throughout plant development to senescence (DC90). There was no significant response to $e[CO_2]$ on plant structural polysaccharides between genotypes except for Yitpi in 2012, where significantly lower %ADF and %NDF were measured in the leaf tissue at DC65 and stems at DC90.

4. Discussion

Based on previous studies it is evident that an escalation in the concentration of atmospheric CO_2 above current ambient levels will have an increased effect on plant growth rates resulting in increased biomass and grain yield, (Fitzgerald, Tausz et al. 2016) and may alter plant composition (Högy, Brunnbauer et al. 2013). Temperature conditions were relatively similar in the two years of this study and both $a[CO_2]$ and $e[CO_2]$ treatments had supplemental irrigation treatments, possible interactive effects of available soil moisture which may occur with climate change could not be examined. This study primarily investigated the implications of $e[CO_2]$ on nutritive quality of wheat-vegetative matter if used as a feed-source for ruminants. While wheat at the vegetative stage is generally not used as a pasture in forage systems, some long-season wheat varieties are adaptable for grazing particularly if sown early; grazed by livestock, and later as plants recover to produce tillers, the grain is harvested for stock-feed. In some cases, particularly for awnless cultivars, wheat crops may be used as a source for hay and therefore the composition of the plant material is important. This study used wheat as a model crop to investigate changes in plant composition grown under $e[CO_2]$ conditions however it is likely that most C_3 cereals will have a similar response in relation to N and WSC concentration although genotypic variability in assimilate translocation-efficiency is expected.

In response to $e[CO_2]$, there was a significant decrease in plant N and the magnitude of the change, was inversely correlated to an increase in WSC. Differences in plant N between ambient and elevated CO_2 treatments were observed as early as DC31 which corresponded to approximately 60 days after sowing. The depression in plant-tissue N due to $e[CO_2]$ is likely to be associated with an increased rate of carboxylation and decreased rates of oxygenation of ribulose-1,5-biphosphate carboxylase/oxygenase (RUBISCO) resulting in increased photosynthesis and hence increased carbohydrate (Stitt and Krapp 1999).

The overall effect of e[CO₂] on C₃ cereals is a reduction in grain N percentage at physiological maturity which is now a widely reported outcome of e[CO₂] (Asseng, Martre et al. 2019; Fernando, Panozzo et al. 2014b; Högy, Brunnbauer et al. 2013; Panozzo, Walker et al. 2014). In this study, a relative reduction in %N of approximately 5-10 % due to elevated CO₂ was measured throughout plant development and 5-7% reduction in the grain. The impact is significant, particularly as grain N has a nutritive value if used as stock-feed.

Conversely, plant WSC are positively affected by e[CO₂] and accumulate in the stems through the early stages of plant development until grain filling when stem-reserve carbohydrates become mobile and partition to the developing grain (Ruuska, Rebetzke et al. 2006). Water-soluble carbohydrates are a source of energy for ruminants and impact on palatability and hence feed-intake. The increase in WSC in response to e[CO₂] at the different growth stages was expected and consistent for all genotypes and in both years which has also been reported by Poorter, Van Berkel et al. (1997). There was however, variation in the magnitude of the response amongst genotypes and significant e[CO₂] x genotype interactions were noted. In this study the largest genotypic response in WSC under e[CO₂] conditions were observed for the genotype SB062, a genotype selected for high WSC content.

As the WSC assay used in this study quantifies the total non-structural carbohydrates (NSC) which include water-soluble sugars and fructans, the measured changes in WSC during plant development cannot differentiate the relative proportions of either soluble sugars or fructans. Logically, it is expected that there is a concomitant increase in both types of NSC although the rate of synthesis for either group of NSC will vary during plant development and particularly post-anthesis. Conversely, structural carbohydrates including lignin, cellulose and hemicellulose were not directly measured *per se*. Proximate analysis used by animal nutritionists to determine for structural carbohydrates, include ADF and NDF. In this study, there were no significant effects due to e[CO₂] in the overall mean values although there was a trend towards an increase in both NDF and ADF during plant development. This trend, although non-significant aligns with the expected compositional changes due to lignification and increases in poly-celluloses during plant development and senescence. The observed values for ADF and NDF in the vegetative tissue were within expected means (AFIA 2014), indicating that future increases in the concentration of CO₂ may not adversely impact on these components of fibre although this may be altered if soil N is limited which will alter the C/N balance. A non-significant response to changes in structural carbohydrates was also reported in other studies, for example, Newman, Abner et al. (2003) reported a slight decrease in lignin and other fibres components due to e[CO₂] and, Porteous, Hill et al. (2009) found no effect of e[CO₂] on NDF, ADF,

