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Feed efficiency in beef cattle: relationship with digestibility, antioxidant activity, oxidative stress, growth performance, and carcass characteristics

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Feed efficiency in beef cattle: Relationship with digestibility, antioxidant activity, oxidative stress, growth performance, and carcass characteristics

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DEDICATION

To my grandparents, Howard & Elinor Russell, Wilbur & Janette Pohlman,

and to my parents, Ron & Alicia Russell…

…continual and unwaivering sources

of encouragement, advice, and inspiration.

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NOMENCLATURE

ADF	Acid detergent fiber
ADG	Average daily gain
ASC	Ascorbate
ATP	Adenosine triphosphate
BF	12^{th} rib backfat
BRD	Bovine respiratory disease
BW	Bodyweight
CP	Crude protein
Cu	Copper
CuZnSOD	Copper-zinc superoxide dismutase
d	Day
DM	Dry matter
DMI	Dry matter intake
DNA	Deoxyribonucleic acid
DNPH	2, 4-dinitrophenylhydrazine
DP	Dressing percent
ETC	Electron transport chain
F:G	Feed:gain
FBW	Final bodyweight
F-Byp	Finishing phase byproduct diet
F-Corn	Finishing phase corn diet

FDA	Food and Drug Administration
FDMdig	Finishing phase dry matter digestibility
FE	Feed efficiency
Fe	Iron
G:F	Gain:feed
G-Corn	Growing phase corn diet
GDMdig	Growing phase dry matter digestibility
GPX	Glutathione peroxidase
G-Rough	Growing phase roughage diet
GSH	Glutathione (reduced)
GSSG	Oxidized glutathione
H	Hydrogen
HCW	Hot carcass weight
HFE	High feed efficiency
IBW	Initial bodyweight
ID	Identification
ISU	Iowa State University
KPH	Kidney, pelvic, heart fat
LFE	Low feed efficiency
MDA	Malondialdehyde
ME	Metabolizable energy
MFE	Middle feed efficiency
MMBW	Metabolic mid-weight

Mn	Manganese
MnSOD	Manganese superoxide dismutase
Mo	Molybdenum
MS	Marbling score
MU	University of Missouri
Na	Sodium
NADPH	Nicotinamide adenine dinucleotide phosphate
NDF	Neutral detergent fiber
NEg	Net energy for gain
NRC	National Research Council
O	Oxygen
OM	Organic matter
PC	Protein carbonyl
PEG	Partial efficiency of growth
PHS	Pulmonary hypertension syndrome
PUFA	Polyunsaturated fatty acids
REA	Ribeye area
RFC	residual feed consumption
RFI	Residual feed intake
RG	Residual gain
RIG	Residual intake and gain
ROS	Reactive oxygen species
S	Sulfur

SD	Standard deviation
Se	Selenium
SE	Standard error
SOD	Superoxide dismutase
tGSH	Total glutathione
TiO_2	Titanium dioxide
TMR	Total mixed ration
USDA	United States Department of Agriculture
YG	Yield grade
Zn	Zinc

ABSTRACT

As production costs increase across the livestock industry, improving feed efficiency (FE) is one of the most crucial tasks for the beef industry to improve economic competitiveness relative to other meat-producing species. Substantial variation in FE may exist between individuals, yet the physiological mechanisms behind this variability are not well characterized. Furthermore, the industry would benefit from a more thorough understanding of the relationships between FE and other traits as well as an evaluation of the repeatability of FE across differing production phases and diet types. Consequently, this research sought to: 1) determine the influence of growing phase FE classification and diet type on performance of steers fed differing finishing phase diets, 2) determine effects of growing phase FE and diet, as well as finishing phase diet on diet digestibility and finishing phase FE, and 3) evaluate the relationship between FE, antioxidant activity and oxidative stress in feedlot steers representing phenotypic extremes for FE. It was hypothesized that relative FE was repeatable across feeding phases and that diet digestibility, antioxidant activity, and oxidative stress may be contributing factors to variation in FE between individuals. Completing the first objective, it was determined that steers classified as highly feed efficient (HFE) in the growing phase maintained greater finishing phase G:F and the relationship was also consistent for mid (MFE) and lowly feed efficient (LFE) growing phase FE classifications. Finishing phase G:F was not directly affected by growing or finishing phase diets but differences in finishing phase performance suggested that differences in finishing phase G:F between FE classifications were driven by differences in ADG among roughage-grown steers, versus differences in DMI that drove G:F variation among corn-

grown steers. Additionally, the roughage growing diet and byproduct finishing diet combination appeared to be most advantageous, as those steers excelled in ADG, generating heavier carcasses with no decrease in G:F or marbling score. After completion of the second objective, it was determined that there were no differences in DM digestibility due to FE classification but fiber digestibility appeared to contribute to FE variation while starch digestibility did not. There was a positive correlation between growing and finishing phase diet DM digestibilities in steers fed similar diet types during both feeding phases, suggesting digestibility measured during one feeding phase may be indicative of digestive capacity during a subsequent phase if the diets are nutritionally similar. At the individual steer level, finishing phase G:F was greater in HFE versus LFE steers, though a negative correlation for G:F was detected between feeding phases when steers were roughage-grown and corn-finished. Finally, completion of the third objective revealed that antioxidant activity may play a role in FE as LFE steers, specifically roughage-grown LFE steers, had greater antioxidant activity than HFE steers, conceivably using a greater proportion of energy otherwise available for tissue accretion. Oxidative stress differences were predominately identified among the roughage-grown steers and in that group, HFE steers appeared to have a greater tolerance for oxidative stress than LFE steers as HFE steers had greater oxidative stress markers. Across the studies, G:F was repeatable from the growing phase to the finishing phase, thus growing phase FE appears to be a reasonable predictor of finishing phase FE. Variation in growth traits, diet digestibility, antioxidant activity, and oxidative stress markers were consistently detected between FE classifications, particularly between FE classifications within the roughage-grown groups.

CHAPTER 1.

GENERAL INTRODUCTION

Maintaining profitability in the beef industry is increasingly challenging as production costs increase, and feed cost is the greatest contributor to production inputs (USDA ERS, 2015). Between 2009 and 2013, annual feed costs increased by over 33% (USDA ERS, 2013) due largely to secondary uses of common feedstuffs such as corn and soybeans by alternative industries and for biofuel production (USDA ERS, 2007). In the United States, corn is a primary energy source in feedlot diets and thus, corn price is a major factor contributing to feedlot profit variability (Langemeier et al., 1992). Even when reliance on grain as a primary energy source is low, as is the case in beef cow-calf operations, total feed costs still account for 70% of the annual cost to maintain a beef cow (USDA, 2010). Thus, feed costs are a critical consideration when determining profitability (Miller et al., 2001). Since cost of gain has the largest impact on the profitability of a feedlot animal after initial purchase price, feed cost is therefore the greatest daily cost. As a result, profitability between animals within a feedlot is a function of feed cost as well as individual performance, a concept referred to as feed efficiency (FE).

It has long been known that FE can vary greatly between individuals (Koch et al., 1963). However, the relationship between FE and other production traits is not well understood and the physiological mechanisms that contribute to beef cattle FE are not yet thoroughly defined (Herd et al., 2004). Although FE has a great deal of economic importance, overall performance and carcass traits are also key profitability drivers. Evaluating the relationship between FE and other production traits could help the industry

ascertain the ideal economic balance depending on the target market. Individual FE measurement can be an expensive and labor-intensive process (Arthur and Herd, 2008). Thus, understanding the relationship between FE measured at different growth stages could provide producers with greater information for selection or management even when FE is tested only once (Durunna et al., 2011). Though a great deal of work has been done to identify the contributors to FE variation, the largest sources of variation may be the least understood, such as variation in metabolism, stress, and other physiological mechanisms (Richardson and Herd, 2004). Considering the potential impact of FE improvement on the sustainability of the industry and the profitability of producers, it is crucial to more thoroughly characterize FE in order to identify opportunities for improvement. The hypothesis is that FE is repeatable from the growing to the finishing phase and that diet digestibility, antioixidant activity, and oxidative stress differ between steers classified as highly or lowly feed efficient.

Dissertation Organization

Chapter 2 is a review of the literature discussing FE and physiological contributions to FE variation. Particular attention is given to oxidative stress, antioxidant function, and the role of micronutrients in the antioxidant system. The next three chapters present research that has been completed on these subjects, including manuscripts submitted or written for submission to the Journal of Animal Science. Chapter 3 investigates the relationship between FE measured during both the feedlot growing and finishing phases, with differing diets fed during the feeding phases, and carcass traits evaluated for the differing FE phenotypes. Chapter 4 focuses on FE repeatability between feeding phases at the individual steer level

and explores the relationship between FE and diet digestibility, especially as diets change between feeding phases. Chapter 5 assesses the metabolic variation between FE phenotypes, specifically the antioxidant activity and oxidative stress measured in highly versus lowly efficient steers. Finally, chapter 6 summarizes the research covered in this dissertation and provides suggestions for the direction of future research.

CHAPTER 2.

REVIEW OF THE LITERATURE

Feed Efficiency in Beef Cattle

Measures of feed efficiency

Feed efficiency is defined as bodyweight gain resulting from a given feed amount (Koch et al., 1963). While producers typically refer to feed conversion, the ratio of feed consumed to weight gained (F:G), conventional research utilizes the FE value, gain:feed (G:F). Thus the preferred F:G is minimal whereas the preferred G:F is maximal. An important factor in the calculation of FE is the use of DMI rather than as-fed feed consumption. With feedstuffs varying widely in water content (NRC, 2000), calculating FE on a dry feed basis is necessary to determine a more accurate and objective measure. Weight gain is the other component in FE determination. Koch et al. (1963) attributed 38% of weight gain variation to differences in inherent FE and 25% of weight gain variation to feed consumption differences. Evaluating up to 13,319 records for a variety of growth, efficiency, and carcass traits measured in bulls between 1991 and 2000, Schenkel et al. (2004) reported G:F was strongly correlated with average daily gain (ADG; r = 0.58) and feed intake (r = -0.47). In terms of herd improvement, G:F is advantageous as it is a moderately heritable trait (0.37 heritability; Schenkel et al. 2004). However, selecting for increased G:F, also results in selection for increased growth rate, and unfortunately, increased mature cow size as well (Herd and Bishop, 2000; Crowley et al., 2011). Although selection for G:F that indirectly results in increased ADG and larger cattle capable of finishing at heavier weights would be

advantageous for the feedlot setting, the indirect selection for larger mature cow size would be disadvantageous for the cow/calf sector.

Besides the rudimentary feed:gain and gain:feed calculations for FE, generally referred to as gross efficiency (Archer et al., 1999), additional systems have been developed to more objectively evaluate performance while accounting for differences between groups of cattle. Partial efficiency of growth (PEG) is calculated as the ratio of weight gain to feed after expected maintenance requirements have been subtracted (Archer et al., 1999). Maintenance requirements can be determined for the individual animal with a metabolic study of energy balance (Archer et al., 1999). To the detriment of this approach, estimating maintenance requirements via metabolic studies is infeasible for large groups of animals. Alternatively, maintenance requirements can be utilized from a population estimate of maintenance requirements in beef cattle (Carstens and Tedeschi, 2006). Compared with G:F, the benefit of PEG is a decreased correlation with ADG ($r = 0.24$; Nkrumah et al., 2004), thus decreasing the potential indirect selection for increased mature cow size. Additionally, although G:F can be increased with no effect on feed intake (Bishop et al., 1991), increased PEG is correlated with decreased feed intake ($r = -0.52$; Nkrumah et al., 2004). The downside of PEG is the failure to account for variation in individual animal energetic efficiencies associated with maintenance; a major downside since maintenance costs are a substantial contributor to differences in efficiency between individuals (Archer et al., 1999). Additionally, although energetic efficiency is consistently greatest for maintenance (Veerkamp and Emmans, 1995a), using a standard maintenance value for every animal in a population fails to account for differences in diet digestibility between individuals.

A more recently developed method, residual gain (RG) reflects the difference in actual and predicted body weight gain of an animal among a group of contemporaries. Residual gain is based on the regression of body weight gain on metabolic mid-weight ([average bodyweight during test]^0.75; MMBW) and feed intake during the test period (Willems et al., 2013). Like G:F, greater positive values are desirable for RG (Crowley et al., 2010). Proponents for RG contend that the measure is associated with greater average daily gain (Berry and Crowley, 2012). Consequently, mature cow size also increases as selection for increased RG progresses (r = 0.67; Crowley et al., 2011), a correlation determined after evaluating several thousand cow performance records. Similar to RG, residual feed intake (RFI) is the difference in actual and predicted feed intake of an animal among a group of contemporaries based on the regression of feed intake on MMBW and ADG during the test period (Arthur et al., 2001a; Robinson and Oddy, 2004). Residual feed intake is also called net feed intake (Arthur et al., 2001b). Unlike RG, a negative RFI value is desirable and variation between animals reflects the difference in energy efficiency for both maintenance and production (Veerkamp et al., 1995b).

Archer et al. (1997) monitored daily intakes for 119 d and measured bodyweights every 14 d for crossbred heifers and bulls. Individual feed intakes could be attained after 35 d however 70 d was required for reliable residual feed intake and daily gain calculations. Similarly, Wang et al. (2006) measured ADG, DMI, G:F and RFI in 456 steers over 91 d and after evaluating a variety of test period durations (7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, or 91 d), determined 63 d as the minimum duration for accurate RFI calculation but only 35 d as the minimum for DMI determination alone. A major shortcoming of RFI, animals gaining poorly but also eating small amounts of feed may result in a desirable RFI value despite

having undesirable ADG (Berry and Crowley, 2012). Residual feed intake benefits include an independence from the component traits used to calculate expected feed intake (Carstens and Tedeschi, 2006; Archer et al., 1999); however, Kennedy et al. (1993) make the point that it is not genetically independent. Thus the interaction of genetics and environment are an important consideration as animals deemed highly efficient in a given environment may not have the same magnitude of variation under different environmental conditions.

Aiming to account for the shortcomings of both RG and RFI, Berry and Crowley (2012) proposed a calculation combining both residual traits as a sum. Residual intake and gain (RIG) is the sum of RG, and RFI multiplied by -1, since desirable RFI is a negative value. The authors utilized existing data from previous investigations utilizing 3,531 purebred bulls tested between 1983 and 2007. Data from bulls not tested for at least 96 days were discarded and the remaining data from 3,153 head were analyzed after accounting for a variety of effects. Residual intake and gain had a correlation of 0.41 with ADG, -0.34 with daily concentrate intake, -0.85 with RFI, and 0.85 with RG. While strong correlations were determined, Berry and Crowley (2012) concluded RIG should be contrasted with other economically valuable traits to ensure no antagonisms exist. Testing the concept of RIG and other FE measures with performance test records from 678 Nellore bull and heifer calves, RIG appeared to be the most advantageous based on selection for increased growth and decreased feed intake Grion et al. (2014). Residual intake and gain could be beneficial to both the feedlot sector as well as the cow/calf sector.

Correlations between feed efficiency measures

With a seemingly diverse set of FE calculations that originate from a common set of measures, there is some correlation between values. Hoque et al. (2009) evaluated growth data on 514 Japanese black bull calves fed roughage and a concentrate supplement for 112 d. They reported a strong negative correlations between RFI and PEG (r = -0.76; Hoque et al., 2009) and a weaker positive correlation between G:F and PEG (r = 0.32). Interestingly, the correlation between G:F and RFI (r = -0.60) reported by Hoque et al. (2009) is markedly greater than correlations reported for RFI and G:F by others (refs). Arthur et al. (2001a) reported a slightly lesser correlation between G:F and RFI (r = -0.53, *P*-value not specified) in 1,180 Angus bulls and heifers tested for 70 d on a 70:30 alfalfa hay:wheat diet. Berry and Crowley (2012) evaluated data from 3,153 bulls fed between 1983 and 2007, reporting a negative correlation between RFI and G:F (r = -0.41, $P < 0.02$) that was even weaker and may have been due to a myriad of factors affecting growth data combined over 24 years. However, following an 82 d growing study (Kelly et al., 2010a), Kelly et al. (2010b) finished 50 crossbred heifers with a 70:30 concentrate:corn silage diet for 84 d and reported a -0.36 correlation coefficient between G:F and RFI. As previously discussed, Berry and Crowley (2012) also examined RG and RIG, reporting strong positive correlations ($P < 0.02$) between G:F and RG (r = 0.71), RG and RIG (r = 0.85), as well as G:F and RIG (r = 0.66). Residual feed intake had a strong negative correlation with RIG (r = -0.85, $P < 0.02$). Ultimately, it appears that additional work may be needed to more precisely determine the relationship between FE measures, especially RFI and G:F phenotypes.

Repeatability in feed efficiency measures

Bodyweight measurements are attainable simply by working cattle across a scale, thus the greatest challenge facing beef producers seeking to measure FE is the ability to measure individual intakes. Though total intake measurements are less feasible in a pasture setting, measuring intakes in the feedlot still requires substantial infrastructure; in either setting, making intake measurements difficult and expensive (Arthur and Herd, 2008). Thus, measuring FE for a limited period would be beneficial if FE is repeatable over multiple feeding phases or can be predicted using one FE evaluation period. Over three years, Durunna et al. (2011) collected growth and intake data on 490 crossbred steers during two consecutive feeding phases growing and finishing phases. Within each year, a feed-swap group (*sic*) was switched from a growing (74% oats, 20% grass hay) to finishing phase diet (56.7% barley, 28.3% oats), a group was fed the growing phase diet during both periods, and a group was fed the finishing phase diet during both periods (Durunna et al., 2011). Steers were classified as low, medium, or highly feed efficient using a 0.5 SD cutoff around the mean for RFI and G:F based on first period performance (Durunna et al., 2011). In the feed swap group, 54.7% changed RFI classification from the first to second feeding period and 61.6% switched G:F classification; however, similar classification changes were also observed in the all growing phase diet-fed group (RFI: 50.7% change, G:F: 53.5% change) and the all finishing phase diet-fed group (RFI: 51.5% change; G:F: 59.1% change; Durunna et al., 2011). Despite a seemingly large movement across classifications, Durunna et al. (2011) reported a far smaller proportion of the total feeding groups that actually moved from the low to high, or high to low FE classification (feed swap: 8.8% RFI, 13.3% G:F; growing diet-fed: 7.0% RFI, 11.2% G:F; finishing diet-fed: 8.0% RFI, 11.2% G:F).

In a similar study, Durunna et al. (2012) fed 190 crossbred heifers the same 90% barley silage, 10% rolled barley diet during consecutive feeding phases and collected growth and intake data throughout. Similar to the previous study, Durunna et al. (2012) classified heifers into low (< 0.5 SD from the RFI mean), medium (± 0.5 SD from the RFI mean), or high (> 0.5 SD from the RFI mean) RFI classifications and reported 51% changed classifications from the first to second feeding period but only 6% changed from low to high RFI classification or vice versa. Kelly et al. (2010b) fed a 70:30 concentrate:corn silage diet to 50 crossbred finishing heifers previously fed the same diet and ranked by FE as yearlings; finishing phase growth and intake was recorded for 84 d. Kelly et al. (2010b) found both RFI ($r = 0.62$) and G:F ($r = 0.37$) to be positively correlated across feeding phases. In all three studies (Kelly et al., 2010b; Durunna et al., 2011; Durunna et al., 2012), RFI rank or classification was more consistent or repeatable between feeding phases than G:F. However, further examination of FE repeatability is needed, especially when diet type changes from one period to the next, as beef cattle routinely receive differing diet types as growth phases change; often from fiber-based to concentrate based diets.

There are a variety of well-accepted FE calculations that can benefit cattle producers, depending on objective. In a terminal setting, use of G:F or RG appears to be most beneficial for improving efficiency of bodyweight gain, though feed intake may be unchanged. However, if used for breeding stock selection, selection for improved G:F or RG may result in increased mature cow size. Developing replacement breeding stock, RFI may be more beneficial for reducing feed intake but if used as the sole selection criteria, growth performance may decrease or remain unchanged. Regardless of the FE measure being utilized, determining the factors contributing to feed efficiency variation between animals is

necessary to improve production and decrease inputs by assessing opportunities for improved management and selection.

Factors Contributing to Feed Efficiency Variation

It is well established that improving feed efficiency is important to the beef industry for both economic and environmental sustainability (Nkrumah et al., 2006). It is also well understood that individuals in a similar environment and even with similar genetics can vary greatly in feed efficiency (Koch et al., 1963). Understanding what contributes to the variation between individuals may afford the opportunity to then evaluate possible means of improving efficiency. Feed efficiency variation contributions can be broadly grouped into five categories: feed intake, diet digestibility and digestion energetics, physical activity, thermoregulation, and energetics of body composition and metabolism (Herd et al., 2004; Herd and Arthur, 2009).

Feed intake

Based on the previous discussion of RFI and other feed efficiency calculations, feed intake understandably contributes to feed efficiency variation (Koch et al., 1963). Besides the direct influence of feed intake on feed efficiency measures, the associated energetics of increased feed intake contribute to energetic efficiency. Greater feed intake requires accommodation via greater gastrointestinal organ size which also results in greater energy expenditure by the organs, an energetic cost called heat increment of feeding (Herd and Arthur, 2009). Evaluating energy expenditure in digestive tract tissues, Webster et al. (1975)

implanted catheters and thermocouples in 13 adult wethers housed in metabolism crates and fed all treatment diets in a Latin square experimental design. When wethers were allowed to consume forage-based or barley-based diets above maintenance requirements, gut tissue expenditure accounted for an average of ~37% of the total heat loss (kJ/MJ gross energy consumed), far greater than the heat loss due to eating (~2%) or fermentation (~11%); the remaining heat loss was attributed to peripheral tissues (Webster et al., 1975). Conversely, Nkrumah et al. (2006) selected 27 crossbred steers from an RFI-tested group of 306 steers (days not specified) and reported no difference in heat increment (kcal/kcal ME) between 11 low feed efficient steers (> 0.5 SD above the RFI mean; 1.25 ± 0.13 kg/d RFI, SE), 8 mid feed efficient steers (± 0.5 SD of the RFI mean; -0.08 ± 0.17 kg/d RFI, SE), and 8 high feed efficient steers (< -0.5 SD below the RFI mean; -1.18 ± 0.16 kg/d RFI, SE). Webster et al. (1975) suggested that energetic use by gastrointestinal tissue was decreased in highly efficient animals while Nkrumah et al. (2006) found no difference. In other vital organs, DiCostanzo et al. (1990) noted a tendency for a correlation between maintenance energy requirements and liver weight ($r = 0.4$, $P \leq 0.16$) but no correlation ($P \geq 0.20$) between maintenance energy and spleen, kidney, lungs, or heart weight in 14 mature Angus cows. More recently, Montanholi et al. (2013) suggested that energetic use in the small intestinal tissue of highly efficient cattle was greater than in lowly efficient cattle. Montanholi et al. (2013) selected the 12 most efficient (-0.53 kg/d RFI) and 12 least efficient (0.64 kg/d RFI) steers from a group of 45 crossbred steers that were finished with a high moisture corn-based diet for 140 d. At harvest, duodenum and ileum tissue were collected on the feed efficiency extremes and upon evaluation, the authors (Montanholi et al., 2013) noted greater cellularity in both the duodenum and ileum of the most versus least efficient steers (33.16 vs. 30.30 and

37.21 vs. 33.65, nuclei number) despite no difference in cell size. Montanholi et al. (2013) concluded that although the increased cellularity would likely increase energetic requirements, but that increases in intestinal metabolic activity likely provide greater energetic benefit than cost, thus improving efficiency. Based on the previous discussion, more work is needed to identify the energetic loss and gain among intestinal tissue as it relates to feed efficiency.

Diet digestibility and digestion energetics

Closely related to feed intake and feeding behavior, diet digestibility and digestion energetics are estimated to account for approximately 10-14% of feed efficiency variation (Richardson and Herd, 2004; Herd et al., 2004). Richardson et al. (1996) fed a 70% hay, 30% wheat diet to two groups of Angus calves for 120 d (group 1: 193 heifers; group 2: 194 heifers and 188 bulls), ranking each group by feed efficiency adjusted for sex and collecting fecal samples on the 10 most and 10 least feed efficient calves from each group (range = 0.147 - 0.075 G:F, specific values not reported; Arthur et al., 1996)). Dry matter digestibility tended to be 1% greater in the most versus least feed efficient calves, and the authors estimated this could account for approximately 14% of the intake difference between the feed efficiency extremes (Richardson et al., 1996). Similarly, after a 120 d RFI test using 79 Angus-sired steer calves divergently bred for RFI and fed a 70% oat diet, Richardson and Herd (2004) evaluated the 16 most feed efficient (RFI = -0.15 ± 0.08 kg/d, SE) and 17 least feed efficient (RFI = 0.16 ± 0.10 kg/d, SE) steers for diet DM digestibility via total fecal collection. The result was a -0.44 correlation between DM digestibility and RFI (Richardson and Herd, 2004); DM digestibility was greater in steers with greater feed efficiency.

Like the Richardson and Herd (2004) investigation, Nkrumah et al. (2006) evaluated 306 crossbred steers for RFI (days not specified) and selected 27 steers at the end of the test period for further evaluation as previously discussed: 11 low feed efficient steers, 8 mid feed efficient steers, and 8 high feed efficient steers. Receiving a concentrate-based diet, the highly efficient steers had greater apparent DM and CP digestibility (75.33 ± 2.10 %; 74.70 ± 2.29%, SE) than the lowly efficient steers (70.87 ± 1.97%; 69.76 ± 2.17%, SE) but neither groups differed from the mid feed efficient group; there were no differences in NDF or ADF digestibility between the feed efficiency groups (Nkrumah et al., 2006), likely because steers were receiving a concentrate-based diet. Digestibility was determined using total fecal collection (Nkrumah et al., 2006).

Unlike the previous investigations, Cruz et al. (2010) noted no differences in apparent DM digestibility (Range: 70-75% DM digestibility, 6.36 SD) between high efficiency (<0.5 SD below the RFI mean; n = 15 total) and low efficiency steers (>0.5 SD above the RFI mean; n = 15 total) identified among 60 Angus-Hereford crossbred steers that were RFI tested for 60 d with a 80% cracked corn diet. The study (Cruz et al., 2010) was split into two 60 d periods such that 30 steers were RFI-tested at a time and the remaining 30 were group housed, resulting in a 120 d total feeding period. Previous research recommends RFI test periods last a minimum of 70 d (Archer et al., 1997), thus the 60 d test periods (Cruz et al., 2010) were less than ideal. Additionally, the previous studies (Richardson and Herd, 2004; Nkrumah et al., 2006) determined digestibility using total fecal collection whereas Cruz et al. (2010) collected fecal grab samples and measured lignin to estimate total fecal output; however, lignin may not be reliable unless complete fecal recovery is conducted (Fahey and Jung, 1983). Thus, DM digestibility generally appears to be greater in cattle displaying

greater feed efficiency; however, the relationship between diet digestibility and FE may not be as consistent in other livestock species.

De Haer and De Vries (1993) calculated RFI in two batches of 90 boars and gilts (180 pigs total), housing sexes separately and using group pens for 80 pigs (8 pigs/pen) with individual housing for the remaining 10 pigs per batch. Intake was automatically monitored using feed stations that detected EID tags on all pigs, the test started when pigs weighed 25-35 kg, and concluded when average live weight reached 100 kg (De Haer and De Vries, 1993). Measuring diet digestibility with use of chromic oxide to estimate total fecal output, De Haer and De Vries (1993) reported that models utilizing RFI did not explain variation in diet digestibility in group housed pigs ($R^2 = 0.19$) or individual housing ($R^2 = 0.45$). The greater relationship between digestibility and RFI in the individually housed pigs was likely due to smaller, more frequent meals, consequently improving digestibility compared with group-housed pigs that ate larger meals prone to more rapid passage rates and decreased digestibility (De Haer and De Vries, 1993).Harris et al. (2012) evaluated diet digestibility in 12 high efficiency crossbred gilts (0. 46 G:F, SE not reported) and 12 low efficiency gilts (0.34 G:F) that resulted from seven generations of divergent RFI selection. Compared to the low efficiency gilts, the high efficiency gilts had greater DM digestibility (87.3 vs. 85.9%, 0.25 SE), greater N digestibility (88.3 vs. 86.1%, 0.47 SE), and greater GE digestibility (86.9 vs. 85.4%, 0.25 SE; Harris et al., 2012). Though De Haer and De Vries (1993) utilized a greater number of animals, Harris et al. (2012) utilized pigs divergently bred for RFI for seven generations, an experimental model that may be more prone to reveal differences due to feed efficiency extremes.

A key physiological difference between monogastrics and ruminants, methane production in the rumen has been identified as a contributor to feed efficiency variation. In the previously discussed steers (n = 27) identified as low (n = 11), mid (n = 8), or high feed efficient (n = 8), Nkrumah et al. (2006) reported lesser methane production (L/kg $BW^{0.75}$) in the high feed efficient steers (1.28 ± 0.14, SE) versus the mid and low feed efficient steers (1.68 ± 0.14 and 1.71 ± 0.11, SE). In a similar study, Hegarty et al. (2007) measured methane production rate (g/d) using 91 Angus steers selected from a divergently RFI bred herd to cover the range of parent RFI estimated breeding values. Steers were fitted with a halter and gas collection apparatus and methane was measured for 10-d periods following 5-d acclimation periods (15 d total) during a 70 d feed efficiency test (Hegarty et al., 2007). Comparing the 10 most efficient and least efficient steers (0.142 vs. 0.088 G:F, 0.006 SE), the most efficient steers had a decreased methane production rate (142.3 ± 16.5 g methane/d, SE) relative to the least efficient steers (190.2 ± 16.5 g methane/d, SE; Hegarty et al., 2007). Since RFI and methane production are feed intake dependent, there were no differences in methane production on a g/kg DMI basis (Hegarty et al., 2007); however, the least efficient steers still tended to produce more methane than the most efficient steers on an ADG basis (173.0 vs. 131.8 g methane/kg ADG, 22.8 SE; Hegarty et al., 2007).

Using respiration calorimeters to measure methane production over 6 hour periods, Freetly and Brown-Brandl (2013) evaluated the most and least feed efficient individuals from 113 crossbred steers fed 83% corn diets and 197 crossbred heifers fed 60% corn silage, 30% alfalfa hay diets. By diet, 37 steers outside the 55% confidence ellipse from BW gain regressed on DMI were selected for evaluation (0.152 ± 0.004 G:F average, SE) and 46 heifers outside the 76% confidence ellipse were selected (0.148 ± 0.005 G:F average, SE;

Freetly and Brown-Brandl, 2013). In the grain-fed steers, G:F and RFI were not related to methane production ($P \geq 0.6$) but in the forage-fed heifers, G:F was positively related to methane production (regression coefficient = 0.30, $P = 0.02$) but RFI was not related ($P =$ 0.5; Freetly and Brown-Brandl, 2013). Thus, Freetly and Brown-Brandl (2013) detected increased methane as G:F increased in forage-fed cattle and the increased methane was likely a result of increased DMI. Additionally, the authors reported limited methane production in cattle fed grain-based diets, likely because decreased methane is characteristic of grain-based diets (Freetly and Brown-Brandl, 2013). The studies disagree on the relationship between FE and methane production though the disagreement may be, in part, due to the use of cattle selectively bred for FE (Nkrumah et al., 2006; Hegarty et al., 2007) versus FE extremes selected from a relatively small population. Nevertheless, additional work would be beneficial to further examine and characterize the relationship between FE and methane, especially due to differences in diet and when growth performance is equivalent.

Physical activity and thermoregulation

Using data from a study by Arthur et al. (2001b) examining bulls and heifers from high and low efficiency divergently selected lines of crossbred cattle outfitted with pedometers, Herd et al. (2004) calculated energy used for various activities by high efficiency (-5.4 MJ ME intake/day RFI) and low efficiency (7.0 MJ ME intake/day RFI) calves. Herd et al. (2004) concluded that greater physical activity in the low efficiency calves amounted to 0.2 more MJ/day for walking, or 1.6% of the additional ME (12.4 MJ/day) consumed by the low versus high efficiency calves. If accounting for increased energetic costs of eating and ruminating in the low efficiency calves as well, total activity accounted

for 5.1% of the greater ME consumption as compared to high efficiency calves (Herd et al., 2004). Using a similar study design and environment as Arthur et al (2001b), Richardson et al. (2000) reported that low efficiency bulls took 6% more steps than high efficiency bulls (measured via pedometer).

Though thermoregulation has been suggested as one of the major contributors to RFI variation (Herd and Arthur, 2009), limited work has been done to evaluate the difference in thermoregulation energetics between animals exhibiting RFI differences. Campos et al. (2014) evaluated performance and thermoregulatory response among 34 growing pigs (50 kg) selected from two lines of Large Whites (17 pigs/line) divergently bred for RFI for seven generations. Pigs were individually housed for 7 d at 24.2°C followed by 14 d at 30.4°C (Campos et al., 2014). The more efficient pigs (0.45 G:F) had greater feed intake than the less efficient pigs (0.40 G:F; 2.138 vs. 2.423 kg feed/d, SE not reported) but did not differ in ADG (0.970 vs. 0.965 kg BW/d; Campos et al., 2014). Campos et al. (2014) concluded that the efficient pigs tended to have a slightly improved heat tolerance relative to the inefficient pigs based on changes in rectal temperature, skin temperature, respiratory rate, and heart rate across 10 measurement days during the test periods; however, energetics were not analyzed to evaluate contribution to feed efficiency differences.

Utilizing a group of 145 White Leghorn hens, Luiting et al. (1991) selected 6 high efficiency and 6 low efficiency layer hens based on residual feed consumption (RFC, feed consumption regressed on egg weight production, ADG, and MMBW over 4 weeks). Luiting et al. (1991) repeated this selection in a second group of 92 hens and in both groups, the selected hens were housed in respiratory cages to evaluate energy metabolism and were evaluated for physical characteristics. Across both groups, the high efficiency hens (-5.7 and

-10.0 g/d RFC) had better plumage quality, lesser nude body areas, and were less active than the low efficiency hens (9.4 and 8.2 g/d RFC), characteristics that were suggested to contribute to more efficient thermoregulation in the high efficiency hens (Luiting et al., 1991). Furthermore, 80% of the difference in RFC was attributed to differences in physical activity level (Luiting et al., 1991). Additional research is undoubtedly needed to more clearly define the relationship between thermoregulation, physical activity, and RFI; perhaps to determine whether ambient temperature differentially impacts animals differing in feed efficiency.

Body composition

Considering that fat deposition has a greater energetic cost/mass than lean deposition, body composition expectedly plays a role in generating feed efficiency variation between individuals (Herd et al., 2004). From an Angus calf crop divergently bred for RFI, Richardson et al. (2001) selected 17 high efficiency-bred steers and 21 low efficiency-bred steers to evaluate body composition as a result of RFI. Steers were fed for 140 d on a 70% oat diet, individual feed intake was measured and at the conclusion of the feeding period, steers were harvested and dissected to measure composition (Richardson et al., 2001). The high efficiency steers (-0.15 ± 0.08 kg/d RFI, SE) had lesser carcass fat/final live weight (9.9 vs. 11.3%, 0.39 SE) than the low efficiency steers (0.16 ± 0.10 kg/d RFI, SE) and retail beef yield from the carcass was greater in the high efficiency steers (63.0 vs. 61.6%, 0.55 SE; Richardson et al., 2001). Evaluating a larger number of steers, Basarab et al. (2003) fed a 73.3% barley diet to 176 crossbred steers for 183 days, randomly selecting steers for slaughter and dissection on d 1, 71, 99, 127, 155, and 183. The conclusion was that empty

body fat gain accounted for 3.9% of feed intake variation whereas empty body water gain accounted for 1.1% (Basarab et al., 2003). Additionally, there was a positive correlation between RFI and empty body fat gain (g fat gained/d; r = 0.26, *P* = 0.002) but no correlation between RFI and empty body protein gain (g protein gained/d; r = -0.11, *P* = 0.2), suggesting that low efficiency steers deposit body fat at a more rapid rate than high efficiency steers (Basarab et al., 2003).

Reaffirming results from Richardson et al. (2001) and Basarab et al. (2003), Nkrumah et al. (2004) measured carcass tissue composition in 150 crossbred cattle (131 steers, 19 bulls) that were performance tested using an 80% dry rolled corn ration fed for 84 d and subsequently classified as high efficiency (-0.95 ± 0.07 kg/d RFI), mid efficiency (-0.14 ± 0.07 kg/d RFI) or low efficiency (0.91 ± 0.08 kg/d RFI). After harvest, carcass measurements among the steers revealed no difference in ribeye area or marbling score, but the high efficiency steers (n = 30) had lesser backfat (0.883 ± 0.071 cm, SE) than the mid efficiency steers (n = 48; 1.055 ± 0.053 cm, SE) and the low efficiency steers (n = 30; 1.156 ± 0.067 cm, SE; Nkrumah et al., 2004). Using ultrasound data gathered every 28 d during the test, change in backfat thickness was determined to be positively correlated with RFI (r = 0.30) such that less efficient steers had greater backfat thickness gain; correlations also revealed that increasing feed efficiency was correlated with increasing lean meat yield (r = -0.22, using RFI) and improved carcass yield grade (r = 0.28, between yield grade and RFI). Feed efficiency and body composition are clearly correlated and it has been previously suggested that body composition differences contribute 5% of the variation in RFI in beef cattle (Herd and Richardson, 2004). Based on the close relationship, RFI may be best estimated by including compositional traits in the model (Basarab et al., 2003).

Ultimately, many factors contribute to feed efficiency but a great deal of work is needed to further clarify what mechanisms contribute to variation and how selection or management can improve feed efficiency though these mechanisms. Herd et al. (2004) argue that metabolic mechanisms are the source of 67% of RFI variation; mechanisms such as protein turnover, ion pumping, and proton leakage. Considering that 90% of cellular oxygen is used for energy production in the mitochondria (Mao et al., 2011), inefficiencies in the mitochondria could contribute to the RFI variation credited to metabolic mechanisms.

Oxidative Stress in Livestock

Reactive oxygen species

Often regarded as the powerhouse of the cell, mitochondria are credited with consuming almost 90% of cells' oxygen to generate ATP via oxidative phosphorylation (Mao et al., 2011). Oxygen is used as an electron acceptor during aerobic metabolism and the backbone of the process is a chain of complexes known as the electron transport chain (ETC). The ETC is located along the inner membrane of the mitochondria where it provides a pathway along which electrons flow, generating energy. The ETC is comprised of 4 multiprotein complexes and ATP synthase (Berg et al., 2007). The energy generated by electron flow through the ETC is used to transport protons across the membrane, creating membrane potential that drives ATP production. However, the process is not 100 percent efficient and as ATP is produced by the mitochondria, electron leakage occurs from the ETC. Electron leakage leads to free radical production, including reactive oxygen species (ROS) that result from oxygen reduction by the free electrons generating species such as the

superoxide (O_2^-), hydroxyl (OH^-), and hydrogen peroxide (H_2O_2; Bayr, 2005) radicals. Though not as reactive as the other species, H_2O_2 can be oxidized to the more powerful OH^- as iron (Fe^{2+}) is reduced via the Fenton reaction (Bottje et al., 2006). It has been suggested that 1% (Mao et al., 2011) or even 2-4% (Bottje et al., 2002; Bottje et al., 2006) of the oxygen consumed by the mitochondria is reduced to ROS.

The effects of increased ROS production can include decreased energetic efficiency leading to decreased feed efficiency. When ROS and other radical generation exceeds the detoxification or antioxidant capacity of a system, it is regarded as oxidative stress (Chirase et al., 2004) and can negatively affect feed efficiency. Antioxidants are substances that delay or inhibit oxidation of a substrate, despite lesser concentrations relative to the oxidizable substrate (Gutteridge, 1995). Comparing isolated liver mitochondria from the eight most and eight least feed efficient broilers (0.83 vs. 0.64 G:F, 0.01 SE) selected from a group of 100 broilers that were feed efficiency tested for 7 d, Bottje et al. (2002) reported greater mitochondrial H_2O_2 production from the lowly efficient broilers relative to the highly efficient broilers (specific means not reported). An increase in H_2O_2 production indicates an increase in electron leak. Grubbs et al. (2013) evaluated H_2O_2 production in isolated mitochondria from liver and muscle collected from gilts divergently selected for feed efficiency over eight generations and feed efficiency tested for 12 weeks. Though results varied depending on tissue, the overall conclusion was that H_2O_2 production was greater in mitochondria isolated from the eight lowly efficient gilts compared to the eight highly efficient gilts (0.41 vs. 0.47 G:F, 0.024 SE; Grubbs et al., 2013).

Dissimilarly, Kolath et al. (2006) compared isolated longissimus dorsi mitochondria from the eight least and nine most feed efficient Angus steers (0.16 vs. 0.20 G:F, 0.01 SE)

selected from a larger group of 40 steers that were paternal siblings and were feed efficiency tested for 158 d (estimated from reported BW and ADG data). The net result was increased H_2O_2 production in muscle mitochondria from the highly versus lowly efficient steers when mitochondria were fed either glutamate (4.16 vs. 2.17 nm $H_2O_2 \cdot minute^{-1} \cdot$mg mitochondrial $protein^{-1}$, 0.46 SE) or succinate (13.95 vs. 6.20 nm $H_2O_2 \cdot minute^{-1} \cdot$mg mitochondrial $protein^{-1}$, 2.25 SE; Kolath et al., 2006). Though the Kolath et al. (2006) study disagreed, overall findings in the remaining studies (Bottje et al., 2002; Grubbs et al., 2013) were in agreement that overall H_2O_2 production in muscle mitochondria was greater in lowly efficient animals, indicating a greater electron leak. The differences between the studies may be due to differences in species and it should be noted that although muscle H_2O_2 production was different, Grubbs et al. (2103) did not detect differences in liver mitochondria yield (μg mitochondrial protein/g tissue) between feed efficiency groups. The loss in energetic efficiency due to increased electron leak, and thus ROS production, is due to a combination of factors: the energetic cost associated with neutralizing ROS to prevent oxidative damage and the energetic cost associated with removal and replacement of biomolecules that have suffered oxidative damage; biomolecules such as proteins and lipids (Figure 1).

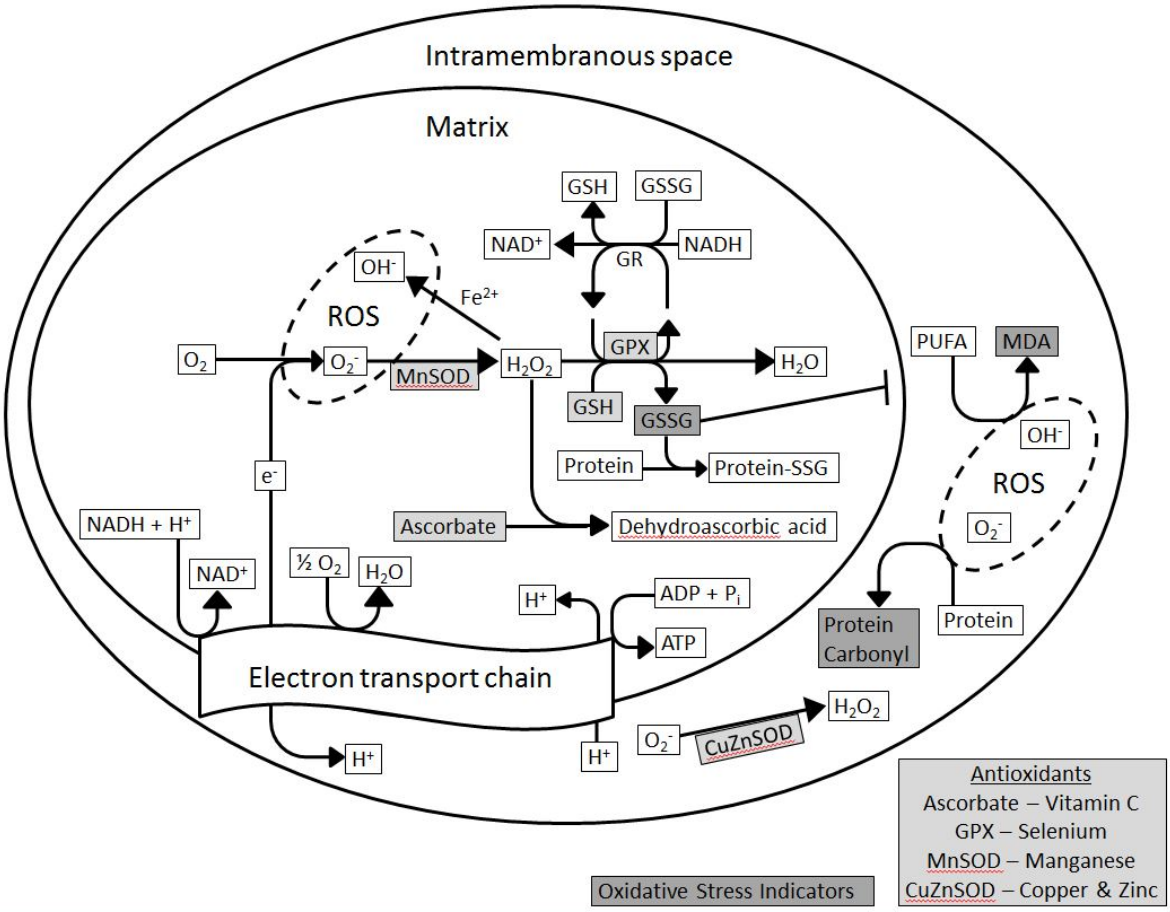

Figure 1. Free radical production and neutralization in the mitochondria. Free electrons lost by the electron transport chain reduce oxygen, generating reactive oxygen species (ROS) that can damage proteins or lipids, resulting in protein carbonyls or malondialdehyde (MDA), respectively. Reactive oxygen species can be neutralized to hydrogen peroxide (H_2O_2) by superoxide dismutases (SOD), specifically Mn-containing mitochondrial SOD (MnSOD) or Cu- and Zn-containing SOD (CuZnSOD). Hydrogen peroxide can be further neutralized by ascorbate or can be converted to water (H_2O) concurrent with the oxidation of glutathione (GSH) to glutathione disulfide (GSSG) via Se-dependent glutathione peroxide (GPX). Adapted from Nordberg and Arner (2001).