cellulose or lignin but the concentration of hemicellulose was less in the e[CO₂] treatment. Similarly, Poorter, Van Berkel et al. (1997) reported for a range of species including wheat, that e[CO₂] had little effect on the structural carbohydrates such as lignin.

In the present study, the effects of e[CO₂] on the *in-vitro* ME value of plant tissue or grain were not consistent between genotypes or years and in part, confirms previous reports by Akin, Kimball et al. (1995); Porteous, Hill et al. (2009). A decrease in *in-vitro* ME due to e[CO₂] was observed in the second year and not for all genotypes. The effect of e[CO₂] reducing the *in-vitro* ME was not evident until anthesis (DC65) and is most likely associated with remobilisation of stem reserves to the spike and the increased lignification of mature leaf tissue. Therefore a possible strategy that livestock producers may adopt to mitigate changes in pastures due to e[CO₂], is to allow ruminants to graze before anthesis when the concentration of WSC is elevated and the risk of low ME reduced. Barbehenn, Chen et al. (2004) investigated a range of C₃ grasses but not *Triticum aestivum*, and demonstrated that C₃ pastures produce a greater ratio of non-structural carbohydrate to nitrogen under e[CO₂] conditions and concluded the grass will be nutritionally superior. Furthermore, it has been established that a higher WSC to N ratio contributes to increased microbiological protein synthesis in the rumen resulting in increased feed digestibility and increased productivity (Hoover and Stokes 1991) and therefore may be a positive outcome of e[CO₂].

5. Conclusion

If projections of increasing levels of atmospheric carbon dioxide follow the predicted models for the next 50 years, a 'positive outcome' for the grain and pasture industry will be increased plant biomass due to increased rates of photosynthesis (Fitzgerald, Tausz, et al. 2016). However, in terms of grain quality, Walker et al. (2017) reported that even with high applications of nitrogen fertiliser (100 kg ha⁻¹), there was an adverse effect on grain protein concentration under e[CO₂]. This will have implication for the stockfeed industry.

The direct impact of e[CO₂] on animal nutrition and subsequently on live-weight gain or milk production may be difficult to quantitate as other interactive factors such as palatability impact on feed in-take. Both palatability and in-take are positively-associated with the water-soluble carbohydrates and negatively-associated with phenolic-type compounds including lignin. For example, Owensby, Cochran et al. (1996) estimated a reduction in live-weight measured over a 150-day period in cattle which consumed forage that was produced under e[CO₂] conditions. These

effects were calculated to be more significant for pastures from e[CO₂] treatments if grazed in the early stages of plant development.

It is clear from this study that e[CO₂] influences the chemical composition of leaf, stem and grain of wheat and hence may impact on the nutritional value predominantly through the alteration of nitrogen content and non-structural carbohydrates which are predominantly WSC. This study confirmed that the concentration of tissue nitrogen content is inversely related to WSC concentration. The implications for increased WSC concentration in the vegetative phase are increased palatability and voluntary feed in-take. Conversely the altered C to N ratio in plant tissue is known to promote an increased synthesis of structural carbohydrates which include lignin, cellulose and hemicellulose. While these compounds were not individually quantified in this study, an increased concentration of lignin is undesirable due to its indigestibility in the rumen and subsequently affect rumen-microbial activity impacting on animal productivity.

The challenge for plant breeders and pasture producers is to maintain quality traits such as plant N which is highly sensitive to the effects of increase carboxylation and limit the synthesis of difficult to digest carbohydrates such as lignin particularly if plant N is reduced. This may be achieved in the absence of minimising climate change impacts through plant breeding or optimised agronomic practices.

Table and Figure Captions:

Table 1. Near-infrared reflectance spectroscopy calibration summary for analysis of the nutritive value of plant and grain for animal feed.