Protein oxidation

Oxidative damage can affect a variety of tissues and biomolecules, including proteins. Oxidative damage is the oxidation of molecules that may render them unusable; such is the case for DNA and proteins. Oxidative damage is associated with age-related dysfunction of the mitochondria (Shigenaga et al., 1994) as well as disease (Bottje et al., 2002). Increased protein oxidation necessitates energy and other resources for repair, decreasing energetic efficiency. When damaged, proteins are no longer usable and are marked for degradation via proteolytic processes such as the ATP-dependent ubiquitin system (Mehlhase and Grune, 2002). The degree of protein oxidation can be determined by measuring protein carbonyls. Carbonyl groups such as aldehydes and ketones are formed on the side chains of proteins, especially arginine, lysine, proline, and threonine, and are stable and easily detected (Dalle-Donne et al., 2003; Figure 1). Carbonyls are produced not only by side chain oxidation but also by oxidative cleavage of proteins, and the result is protein with blockage at the N-terminal amino acid by a ketoacyl group, thus preventing further utilization of the proteins as the reaction is irreversible. Carbonyl concentrations are commonly determined via derivatization with 2, 4-dinitrophenylhydrazine (DNPH), resulting in a hydrazone that can be measured spectrophotometrically (Castegna et al., 2003).

The energetic cost of protein oxidation has been examined in domestic livestock by comparing protein carbonyls across efficient and inefficient cohorts. After determining feed efficiency in 100 broilers from a single genetic line over a 7 d feed efficiency test, Iqbal et al. (2004) selected the eight most and eight least efficient birds (0.80 vs 0.62 G:F, 0.01 SE), isolated breast muscle mitochondria, and analyzed for protein carbonyl concentrations. The result was 81% greater protein carbonyl concentrations in the lowly efficient broilers as

compared to the highly efficient broilers (specific means not reported), nearly double the protein oxidation; a likely contributor to the decreased efficiency observed in those birds. In a similar investigation, Iqbal et al. (2005) selected the eight most and eight least feed efficient broilers (0.80 vs. 0.62 G:F, 0.01 SE) from a feed efficiency tested (7 d) group of one hundred broilers and reported a 91% increase in protein carbonyls in liver of lowly versus highly efficient birds. Likewise, Ojano-Dirain et al. (2007) selected the eight most and eight least feed efficient broilers (0.72 vs. 0.55 G:F, 0.01 SE) from a total group of 100 broilers that were feed efficiency tested for 7 d, analyzed duodenal mucosa and mitochondria for protein carbonyls, and reported greater protein carbonyls in the lowly feed efficient broilers (specific means not reported). Finally, Bottje et al. (2006) summarized multiple poultry feed efficiency investigations, finding that protein carbonyl concentrations were consistently greater in lymphocytes, breast, leg, heart, liver, and gut tissue gathered from lowly versus highly efficient birds (specific means not reported); the greatest difference was present in breast muscle tissue. However, indications of greater protein oxidation in lowly versus highly feed efficient individuals are not isolated to poultry alone. Sandelin et al. (2005) measured feed efficiency in 93 purebred Angus steers over 130 d and upon harvest, collected muscle samples from the eight most and eight least feed efficient steers (0.252 vs. 0.154 G:F, 0.02 SE); protein carbonyl content was markedly greater in the lowly efficient steers as compared to the highly efficient steers (specific means not reported). Proteins can be readily oxidized and the energetic cost associated with the cellular resolution of these oxidized proteins appears to be associated with a decrease in feed efficiency in livestock but additional work is needed to more clearly demonstrate this relationship.

Lipid oxidation

Lipids, especially polyunsaturated fatty acids (PUFA) are also prone to oxidative damage. The principle result of PUFA oxidation, malondialdehyde (MDA; Figure 1) is a toxic aldehyde that is commonly measured as a marker of lipid oxidation (Del Rio et al., 2005) and an indicator of oxidative stress. As is the case for oxidatively damaged proteins, MDA is not simply a damaged molecule that must be degraded but also acts in a toxic manner, causing further oxidative damage inside and outside of cells by oxidizing proteins and DNA (Marnett, 1999). As a result, oxidative stress can be assessed intracellularly or extracellularly via MDA determination, a process that condenses MDA with thiorbarbituric acid to produce an adduct that can be measured spectrophotometrically (Janero, 1990; Draper et al., 1993; Del Rio et al., 2005). Effects of MDA include impacts on cell permeability due to the bi-lipid nature of cell membranes (Rezaei and Dalir-Naghadeh, 2006). As previously mentioned, oxidative stress is associated with disease and this relationship is due, in part, to fragility of membranes that results from oxidation of lipids. As a result, damaged cells must be removed by macrophages; thus, lipid oxidation generates energetic costs due to cellular degradation of cells (Chacko et al., 2013). Additionally, the propensity of MDA to react with DNA or proteins results in impairments or inefficiencies in a variety of tissues (Del Rio et al., 2005). Regardless of the tissue affected, increased MDA causes oxidative stress that results in energetic inefficiencies.

Oxidative stress has been measured, via MDA, in many livestock species due to multiple environmental stressors that decrease animal performance. Disease challenges generate oxidative stress, as was the case in an investigation of bovine theileriosis in crossbred cattle of mixed ages and sexes (Rezaei and Dalir-Naghadeh, 2006). The

researchers reported greater oxidative stress in infected cattle versus healthy controls, finding greater erythrocyte MDA concentrations in the infected cattle (Rezaei and Dalir-Naghadeh, 2006). The investigators also reported increasing MDA concentrations associated with increasing disease severity based on packed cell volume (uninfected control, mild-moderate anemia, severe anemia: 25.47, 95.78, 138.81 nmol MDA/g hemoglobin, 3.003 SE), suggesting increased oxidative stress as the challenge increased in severity (Rezaei and Dalir-Naghadeh, 2006). Oxidative stress due to transit has also been documented in cattle; Chirase et al. (2004) transported 105 crossbred beef calves from Tennessee to Texas (1930 km), measuring serum MDA before and after transit. Serum MDA was nearly three-times greater in calves post-transport compared to baseline values (30.2 vs. 10.9, μg/ml) and comparing calves based on post-travel mortality, calves that died had 43% greater serum MDA concentrations than calves that lived (49.4 vs. 42.2, μg/ml; Chirase et al., 2004). Furthermore, the calves that showed signs of bovine respiratory disease (BRD) post-transit had two-fold greater serum MDA concentrations than calves not exhibiting BRD (Chirase et al., 2004); thus, the increase in oxidative stress due to transport may have been largely disease-driven in that case. Along with disease and transport stressors, dietary influences such as high sulfur can increase oxidative stress in beef feedlot steers (Pogge et al., 2015). Although other oxidative stress markers were increased and ADG linearly decreased in Angus-cross steers fed conventional feedlot finishing diets when dietary S increased from 0.22 to 0.55% (Pogge and Hansen, 2013b), longissimus dorsi MDA concentrations in the subsequent carcass analysis did not respond to the increased dietary S consumed by the steers (Pogge et al., 2013). Consequently, MDA may not be suitable as a sole indicator of oxidative stress in all cases.

Inefficiencies in the ETC lead to increased electron leak, ROS production, and subsequent oxidative damage to biomolecules. Based on relative protein carbonyl concentrations in highly and lowly feed efficient cohorts, it is clear that oxidative stress can impact feed efficiency. Based on the presence of MDA in animals subjected to disease and transport stress, ROS and other free radicals are generated amid a variety of environmental stressors. The energetic cost associated with neutralizing ROS as well as degrading and replacing oxidatively damaged biomolecules is one of the underlying causes of feed efficiency losses. Thus, a multi-faceted and cooperative antioxidant system is required to decrease oxidative stress and ameliorate the oxidative balance to prevent loss of performance in livestock.

Antioxidant Functions

As previously discussed, ROS are generated in a variety of situations (Lykkesfeldt and Svendsen, 2007). Neutralizing these ROS and degrading/replacing oxidatively damaged biomolecules or the cells containing the damaged biomolecules is energetically costly, leading to decreased energy efficiency. A multi-faceted antioxidant system inside and outside of the mitochondria is responsible for mitigating oxidant pressure and preventing oxidative damage. Therefore, the role of the antioxidant system is to adapt to changing needs and delay or inhibit oxidation of another substrate (Sies, 1997).

Superoxide dismutase

The primary line of defense against ROS pressure in eukaryotes is a family of superoxide dismutase enzymes (SOD), aptly named for the metals necessary for their

biological function, a Mn-containing SOD (MnSOD) as well as a Cu- and Zn-containing SOD (CuZnSOD; Nordberg and Arner, 2001). The SOD serve to reduce OH^- to H_2O_2 (Nordberg and Arner, 2001; Figure 1) as well as convert O_2^- to O_2 and H_2O_2. Specifically, two molecules of O_2^- are used by SOD to generate one H_2O_2 molecule. In the cytoplasm, CuZnSOD is the sole SOD present (Crapo et al., 1992); however, within the mitochondria, CuZnSOD acts in the inter-membranous space while MnSOD works in the matrix (Weisiger and Fridovich, 1973; Okado-Matsumoto and Fridovich, 2001). The Cu and Zn play differing roles in CuZnSOD, as the Cu is catalytic while the Zn provides structural stability (Paynter et al., 1979). Nordberg and Arner (2001) suggested that unlike CuZnSOD, MnSOD expression was induced by oxidative stress, providing a metered response as oxidative challenges increased in severity. More recently however, Huang et al. (2015) reported that heat exposure caused oxidative stress-induced increases in plasma SOD activity (CuZnSOD) and mitochondrial SOD activity (specific means not reported), suggesting that both SOD types may be upregulated due to oxidative stress. Huang et al. (2015) evaluated SOD activity in 24 broiler chicks (35 d old) after 12 were exposed to 32°C and 12 were exposed to 21°C for 7 d. Evaluating seasonal temperature effects on Holstein cows prior to- and post-calving in spring versus summer, Bernabucci et al. (2002) measured greater erythrocyte SOD activity at 3 d prior to calving, as well as 1 d and 15 d post-calving in the summer calving cows experiencing moderate heat stress (39.5 ± 0.2°C rectal temperatures), likely a response to increased oxidative stress that is characteristic of heat stress (Altan et al., 2003). Iqbal et al. (2002) evaluated liver SOD activity in broilers suffering from pulmonary hypertension syndrome (PHS) and fed supplementary vitamin E, noting no differences in SOD activity despite differences in other antioxidant activities. Reports of the relationship between live

performance and SOD activity in livestock are quite limited, thus a great deal of opportunity exists for novel investigations to evaluate what influence SOD activity may have on feed efficiency and other traits. Electron transport chain complex activities and H_2O_2 production in livestock representing feed efficiency extremes have been extensively analyzed (Bottje et al., 2002; Ojano-Dirain et al., 2004; Kolath et al., 2006; Bottje and Carstens, 2009). Despite the intermediary role of SOD in reducing ROS to H_2O_2, SOD activity has not been evaluated at any great length in feed efficient and inefficient livestock to determine what role the enzyme may play, and the same can be said for other important antioxidants.

Glutathione system

Manganese SOD is responsible for reducing O_2^- to H_2O_2 which is further converted to H_2O. The conversion to H_2O is accomplished as reduced glutathione (GSH) is oxidized to glutathione disulfide (GSSG) by glutathione peroxidase (GPX; Figure 1). Consequently, the GSSG:GSH ratio is an indicator of oxidative stress as a greater ratio indicates a greater degree of oxidative stress (Ojano-Dirain et al., 2005; Bottje and Carstens, 2009). The glutathione system is incredibly important for cellular detoxification, such as excessive H_2S in hepatocytes (Truong et al., 2006), and to the total antioxidant system as GSH is the most abundant intracellular antioxidant (Nordberg and Arner, 2001; Kondoh et al., 2003). Along with SOD activity, the glutathione ratio and measure of glutathione peroxidase activity both serve to further articulate the antioxidant capacity in each step from the conversion of ROS to H_2O. Glutathione peroxidase is a selenium dependent enzyme (Anderson et al., 1978) that contains seleno-cysteine (Nordberg and Arner, 2001). Glutathione peroxidase catalyzes the conversion of two GSH to one GSSG via the selenolate in GPX that temporarily accepts the

hydroxyl group (Engman et al., 1994; Nordberg and Arner, 2001). The GSSG can be reduced back to two GSH by glutathione reductase to replenish antioxidant capacity. However, glutathione reductase is NADPH-dependent (Nordberg and Arner, 2001) and thus, replenishing GSH is energetically costly, decreasing energy efficiency when increased oxidative stress is present. Although comparisons of GPX between feed efficiency groups are limited in livestock, a tendency for greater GSSG:GSH has been observed in animals with poorer feed efficiency (Ojano-Dirain et al., 2005). Ojano-Dirain et al. (2005) compared duodenal mucosa glutathione concentrations and GPX activity in the eight most and eight least feed efficient broilers identified from an original group of 100 broilers. Despite the tendency for glutathione ratio to differ (0.093 vs. 0.070 GSSG:GSH, 0.008 SE) between the feed efficiency groups (0.62 vs. 0.80 G:F, 0.01 SE), no differences were noted in GPX activity (Ojano-Dirain et al., 2005). Increases in oxidative stress have been noted in lowly efficient individuals as well as those suffering from immune challenges like PHS (Ojano-Dirain et al., 2005), and GPX may respond to increases in oxidative stress. In broilers suffering from PHS, Iqbal et al. (2002) reported increased liver GPX activity when birds were not fed supplemental vitamin E (specific means not reported), thus the GPX response was likely due to increased oxidative stress. Furthermore, Iqbal et al. (2002) also measured lung mitochondrial glutathione concentrations in birds selected or not-selected for resistance to PHS. They noted greater GSSG:GSH in the broilers not selected for PHS resistance and the ratio was driven by increased GSSG since there were no differences in GSH concentrations (Iqbal et al., 2002). The assessment of GSSG:GSH in blood or tissues provides a valuable but underutilized means for evaluating oxidative status and identifying oxidative stress in livestock. However, evaluating GPX activity and glutathione

concentrations along with SOD activity only provides a glimpse into a complex and beneficially redundant antioxidant system.

Other antioxidants

Working in conjunction with SOD, the heme-containing protein, catalase, is largely located in cellular peroxisomes where it neutralizes two H_2O_2 molecules, converting them to one O_2 molecule and two H_2O molecules (Nordberg and Arner, 2001). The value of catalase is especially notable in neutralizing H_2O_2 to prevent the previously discussed Fenton reaction that yields the more harmful hydroxyl radicals. Furthermore, compared to GPX in erythrocytes, analysis has shown that catalase provides equal or greater H_2O_2 neutralization (Gaetani et al., 1989). Although superoxide inhibits catalase, SOD works in positive synergism with catalase, relieving catalase inhibition (Kono and Fridovich, 1982). The water-soluble antioxidant, ascorbate, also serves as a scavenger of oxidative species as the ROS more readily oxidize ascorbate than GSH (Li et al., 2001; Figure 1), thereby preserving GSH antioxidant capacity. Therefore, determining ascorbate concentration in the mitochondria is important when GSH is being determined as an indicator of oxidative stress. Ascorbate also regenerates α-tocopherol by donating a proton to the oxidized form of tocopherol, semiquinone (Li et al., 2001; Nordberg and Arner, 2001). By donating a proton, ascorbate is oxidized to an ascorbyl radical that can be directly reduced back to ascorbate by GSH or converted to dehydroascorbate that can then be reduced to ascorbate via a NADPH-dependent reaction with thioredoxireductase. Consequently, reducing oxidized ascorbate back to ascorbate is an energetically costly process, directly due to the dehydroascorbate to ascorbate reaction, or indirectly due to the energetic cost of replenishing GSH following its

use in reducing the ascorbyl radical. Regenerated by ascorbate, tocopherol is a well-known non-enzymatic antioxidant that is lipid soluble (Sies, 1997). Tocopherol accepts oxidation by donating a proton to free radicals (Nordberg and Arner, 2001). Like tocopherol, beta-carotene and other carotenoids are lipid soluble and have plant origins. Beta-carotene participates in the antioxidant system by reacting with peroxyl radicals, forming a stabilized carbon-centered radical within its alkyl structure, thus preventing further ROS proliferation (Fang et al., 2002). Carrying out the majority of cellular oxygen reduction, cytochrome oxidase is an advantageous member of the antioxidant system because it does not release superoxide or other radicals as is the case with SOD and other antioxidants (Sies, 1997). Binding metal ions can also provide an effective means for preventing oxidation and as such, proteins responsible for binding metals can help to prevent oxidative stress, proteins like ceruloplasmin, ferritin, and transferrin (Sies, 1997). By chelating and transporting metals, substrates for oxidation can be decreased, particularly Fe and Cu. Iron especially requires a chaperone as it is easily oxidized, and as such, systems are in place to prevent free Fe. Ferritin serves to sequester and chaperone Fe intracellularly, decreasing Fe availability as a pro-oxidant (Balla et al., 1992). Similarly, transferrin is responsible for binding and transporting Fe intercellularly. In addition to intercellular transport of copper that prevents oxidation of the trace metal, ceruloplasmin is also independently capable of scavenging superoxide or other ROS (Goldstein et al., 1979). Best known for binding zinc and copper, metallothionein has also been shown to scavenge ROS (Kondoh et al., 2003). In fact, the ability of metallothionein to react with and neutralize hydroxyl radicals has been reported as ~350-times greater than that of glutathione (Sato, 1992). Therefore, metallothionein serves to

decrease oxidative stress by chelating metals to decrease available substrates for oxidation and by directly neutralizing ROS.

The antioxidant system benefits from cyclic regenerative pathways, redundancy, and cooperative activities to neutralize ROS and free radicals under a variety of conditions. Understanding the antioxidant systems and their roles in feed efficiency will likely be beneficial for identifying energetic inefficiencies as well as opportunities to impact and decrease these inefficiencies. Therefore, further exploration is needed to investigate the relationships between antioxidant systems and feed efficiency in livestock. A challenge for investigators moving forward is to work toward consistency in analysis of antioxidant activities as well as the interrelationships between antioxidants. Antioxidant activities have been discussed in a variety of blood constituents, liver, and other tissues; thus, a greater degree of consistency in tissue analysis would help researchers more clearly elucidate how animal production stage, health status, environment, or nutritional status may impact antioxidant systems. More specifically, understanding how nutrition impacts antioxidant capacities and activities may reveal options for maintaining or improving production efficiency and performance, potentially through trace mineral and vitamin nutrition.

Antioxidants and Micronutrients

The importance of the antioxidant system for neutralizing ROS and other free radicals is clear, and nutrition plays a key role, especially trace mineral and vitamin nutrition. Although trace minerals can potentiate oxidative damage to lipids and proteins (Sies, 1997), they also play integral roles as part of antioxidant structures and thus, antioxidants can be

impacted by trace mineral status and dietary mineral intake. Antioxidant vitamins are also key players in inhibiting or delaying oxidative damage to biomolecules.

Copper

Copper plays a major role in the antioxidant system through CuZnSOD (Figure 1), ceruloplasmin (Spears and Weiss, 2008), and cytochrome oxidase (Sies, 1997). Copper and Zn work in conjunction in CuZnSOD, with Zn serving a structural role and Cu serving a catalytic role (Paynter et al., 1979). The minimum recommended Cu concentration for growing and finishing cattle diets is 10 mg/kg DM (NRC, 2000) though nutritionists routinely feed 20 mg/kg DM feedlot diets (Vasconcelos and Galyean, 2007). Deficiencies occur most often due to antagonists such as high dietary Fe, S, or Mo (Spears, 2003) though a primary deficiency can occur due to limited dietary Cu intake (Gengelbach et al., 1994). Deficiency can result from a variety of underlying factors, from differences in Cu requirement due to breed variation or regional differences in soil mineral concentrations (Gooneratne et al., 1989) that translate to differences in forage mineral concentrations. In the feedlot, Cu deficiency is more likely in conventional diets with increased byproduct inclusions that often contain greater S. The result of Cu deficiency, antagonist-driven or not, can be decreased antioxidant activity. Deficient Cu status has been shown to decrease CuZnSOD activity in steers (Xin et al., 1991). For eight months, researchers fed Holstein steers a 35:65, concentrate:forage diet supplemented with either 20 mg Cu/kg diet DM from $CuSO_4$ to improve Cu status or 10 mg Mo/kg diet DM to deplete Cu (Xin et al., 1991). Briefly, sulfates are reduced to sulfides in the rumen; Mo combines with sulfides to form thiomolybdates that bind Cu in an insoluble complex, decreasing bioavailable Cu (Spears,

2003). After 8 months, Mo-supplemented steers had markedly decreased liver Cu compared with Cu-supplemented steers (18.1 vs. 305.8 mg Cu/kg DM, no SE reported; Xin et al., 1991) thus the Mo-supplemented steers were clinically deficient whereas the Cu-supplemented steers had adequate status (Kincaid, 1999). Consequently, the Cu-adequate steers had greater CuZnSOD activity than Cu-deficient steers in red blood cell lysate (0.60 vs. 0.36 U activity/mg hemoglobin, no SE reported), neutrophils (0.65 vs. 0.31 U activity/10^6 cells, no SE reported), and whole blood lysate (20.4 vs. 15.4 U activity/ml, no SE reported; Xin et al., 1991). Furthermore, Cu deficiency impacted neutrophil bactericidal capacity; faced with an *in vitro Staphylococcus aureus* challenge, the percentage of *S. aureus* killed by neutrophils from Cu-deficient steers was decreased relative to neutrophils isolated from the Cu-adequate steers (17.3 vs. 27.7%, no SE reported), potentially a result of decreased antioxidant protection in the neutrophils during the bactericidal process (Xin et al., 1991). The decrease in neutrophil bactericidal capacity may be partially explained by the reduction of superoxide to H_2O_2 by CuZnSOD, since the neutrophils' bactericidal process is facilitated by a bacteria-damaging respiratory burst, a release of superoxide and H_2O_2 (Carreras et al., 1994). Conversely, when Arthur and Boyne (1985) evaluated dietary Cu effects on neutrophil CuZnSOD activity in Friesian calves fed diets containing a total of 1.8 or 12 mg Cu/kg DM, they reported decreasing CuZnSOD activity throughout the 24 week trial, regardless of dietary Cu concentration. However, the authors also indicated that final CuZnSOD activity values at the end of the 24 week trial were similar to CuZnSOD activity observed in other Cu-supplemented adult cattle (Arthur and Boyne, 1985), thus the decrease in CuZnSOD activity may be age-related rather than a result of decreased dietary Cu. Dietary Cu concentration also had no effect on glutathione peroxidase activity (Arthur and Boyne, 1985).