Table 2. Pooled cultivar/genotype (G) significance effects for each measured trait arising from CO₂ and interactions. (Significant effects (P<0.05) are highlighted).

Figure 1. Nitrogen (%) in the leaves, stems (DC31 and DC65), spikes (DC65), stems and leaves (DC90) and grain (DC90) at a[CO₂] (—◆—) and e[CO₂] (---■---) for 2011 and 2012. Error bars are LSD=0.05.

Figure 2. Water-soluble carbohydrate (%) in the leaves, stems (DC31 and DC65), spikes (DC65), stems and leaves (DC90) at a[CO₂] (—◆—) and e[CO₂] (---■---) for 2011 and 2012. Error bars are LSD=0.05.

Figure 3. *Invitro* metabolisable energy (%ME) in the leaves, stems (DC31 and DC65), spikes (DC65), stems and leaves (DC90) and grain (DC90) at a[CO₂] (—◆—) and e[CO₂] (---■---) for 2011 and 2012. Error bars are LSD=0.05.

Acknowledgements

Research within the Australian Grains Free Air CO₂ Enrichment (AGFACE) facility was funded by the Department of Jobs, Precincts and Regions (DJPR), Grains Research and Development Corporation (GRDC), and the University of Melbourne with support from and the Australian Commonwealth Department of Agriculture. The authors acknowledge Dr Glenn Fitzgerald and Dr Michael Tausz as project leaders for AGFACE. The technical support Russel Argall and his team for field management, the AGFACE technical group, Dr. Mahabubur Mollah (AGFACE engineer) and the Grains and Forage Chemistry Laboratory for technical support are gratefully acknowledged.

References

- AFIA (2014). AFIA - Laboratory Methods Manual, Publication No. 03/001 *A reference manual of standard methods for the analysis of fodder, version 8*. Australian Fodder Industry Association Inc, Melbourne Australia.
- Akin, D. E., B. A. Kimball, W. R. Windham, P. J. Pinter, G. W. Wall, R. L. Garcia, R. L. LaMorte and W. H. Morrison (1995). Effect of free-air CO₂ enrichment (FACE) on forage quality of wheat. *Animal Feed Science and Technology*, 53, 29-43. [http://dx.doi.org/10.1016/0377-8401\(94\)00736-5](http://dx.doi.org/10.1016/0377-8401(94)00736-5)
- Asseng, S., P. Martre, A. Maiorano and et al. (2019). Climate change impact and adaptation for wheat protein. *Global Change Biology*, 25, 155-173. <https://doi.org/10.1111/gcb.14481>
- Barbehenn, R. V., Z. Chen, D. N. Karowe and A. Spickard (2004). C3 grasses have higher nutritional quality than C4 grasses under ambient and elevated atmospheric CO₂. *Global Change Biology*, 10, 1565-1575. <https://doi.org/10.1111/J.1365-2486.2004.00833.X>
- Carter, T. R., R. N. Jones, X. Lu, S. Bhadwal, C. Conde, L. O. Mearns, B. C. O'Neill, M. D. A. Rounsevell and M. B. Zurek (2007). New assessment methods and the characterisation of future conditions. In: M. L. Parry, O. F. Canziani, J. P. Palutikof, P. J. van der Linden and C. E. Hanson eds. *Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. pp. 133-171. Cambridge University Press, Cambridge, UK.
- CCD-Method-02-03 (2003). Dumas (Combustion) total nitrogen determination *Official Testing Methods*. Official Testing Methods of the Cereal Chemistry Division, Melbourne, Victoria.
- Conroy, J. (1992). Influence of elevated atmospheric CO₂ concentrations on plant nutrition. *Australian Journal of Botany*, 40, 445-456. <http://dx.doi.org/10.1071/BT9920445>
- Dellar, M., C. F. E. Topp, G. Banos and E. Wall (2018). A meta-analysis on the effects of climate change on the yield and quality of European pastures. *Agriculture, Ecosystems & Environment*, 265, 413-420. <https://doi.org/10.1016/j.agee.2018.06.029>
- Dietterich, L. H., A. Zanobetti, I. Kloog, P. Huybers, A. D. B. Leakey, A. J. Bloom, E. Carlisle, N. Fernando, G. Fitzgerald, T. Hasegawa, N. M. Holbrook, R. L. Nelson, R. Norton, M. J. Ottman, V. Raboy, H. Sakai, K. A. Sartor, J. Schwartz, S. Seneweera, Y. Usui, S. Yoshinaga and S. S. Myers (2015). Impacts of elevated atmospheric CO₂ on nutrient content of important food crops. *Scientific Data*, 2, 150036. <https://doi.org/10.1038/sdata.2015.36>
- Dreccer, M. F., A. F. van Herwaarden and S. C. Chapman (2009). Grain number and grain weight in wheat lines contrasting for stem water soluble carbohydrate concentration. *Field Crops Research*, 112, 43-54. <http://dx.doi.org/10.1016/j.fcr.2009.02.006>