Along with CuZnSOD activity, Cu deficiency can impact cytochrome oxidase activity as well. For six weeks, Paynter et al. (1979) fed weanling rats a basal diet containing 0.8 mg Cu/kg diet, supplemented with 0, 4, or 24 mg Cu/kg as $CuSO_4$; the 0 mg Cu/kg diet served as a depletion diet, though the 4 mg Cu/kg diet was still slightly below the current recommendation of 5 mg Cu/kg for growing rats (NRC, 1995). After 6 weeks, rats receiving the depletion diet were fed the 24 mg Cu/kg diet to replete Cu for 10 d (Paynter et al., 1979). At the conclusion of the initial 6 week period, no differences were detected in CuZnSOD or cytochrome oxidase activities between the Cu-supplemented groups in lung, testis, muscle, heart, kidney, liver, brain, or erythrocytes; however, the Cu-depleted rats had lesser CuZnSOD activity than supplemented rats in all tissues except brain and muscle, and had lesser cytochrome oxidase activity in all tissues except brain and lung (Paynter et al., 1979). Thus, it appears that antioxidant activity is preferentially spared in the brain. After the 10 d repletion in the depleted rats, CuZnSOD and cytochrome oxidase activity had nearly returned to baseline values in liver, with antioxidant activity in other tissues returning more slowly. The concentration of ceruloplasmin is also closely correlated with circulating Cu, so closely that ceruloplasmin concentrations are routinely used diagnostically to assess Cu status (Blakley and Hamilton, 1985), though ceruloplasmin may not be an ideal Cu status marker relative to liver Cu concentrations. Ceruloplasmin and Cu have been shown to be positively correlated in the serum of 116 cattle ($r = 0.83$), the serum of 45 sheep ($r = 0.92$), and the plasma of 87 cattle ($r = 0.60$; Blakley and Hamilton, 1985). Evaluating the correlation between liver Cu concentration and serum ceruloplasmin in 72 cattle, the relationship was not as strong ($r = 0.35$), indicating that ceruloplasmin may be a poor indicator of Cu storage in the liver (Blakley and Hamilton, 1985). Although Cu deficiency decreases Cu-dependent

antioxidant activity, the work by Paynter et al. (1979) would suggest that exceeding Cu requirements several-fold does not greatly enhance antioxidant activity when animals are already Cu-adequate.

Zinc

Evaluations of CuZnSOD activity due to Zn status in ruminants are limited, but Zn deficiency has been shown to decrease CuZnSOD activity in mice (Cao and Chen, 1991). In that study, sedentary and exercised mice were fed a Zn-deficient diet (1.6 mg total Zn/kg) or a $ZnSO_4$-supplemented diet (51.6 mg total Zn/kg) that exceeded requirements (10 mg Zn/kg; NRC, 1995). The authors noted decreased CuZnSOD activity in blood and liver in the Zn-deficient mice relative to the Zn-supplemented mice, both sedentary and exercised (Cao and Chen, 1991). It is unclear from the Cao and Chen (1991) work whether the greater CuZnSOD activity was a result of meeting or exceeding dietary Zn requirements. Shaheen and El-Fattah (1995) also noted decreased cytosolic SOD (CuZnSOD) activity in Zn-deficient (0.5 mg Zn/kg diet) versus Zn adequate (30 mg Zn/kg diet, with Zn supplemented as $ZnSO_4$) rats fed treatment diets for 3 weeks. Differences were detected in liver (2.95 vs. 5.14 U mg/protein, 0.34 SE), and whole blood (253 vs. 314 U/ml, 7.85 SE) but not pancreas (2.49 vs. 2.45 U/mg protein, 0.19 SE; Shaheen and El-Fattah, 1995). Intriguingly, Shaheen and El-Fattah (1995) also reported decreased total glutathione concentrations in Zn-deficient versus Zn-adequate rat blood (23 vs. 38 mg/dL, 1.9 SE) and liver (2.29 vs. 3.34 mg/g wet wt, 0.18 SE), but increased pancreas glutathione (2.16 vs. 1.77 mg/g wet wt, 0.09 SE). Though total glutathione is not indicative of oxidative stress, the greater total capacity does suggest a greater antioxidant capacity. When fed a diet containing 1000 mg Zn/kg diet for another 3

weeks, the previously Zn-deficient rats regained CuZnSOD activity and glutathione concentrations relative to the Zn-adequate rats (Shaheen and El-Fattah, 1995). Metallothionein concentrations are also highly responsive to Zn status in the animal (Sato et al., 1984) and have been correlated with Zn in a dose-dependent manner (Cousins, 1985). Comparing rats fed a Zn-deficient diet (<1 mg Zn/kg) and rats fed a Zn-adequate diet (40 mg Zn/kg diet, Zn supplemented as $ZnSO_4$) for 15 d, Sato et al. (1984) reported decreased plasma Zn (50% decrease in Zn-deficient rats, exact means not reported), liver metallothionein, and plasma metallothionein in the Zn-deficient rats, with both metallothionein measures in the Zn-deficient rats nearly undetectably low after 5 d on treatment diets. It is difficult to fully relate these results (Sato et al., 1984) to cattle as such low dietary Zn concentrations are unlikely in beef cattle diets since the majority of typical cattle feedstuffs contain at least 20-30 mg Zn/kg DM (NRC, 2000). However, considering plants can grow normally despite decreased soil trace mineral concentrations (McDowell, 1996), cattle on high forage diets are still at risk of insufficient Zn intake. Thus, additional work is needed to specifically evaluate CuZnSOD activity differences in cattle, as well as dietary Zn effects on CuZnSOD in cattle.

Manganese

Along with Cu and Zn, Mn also plays an integral role in the antioxidant system, as part of MnSOD. The NRC (2000) recommendation for growing and finishing cattle diets is 20 mg Mn/kg diet DM, as is the requirement for sheep (NRC, 2007). A Mn deficiency in cattle can lead to decreased fertility as well as decreased growth, but with the exception of a few regions with Mn-deficient soils, grains and forage typically contain adequate Mn

(Ammerman and Goodrich, 1983), making a Mn deficiency less likely in cattle. Unlike Cu and Zn, there is no well accepted concentration in plasma or liver for classifying cattle as deficient or marginally-deficient (Hansen et al., 2006) though Kincaid (1999) suggests < 7 mg Mn/kg liver as deficient, 7-15 mg Mn/kg liver as marginal, and >13 mg Mn/kg as adequate. Though investigations of the relationship between Mn status and antioxidant activity in ruminants are limited, Masters et al. (1988) evaluated the effects of 13, 19, 30, or 45 mg Mn/kg DM diets on tissue MnSOD activity in 30 Merino rams (n/diet not reported) fed for 84 d. Manganese SOD activity in the heart was positively correlated with Mn concentration in the heart ($r = 0.77$, specific means not reported) as well as dietary Mn, but there were no correlations for liver, kidney, testes, or muscle MnSOD activity and dietary Mn (Masters et al., 1988); thus dietary Mn had limited effects on MnSOD activity and was not enhanced by dietary concentrations that were more than double the NRC (2007) recommendations.

Rosa et al. (1980) investigated the relationship between MnSOD and dietary Mn in rats, mice, and chickens. In the mouse experiment, Rosa et al. (1980) compared MnSOD and CuZnSOD activities in brain, heart, lung, and liver of mice fed either a deficient 1 mg Mn/kg diet or a control 45 mg Mn/kg diet, well exceeding the 10 mg Mn/kg requirement (NRC, 1995). Most notable at the end of the 21 d trial, liver MnSOD activity in the deficient mice was only 17% of the MnSOD activity in the control mice (48 ± 2.84 vs. 286.8 ± 10.0 U/g fresh liver, SE) whereas brain MnSOD appeared to be more preferentially spared with deficient mice maintaining 50% of control activity (90 ± 1.4 vs. 188 ± 12.8 U/g fresh brain, SE; Rosa et al., 1980). Heart (control vs. deficient: 136 ± 5.04 vs. 102 ± 4.24 U/g wet wt., SE) and lung (control vs. deficient: 70.8 ± 3.36 vs. 54 ± 7.20 U/g wet wt., SE) MnSOD

activity were also decreased in the Mn-deficient mice. Thus, heart MnSOD activity was decreased due to decreased dietary Mn in both studies (Rosa et al., 1980; Masters et al., 1988). Rosa et al. (1980) also fed either a deficient 1 mg Mn/kg diet or a control 45 mg Mn/kg diet to Leghorn chicks for 21 d; 20 mg Mn/kg diet is the NRC recommendation (NRC, 1994). At 7, 14, and 21 d of age, the control chicks had greater liver MnSOD activity than the deficient chicks but at 21 d, the Mn-deficient chicks had greater liver CuZnSOD activity than the control chicks (847.5 ± 41.6 vs. 700.5 ± 36.2 U/g fresh liver, SE; Rosa et al., 1980). The results (Rosa et al., 1980) suggest the possibility of a complementary and compensatory relationship between MnSOD and CuZnSOD, though such a relationship has not been reported elsewhere.

Investigating the possibility of increasing MnSOD activity above that normally found in adequate animals, Rosa et al. (1980) also provided chicks with diets providing 1 mg Mn/kg diet or 45 mg Mn/kg diet (control) for 14 d and then provided a diet containing 1,000 mg Mn/kg diet for d 15-21. Despite greater liver MnSOD activity in control chicks at d 7 and 14 with no differences in CuZnSOD, at d 21 there were no differences between diet groups for MnSOD or CuZnSOD (Rosa et al., 1980), indicating that deficient chicks fed the supranutritional Mn diet for 7 d could replenish mineral status and SOD activity. However, the MnSOD activities did not differ at d 21 between control chicks and either group supplemented with 1,000 mg Mn/kg, nor was there a difference in CuZnSOD activity between groups fed the same diet for the first 14 d (Rosa et al., 1980), indicating that the supranutritional supplementation may have been sufficient to regain mineral status and SOD activity but did not increase SOD activity above and beyond normal values. An assessment of fecal Mn would have been beneficial to determine apparent Mn absorption since 1,000 mg

Mn/kg diet may have exceeded the Mn-absorptive capacity of the chick, thereby lessening the possibility of a dose-response to such a high Mn supplementation rate. Unlike the mouse trials, Rosa et al. (1980) reported no differences in MnSOD between rats fed the same Mn-deficient or control diets.

Following a Cu and Mn study in Angus cows, Hansen et al. (2009) compared gene expression in the offspring of these cows after a 422 d feeding period in which calves were fed a Cu-adequate diet (10 mg supplemental Cu/kg DM, 20 mg supplemental Mn/kg DM), a Cu-deficient diet (no supplemental Cu, 20 mg supplemental Mn/kg DM, 2 mg supplemental Mo/kg DM) or a Cu-deficient, supranutritional-Mn diet (no supplemental Cu, 500 mg supplemental Mn/kg DM, 2 mg supplemental Mo/kg DM). At harvest (d 422), no differences were detected in liver SOD gene expression due to diets but calves fed the Cu-deficient, supranutritional-Mn diet had liver cytochrome oxidase gene expression that was greater than the Cu-deficient calves and equivalent to the Cu-adequate calves (Hansen et al., 2009), suggesting that supranutritional Mn could have a positive effect on cytochrome oxidase activity despite Cu deficiency. Though limited research is available, it appears that Mn storage in liver is limited (Masters et al., 1988) and once absorbed, excess Mn is excreted in the bile (Suttle, 2010); thus, consistent intake/supplementation is necessary to prevent deficiency and the subsequent decrease in MnSOD activity. Exceeding recommended dietary Mn by more than double does not enhance MnSOD activity (Masters et al., 1988) though it may have an impact on cytochrome oxidase gene expression in Cu-deficient calves (Hansen et al., 2009).

Selenium

Selenium-dependent glutathione peroxidase has been well documented to reflect Se status of the animal. Providing Friesian calves with diets containing inadequate (0.01 mg/kg DM) or NRC (2000) recommended (0.1 mg/kg DM, from Na_2SeO_3) Se-containing diets for 24 weeks, Arthur and Boyne (1985) reported a decrease in neutrophil glutathione peroxidase activity to undetectable values in the inadequately supplemented calves within 9 weeks of the trial start, whereas the calves receiving 0.1 mg Se/ kg diet DM showed no change in neutrophil glutathione peroxidase activity throughout the trial. Dietary Se concentration had no effect on CuZnSOD activity (Arthur and Boyne, 1985). Similarly, Anderson et al. (1978) evaluated the relationship between Se status and glutathione peroxidase activity in erythrocytes and muscle of cattle and sheep, noting positive correlations (correlation coefficients not reported) between erythrocyte glutathione peroxidase activity and whole blood Se concentrations in 191 cattle and 43 sheep. Anderson et al. (1978) also monitored erythrocyte glutathione peroxidase activity and whole blood Se concentrations in 15 Friesian calves receiving a 0.01 mg Se/kg diet DM, with five of the calves receiving 2.5 mg Se injections (as Na_2SeO_3) every 2 weeks over a 10 week trial. The calves receiving Se injections had progressively increasing erythrocyte glutathione peroxidase activity and whole blood Se concentrations throughout the 10 week trial, while the uninjected calves had progressively decreasing glutathione peroxidase activity and relatively constant whole blood Se (Anderson et al., 1978). In those same calves, Anderson et al. (1978) measured glutathione peroxidase activity in 10 muscles after the 10 week period, reporting greater glutathione peroxidase activity for injected calves relative to uninjected calves in all ten muscles measured; the extremes in glutathione peroxidase activity ratios ranged from 5.8:1

activity in heart (injected:uninjected) to 85:1 activity in semimembranosus (injected:uninjected).

Though not all antioxidants respond to increased circulating concentrations of the minerals on which they are dependent, previous work has shown a glutathione peroxidase response to increased circulating Se. Pogge et al. (2012) fed corn silage-based diets meeting or exceeding NRC (2000) mineral recommendations to Angus and Simmental steers and injected the steers with saline or a commercial injectable mineral product containing Cu, Zn, Mn, and Se (Multimin 90, Multimin USA, Fort Collins, CO). Steers injected with the Multimin product had greater erythrocyte glutathione peroxidase activity than steers injected with saline (226.2 vs. 160.4 U/mg hemoglobin, 15.5 SE) despite consuming a common diet that provided 0.37 mg Se/kg DM (Pogge et al., 2012), exceeding NRC (2000) recommendations. Highlighting the interrelationship between glutathione peroxidase and other antioxidants, Mercier et al. (2004) reported inverse responses of muscle glutathione peroxidase and total SOD (MnSOD + CuZnSOD) activity in Charolais cows finished on pasture or a mixed diet, though neither diet or tissue mineral content were measured. Cows finished on grass pasture for 100 d had lesser muscle glutathione peroxidase activity (63.6 ± 17.9 vs. 197.8 ± 39.6 nmol oxidized NADPH·min^{-1}·mg protein^{-1}, SD) and greater muscle total SOD activity (3.65 ± 0.68 vs. 0.58 ± 0.10 U, SD) than cows fed mixed diets of grain, silage and cattle-cake (Mercier et al., 2004). Ultimately, glutathione peroxidase responds to Se supplementation, even when Se requirements are exceeded. Though Se supplementation in cattle is regulated by the FDA due to toxicity concerns, grazing cattle are typically at a greater risk of deficiency than toxicity with the exception of a few regions (Ammerman and

Goodrich, 1983). Providing inorganic Se supplementation is typically sufficient to meet NRC (2000) requirements, thereby preventing deficiency.

Vitamins

The regenerative relationship between the antioxidant vitamins C and E is well known (Willson, 1987; Chew, 1996; Combs Jr., 2012). However, there is no established NRC (2000) requirement for vitamin E in growing cattle. Previous investigations of the relationship between plasma or tissue α-tocopherol and dietary vitamin E supplementation in cattle have shown consistent increases in tocopherol when vitamin E intake is increased (Hidiroglou et al., 1988; Hidiroglou et al., 1995; Realini et al., 2003; Cusack et al., 2005). Pasture forages are rich in vitamin E but the same cannot be said for concentrates and stored forages (NRC, 2000). Muscle α-tocopherol concentrations were compared in Hereford steers that were pasture-finished, concentrate-finished, or concentrate-finished with supplemental vitamin E (1,000 IU vitamin E·steer^{-1}·d^{-1}) for 100 d (Realini et al., 2004). Similar concentrations were detected between pasture-finished steers (3.91 mg α-tocopherol/kg wet wt) and vitamin E-supplemented concentrate-finished steers (3.74 mg α-tocopherol/kg wet wt) but unsupplemented concentrate-finished steers (2.92 mg α-tocopherol/kg wet wt) had decreased muscle α-tocopherol.

Because ruminants can endogenously produce ascorbate in their liver (Matsui, 2012), there is no established dietary vitamin C requirement for beef cattle (NRC, 2000). However, previous investigations consistently show a positive effect of vitamin C supplementation on plasma ascorbate concentrations (Hidiroglou et al., 1995; Weiss, 2001; Padilla et al., 2007; Pogge and Hansen, 2013a, b). As part of the antioxidant system, ascorbate also has a

cooperative relationship with glutathione (Johnston et al., 1993). Vitamin C supplementation in feedlot cattle has shown no effect on oxidized:reduced liver glutathione ratios in steers fed diets containing 0.22% (Pogge and Hansen, 2013b) or 0.31% dietary S (Pogge et al., 2015). However, supplementing 5 g rumen bypass vitamin C·steer^{-1}·d^{-1} does appear to decrease glutathione ratios and spare glutathione antioxidant capacity in high S finishing steers fed diets containing 0.55% S (ratios: no vitamin C = 0.28, VC = 0.07; Pogge and Hansen 2013b) or 0.59% S (ratios: no VC = 0.243, VC = 0.196-0.232; Pogge et al., 2015). The effects of supplemental vitamin C on antioxidant capacity in cattle appear limited to improved plasma ascorbate and tissue glutathione concentrations though, as Cusack et al. (2005) and Weiss (2001) have reported no impact of supplemental vitamin C on plasma α-tocopherol.

Nutrition is a tool for generating predictable performance despite variation in production environments. Trace mineral nutrition can provide an opportunity to maintain or improve health and performance through a variety of roles, including antioxidant systems. The difficulty in interpreting much of the current literature is in dealing with inconsistencies such as duration of dietary treatments, variation between analyzed tissue types, antioxidants/markers measured, and assay methodologies. More consistency is needed to truly understand the role and opportunities for trace mineral and vitamin nutrition in antioxidants. Taking a more systematic approach may be especially valuable: measuring multiple interrelated antioxidants and oxidative stress markers in the same subjects to paint a more clear picture of the antioxidant process and its effect on animal health and performance.

LITERATURE CITED

Altan, Ö., A. Pabuçcuoğlu, A. Altan, S. Konyalioğlu, and H. Bayraktar. 2003. Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. Brit. poultry sci. 44(4):545-550. doi:10.1080/00071660310001618334.

Ammerman, C. B., and R. D. Goodrich. 1983. Advances in mineral nutrition in ruminants. J. Anim. Sci. 57(2):519-533.

Anderson, P. H., S. Berrett and D. S. P. Patterson. 1978. Glutathione peroxidase activity in erythrocytes and muscle of cattle and sheep and its relationship to selenium. J. Comp. Path. 88(2):181-189.

Archer, J. A., P. F. Arthur, R. M. Herd, P. F. Parnell, and W. S. Pitchford. 1997. Optimum postweaning test for measurement of growth rate, feed intake, and feed efficiency in British breed cattle. J. Anim. Sci. 75: 2024-2032.

Archer, J. A., E. C. Richardson, R. M. Herd and P. F. Arthur. 1999. Potential for selection to improve efficiency of feed use in beef cattle: A review. Australian Journal of Agricultural Research. 50: 147-161.

Arthur, J. R., and R. Boyne. 1985. Superoxide dismutase and glutathione peroxidase activities in neutrophils from selenium deficient and copper deficient cattle. Life Sci. 36(16):1569-1575.

Arthur, P. F., R. M. Herd, J. H. Wright, G. Xu, K. C. P. Dibley, and E. C. Richardson. 1996. Net feed conversion efficiency and its relationship with other traits in beef cattle. Proc. Aust. Soc. Anim. Prod. 21:107–110.

Arthur, P. F., J. A. Archer, D. J. Johnston, R. M. Herd, E. C. Richardson and P. F. Parnell. 2001a. Genetic and phenotypic variance and covariance components for feed intake, feed efficiency, and other postweaning traits in Angus cattle. J. Anim. Sci. 79: 2805-2811.

Arthur, P. F., J. A. Archer, R. M. Herd and G. J. Melville. 2001b. Response to selection for net feed intake in beef cattle. Proc. Adv. Anim. Breed. Genet. 14:135–138.

Arthur, P. F., and R. M. Herd. 2008. Residual feed intake in beef cattle. Revista Brasileira de Zootecnia. 37:269-279.

Balla, G., H. S. Jacob, J. Balla, M. Rosenberg, K. Nath, F. Apple, J. W. Eaton, and G. M. Vercellotti. 1992. Ferritin: a cytoprotective antioxidant strategem of endothelium. Journal of Biological Chemistry. 267(25):18148-18153.

Basarab, J. A., M. A. Price, J. L. Aalhus, E. K. Okine, W. M. Snelling, and K. L. Lyle. 2003. Residual feed intake and body composition in young growing cattle. Can. J. Anim. Sci. 83(2):189-204.

Bayr, H. 2005. Reactive oxygen species. Critical care medicine. 33(12):S498-S501.

Berg, J. M., J. L. Tymoczko, and L. Stryer. 2007. Biochemistry. 6th ed. W. H. Freeman and Co. New York, NY.

Bernabucci, U., B. Ronchi, N. Lacetera, and A. Nardone. 2002. Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. J. Dairy Science. 85(9):2173-2179. doi:10.3168/jds.S0022-0302(02)74296-3.

Berry, D. P., and J. J. Crowley. 2012. Residual intake and body weight gain: a new measure of efficiency in growing cattle. J. Anim. Sci. 90(1): 109-115.

Bishop, M. D., M. E. Davis, W. R. Harvey, G. R. Wilson, and B. D. VanStavern. 1991. Divergent selection for postweaning feed conversion in Angus beef cattle: I. Mean comparisons. J. Anim. Sci. 69(11):4348-4359.

Blakley, B. R., and D. L. Hamilton. 1985. Ceruloplasmin as an indicator of copper status in cattle and sheep. Can. J. Comp. Med. 49(4):405-408.

Bottje, W., Z. X. Tang, M. Iqbal, D. Cawthon, R. Okimoto, T. Wing and M. Cooper. 2002. Association of Mitochondrial Function with Feed Efficiency within a Single Genetic Line of Male Broilers. Poultry Sci. 81:546-555.

Bottje, W., N. R. Pumford, C. Ojano-Dirain, M. Iqbal, and K. Lassiter. 2006. Feed Efficiency and Mitochondrial Function. Poultry Sci. 85(1):8-14. doi:10.1093/ps/85.1.8

Bottje, W. G. and G. E. Carstens. 2009. Association of mitochondrial function and feed efficiency in poultry and livestock. J. Anim. Sci. 87:E48-E63. doi:10.2527/jas.2008-1379.

Campos, P. H., J. Noblet, Y. Jaguelin-Peyraud, H. Gilbert, P. Mormede, R. F. M. de Oliveira Donzele, J. L. Donzele, and D. Renaudeau. 2014. Thermoregulatory responses during thermal acclimation in pigs divergently selected for residual feed intake. Int. J. Biometeorol. 58:1545–1557.

Cao, G., and J. Chen. 1991. Effects of dietary zinc on free radical generation, lipid peroxidation, and superoxide dismutase in trained mice. Arch. Biochem.and Biophys. 291(1):147-153.

Carreras, M. C., G. A. Pargament, S. D. Catz, J. J. Poderoso, and A. Boveris. 1994. Kinetics of nitric oxide and hydrogen peroxide production and formation of peroxynitrite during the respiratory burst of human neutrophils. FEBS letters. 341(1): 65-68.

Carstens, G. E. and L. O. Tedeschi. 2006. Defining feed efficiency in beef cattle. Proceedings of the Beef Improvement Federation: 38th Annual Research Symposium and Annual Meeting. 12-21.

Castegna, A., J. Drake, C. Pocernich, and D. A. Butterfield. 2003. Protein carbonyl levels—an assessment of protein oxidation. Methods in Biological Oxidative Stress. Humana Press. Totowa, NJ.

Chacko, B. K., P. A. Kramer, S. Ravi, M. S. Johnson, R. W. Hardy, S. W. Ballinger, and V. M. Darley-Usmar. 2013. Methods for defining distinct bioenergetic profiles in platelets, lymphocytes, monocytes, and neutrophils, and the oxidative burst from human blood. Lab. Investigation. 93(6):690-700. doi:10.1038/labinvest.2013.53.

Chew, B. P. 1996. Importance of antioxidant vitamins in immunity and health in animals. Anim. Feed Sci. and Tech. 59(1):103-114.

Chirase, N. K., L. W. Greene, C. W. Purdy, R. W. Loan, B. W. Auvermann, D. B. Parker, E. F. Walborg Jr, D. E. Stevenson, Y. Xu, and J. E. Klaunig. 2004. Effect of transport stress on respiratory disease, serum antioxidant status, and serum concentrations of lipid peroxidation biomarkers in beef cattle. American journal of veterinary research. 65(6):860-864. doi: 10.2460/ajvr.2004.65.860.

Combs Jr., G. F., 2012. The vitamins: fundamental aspects in nutrition and health. 4th ed. Academic Press, San Diego, CA.

Cousins, R. J. 1985. Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. Physiol Rev. 65(2):238-309.

Crapo, J. D., T. Oury, C. Rabouille, J. W. Slot and L. Chang. 1992. Copper, zinc superoxide dismutase primarily a cytosolic protein in human cells. Proc. Natl. Acad. Sci. 89:10405-10409.

Crowley, J. J., M. McGee, D. A. Kenny, D. H. Crews, Jr., R. D. Evans and D. P. Berry. 2010. Phenotypic and genetic parameters for different measures of feed efficiency in different breeds of Irish performance-tested beef bulls. Journal of Animal Science 88: 885-894.

Crowley, J. J., R. D. Evans, N. McHugh, D. A. Kenny, M. McGee, D. H. Crews, and D. P. Berry. 2011. Genetic relationships between feed efficiency in growing males and beef cow performance. J. Anim. Sci. 89(11):3372-3381.

Cruz, G. D., J. A. Rodríguez-Sánchez, J. W. Oltjen, and R. D. Sainz. 2010. Performance, residual feed intake, digestibility, carcass traits, and profitability of Angus-Hereford steers housed in individual or group pens." J. Anim. Sci. 88(1):324-329.

Cusack, P. M. V., N. P. McMeniman, and I. J. Lean. 2005. The physiological and production effects of increased dietary intake of vitamins E and C in feedlot cattle challenged with bovine herpesvirus 1. J. Anim. Sci. 83(10):2423-2433. doi:/2005.83102423x

Dalle-Donne, I., R. Rossi, D. Giustarini, A. Milzani, and R. Colombo. 2003. Protein carbonyl groups as biomarkers of oxidative stress. Clinica chimica acta. 329(1):23-38. doi:10.1016/S0009-8981(03)00003-2.

De Haer, L. C. M., and A. G. De Vries. 1993. Feed intake patterns of and feed digestibility in growing pigs housed individually or in groups. Lvstk Prod. Sci. 33(3):277-292.

Del Rio, D., A. J. Stewart, and N. Pellegrini. 2005. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutrition, Metabolism and Cardiovascular Diseases. 15(4):316-328. doi: 10.1016/j.numecd.2005.05.003.

DiCostanzo, A., J. C. Meiske, S. D. Plegge, T. M. Peters, and R. D. Goodrich. 1990. Within-herd variation in energy utilization for maintenance and gain in beef cows. J. Anim. Sci. 68(7):2156-2165. doi:/1990.6872156x.

Draper, H. H., E. J. Squires, H. Mahmoodi, J. Wu, S. Agarwal, and M. Hadley. 1993. A comparative evaluation of thiobarbituric acid methods for the determination of malondialdehyde in biological materials. Free Radical Biology and Medicine. 15(4):353-363.

Durunna, O. N., F. D. N. Mujibi, L. Goonewardene, E. K. Okine, J. A. Basarab, Z. Wang, and S. S. Moore. 2011. Feed efficiency differences and reranking in beef steers fed grower and finisher diets. J. Anim. Sci. 89:158-167. doi: 10.2527/jas.2009-2514.

Durunna, O. N., M. G. Colazo, D. J. Ambrose, D. McCartney, V. S. Baron, and J. A. Basarab. 2012. Evidence of residual feed intake reranking in crossbred replacement heifers. J. Anim. Sci. 90(3):734-741.

Engman, L., C. Andersson, R. Morgenstern, I. A. Cotgreave, C. M. Andersson, and A. Hallberg. 1994, Evidence for a common selenolate intermediate in the glutathione peroxidase-like catalysis of α-(phenylselenenyl) ketones and diphenyl diselenide. Tetrahedron. 50(9):2929-2938. doi:10.1016/S0040-4020(01)87004-6.

Fahey, G. C., and H. G. Jung. 1983. Lignin as a marker in digestion studies: a review. J. Anim. Sci. 57(1):220-225.

Fang, Y., S. Yang, and G. Wu. 2002. Free radicals, antioxidants, and nutrition. Nutrition. 18(10): 872-879. doi: 10.1016/S0899-9007(02)00916-4.

Freetly, H. C., and T. M. Brown-Brandl. 2013. Enteric methane production from beef cattle that vary in feed efficiency. J. Anim. Sci. 91(10):4826-4831.

Gaetani, G. F., S. Galiano, L. Canepa, A. M. Ferraris, and H. N. Kirkman. 1989. Catalase and glutathione peroxidase are equally active in detoxification of hydrogen peroxide in human erythrocytes. Blood. 73(1):334-339.

Gengelbach, G. P., J. D. Ward, and J. W. Spears. 1994. Effect of dietary copper, iron, and molybdenum on growth and copper status of beef cows and calves. J. Anim. Sci. 72(10): 2722-2727.

Goldstein, I. M., H. B. Kaplan, H. S. Edelson, and G. Weissmann. 1979. Ceruloplasmin. A scavenger of superoxide anion radicals. Journal of Biological Chemistry. 254(10):4040-4045.

Gooneratne, S. R., W. T. Buckley, and D. A. Christensen. 1989. Review of copper deficiency and metabolism in ruminants. Can. J. Anim. Sci. 69(4):819-845.

Grion, A. L., M. E. Z. Mercadante, J. N. S. G. Cyrillo, S. F. M. Bonilha, E. Magnani, and R. H. Branco. 2014. Selection for feed efficiency traits and correlated genetic responses in feed intake and weight gain of Nellore cattle. J. Anim. Sci. 92(3):955-965.

Grubbs, J. K., A. N. Fritchen, E. Huff-Lonergan, J. C. M. Dekkers, N. K. Gabler, and S. M. Lonergan. 2013. Divergent genetic selection for residual feed intake impacts mitochondria reactive oxygen species production in pigs. J. Anim. Sci. 91(5):2133-2140. doi:10.2527/jas2012-5894.

Gutteridge, J. M. 1995. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin. chem. 41(12):1819-1828.

Hansen, S. L., J. W. Spears, K. E. Lloyd and C. S. Whisnant. 2006. Growth, reproductive performance and manganese status of heifers fed varying concentrations of manganese. J. Anim. Sci. 84(12): 3375-3380. doi:10.2527/jas.2005-667.

Hansen, S. L., M. S. Ashwell, L. R. Legleiter, R. S. Fry, K. E. Lloyd, and J. W. Spears. 2009. The addition of high manganese to a copper-deficient diet further depresses copper status and growth of cattle. Brit. J. Nutr. 101(7):1068-1078. doi:10.1017/S0007114508057589.

Harris, A. J., J. F. Patience, S. M. Lonergan, C. J. M. Dekkers, and N. K. Gabler. 2012. Improved nutrient digestibility and retention partially explains feed efficiency gains in pigs selected for low residual feed intake. J. Anim. Sci. 90:164-166. doi:10.2527/jas53855.

Hegarty, R. S., J. P. Goopy, R. M. Herd, and B. McCorkell. 2007. Cattle selected for lower residual feed intake have reduced daily methane production. J. Anim. Sci. 85(6):1479-1486. doi:10.2134/jas1963.222486x.

Herd, R. M., and S. C. Bishop. 2000. Genetic variation in residual feed intake and its association with other production traits in British Hereford cattle. Livest Prod. Sci. 63(2):111-119.

Herd, R. M., J. A. Archer and P. F. Arthur. 2003. Reducing the cost of beef production through genetic improvement in residual feed intake: Opportunity and challenges to application. J. Anim. Sci. 81: E9-17.

Herd, R. M., V. H. Oddy, and E. C. Richardson. 2004. Biological basis for variation in residual feed intake in beef cattle. 1. Review of potential mechanisms. Aust. J. Exp. Agric. 44(5):423-430. doi:10.1071/EA02220.

Herd, R., and P. F. Arthur. 2009. Physiological basis for residual feed intake. J. Anim. Sci. 87(E Suppl.):E64–E71.

Hidiroglou, N., L. F. Laflamme, and L. R. McDowell. 1988. Blood plasma and tissue concentrations of vitamin E in beef cattle as influenced by supplementation of various tocopherol compounds. J. Anim. Sci. 66(12):3227-3234. doi:10.2134/jas1988.66123227x.

Hidiroglou, M., T. R. Batra, and M. Ivan. 1995. Effects of supplemental vitamins E and C on the immune responses of calves. J. Dairy Sci. 78:1578-1583.

Hoque, M. A., M. Hosono, T. Oikawa, and K. Suzuki. 2009. Genetic parameters for measures of energetic efficiency of bulls and their relationships with carcass traits of field progeny in Japanese Black cattle. J. Anim. Sci. 87(1):99-106.

Huang, C., H. Jiao, Z. Song, J. Zhao, X. Wang, and H. Lin. 2015. Heat stress impairs mitochondria functions and induces oxidative injury in broiler chickens. J. Anim. Sci. 93(5):2144-2153.

Iqbal, M., D. Cawthon, K. Beers, R. F. Wideman, Jr., and W. G. Bottje. 2002. Antioxidant enzyme activities and mitochondrial fatty acids in pulmonary hypertension syndrome (PHS) in broilers. Poultry Sci. 81:252–260.

Iqbal, M., N. R. Pumford, Z. X. Tang, K. Lassiter, T. Wing, M. Cooper and W. Bottje. 2004. Low Feed Efficient Broilers Within a Single Genetic Line Exhibit Higher Oxidative Stress and Protein Expression in Breast Muscle with Lower Mitochondrial Complex Activity. Poultry Sci. 83:474-484. doi:10.1093/ps/83.3.474.

Iqbal, M., N. R. Pumford, Z. X. Tang, K. Lassiter, C. Ojano-Dirain, , T. Wing, M. Cooper, and W. Bottje. 2005. Compromised liver mitochondrial function and complex activity in low feed efficient broilers are associated with higher oxidative stress and differential protein expression. Poultry sci. 84(6):933-941. doi: 10.1093/ps/84.6.933.

Janero, D. R. 1990. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radical Biology and Medicine. 9(6):515-540.

Johnston, C. S., C. G. Meyer, and J. C. Srilakshmi. 1993. Vitamin C elevates red blood cell glutathione in healthy adults. Amer. J. Clin. Nutr. 58(1):103-105.

Kelly, A. K., M. McGee, D. H. Crews, A. G. Fahey, A. R. Wylie, and D. A. Kenny. 2010a. Effect of divergence in residual feed intake on feeding behavior, blood metabolic variables, and body composition traits in growing beef heifers. J. Anim. Sci. 88(1):109-123.

Kelly, A. K., M. McGee, D. H. Crews, Jr., T. Sweeney, T. M. Boland, and D. A. Kenny. 2010b. Repeatability of feed efficiency, carcass ultrasound, feeding behavior, and blood metabolic variables in finishing heifers divergently selected for residual feed intake. J. Anim. Sci. 88:3214-3225. doi: 10.2527/jas.2009-2700.

Kennedy, B. W., J. H. van der Werf, and T. H. Meuwissen. 1993. Genetic and statistical properties of residual feed intake. J. Anim. Sci. 71: 3239-3250.

Kincaid, R. L. 1999. Assessment of trace mineral status of ruminants: a review. Proceedings of ASAS. 1–10.

Koch, R. M., L. A. Swiger, D. Chambers and K. E. Gregory. 1963. Efficiency of feed use in beef cattle. J. Anim. Sci. 22(2): 486-494.

Kolath, W. H., M. S. Kerley, J. W. Golden, and D. H. Keisler. 2006. The relationship between mitochondrial function and residual feed intake in Angus steers. J. Anim. Sci. 84(4):861-865. doi:/2006.844861x.

Kondoh, M., K. Kamada, M. Kuronaga, M. Higashimoto, M. Takiguchi, Y. Watanabe, and M. Sato. 2003. Antioxidant property of metallothionein in fasted mice. Toxicology letters. 143(3): 301-306. doi:10.1016/S0378-4274(03)00195-4.

Kono, Y., and I. Fridovich. 1982. Superoxide radical inhibits catalase. J. Biological Chemistry. 257(10):5751-5754.

Langemeier, M., T. Schroeder and J. Mintert. 1992. Determinants of cattle finishing profitability. Southern J.Agric. Econ. 24: 41-41.

Li, X., C. E. Cobb, K. E. Hill, R. F. Burk and J. M. May. 2001. Mitochondrial uptake and recycling of ascorbic acid. Arch Biochem Biophys. 387:143–153.

Luiting, P., J. W. Schrama, W. Van der Hel, and E. M. Urff. 1991. Metabolic differences between White Leghorns selected for high and low residual food consumption. Brit. Poultry Sci. 32(4):763-782.

Lykkesfeldt, J., and O. Svendsen. 2007. Oxidants and antioxidants in disease: oxidative stress in farm animals. Vet. J. 173(3):502-511. doi:10.1016/j.tvjl.2006.06.005.

Mao, G., G. A. Kraus, I. Kim, M. E. Spurlock, T. B. Bailey, and D. C. Beitz. 2011. Effect of a mitochondria-targeted vitamin E derivative on mitochondrial alteration and systemic oxidative stress in mice. British J Nutr. 106:87-95. doi: 10.1017/S0007114510005830.

Marnett, L. J. 1999. Lipid peroxidation—DNA damage by malondialdehyde. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 424(1):83-95. doi:10.1016/S0027-5107(99)00010-X.

Masters, D. G., D. I. Paynter, J. Briegel, S. K. Baker, and D. B. Purser. 1988. Influence of manganese intake on body, wool and testicular growth of young rams and on the concentration of manganese and the activity of manganese enzymes in tissues. Crop and Pasture Sci. 39(3):517-524. doi:10.1071/AR9880517.

Matsui, T. 2012. Vitamin C nutrition in cattle. Asian-Australasian J. Anim. Sci. 25(5):597-605. doi:10.5713/ajas.2012.r.01.

McDowell, L. R. 1996. Feeding minerals to cattle on pasture. Anim. Feed Sci. and Tech. 60(3):247-271.

Mehlhase, J. and T. Grune. 2002. Proteolytic response to oxidative stress in mammalian cells. Biol. Chem. 383:559-567. doi:10.1515/BC.2002.057.

Mercier, Y., P. Gatellier, and M. Renerre. 2004. Lipid and protein oxidation in vitro, and antioxidant potential in meat from Charolais cows finished on pasture or mixed diet. Meat Sci. 66(2):467-473. doi:10.1016/S0309-1740(03)00135-9.

Miller, A. J., D. B. Faulkner, R. K. Knipe, D. R. Strohbehn, D. F. Parrett, and L. L. Berger. 2001. Critical control points for profitability in the cow-calf enterprise. Prof. Anim. Sci. 17(4):295-302.

Montanholi, Y., A. Fontoura, K. Swanson, B. Coomber, S. Yamashiro, and S. Miller. 2013. Small intestine histomorphometry of beef cattle with divergent feed efficiency. Acta Vet. Scan. 55(1):1-6.

Nkrumah, J. D., J. A. Basarab, M. A. Price, E. K. Okine, A. Ammoura, S. Guercio, C. Hansen, B. Benkel, and S. S. Moore. 2004. Different measures of energetic efficiency and their phenotypic relationships with growth, feed intake, and ultrasound and carcass merit in hybrid cattle. J. Anim. Sci. 82(8):2451-2459.

Nkrumah, J. D., E. K. Okine, G. W. Mathison, K. Schmid, C. Li, J. A. Basarab, M. A. Price, Z. Wang, and S. S. Moore. 2006. Relationships of feedlot feed efficiency, performance, and feeding behavior with metabolic rate, methane production, and energy partitioning in beef cattle. J. Anim. Sci. 84:145-153. doi:/2006.841145x.

Nordberg, J. and E. S. J. Arner. 2001. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free radical biology and medicine. 31(11):1287-1312.

NRC. 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.

NRC. 1995. Nutrient Requirements of Laboratory Animals. 4th rev. ed. National Academy Press, Washington, DC.

NRC. 2000. Nutrient requirements of beef cattle. 7th rev. ed. National Academy Press, Washington, D.C.

NRC. 2007. Nutrient requirements of Small Ruminants. Natl. Acad. Press, Washington, DC.

Ojano-Dirain, C. P., M. Iqbal, D. Cawthon, S. Swonger, T. Wing, M. Cooper, and W. Bottje. 2004. Determination of mitochondrial function and site-specific defects in electron transport in duodenal mitochondria in broilers with low and high feed efficiency. Poultry science. 83(8):1394-1403.

Ojano-Dirain, C., M. Iqbal, T. Wing, M. Cooper, and W. Bottje. 2005. Glutathione and respiratory chain complex activity in duodenal mitochondria of broilers with low and high feed efficiency. Poultry science. 84(5):782-788. doi:10.1093/ps/84.5.782.

Ojano-Dirain, C., M. Toyomizu, T. Wing, M. Cooper, and W. G. Bottje. 2007. Gene Expression in Breast Muscle and Duodenum from Low and High Feed Efficient Broilers. Poultry Sci. 86(2):372-381. doi:10.1093/ps/86.2.372.

Okado-Matsumoto, A. and I. Fridovich. 2001. Subcellular Distribution of Superoxide Dismutases (SOD) in Rat Liver. J. Biol. Chem. 276(42):38388-38393.

Padilla, L., T. Matsui, K. Shibano, H. Katamoto, and H. Yano. 2007. The effect of vitamin C supplementation on plasma concentration and urinary excretion of vitamin C in cattle. J. Anim. Sci. 85:3367–3370. doi:10.2527/jas.2007-0060.

Paynter, D. I,. R. J. Moir, and E. J. Underwood. 1979. Changes in activity of the Cu-Zn superoxide dismutase enzyme in tissues of the rat with changes in dietary copper. J. Nutr. 109:1570-1576.

Pogge, D. J., E. L. Richter, M. E. Drewnoski, and S. L. Hansen. 2012. Mineral concentrations of plasma and liver after injection with a trace mineral complex differ among Angus and Simmental cattle. J. Anim. Sci. 90(8):2692-2698. doi:10.2527/jas.2011-4482.

Pogge, D. J. and S. L. Hansen. 2013a. Effect of varying concentrations of vitamin C on performance, blood metabolites, and carcass characteristics of steers consuming a common high-sulfur (0.55% S) diet. J. Anim. Sci. 91(12):5754-5761. doi:10.2527/jas.2013-6909.

Pogge, D. J. and S. L. Hansen. 2013b. Supplemental vitamin C improves marbling in feedlot cattle consuming high sulfur diets. J. Anim. Sci. 91:4303–4314. doi:10.2527/jas.2012-5638.

Pogge, D. J., S. M. Lonergan, and S. L. Hansen. 2013. Influence of supplemental vitamin C on postmortem protein degradation and fatty acid profiles of the longissimus thoracis

of steers fed varying concentrations of dietary sulfur. Meat Science. 96:956–963. doi: 10.1016/j.meatsci.2013.08.031.

Pogge, D. J., S. M. Lonergan, and S. L. Hansen. 2015. Impact of supplementing vitamin C for 56, 90, or 127 days on growth performance and carcass characteristics of steers fed a 0.31 or 0.59% sulfur diet. J. Anim. Sci. 93(5):2297-2308. doi:10.2527/jas.2014-7913.

Realini, C. E., S. K. Duckett, G. W. Brito, M. Dalla Rizza, and D. De Mattos. 2004. Effect of pasture vs. concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. Meat Sci. 66(3):567-577. doi:10.1016/S0309-1740(03)00160-8.

Rezaei, S. A., and B. Dalir-Naghadeh. 2006. Evaluation of antioxidant status and oxidative stress in cattle naturally infected with *Theileria annulata*. Veterinary parasitology. 142(1):179-186. doi:10.1016/j.vetpar.2006.05.033.