- Ehleringer, J. R., T. E. Cerling and M. D. Dearing (2002). Atmospheric CO₂ as a Global Change Driver Influencing Plant-Animal Interactions¹. *Integrative and Comparative Biology*, 42, 424-430. 10.1093/icb/42.3.424
- Erbs, M., R. Manderscheid, G. Jansen, S. Seddig, A. Pacholski and H.-J. Weigel (2010). Effects of free-air CO₂ enrichment and nitrogen supply on grain quality parameters and elemental composition of wheat and barley grown in a crop rotation. *Agriculture, Ecosystems and Environment*, 136, 59-68.
- Fernando, N., J. Panozzo, M. Tausz, R. M. Norton, G. J. Fitzgerald, S. Myers, M. E. Nicolas and S. Seneweera (2014a). Intra-specific variation of wheat grain quality in response to elevated [CO₂] at two sowing times under rain-fed and irrigation treatments. *Journal of Cereal Science*, 59, 137-144. <http://dx.doi.org/10.1016/j.jcs.2013.12.002>
- Fernando, N., J. Panozzo, M. Tausz, R. M. Norton, N. Neumann, G. J. Fitzgerald and S. Seneweera (2014b). Elevated CO₂ alters grain quality of two bread wheat cultivars grown under different environmental conditions. *Agriculture, Ecosystems & Environment*, 185, 24-33. <http://dx.doi.org/10.1016/j.agee.2013.11.023>
- Fitzgerald, G. J., M. Tausz, G. O'Leary, M. R. Mollah, S. Tausz-Posch, S. Seneweera, I. Mock, M. Löw, D. L. Partington, D. McNeil and R. M. Norton (2016). Elevated atmospheric [CO₂] can dramatically increase wheat yields in semi-arid environments and buffer against heat waves. *Global Change Biology*, 22, 2269-2284. <https://doi.org/10.1111/gcb.13263>
- Hartley, S. E., C. G. Jones, G. C. Couper and T. H. Jones (2000). Biosynthesis of plant phenolic compounds in elevated atmospheric CO₂. *Global Change Biology*, 6, 497-506. <https://doi.org/10.1046/j.1365-2486.2000.00333.x>
- Högy, P., M. Brunnbauer, P. Koehler, K. Schwadorf, J. Breuer, J. Franzaring, D. Zhunusbayeva and A. Fangmeier (2013). Grain quality characteristics of spring wheat (*Triticum aestivum*) as affected by free-air CO₂ enrichment. *Environmental and Experimental Botany*, 88, 11-18. <http://dx.doi.org/10.1016/j.envexpbot.2011.12.007>
- Högy, P. and A. Fangmeier (2008). Effects of elevated atmospheric CO₂ on grain quality of wheat. *Journal of Cereal Science*, 48, 580-591.
- Hoover, W. H. and S. R. Stokes (1991). Balancing Carbohydrates and Proteins for Optimum Rumen Microbial Yield¹. *Journal of Dairy Science*, 74, 3630-3644. [http://dx.doi.org/10.3168/jds.S0022-0302\(91\)78553-6](http://dx.doi.org/10.3168/jds.S0022-0302(91)78553-6)
- IPCC (2013). Technical summary. In: T. F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P. M. Midgley eds. *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the*

- Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK and New York, NY, USA.
- Kimball, B. A. (2016). Crop responses to elevated CO₂ and interactions with H₂O, N, and temperature. *Current Opinion in Plant Biology*, 31, 36-43. <http://dx.doi.org/10.1016/j.pbi.2016.03.006>
- Lundberg, K. M., P. C. Hoffman, L. M. Bauman and P. Berzaghi (2004). Prediction of Forage Energy Content by Near Infrared Reflectance Spectroscopy and Summative Equations. *The Professional Animal Scientist*, 20, 262-269. [http://dx.doi.org/10.15232/S1080-7446\(15\)31309-7](http://dx.doi.org/10.15232/S1080-7446(15)31309-7)
- Manderscheid, R., J. Bender, H. J. Jäger and H. J. Weigel (1995). Effects of season long CO₂ enrichment on cereals. II. Nutrient concentrations and grain quality. *Agriculture, Ecosystems & Environment*, 54, 175-185. [http://dx.doi.org/10.1016/0167-8809\(95\)00602-O](http://dx.doi.org/10.1016/0167-8809(95)00602-O)
- Mollah, M., R. Norton and J. Huzzey (2009). Australian grains free-air carbon dioxide enrichment (AGFACE) facility: design and performance. *Crop and Pasture Science*, 60, 697-707. <http://dx.doi.org/10.1071/CP08354>
- Mollah, M., D. Partington and G. Fitzgerald (2011). Understand distribution of carbon dioxide to interpret crop growth data: Australian grains free-air carbon dioxide enrichment experiment. *Crop and Pasture Science*, 62, 883-891. <http://dx.doi.org/10.1071/CP11178>
- Muir, S. K., G. N. Ward and J. L. Jacobs (2014). Milk production and composition of mid-lactation cows consuming perennial ryegrass-and chicory-based diets. *Journal of Dairy Science*, 97, 1005-1015. <http://dx.doi.org/10.3168/jds.2013-7183>
- Newman, J. A., M. L. Abner, R. G. Dado, D. J. Gibson, A. Brookings and A. J. Parsons (2003). Effects of elevated CO₂, nitrogen and fungal endophyte-infection on tall fescue: growth, photosynthesis, chemical composition and digestibility. *Global Change Biology*, 9, 425-437. [10.1046/j.1365-2486.2003.00601.x](http://dx.doi.org/10.1046/j.1365-2486.2003.00601.x)
- Olivares-Villegas, J. J., M. P. Reynolds and G. K. McDonald (2007). Drought-adaptive attributes in the Seri/Babax hexaploid wheat population. *Functional Plant Biology*, 34, 189-203. <http://dx.doi.org/10.1071/FP06148>
- Owensby, C. E., R. C. Cochran and L. M. Auen (1996). Effects of elevated carbon dioxide on forage quality for ruminants. In: C. Koerner and F. Bazzaz eds. *Carbon dioxide, populations, and communities*. pp. 363-371. Physiologic Ecology Series. Academic Press., San Diego, California.
- Panozzo, J. F., H. A. Eagles and M. Wootton (2001). Changes in protein composition during grain development in wheat. *Australian Journal of Agricultural Research*, 52, 485-493.
- Panozzo, J. F., C. K. Walker, D. L. Partington, N. C. Neumann, M. Tausz, S. Seneweera and G. J. Fitzgerald (2014). Elevated carbon dioxide changes grain protein concentration and

composition and compromises baking quality. A FACE study. *Journal of Cereal Science*, 60, 461-470. <http://dx.doi.org/10.1016/j.jcs.2014.08.011>

Payne, R., S. Welham and S. Harding (2013). A Guide to REML in GenStat®, 16th ed. VSN International, Hemel Hempstead, UK.