Richardson, E. C., R. M. Herd, P. F. Arthur, J. Wright, G. Xu A, K. Dibley, and V. H. Oddy. 1996. Possible physiological indicators for net feed conversion efficiency in beef cattle. Proc. Aust. Soc. Anim. Prod. 21:103-106.

Richardson, E. C., R. M. Herd, and V. H. Oddy. 2000. Variation in body composition, activity and other physiological processes and their associations with feed efficiency. Feed efficiency in beef cattle - Proceedings of the feed efficiency workshop. Ed. J.A. Archer, R. M. Herd, and P. F. Arthur). 46-50.

Richardson, E. C., R. M. Herd, V. H. Oddy, J. M. Thompson, J. A. Archer, and P. F. Arthur. 2001. Body composition and implications for heat production of Angus steer progeny of parents selected for and against residual feed intake. Anim. Prod. Sci. 41(7):1065-1072.

Richardson, E. C. and R. M. Herd. 2004. Biological basis for variation in residual feed intake in beef cattle. 2. Synthesis of results following divergent selection. Aust. J. Exp. Agric. 44(5):431-440. doi:10.1071/EA02221.

Robinson, D. L., and V. H. Oddy. 2004. Genetic parameters for feed efficiency, fatness, muscle area and feeding behaviour of feedlot finished beef cattle. Livest. Prod. Sci. 90(2):255-270.

Rosa, G., C. L. Keen, R. M. Leach and L. S. Hurley. 1980. Regulation of superoxide dismutase activity by dietary manganese. J. Nutr. 110: 795-804.

Sandelin, B. A., A. H. Brown, Jr., M. A. Brown, C. Ojano-Dirain, and R. T. Baublits. 2005. Association of Mitochondrial Function with Feed Efficiency in Angus Cattle. Univ. Arkansas, Fayetteville. http://arkansasagnews.uark.edu/535-30.pdf (Accessed 20 September, 2015).

Sato, M., R. K. Mehra, and I. Bremner. 1984. Measurement of plasma metallothionein-I in the assessment of the zinc status of zinc-deficient and stressed rats. J. Nutr. 114(9):1683-1689.

Schenkel, F. S., S. P. Miller, and J. W. Wilton. 2004. Genetic parameters and breed differences for feed efficiency, growth, and body composition traits of young beef bulls. Can. J. Anim. Sci. 84(2):177-185.

Shaheen, A. A., and A. A. A. El-Fattah. 1991. Effect of dietary zinc on lipid peroxidation, glutathione, protein thiols levels and superoxide dismutase activity in rat tissues. Int J. Biochem. & Cell Bio. 27(1):89-95.

Shigenaga, M. K., T. M. Hagen and B. N. Ames. 1994. Oxidative damage and mitochondrial decay in aging. Proc. Natl. Acad. Sci. 91: 10771-10778.

Sies, H. 1997. Oxidative stress: oxidants and antioxidants. Experimental physiology. 82(2):291-295. doi: 10.1113/expphysiol.1997.sp004024.

Spears, J. W. 2003. Trace mineral bioavailability in ruminants. J. Nutr. 133(5):1506S-1509S.

Spears, J. W., and W. P. Weiss. 2008. Role of antioxidants and trace elements in health and immunity of transition dairy cows. Vet. J. 176(1):70-76. doi:10.1016/j.tvjl.2007.12.015.

Suttle, N. F. 2010. Mineral Nutrition of Livestock. 4th ed. CABI Publishing, New York.

Truong, D. H., M. A. Eghbal, W. Hindmarsh, S. H. Roth, and P. J. O'Brien. 2006. Molecular mechanisms of hydrogen sulfide toxicity. Drug Metab. Rev. 38(4):733-744. doi:10.1080/03602530600959607.

USDA ERS. 2007. Ethanol Expansion in the United States: How Will the Agricultural Sector Adjust.

USDA ERS. 2010. Commodity costs and returns: Data.

USDA ERS. 2013. Farm production expenses, 2009-2013F.

USDA ERS. 2015. Farm production expenses, 2014-2015F.

Vasconcelos, J. T., and M. L. Galyean. 2007. Nutritional recommendations of feedlot consulting nutritionists: The 2007 Texas Tech University survey. J. Anim. Sci. 85(10):2772-2781. doi:10.2527/jas.2007-0261.

Veerkamp, R. F. and G.C. Emmans. 1995a. Sources of genetic variation in energetic efficiency of dairy cows. Livest. Prod. Sci. 44: 87-97.

Veerkamp, R. F., G.C. Emmans, A.R. Cromie and G. Simm. 1995b. Variance components for residual feed intake in dairy cows. Livest. Prod. Sci. 41: 111-120.

Wang, Z., J. D. Nkrumah, C. Li, J. A. Basarab, L. A. Goonewardene, E. K. Okine, D. H. Crews, Jr. and S. S. Moore. 2006. Test duration for growth, feed intake, and feed efficiency in beef cattle using the GrowSafe system. J. Anim. Sci. 84: 2289-2298

Webster, A. J. F., P. O. Osuji, F. White, and J. F. Ingram. 1975. The influence of food intake on portal blood flow and heat production in the digestive tract of sheep. Brit. J. Nutr. 34(1):125-139.

Weisiger, R. A. and I. Fridovich. 1973. Mitochondrial superoxide dismutase – Site of synthesis and intramitochondrial localization. J. Biol. Chem. 248(13):4793-4796.

Weiss, W. P. 2001. Effect of dietary vitamin C on concentrations of ascorbic acid in plasma and milk. J. Dairy Sci. 84:2302-2307.

Willems, O. W., S. P. Miller, and B. J. Wood. (2013). Assessment of residual body weight gain and residual intake and body weight gain as feed efficiency traits in the turkey (*Meleagris gallopavo*). Genetics Selection Evolution. 45(1): 1-8.

Willson, R. L. 1987. Vitamin, selenium, zinc and copper interactions in free radical protection against ill-placed iron. Proc. Nutr. Soc. 46:27-34.

Xin, Z., D. F. Waterman, R. W. Hemken, and R. J. Harmon. 1991. Effects of copper status on neutrophil function, superoxide dismutase, and copper distribution in steers. J. Dairy Sci. 74(9):3078-3085. doi: 10.3168/jds.S0022-0302(91)78493-2.

CHAPTER 3.

INFLUENCE OF GROWING PHASE FEED EFFICIENCY CLASSIFICATION ON FINISHING PHASE GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS OF BEEF STEERS FED DIFFERENT DIET TYPES

A paper to be submitted for publication to *The Journal of Animal Science*

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Abstract

A five-year study was conducted using 985 crossbred steers (464 ± 32 kg, SD) fed in six separate groups to determine the influence of growing phase (GP) feed efficiency (FE) classification and diet type on finishing phase (FP) FE of steers. At University of Missouri, steers were fed GP whole shell corn (G-Corn; 528 steers) or roughage-based (G-Rough; 457 steers) diets using GrowSafe feed bunks to measure DMI for 69-89 d. At the end of the GP, steers were ranked by residual feed intake (RFI), shipped to Iowa State University, and blocked into FP pens (5-6 steers/pen) by GP diet and RFI rank (upper, middle, or lower one-third). Steers were transitioned to either FP cracked corn or byproduct-based diets and fed until 1.27 cm backfat was reached, with Optaflexx (200 mg/d) fed for the last 28-32 d prior to harvest. After completion of the sixth group, average growing phase G:F within GP diet was calculated for each FP pen (168 total pens) using GP initial BW as a covariate (G-Corn: 0.207 ± 0.038, SD; G-Rough: 0.185 ± 0.036, SD). Pens were classified as highly (HFE; > 0.5

SD from the G:F mean; 58 pens), mid (MFE; ± 0.5 SD from the G:F mean; 60 pens), or lowly (LFE; < 0.5 SD from the G:F mean; 50 pens) feed efficient. Data were analyzed using PROC MIXED of SAS. Experimental unit was FP pen and the model included the fixed effects of GP diet, FE classification, FP diet, and the interactions. Group (1-6) was included as a fixed effect. There were no three-way interactions ($P \geq 0.2$) for any measured traits. Finishing phase G:F was not affected by any interactions ($P \geq 0.5$) but was greater ($P \leq 0.03$) for HFE versus MFE and LFE and greater ($P = 0.02$) for MFE versus LFE. Growing phase diet × FE classification effects were detected ($P \leq 0.01$) for FP final BW (FBW), ADG, and DMI. Among G-Rough steers, HFE and MFE had greater ($P \leq 0.04$) FBW and ADG than LFE but among the G-Corn steers, LFE had heavier ($P = 0.03$) FBW than HFE while ADG was unaffected ($P \geq 0.2$) by FE classification. Dry matter intake was unaffected ($P \geq 0.3$) by FE classification among G-Rough steers but among G-Corn steers LFE had greater ($P \leq 0.003$) DMI than MFE and HFE. Overall, differences in finishing phase G:F between FE classifications were driven by different factors depending on diet; ADG differed among roughage-grown steers and DMI differed among corn-grown steers. Ultimately, steers classified as highly feed efficient during the GP still had superior FE during the FP.

Introduction

As production costs increase across the beef industry, improving feed efficiency (FE) is crucial to profitability and economic sustainability. Feed efficiency appears to be moderately heritable (Arthur et al., 2001), thus genetic improvement can be accomplished across the industry by identifying and selecting cattle based on individual FE. Due to the cost and infrastructure required to measure individual intake for FE calculations (Arthur and

Herd, 2008), FE is often measured during a single feeding phase in cattle fed one diet type (Arthur et al., 2001; Nkrumah et al., 2004). However, cattle fed for conventional quality-based markets are often grown on roughage-based diets and then transitioned to more energy-dense finishing diets. Consequently, measuring FE for a limited period would be beneficial if FE is repeatable over multiple feeding phases and can be predicted using one FE evaluation period. Previous work evaluating FE repeatability across consecutive feeding phases and differing diets is limited but has shown FE to be positively correlated across phases (Durunna et al., 2011). As grain-based growing phase diets are increasingly explored (Ridenour et al., 1982; Schoonmaker et al., 2003), it may be beneficial to examine FE in cattle grown on roughage as well as grain-based diets. Additionally, with the increased prevalence of grain byproducts in conventional feedlot diets, finishing diets may vary greatly in chemical composition. Thus, comparisons of FE phenotypes in both corn-based and fibrous byproduct-based diets are important to evaluate how these diets may influence FE. The objective of the current study was to determine the influence of growing phase FE classification and diet type on FE and growth performance of steers finished on corn or byproduct-based diets. The hypothesis was that steers classified as highly feed efficient during the growing phase would be more feed efficient during the finishing phase, regardless of finishing phase diet type.

Materials and Methods

Experimental Animals

All procedures and protocols were approved by the University of Missouri and Iowa State University animal care and use committees. A five-year study was conducted using 985

crossbred steers (464 ± 32 kg, SD) fed in six separate groups (167, 82, 190, 188, 179, and 179 total steers for groups 1-6, respectively). Steers were purchased from Missouri and surrounding states and 74% were black-hided. Steers were fed at University of Missouri (MU) for the growing phase, fed at Iowa State University (ISU) for the finishing phase, and harvested at a commercial packing facility in Denison, IA (Tyson Fresh Meats).

Growing Phase

Following a minimum 21 d receiving phase at MU, steers were weighed prior to feeding on two consecutive d to establish a growing phase initial BW. Steers were stratified by initial BW across one of two growing phase diets formulated to meet or exceed NRC recommendations for growing cattle (NRC, 2000; Table 1); a whole shell corn-based diet (G-Corn; 528 steers) or a roughage-based diet (G-Rough; 457 steers). All steers received an electronic ID tag (Allflex US Inc., Dallas-Fort Worth Airport, TX), a pour-on (Cydectin, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) to control external and internal parasites, and vaccinations (Bovi Shield Gold 5 and Ultrabac 7, Zoetis, Florham Park, NJ). Steers were implanted with a combination implant containing 16 mg estrogen and 80 mg trenbolone acetate (Component TE-IS, Elanco Animal Health, Greenfield, IN or Revalor IS, Merck Animal Health, Summit, NJ). Steers were housed in earthen lots with access to shelter, *ad libitum* water access, and fed using Growsafe feed bunks (GrowSafe Systems Ltd., Airdrie, AB, Canada) capable of measuring individual feed intake. Feed ingredients were blended and fed once daily using a truck-mounted mixer. Feed calls were made each morning and daily feed delivery was targeted at 5% more than *ad libitum* intake. Intermediate BW measurements were taken prior to feeding every 14 to 28 d and at the

conclusion of the growing phase (69 to 89 d), steers were weighed prior to feeding on two consecutive d to establish growing phase final BW. Total mixed ration (TMR) samples were collected weekly and dried at 55°C to determine DM content for DMI calculations. The TMR samples were analyzed for NDF (α-amylase included; Van Soest et al., 1991) and ADF (Goering and Van Soest, 1970) using an ANKOM200 Fiber Analyzer (ANKOM Technology, Macedon, NY), and were analyzed for CP via combustion (AOAC, 1990; LECO Tru-Mac, LECO Corporation, St. Joseph, MI). At the conclusion of the growing phase, residual feed intake (RFI; Basarab et al., 2003) was calculated for steers within diet by regressing DMI on ADG and metabolic mid-BW (MMBW; [average growing phase BW] ^ 0.75) and steers were ranked by RFI within growing phase diet (G-Corn or G-Rough) for use in finishing phase pen assignments.

Finishing Phase

After the growing phase, steers were trucked 435 km to ISU and blocked into finishing phase pens (5-6 steers/pen) by growing phase diet and RFI ranking (upper, middle, or lower one-third). Steers were fed receiving diets nutritionally similar to their growing phase diets; four of the 6 groups were fed receiving diets for 5 d while two of the groups were fed receiving diets for 17 d to facilitate diet digestibility analysis (data reported elsewhere). Steers were then transitioned over 14 to 21 d to either a cracked corn-based diet (F-Corn; Table 2) or a byproduct-based diet (F-Byp) formulated to meet or exceed NRC recommendations for growing cattle (NRC, 2000). Each morning, bunks were scored prior to feeding and pen feed delivery was determined using a modified slick bunk practice previously described by Drewnoski et al. (2014). Weekly TMR samples and monthly orts

were collected and dried at 70°C for 48 h to determine DM content. Finishing phase TMR samples were analyzed for NDF, ADF, and CP using methods described for growing phase TMR analysis. Dry matter intake was calculated using feed deliveries and orts, corrected for DM. Bodyweight was measured prior to feeding on two consecutive d to determine finishing phase initial BW (IBW). Intermediate finishing phase BW were measured every 28 d and steers were implanted on d 28 with a combination implant containing 120 mg trenbolone acetate, 24 mg estrogen, and 29 mg tylosin tartrate (Component TE-S with Tylosin, Elanco Animal Health, Greenfield, IN). Ractopamine hydrochloride (Optaflexx, Elanco Animal Health, Greenfield, IN) was fed for the final 28 to 32 d at a rate of 200 $mg \cdot steer^{-1} \cdot d^{-1}$ and steers were harvested when the majority of steers had an estimated 1.27 cm backfat depth by visual appraisal. Steers were weighed prior to feeding on the final two d of the finishing phase to determine final BW (FBW). All live BW were pencil-shrunk 4%. At harvest, carcasses were chilled for 24 h at the processing plant and then ribbed between the 12th and 13th ribs. Representatives of Tri-County Steer Carcass Futurity Cooperative (Lewis, IA) collected carcass data at the plant and were masked to treatments. Carcass data included HCW, 12th rib backfat, ribeye area (REA), KPH, calculated yield grade, and marbling score (300 = slight, 400 = small, 500 = modest). Dressing percent was calculated (HCW/FBW).

Feed efficiency classification

Within each group, cattle were blocked to finishing phase pens based on growing phase RFI rankings within growing diet. However, after data were collected for all six groups, average growing phase G:F was calculated for each set of steers (5-6 steers/pen) assigned to a particular finishing phase pen (985 total steers, 168 total pens). Using this

information, a growing phase G:F value for each finishing phase pen of steers was calculated using growing phase initial BW as a covariate in the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). To assess the impact of growing phase feed efficiency on subsequent finishing phase feed efficiency, live growth and carcass performance were compared between finishing phase pens classified by average growing phase G:F within diet (G-Corn: 0.207 ± 0.038, SD; G-Rough: 0.185 ± 0.036, SD). Pens were classified as highly (HFE; > 0.5 SD from the G:F mean; Table 3), mid (MFE; ± 0.5 SD from the G:F mean), or lowly (LFE; < 0.5 SD from the G:F mean) feed efficient. In total, there were 58 HFE (G-Corn: 33 pens, G-Rough: 25 pens), 60 MFE (G-Corn: 30 pens, G-Rough: 30 pens), and 50 LFE (G-Corn: 27 pens, G-Rough: 23 pens) classified finishing phase pens. Using equations proposed by Plascencia et al. (1999) and finishing phase growth data for each finishing phase pen, finishing phase dietary NE_g values were calculated for each pen.

Statistical Analysis

Finishing phase growth and carcass data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with finishing phase pen as the experimental unit. The model included the fixed effects of growing phase diet (G-Corn, G-Rough), growing phase feed efficiency classification (HFE, 44 pens; MFE, 75 pens; LFE, 49 pens), finishing phase diet (F-Corn, F-Byp; 84 pens/diet), and the interactions. Group (1-6) was also included in the model as a fixed effect. Interactions were removed from the model if the interaction *P* value was > 0.20. No three-way interactions were significant for finishing phase growth or carcass traits. Finishing phase IBW was tested and included in the model as a covariate for finishing phase FBW, DMI, G:F, and HCW. A Cook's D outlier test was used to identify outliers prior

to data analysis but none were identified. Significance was declared at $P \leq 0.05$, with tendencies declared when $0.06 < P < 0.10$. The values reported are least square means and standard errors of the means generated with the lsmeans command and compared using the pdiff command.

Results

Finishing phase growth and carcass traits

There were no growing phase diet × FE classification × finishing phase diet effects ($P \geq 0.2$) on finishing phase growth or carcass traits. Additionally, there were no growing phase FE classification × finishing phase diet interaction effects ($P \geq 0.1$) on growth or carcass traits with the exception of dressing percent ($P = 0.04$). Although dressing percent within byproduct-finished steers did not differ ($P \geq 0.7$) due to FE classification (63.7 ± 0.18 average % dress, SE; data not shown), the corn-finished HFE steers (62.4 ± 0.19 % dress, SE) had lesser ($P \leq 0.01$) dressing percent than the corn-finished MFE and LFE steers (63.1 ± 0.17 average % dress, SE).

There were growing phase diet × finishing phase diet interactions ($P \leq 0.02$; Table 5) for finishing phase FBW, ADG, and DMI. The G-Rough/F-Byp steers had greater ($P \leq 0.04$) FBW and DMI than any other growing phase diet × finishing phase diet combination. The G-Rough/F-Byp steers had greater ($P \leq 0.05$) ADG than the corn-finished steers, while the G-Rough/F-Corn steers had lesser ($P = 0.04$) ADG than the G-Corn/F-Byp steers. There were no ADG differences ($P = 0.13$) among the byproduct-finished steers and no ADG differences ($P = 0.6$) among the corn-grown steers. Finishing phase G:F was not affected by the growing phase diet × finishing phase diet interaction ($P = 0.5$) or the growing phase diet × FE

classification interaction ($P = 0.8$), but was impacted by the main effect of growing phase FE classification ($P = 0.002$; Figure 1). Finishing phase G:F was greater ($P \leq 0.03$) for HFE steers than steers classified as MFE or LFE; MFE steers had greater ($P = 0.02$) finishing phase G:F than LFE steers. Calculated finishing phase NE_g values were affected ($P = 0.001$; Figure 2) by FE classification but were unchanged ($P \geq 0.2$) due to finishing phase diet or any interactions. The HFE steers had greater ($P \leq 0.04$) calculated finishing phase NE_g than MFE and LFE steers; NE_g values were also greater ($P = 0.01$) for MFE steers relative to LFE steers. Finally, finishing phase NE_g values were greater ($P = 0.01$; data not shown) for corn-grown steers (1.66 ± 0.009 Mcal NE_g/kg DM, SE) versus roughage-grown steers (1.62 ± 0.011 Mcal NE_g/kg DM, SE).

Carcass traits were also affected by growing phase and finishing phase diets. There tended ($P = 0.06$; Table 5) to be a growing phase diet × finishing phase diet interaction for HCW; among the byproduct-finished steers, the roughage-grown steers had heavier HCW than the corn-grown steers but there were no differences among the corn-finished steers. There was a growing phase diet × finishing phase diet interaction ($P = 0.003$) for backfat in which the G-Rough/F-Byp steers had thicker ($P \leq 0.002$) backfat than any other growing phase diet × finishing phase diet combination. There were no differences ($P \geq 0.1$) in backfat among the corn-grown steers, and no differences ($P = 0.3$) in backfat among the corn-finished steers. The G-Rough/F-Corn steers had lesser ($P = 0.01$) backfat than the G-Corn/F-Byp steers. There was a growing phase diet × finishing phase diet interaction ($P = 0.006$) for yield grade in which the G-Rough/F-Byp steers had the greater ($P \leq 0.01$) yield grade and there were no yield grade differences ($P \geq 0.1$) detected among the G-Rough/F-Corn steers and the corn-grown steers. There were no growing phase diet × finishing phase diet

interactions ($P \geq 0.5$) for REA, dressing percent, or KPH. The byproduct-finished steers had greater ($P = 0.01$) REA than the corn-finished steers (89.5 vs. 88.1 cm^2 REA). The byproduct-finished steers had greater ($P = 0.03$) KPH than the corn-finished steers (2.35 vs. 2.30%) but KPH was unaffected ($P = 0.2$) by growing phase diet.

Growing phase diet × FE classification effects were detected ($P \leq 0.01$; Table 6) for finishing phase FBW, ADG, DMI as well as HCW and REA. Among the roughage-grown steers, the HFE and MFE steers had heavier ($P \leq 0.04$) FBW than the LFE steers but among the corn-grown steers, the LFE steers had heavier ($P = 0.03$) FBW than the HFE steers. Among the roughage-grown steers, the HFE and MFE steers also had ($P \leq 0.01$) greater ADG than the LFE steers but among the corn-grown steers, ADG was unaffected ($P \geq 0.2$) by FE classification. Dry matter intake was unaffected ($P \geq 0.3$) by FE classification within the roughage-grown groups but within the corn-grown groups, the LFE steers had greater ($P \leq 0.003$) DMI than the MFE and HFE steers. There were no differences ($P \geq 0.1$) in HCW among the roughage-grown steers due to FE classification but among the corn-grown steers, the HFE steers had lighter ($P \leq 0.01$) HCW than the LFE and MFE steers. Roughage-grown HFE steers had larger ($P \leq 0.04$) REA than any other growing phase diet × FE classification combination. The MFE steers, regardless of diet, had larger ($P \leq 0.05$) REA than the corn-grown LFE steers, but there were no differences ($P \geq 0.1$) in REA among the roughage-grown MFE, roughage-grown LFE, corn-grown HFE steers, and corn-grown MFE steers. Marbling score was unaffected ($P \geq 0.2$; data not shown) by any diet or interaction effects but the HFE steers (417 ± 5.6, SE) had lesser ($P \leq 0.01$) marbling score than the MFE (433 ± 4.3, SE) and LFE (439 ± 5.1, SE) steers; marbling score did not differ ($P = 0.4$) between MFE and LFE steers.

Discussion

In the current study, pens of steers classified as highly feed efficient based on growing phase G:F still had greater G:F during the finishing phase, a pattern that was consistent for the pens classified as mid feed efficient and lowly feed efficient as well. Furthermore, the growing phase FE classification relationship with finishing phase G:F was independent of dietary effects as there were no diet or diet × FE classification interactions. This G:F repeatability between phases is similar to results reported by Durunna et al. (2011; 2012) in which G:F was positively correlated between growing and finishing phases in crossbred steers and heifers fed differing or similar diets during each phase. Durunna et al. (2011) also classified steers by FE based on a similar 0.5 SD cutoff as utilized in the current study. Durunna et al. (2011) also reported relative movement of steers from one FE classification to another, noting that of the 331 steers fed differing diets during the growing and finishing phase, 160 (48.3%) steers moved one FE classification and 44 (13.3%) moved two classifications. Reclassifying pens by finishing phase G:F within finishing phase diet, FE classification repeatability was slightly better in the current study; of the 168 pens fed over the course of the current study, 70 pens (41.7%, data not shown) moved one classification and 10 pens (11.9%) moved two classifications. Kelly et al. (2010) reported a positive correlation between FE measured during a yearling feeding period and a finishing period in beef heifers fed similar high fiber diets during both periods. Interestingly, the differences in finishing phase G:F between FE classifications in the current study appear to be driven by different factors depending on growing phase diet.

The most striking effects of growing phase diet × FE classification on finishing phase performance are noted in ADG and DMI. Within both diets, differences in FBW were

congruent with ADG differences. Among the corn-grown steers, ADG did not differ between FE classifications, but DMI was lesser for the HFE and MFE steers than the LFE steers; thus, DMI drove the differences in finishing phase G:F among FE classifications in the corn-grown steers. Conversely, among the roughage-grown steers there was no difference in DMI between FE classifications but ADG was greater for the HFE and MFE steers than LFE steers; suggesting ADG drove finishing phase G:F differences among FE classifications in the roughage-grown steers. Greater fiber utilization has previously been noted in roughage-grown steers classified as highly efficient (Russell, 2015) and this digestibility advantage likely contributed to the growth advantage in the highly versus lowly feed efficient roughage-grown steers in the present study. During the growing phase, though DMI did not differ due to growing phase diet × FE classification ($P = 0.8$; data not shown), ADG tended to differ ($P = 0.09$) among the FE classifications within the roughage-grown group (HFE: 1.96 ± 0.030; MFE: 1.89± 0.023; LFE: 1.78 ± 0.029 kg/d, SE). Average daily gain did not differ ($P \geq 0.3$, data not shown) among FE classifications within the corn group during the growing phase (1.86 ± 0.027 kg/d, SE). Consistent differences in ADG among the roughage-grown steers across both phases are indicative of the inherent genotypic variation in the steers that lead to phenotypic FE variation. Although not reported here, all steers in the current study were genotyped with the Illumina BovineSNP50 assay (Matukumalli et al., 2009) to evaluate the relationship between FE phenotype and genotype with the goal of generating tools that can identify the ideal diet type for individual animals.

The differences in finishing phase efficiency between the growing phase FE classifications are well illustrated in NE_g values that were generated using finishing phase pen growth data from the current study. Differences in calculated finishing phase NE_g were

detected due to growing phase diet and FE classification but not for the interaction, though NE_g differences are likely not as reflective of FE variation as the contributing performance variables (Vasconcelos and Galyean, 2008). Nevertheless, NE_g was expectedly greater in the HFE steers and was poorest among the LFE steers. Interestingly, the greater finishing phase NE_g calculated for corn-grown steers compared with roughage-grown steers, regardless of finishing phase diet, suggests that growing phase diet may have implications on finishing phase energetics. Numerical differences in finishing phase NE_g were more pronounced among the roughage-grown steers (HFE: 1.66, MFE:1.62, LFE: 1.57 Mcal NE_g/kg DM; data not shown) than among the corn-grown steers (HFE: 1.69, MFE: 1.67, LFE: 1.62 Mcal NE_g/kg DM). This increased NE_g variation suggests that variation in growing phase FE may have a greater impact on finishing phase performance in cattle grown on roughage-based diets, reasons for which are unclear. Jones et al. (1985) found no difference in reticulo-rumen size in concentrate versus forage-fed steers, and in the current study there was no consistent difference in finishing phase DMI between roughage and corn-grown cattle. Accordingly, it is unlikely that finishing phase ME differences were due to variations in rumen capacity or DMI resulting from growing phase diet type. Further research would be beneficial to compare steers representing phenotypic FE extremes and evaluate the effects of diet type on internal organ sizes and volumes in an attempt to better explain the greater variation among roughage-grown steers of differing FE phenotypes.

Dietary effects on finishing phase performance were overwhelmingly driven by the performance of the roughage-grown byproduct-finished steers. The roughage-grown byproduct-finished steers were slightly heavier at the beginning of the finishing phase but after applying IBW as a covariate, still had greater average FBW. The roughage-grown

byproduct-finished steers had increased ADG and DMI compared to the other diet combinations but no differences were detected in G:F due to any growing phase × finishing phase diet combination. In a study employing diets similar to finishing phase diets fed in the current study, Trejo et al. (2010) reported increased final BW, ADG, and DMI but no difference in G:F in steers finished with a dry byproduct-based diet (40% dried distillers grain, 35% soybean hulls) versus steers finished with a 75% dry-rolled corn diet. Also similar to the current study, Schoonmaker et al. (2003) fed crossbred steers one of two growing phase diets, either a 71% corn diet or a 55% soybean hulls and 30% hay diet, followed by a common 76% corn finishing phase diet until a common backfat was reached. Schoonmaker et al. (2003) reported no differences in finishing phase FBW, ADG, DMI, or G:F due to growing phase diet. Distillers grains are typically regarded as energetically greater than corn (Klopfenstein et al., 2008) but in spite of increased distillers grain inclusion in the byproduct finishing diet, calculated NE_g was unaffected by finishing phase diet. The equivalent energetic value of the finishing phase diets may have resulted from a negative effect of corn inclusion on fiber digestibility (Grigsby et al., 1993) in the byproduct-based diet, especially due to 20% DM inclusion of soybean hulls, a feedstuff that tends to decrease diet digestibility in corn-containing diets (Ferreria, 2011). Alternatively, since NE_g calculations have a greater sensitivity to DMI changes than ADG changes (Vaconcelos and Galyean, 2008), anticipated improvements in NE_g due to ADG in the byproduct-finished steers may have simply been counteracted by increased DMI in the NE_g calculation.

Carcass trait differences due to FE classification were limited. The HFE steers had decreased marbling scores but all three FE classifications had sufficient marbling score to grade low choice on the USDA Quality Grade grid. Hot carcass weight was largely

unchanged due to FE classification though corn-grown HFE steers had lighter carcasses than the other corn-grown steers. In grain-finished steers classified by FE using the same 0.5 SD cutoff used in the current study, Nkrumah et al. (2004; 2007) observed no difference in HCW or marbling score between FE classifications. The roughage-grown HFE steers in the current study had greater REA than the other growing phase diet × FE classification groups, but effects of FE classification on REA were mixed within the corn-grown steers, with MFE generating larger REA than the LFE. Among the roughage-grown steers, ADG, REA, and growing phase FE appear to have a numerically linear relationship, such that the decreased finishing phase ADG noted in the less efficient steers may be due to decreased muscle accretion since backfat was unaffected by FE classification. Although factors affecting muscle accretion were not explored in the current study, previous FE investigations have reported increased N retention (Harris et al., 2012) and decreased protein turnover (Cruzen et al., 2013) associated with highly feed efficient pigs. Nkrumah et al. (2004; 2007) reported thicker backfat and increased yield grade, but no difference in REA in lowly versus highly efficient steers. Interestingly, dressing percent varied due to FE classification among the corn-finished steers but not among the byproduct-finished steers in the current study. The underlying reasons for this observation are unclear as the corn-fed HFE steers had lesser DMI which would expectedly lead to less gut fill and improved dressing percent, yet dressing percent was poorest for these steers. Ultimately, FE classification appears to have limited effects on carcass traits when steers are fed to a common degree of finish; consequently, selection for improved FE will likely have minimal impacts on the consumer product.

Like growth performance in the current study, carcass traits measured in the roughage-grown byproduct-finished steers generally differed from traits measured in steers

fed the other diet combinations. The roughage-grown byproduct-finished steers had thicker backfat, greater yield grade, and heavier HCW. The increased backfat and fat accretion observed in the roughage-grown byproduct-finished steers is likely a result of greater energy intake as those steers also had markedly increased DMI. Similarly, Trejo et al. (2010) reported heavier HCW from steers finished with a byproduct-based diet versus steers finished with a 75% dry-rolled corn diet, but conversely, found no effect of finishing phase diet on backfat or yield grade. Also similar to the current study, Trejo et al. (2010) reported no difference REA, marbling score, or KPH due to finishing phase diet. Regardless of growing phase diet, steers finished with the corn-based did not differ for any carcass traits measured. Likewise, Schoonmaker et al. (2003) reported no differences in HCW, backfat, REA, KPH, yield grade, or marbling score between crossbred steers fed corn or roughage-based growing diets, fed a common corn-based finishing diet, and slaughtered when a common backfat thickness was reached. Consequently, it appears that corn-based finishing diets alleviate differences that may otherwise arise due to growing phase diet. Ultimately, effects of growing and finishing phase diets on carcass traits were consistent with previous research. Limited work has evaluated growth and carcass traits due to multiple growing phase diets and multiple finishing phase diets, especially as new industry byproducts become available as feedstuffs for cattle. Thus, further work would be beneficial to provide producers with a more complete evaluation of conceivable diet combinations that can be used in feedlot cattle production.

Implications

As feed costs increase, the economic sustainability of the beef industry will be increasingly dependent on improving FE via selection against inefficient cattle. Previous investigations into the repeatability of FE across feeding phases are limited, especially when diet types change from one phase to the next. In the current study, pens classified as highly feed efficient in the growing phase maintained greater G:F in the finishing phase and the relationship was consistent for mid and lowly feed efficient pens. The relationship between diet and growing phase FE phenotype is complex and affects both finishing phase performance and post-harvest carcass traits. Further research would be valuable to investigate the underlying causes of variation in FE in roughage-grown steers versus corn-grown steers, especially as the underlying drivers behind FE variation differed depending on diet type. Despite no differences in finishing phase G:F due to diet, the roughage-grown byproduct-finished steers excelled in ADG due to increased DMI and generated heavier carcasses with no decrease in marbling score; an economically advantageous diet combination under current market conditions. At the same time, differences were limited in corn-finished steers, regardless of growing phase diet; thus growth and carcass variation was largely driven by steers fed more fibrous diets. Overall, FE appears relatively repeatable across consecutive feeding phases. Future research evaluating cattle performance using multiple growing and finishing phase diet combinations would be advantageous, with a particular focus on cattle fed fibrous diets.

Literature Cited

AOAC. 1990. Official methods of analysis. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Arthur, P. F., J. A. Archer, D. J. Johnston, R. M. Herd, E. C. Richardson and P. F. Parnell. 2001. Genetic and phenotypic variance and covariance components for feed intake, feed efficiency, and other postweaning traits in Angus cattle. J. Anim. Sci. 79: 2805-2811. doi:/2001.79112805x.

Arthur, P. F., and R. M. Herd. 2008. Residual feed intake in beef cattle. Revista Brasileira de Zootecnia. 37:269-279.

Basarab, J. A., M. A. Price, J. L. Aalhus, E. K. Okine, W. M. Snelling, and K. L. Lyle. 2003. Residual feed intake and body composition in young growing steers. Can. J. Anim. Sci. 83:189.-204. doi:10.4141/A02-065.

Cruzen, S. M., A. J. Harris, K. Hollinger, R. M. Punt, J. K. Grubbs, J. T. Selsby, J. C. M. Dekkers, N. K. Gabler, S. M. Lonergan, and E. Huff-Lonergan. 2013. Evidence of decreased muscle protein turnover in gilts selected for low residual feed intake. J. Anim. Sci. 91(8):4007-4016. doi:10.2527/jas2013-6413.

Drewnoski, M. E., P. Doane, and S. L. Hansen. 2014. Ferric citrate decreases ruminal hydrogen sulphide concentrations in feedlot cattle fed diets high in sulphate. Brit. J. Nutr. 111(2):261-269. doi:10.1017/S0007114513002304.

Durunna, O. N., F. D. N. Mujibi, L. Goonewardene, E. K. Okine, J. A. Basarab, Z. Wang, and S. S. Moore. 2011. Feed efficiency differences and reranking in beef steers fed grower and finisher diets. J. Anim. Sci. 89:158-167. doi: 10.2527/jas.2009-2514.

Durunna, O. N., M. G. Colazo, D. J. Ambrose, D. McCartney, V. S. Baron, and J. A. Basarab. 2012. Evidence of residual feed intake reranking in crossbred replacement heifers. J. Anim. Sci. 90(3):734-741. doi:10.2527/jas.2011-4264.

Ferreira, E. M., A. V. Pires, I. Susin, C. Q. Mendes, M. A. A. Queiroz, R. C. Araujo, R. S. Gentil and S. C. Loerch. 2011. Apparent digestibility, nitrogen balance, and ruminal constituents in ram lambs fed high-concentrate diets containing soybean hulls. J. Anim. Sci. 89: 4127-4133. doi:10.2527/jas.2010-3419.

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analyses (apparatus, reagents, procedures, and applications). Agric. Handbook No. 379. ARS-USDA, Washington, DC.

Grigsby, K. N., M. S. Kerley, J. A. Paterson and J. C. Weigel. 1993. Combinations of starch and digestible fiber in supplements for steers consuming a low-quality bromegrass hay diet. J. Anim. Sci. 71: 1057-1064. doi:/1993.7141057x.

Harris, A. J., J. F. Patience, S. M. Lonergan, C. J. M. Dekkers, and N. K. Gabler. 2012. Improved nutrient digestibility and retention partially explains feed efficiency gains in pigs selected for low residual feed intake. J. Anim. Sci. 90:164-166. doi:10.2527/jas53855.

Jones, S. D. M., R. E. Rompala, and L. E. Jeremiah. 1985. Growth and composition of the empty body in steers of different maturity types fed concentrate or forage diets. J. Anim. Sci. 60(2):427-433. doi:10.2134/jas1985.602427x.

Kelly, A. K., M. McGee, D. H. Crews, Jr., T. Sweeney, T. M. Boland, and D. A. Kenny. 2010. Repeatability of feed efficiency, carcass ultrasound, feeding behavior, and blood metabolic variables in finishing heifers divergently selected for residual feed intake. J. Anim. Sci. 88:3214-3225. doi: 10.2527/jas.2009-2700.

Klopfenstein, T. J., G. E. Erickson, and V. R. Bremer. 2008. BOARD-INVITED REVIEW: Use of distillers by-products in the beef cattle feeding industry. J. Anim. Sci. 86(5):1223-1231. doi:10.2527/jas.2007-0550.

Matukumalli, L. K., C. T. Lawley, R. D. Schnabel, J. F. Taylor, M. F. Allan, M. P. Heaton, J. O'Connell, S. S. Moore, T. P. L. Smith, T. S. Sonstegard, and C. P. Van Tassell. 2009. Development and characterization of a high density SNP genotyping assay for cattle. PLoS One 4: e5350.

Nkrumah, J. D., J. A. Basarab, M. A. Price, E. K. Okine, A. Ammoura, S. Guercio, C. Hansen, C. Li, B. Benkel, B. Murdoch, and S. S. Moore. 2004. Different measures of energetic efficiency and their phenotypic relationships with growth, feed intake, and ultrasound and carcass merit in hybrid cattle. J. Anim. Sci. 82:2451–2459. doi:/2004.8282451x.

Nkrumah, J. D., J. A. Basarab, Z. Wang, C. Li, M. A. Price, E. K. Okine, D. H. Crews, and S. S. Moore. 2007. Genetic and phenotypic relationships of feed intake and measures of efficiency with growth and carcass merit of beef cattle. J. Anim. Sci. 85(10):2711-2720. doi:10.2527/jas.2006-767.

NRC. 2000. Nutrient requirements of beef cattle. 7th rev. ed. Natl. Acad. Press, Washington, D.C.

Plascencia, A., M. Estrada, and R. A. Zinn. 1999. Influence of free fatty acid content on the feeding value of yellow grease in finishing diets for feedlot cattle. J. Anim. Sci. 77(10):2603-2609. doi:/1999.77102603x.

Ridenour, K. W., H. E. Kiesling, G. P. Lofgreen, and D. M. Stiffler. 1982. Feedlot performance and carcass characteristics of beef steers grown and finished under different nutrition and management programs. J. Anim. Sci. 54(6):1115-1119. doi:10.2134/jas1982.5461115x.

Russell, J. R. 2015. Feed efficiency in beef cattle: Relationship with digestibility, antioxidant activity, oxidative stress, growth performance, and carcass characteristics. PhD Diss. Iowa State Univ., Ames.

Schoonmaker, J. P., M. J. Cecava, D. B. Faulkner, F. L. Fluharty, H. N. Zerby, and S. C. Loerch. 2003. Effect of source of energy and rate of growth on performance, carcass characteristics, ruminal fermentation, and serum glucose and insulin of early-weaned steers. J. Anim. Sci. 81(4):843-855. doi:/2003.814843x.

Trejo, C. O., D. B. Faulkner, A. Shreck, J. W. Homm, T. G. Nash, S. L. Rodriguez-Zas, and L. L. Berger. 2010. Effects of co-products and breed of sire on the performance, carcass characteristics, and rates of ultrasound backfat and marbling deposition in feedlot cattle. Prof. Anim. Sci. 26(6):620-630.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597. doi:10.3168/jds.S0022-0302(91)78551-2.

Vasconcelos, J. T., and M. L. Galyean. 2008. Technical Note: Do dietary net energy values calculated from performance data offer increased sensitivity for detecting treatment differences?. J. Anim. Sci. 86(10):2756-2760. doi:10.2527/jas.2008-1057.

Table 1. Composition and analysis of growing phase whole-shell corn-based diets (G-Corn) fed to steers

	Group			
Ingredient, % DM	1, 2, 3	4	5	6
Whole shell corn	78.59	70.92	65.10	64.26
Dried distillers grains	9.72	17.00	24.50	26.07
Soyplus[1]	6.25	6.38	4.51	4.96
Wheat middlings	2.65	2.00	-	-
Porcine blood meal	-	1.30	3.50	2.52
Limestone	1.50	1.40	1.21	1.09
Urea	0.39	0.60	0.47	0.19
Choice white grease	0.20	0.12	0.10	0.19
Salt	0.17	0.04	0.13	0.22
Vitamin premix[2]	0.17	0.16	0.25	0.23
Trace mineral premix[3]	0.17	0.07	0.09	-
Potassium chloride	0.17	-	-	-
Pellet binder	-	-	0.13	0.19
Rumensin 90[4]	0.01	0.01	0.01	0.01
Nutritional analysis[5]				
DM, % as-fed basis	90.7	90.3	88.3	85.1
NDF, % DM	17.8	20.2	21.1	26.4
ADF, % DM	4.4	5.0	4.9	6.5
CP, % DM	17.2	17.9	23.1	20.5

[1] Soyplus (West Central Cooperative, Ralston, IA).
[2] Vitamin premix fulfills 2,200 IU vitamin A, 275 IU vitamin D, 100 IU vitamin E per kg of diet.
[3] Trace mineral premix fulfills 10 mg Cu, 50 mg Fe, 20 mg Mn, 30 mg Zn, 0.1 mg Co, 0.1 mg Se, 0.5 mg I per kg diet.
[4] Provided Monensin at 150 $mg \cdot steer^{-1} \cdot d^{-1}$, Elanco Animal Health, Indianapolis, IN.
[5]Determined from analysis of total mixed rations.

Table 2. Composition and analysis of growing phase forage and soybean hull-based diets (G-Rough) fed to steers

	Group[1]			
Ingredient, % DM	1, 3	4	5	6
Soybean hull pellets	40.81	36.57	38.16	36.84
Alfalfa/grass baleage	34.21	-	-	-
Corn Silage	-	36.00	-	-
Rye baleage	-	-	32.49	-
Sudan baleage	-	-	-	36.25
Dried distillers grains	15.13	15.00	22.24	22.70
Soyplus[2]	-	5.50	4.05	1.75
Porcine blood meal	-	0.80	2.02	1.65
Ground corn	8.62	5.00	-	-
Limestone	0.57	0.70	0.61	0.35
Salt	0.25	0.07	0.11	0.18
Vitamin premix[3]	0.20	0.20	0.20	0.18
Trace mineral premix[4]	0.20	0.13	0.07	0.07
MFP[5]	-	0.03	0.05	0.03
Rumensin 90[6]	0.01	0.01	0.01	0.01
Nutritional analysis[7]				
DM, % as-fed basis	79.4	68.9	68.3	66.8
NDF, % DM	50.1	46.9	52.3	57.5
ADF, % DM	32.5	26.5	29.0	31.5
CP, % DM	17.2	16.0	22.3	20.8

[1] Forage and soybean hull-based diet was not fed during group 2.
[2] Soyplus (West Central Cooperative, Ralston, IA).
[3] Vitamin premix fulfills 2,200 IU vitamin A, 275 IU vitamin D, 100 IU vitamin E per kg of diet.
[4] Trace mineral premix fulfills 10 mg Cu, 50 mg Fe, 20 mg Mn, 30 mg Zn, 0.1 mg Co, 0.1 mg Se, 0.5 mg I per kg diet.
[5] DL-methionine hydroxy analogue calcium (84% methionine, Novus International, Saint Charles, MO).
[6] Provided Monensin at 150 $mg \cdot steer^{-1} \cdot d^{-1}$, Elanco Animal Health, Indianapolis, IN.
[7] Determined from analysis of total mixed rations.