Poorter, H. (1993). Interspecific Variation in the Growth Response of Plants to an Elevated Ambient CO₂ Concentration. *Vegetatio*, 104/105, 76-97. <http://www.jstor.org/stable/20029739>

Poorter, H., Y. Van Berkel, R. Baxter, J. Den Hertog, P. Dijkstra, R. M. Gifford, K. L. Griffin, C. Roumet, J. Roy and S. C. Wong (1997). The effect of elevated CO₂ on the chemical composition and construction costs of leaves of 27 C₃ species. *Plant, Cell & Environment*, 20, 472-482. <http://dx.doi.org/10.1046/j.1365-3040.1997.d01-84.x>

Porteous, F., J. Hill, A. S. Ball, P. J. Pinter, B. A. Kimball, G. W. Wall, F. J. Adamsen, D. J. Hunsaker, R. L. LaMorte, S. W. Leavitt, T. L. Thompson, A. D. Matthias, T. J. Brooks and C. F. Morris (2009). Effect of Free Air Carbon dioxide Enrichment (FACE) on the chemical composition and nutritive value of wheat grain and straw. *Animal Feed Science and Technology*, 149, 322-332. <http://dx.doi.org/10.1016/j.anifeedsci.2008.07.003>

Ruuska, S. A., G. J. Rebetzke, A. F. van Herwaarden, R. A. Richards, N. A. Fettell, L. Tabe and C. L. D. Jenkins (2006). Genotypic variation in water-soluble carbohydrate accumulation in wheat. *Functional Plant Biology*, 33, 799-809. <http://dx.doi.org/10.1071/FP06062>

Seneweera, S. and R. M. Norton (2011). Plant Responses to Increased Carbon Dioxide *Crop Adaptation to Climate Change*. pp. 198-217. Wiley-Blackwell.

Stitt, M. and A. Krapp (1999). The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. *Plant, Cell & Environment*, 22, 583-621. <http://dx.doi.org/10.1046/j.1365-3040.1999.00386.x>

Taub, D. R., B. Miller and H. Allen (2008). Effects of elevated CO₂ on the protein concentration of food crops: a meta-analysis. *Global Change Biology*, 14, 565-575. <http://dx.doi.org/10.1111/j.1365-2486.2007.01511.x>

Tava, A., N. Berardo, C. Cunico, M. Romani and M. Odoardi (1995). Cultivar Differences and Seasonal Changes of Primary Metabolites and Flavor Constituents in Tall Fescue in Relation to Palatability. *Journal of Agricultural and Food Chemistry*, 43, 98-101. <http://dx.doi.org/10.1021/jf00049a018>

Taweel, H. Z., B. M. Tas, H. J. Smit, A. Elgersma, J. Dijkstra and S. Tamminga (2005). Effects of feeding perennial ryegrass with an elevated concentration of water-soluble carbohydrates on intake, rumen function and performance of dairy cows. *Animal Feed Science and Technology*, 121, 243-256. <http://dx.doi.org/10.1016/j.anifeedsci.2005.02.024>

572 Walker, C., R. Armstrong, J. Panozzo, D. Partington and G. Fitzgerald (2017). Can nitrogen fertiliser
 573 maintain wheat (*Triticum aestivum*) grain protein concentration in an elevated CO₂
 574 environment? *Soil Research*, 55, 518-523. <https://doi.org/10.1071/SR17049>

575 Van Soest, P. J., J. B. Robertson and B. A. Lewis (1991). Methods for Dietary Fiber, Neutral Detergent
 576 Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *Journal of Dairy*
 577 *Science*, 74, 3583-3597. [http://dx.doi.org/10.3168/jds.S0022-0302\(91\)78551-2](http://dx.doi.org/10.3168/jds.S0022-0302(91)78551-2)

578 Zadoks, J. C., T. T. Chang and C. F. Konzak (1974). A decimal code for the growth stages of cereals.
 579 *Weed Research*, 14, 415-421. [10.1111/j.1365-3180.1974.tb01084.x](https://doi.org/10.1111/j.1365-3180.1974.tb01084.x)

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Panozzo, JF;Walker, CK;Maharjan, P;Partington, DL;Korte, CJ

Title:

Elevated CO₂ affects plant nitrogen and water-soluble carbohydrates but not in vitro metabolisable energy

Date:

2019-07-22

Citation:

Panozzo, J. F., Walker, C. K., Maharjan, P., Partington, D. L. & Korte, C. J. (2019). Elevated CO₂ affects plant nitrogen and water-soluble carbohydrates but not in vitro metabolisable energy. JOURNAL OF AGRONOMY AND CROP SCIENCE, 205 (6), pp.647-658. <https://doi.org/10.1111/jac.12359>.

Persistent Link:

<http://hdl.handle.net/11343/286195>