Table 3. Composition and analysis of finishing phase diets fed to steers[1]

Ingredient, % DM	F-Corn[2]	F-Byp[2]
Cracked corn	75	30
Dried distillers grains	14.99	39.99
Soybean hull pellets	-	20
Bromegrass hay	8	8
Limestone	1.54	1.54
Salt	0.31	0.31
Vitamin A premix[3]	0.11	0.11
Trace mineral premix[4]	0.035	0.035
Rumensin 90[5]	0.013	0.013
Nutritional analysis[6]		
DM, % as-fed basis	84.5	84.1
NDF, % DM	24.4	42.7
ADF, % DM	8.0	18.7
CP, % DM	11.2	18.4

[1] Ingredient composition of finishing phase diets was consistent across all six groups.
[2] Finishing phase diets: F-Corn = cracked corn-based; F-Byp = dried distillers grains and soybean hull-based.
[3] Vitamin A premix contained 4,400,000 IU/kg.
[4] Provided per kilogram of diet (from inorganic sources): 30 mg Zn, 20 mg Mn, 0.5 mg I, 0.1 mg Se, 10 mg Cu, 0.1 mg Co.
[5] Provided Monensin at 200 $mg \cdot steer^{-1} \cdot d^{-1}$, Elanco Animal Health, Indianapolis, IN.
[6] Determined from analysis of total mixed rations.

Table 4. Descriptive statistics of growing phase feed efficiency classifications calculated for finishing phase pens across all groups

	G-Corn[1]			G-Rough[1]		
	HFE[2]	MFE[2]	LFE[2]	HFE[2]	MFE[2]	LFE[2]
Pens (n)	24	41	25	20	34	24
G:F[3]						
Average	0.258	0.218	0.180	0.228	0.196	0.169
Minimum	0.235	0.203	0.141	0.211	0.185	0.144
Maximum	0.298	0.233	0.202	0.262	0.208	0.183

[1] Growing phase diets: G-Corn = whole shell corn-based; G-Rough = forage and soybean hull-based.

[2] Feed efficiency classifications: HFE = highly feed efficient (> 0.5 SD from the G:F mean); MFE = mid feed efficiency (± 0.5 SD from the G:F mean); LFE = lowly feed efficient (< 0.5 SD from the G:F mean).

[3] Growing phase G:F for each finishing phase pen calculated using individual BW and DMI data for each steer housed in a finishing phase pen, and utilizing growing phase initial BW as a covariate in the MIXED procedure of SAS 9.3 (SAS Institute Inc., Cary, NC).

Table 5. Effect of growing phase and finishing phase diets on finishing phase growth performance and carcass traits

	G-Corn[1]		G-Rough[1]			*P*-values[3, 4]		
	F-Corn[2]	F-Byp[2]	F-Corn[2]	F-Byp[2]	SEM	G Diet	F Diet	G*F Diet
Live performance								
Initial BW[5], kg	453	456	464	467	-	-	-	-
Final BW[6,7], kg	609[b]	611[b]	606[b]	617[a]	2.0	0.4	0.001	0.02
ADG, kg/d	1.80[bc]	1.81[ab]	1.75[c]	1.86[a]	0.022	1.0	0.003	0.02
DMI[7], kg/d	10.8[b]	11.0[b]	10.7[b]	11.5[a]	0.09	0.02	<0.001	0.002
G:F[7]	0.167	0.166	0.163	0.164	0.0015	0.04	0.7	0.5
Carcass traits[8,9]								
HCW[7], kg	382[z]	389[y]	382[z]	394[x]	1.4	0.14	<0.001	0.06
DP, %	62.7	63.7	63.0	63.8	0.14	0.1	<0.001	0.6
BF, cm	1.32[bc]	1.39[b]	1.28[c]	1.53[a]	0.030	0.12	<0.001	0.003
KPH, %	2.29	2.33	2.31	2.38	0.025	0.2	0.03	0.6
REA, cm^2	87.1	88.9	89.1	90.0	0.58	0.02	0.01	0.5
YG	3.08[b]	3.13[b]	3.01[b]	3.32[a]	0.049	0.3	<0.001	0.006

[a, b, c] Least squares means in a row without common superscript differ ($P \leq 0.05$).

[x, y, z] Least squares means in a row without common superscript tend to differ ($P < 0.1$)

[1] Growing phase diets: G-Corn = whole shell corn-based; G-Rough = forage and soybean hull-based.

[2] Finishing phase diets: F-Corn = cracked corn-based; F-Byp = dried distillers grains and soybean hull-based.

[3] *P*-values: G Diet = main effect of growing phase diet; F Diet = main effect of finishing phase diet; G*F Diet = interaction effect of growing and finishing phase diets.

[4] Three-way interaction between growing phase diet, finishing phase diet, and growing phase feed efficiency classification was not significant ($P > 0.2$).

[5] Initial BW, pencil shrunk 4%.

[6] Final BW, pencil shrunk 4%.

[7] Initial BW applied as a covariate.

[8] Carcass traits: DP = dressing percent; BF = 12th rib backfat thickness; REA = ribeye area; YG = yield grade.

[9] Growing diet, finishing diet, and the interaction were not significant ($P \geq 0.2$) for marbling score.

Table 6. Effect of growing phase diet and feed efficiency classification on finishing phase growth performance and carcass traits

	G-Corn[1]			G-Rough[1]				
	LFE[2]	MFE[2]	HFE[2]	LFE[2]	MFE[2]	HFE[2]	SEM	*P*-value[3,4]
Live performance								
Initial BW[5], kg	448	457	459	460	462	475	-	-
Final BW[6, 7], kg	615[ab]	609[bc]	605[c]	605[c]	612[ab]	618[a]	2.6	0.001
ADG, kg/d	1.85[ab]	1.79[bc]	1.78[bc]	1.72[c]	1.82[ab]	1.87[a]	0.029	0.005
DMI[7], kg/d	11.3[a]	10.7[bc]	10.6[c]	11.0[ab]	11.1[a]	11.2[a]	0.12	0.002
Carcass traits								
HCW[7], kg	389[a]	386[a]	381[b]	385[ab]	387[a]	390[a]	1.9	0.003
REA[8], cm^2	86.6[c]	89.6[b]	87.9[bc]	87.9[bc]	89.1[b]	91.7[a]	0.78	0.01

[a, b, c] Least squares means in a row without common superscript differ ($P < 0.05$).

[1] Growing phase diets: G-Corn = whole shell corn-based; G-Rough = forage and soybean hull-based.

[2] Growing phase feed efficiency classifications: HFE = highly feed efficient (> 0.5 SD from the G:F mean); MFE = mid feed efficiency (± 0.5 SD from the G:F mean); LFE = lowly feed efficient (< 0.5 SD from the G:F mean).

[3] Interaction effect of growing phase diet and feed efficiency classification.

[4] Growing phase diet × feed efficiency classification interaction was not significant ($P \geq 0.14$) for G:F, dressing percent, backfat, KPH, yield grade, or marbling score; Three way interaction between growing phase diet, finishing phase diet, and growing phase feed efficiency classification was not significant ($P > 0.2$).

[5] Initial BW pencil shrunk 4%.

[6] Final BW, pencil shrunk 4%.

[7] Initial BW applied as a covariate.

[8] Ribeye area.

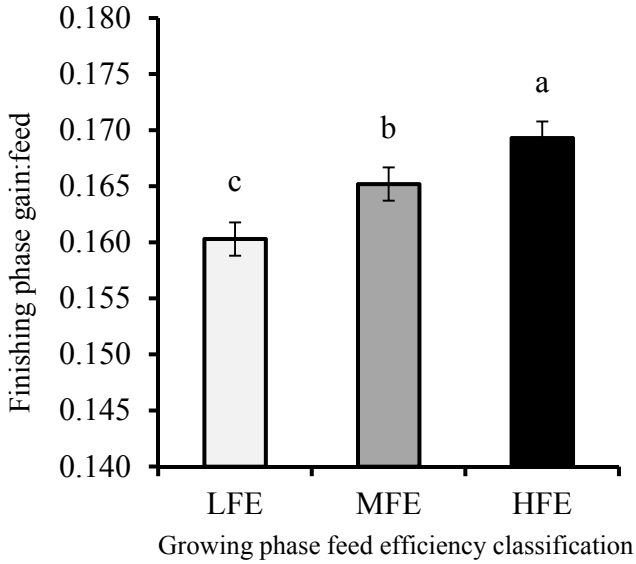

Figure 1. Finishing phase G:F in steers due to growing phase feed efficiency classification: HFE = highly feed efficient (> 0.5 SD from the growing phase G:F mean; n = 44 pens); MFE = mid feed efficiency (± 0.5 SD from the growing phase G:F mean; n = 75 pens); LFE = lowly feed efficient (< 0.5 SD from the growing phase G:F mean; n = 49 pens). Finishing phase initial BW applied as covariate. Values are means ± 0.0015, SEM. Means without common superscript differ ($P \leq 0.05$).

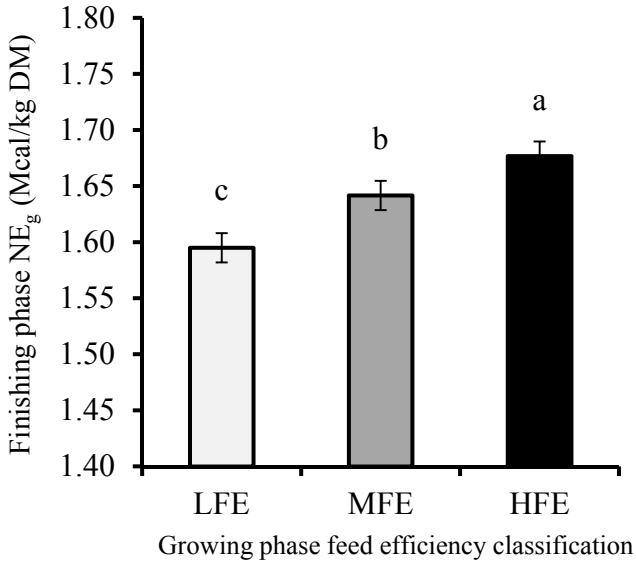

Figure 2. Finishing phase net energy for gain (NE_g; Mcal/kg DM) in steers due to growing phase feed efficiency classification: HFE = highly feed efficient (> 0.5 SD from the growing phase G:F mean; n = 44 pens); MFE = mid feed efficiency (± 0.5 SD from the growing phase G:F mean; n = 75 pens); LFE = lowly feed efficient (< 0.5 SD from the growing phase G:F mean; n = 49 pens). Net energy values calculated using equations from Plascencia et al. (1999) and finishing phase pen live performance. Values are means ± 0.013, SEM. Means without common superscript differ ($P \leq 0.05$).

CHAPTER 4.

INFLUENCE OF FEED EFFICIENCY CLASSIFICATION ON DIET DIGESTIBILITY AND GROWTH PERFORMANCE OF BEEF STEERS

A paper submitted to *The Journal of Animal Science*

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Abstract

The diet digestibility and feed efficiency (FE) relationship is not well characterized in cattle. The objective of the study was to determine effects of growing phase FE and diet, as well as finishing phase diet on diet digestibility and finishing phase FE. Two groups, totaling 373 crossbred steers, were fed for 70 d at the University of Missouri for the growing phase and then shipped to Iowa State University (ISU) for finishing. GrowSafe feed bunks were used during both feeding phases. Steers were fed either growing phase whole shell corn (G-Corn) or roughage-based (G-Rough) diets. Within each group, the 12 greatest and 12 least feed efficient steers from each growing diet (n = 96 total; 48 steers/group; 488 ± 5 kg) were selected for further evaluation. At ISU, steers were fed an average of 10 g titanium dioxide·steer^{-1}·d^{-1} (TiO_2) in receiving phase diets similar to growing diets for 15 d with fecal grab samples collected on d 14 and 15 to determine diet DM digestibility during receiving (GDMdig). For finishing, steers were transitioned to byproduct (F-Byp) or corn-based diets (F-Corn; 12 steers·growing×finishing diet combination^{-1}·group^{-1}). Optaflexx (200 mg/d) was

fed for 28 d prior to harvest and the TiO_2 protocol was repeated immediately before introducing Optaflexx to determine diet DM digestibility during finishing (FDMdig). Data from the two groups (96 steers) were pooled, steers were ranked by growing phase G:F, and then classified as the 24 greatest (HFE) or 24 least (LFE) feed efficient steers from each growing diet. Data were analyzed using PROC MIXED of SAS with group applied as a fixed effect. There was a positive correlation between GDMdig and FDMdig for steers fed similar diets during both feeding phases, G-Rough/F-Byp steers ($r = 0.68$, $P < 0.01$) and G-Corn/F-Corn steers ($r = 0.49$, $P = 0.02$) but a negative correlation for G:F between phases in G-Rough/F-Corn steers ($r = -0.57$, $P < 0.01$). Finishing G:F was greater in HFE versus LFE steers ($P = 0.04$) but there was no difference ($P \geq 0.5$) in GDMdig or FDMdig due to FE classification . There was a positive correlation for DM digestibility between feeding phases when steers were grown and finished on similar diets. Overall, FE was repeatable but was negatively correlated between phases when steers were roughage-grown and corn-finished, reinforcing the idea that cattle should be FE tested using diet types similar to the production environment of interest.

Introduction

Cost of gain is a major contributor to feedlot profitability and is predominately driven by feed conversion, or feed efficiency (FE). Feed efficiency can vary greatly between individuals but the underlying sources of variation are not well characterized. Activity level, methane production, tissue metabolism, and diet digestibility have all been credited with contributing to FE variation (Nkrumah et al., 2006; Richardson and Herd, 2004; Herd et al., 2004). The current investigation was focused on the contribution of diet digestibility, which

is estimated to be responsible for 10-14% of FE variation (Richardson and Herd, 2004; Herd et al., 2004). Comparing phenotypic FE extremes, previous work showed that cattle with greater FE had greater DM utilization than less efficient contemporaries (Nkrumah et al., 2006; Richardson et al., 1996). In a typical U.S. beef system targeting the quality-based market, animals are grown on roughage-based diets and then transitioned to grain-based finishing diets. Cattle are often only FE phenotyped during the growing phase, thus there is a need for investigation of the relationship between digestibility and FE phenotype as diets change, as well as whether FE phenotype is repeatable across diet types. Though limited work exists, Kelly et al. (2010) found FE to be positively correlated between yearling and finishing periods when beef heifers were fed similar high fiber diets. Likewise, Durunna et al. (2011) found FE to be positively correlated when cattle were fed the same diet during both feeding periods, as well as when diet changed between periods. The objective of the current study was to determine effects of growing phase diet, growing phase feed efficiency, and finishing phase diet on growing and finishing phase diet digestibilities as well as finishing phase FE. It was hypothesized that diet digestibility would be greater in cattle with a favorable FE phenotype.

Materials and Methods

Growing Phase

All procedures and protocols were approved by the University of Missouri and Iowa State University animal care and use committees. Two groups of crossbred steers (192 steers, started Fall 2013; 181 steers, started Spring 2014) were fed growing diets, and growth and feed intake were measured at the University of Missouri (Columbia, MO). All steers received

an electronic ID tag (Allflex US Inc., Dallas-Fort Worth Airport, TX) in the left ear to facilitate intake measurement using a Growsafe FI System (GrowSafe Systems Ltd., Airdrie, AB, Canada). A pour-on (Cydectin, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) was administered to control internal and external parasites and steers were vaccinated against viral and clostridial infections (Bovi Shield Gold 5 and Ultrabac 7, Zoetis, Florham Park, NJ). Steers were implanted with a combination implant containing 80 mg trenbolone acetate and 16 mg estrogen (Component TE-IS, Elanco Animal Health, Greenfield, IN; Revalor IS, Merck Animal Health, Summit, NJ). The second group was also treated with Excede on arrival. The steers were housed on earthen lots with access to shelter and *ad libitum* water access provided by automatic waterers. Feed ingredients were blended in a truck-mounted mixer and fed once daily. Following a minimum 21 d receiving phase, steers were weighed on two consecutive days prior to feeding to establish an initial BW and stratified by weight across one of two growing phase diets. Experimental diets were composed primarily of roughage (G-Rough; Group 1: alfalfa/grass baleage and soybean hulls; Group 2: rye baleage and soybean hulls) or whole-shell corn (G-Corn). In both groups, steers were on the growing phase test for 70 d with intermediate BW measurements taken every 21-28 d. At the completion of the growing phase, steers were weighed on two consecutive days to determine final growing phase BW, individual growth and residual feed intake (RFI; Basarab et al., 2003) values within diet were calculated, and steers were ranked by FE (as RFI) within diet. Steers were then trucked 445 km to Iowa State University for the finishing phase.

Receiving and Finishing Phase

The 12 greatest and 12 least feed efficient steers from each growing phase diet were selected from group 1 (n = 48, 509 ± 7 kg, SD) and group 2 (n = 48, 467 ± 7 kg) for determination of growing and finishing diet digestibility as well as performance analysis. Upon arrival at ISU, steers were grouped by FE within growing phase diet and housed in six-head pens with concrete flooring, partial roof, and Growsafe feed bunks capable of measuring individual feed intake. Steers were fed receiving diets (Table 1) similar in nutrient composition to their respective growing phase diets for 15 d. Titanium dioxide was included in the receiving diets at an average of 10 g per steer daily as an indigestible marker. Fecal grab samples were collected on d 14 and 15 of the titanium dioxide feeding period and were dried for two weeks at 55°C for further analyses. After the receiving period and fecal collection, steers were transitioned over 18 days to finishing diets (Table 1) composed largely of corn (F-Corn) or grain byproducts (F-Byp). At the start of the finishing period, steers were weighed on two consecutive days prior to feeding to determine finishing phase initial BW (IBW). On d 28 of the finishing phase steers were implanted with Component TE-S (Elanco Animal Health, Greenfield, IN). Steers were fed finishing diets until an estimated average 1.27 cm backfat depth was reached, receiving Optaflexx ($200 \cdot mg \cdot steer^{-1} \cdot day^{-1}$, Elanco Animal Health, Greenfield, IN) for the final 27 (group 1) or 28 d (group 2) of the finishing test period prior to harvest. Due to differences in average finishing phase IBW for the finishing phase, group 1 had a 56 d total finishing phase whereas group 2 had a 97 d total finishing phase. Finishing phase diet digestibility was determined by repeating the 15 d titanium dioxide feeding and fecal collection protocol immediately prior to Optaflexx introduction. Therefore, finishing phase fecal collection was completed on d 28 and 29 for

group 1 and d 68 and 69 for group 2 for the respective finishing phases. Intermediate weights were measured every 28 d and steers were weighed for two consecutive days prior to feeding at the conclusion of the finishing phase to determine final BW (FBW); a four percent pencil-shrink was applied to all weights. All diets were formulated to meet or exceed NRC recommendations for growing cattle (NRC, 2000).

Sample Analysis

Ingredient, total mixed ration, and fecal samples were dried, ground through a 2 mm screen in a Retsch ZM 100 mill, (Retsch GmbH, Haan, Germany) and analyzed for DM, OM, CP, NDF, ADF, and titanium dioxide content. The DM was determined by drying 1 g replicates of each sample at 105°C for 24 hours while OM was determined by incinerating the dried samples at 600°C for 4 hours. Crude protein was calculated as N × 6.25, and N was determined by combustion (AOAC, 1990; LECO Tru-Mac, LECO Corporation, St. Joseph, MI) using EDTA as a calibration standard after every 10 samples. Analysis for NDF (Van Soest et al., 1991) and ADF (Goering and Van Soest, 1970) concentrations utilized an ANKOM^{200} Fiber Analyzer (ANKOM Technology, Macedon, NY), with alpha-amylase used during the NDF procedure. Starch content was determined colorimetrically using a glucose oxidase/peroxidase assay (D-Glucose Assay Kit, Megazyme International Ireland, Wicklow, Ireland) after further grinding (1 mm screen; Retsch ZM 100 mill, Retsch GmbH, Haan, Germany) and preparing samples as previously described (Hall, 2009) using α-amylase and amyloglucosidase (Megazyme International Ireland, Wicklow, Ireland). For titanium dioxide content determination, samples were prepared using methods outlined by Myers et al. (2004) and titanium dioxide concentration was determined colorimetrically (Eon Microplate

Spectrophotometer, BioTek, Winooski, VT). Individual feed intake (DM basis) and the dietary titanium inclusion (g titanium dioxide / g diet) were multiplied to determine individual titanium dioxide intake (g, DM basis) for each steer. Fecal output (g, DM basis) was calculated by dividing individual titanium dioxide intake (g, DM basis) by the fecal titanium dioxide concentration (g titanium dioxide / g dry feces). Fecal output (g, DM basis) and fecal nutrient concentrations (DM, OM, NDF, ADF, CP, starch) were multiplied to calculate nutrient outputs for each steer (g, DM basis). Similarly, individual feed intake (g, DM basis) and analyzed diet nutrient concentrations were multiplied to determine nutrient intake (g, DM basis). Apparent digestibility (%) for each nutrient was calculated as: [1 – (output /input)] × 100. Fecal samples for each 2 d collection period were analyzed separately for fecal nutrient and titanium dioxide concentrations; the 2 d average for each concentration was used in digestibility calculations.

Statistical Analysis

The 96 steers were ranked by growing phase G:F and categorized as the 24 greatest (HFE) or 24 least (LFE) feed efficient steers from each growing phase diet. Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). The receiving phase diet digestibility model included the fixed effects of growing phase diet (G-Corn, G-Rough), growing phase feed efficiency classification (HFE, LFE), and the interaction. The finishing phase diet digestibility and growth performance models included the fixed effects of growing phase diet, growing phase feed efficiency classification, finishing phase diet (F-Byp, F-Corn), and the interactions. Group (1, 2) was included in the models as a fixed effect for receiving and finishing phase analyses. If the *P*-value associated with an interaction exceeded

0.20, the interaction was removed from the model. No three-way interactions were significant during the finishing phase. Finishing phase IBW was applied as a covariate for finishing growth traits. The DMI for the respective titanium dioxide feeding periods were applied as covariates but removed from the model if $P > 0.20$. A Cook's D outlier test was used to identify outliers that were subsequently removed prior to data analysis. Because individual BW and intake were measured, steer was the experimental unit. Correlations were determined using the CORR procedure of SAS to generate Pearson's correlation coefficients denoted as r. Significance was declared at $P \leq 0.05$, with tendencies declared when $P = 0.06$ to 0.15. The values reported are least square means and standard errors of the means compared using the pdiff command in the MIXED procedure of SAS.

Results

Finishing phase growth performance

There were no interactions ($P \geq 0.2$) between FE classification and diet for any finishing phase performance measures. There were no differences ($P \geq 0.5$) in final BW, ADG, DMI, or G:F due to growing phase diet (Table 2). Within the steers grown on roughage, steers finished on the byproduct diet had greater ($P \leq 0.01$) final BW, ADG, and DMI than corn-finished steers, while regardless of finishing diet the steers grown on corn were intermediate for both measures. Finishing phase G:F was unchanged ($P \geq 0.4$) due to growing or finishing phase diet. Comparing the LFE and HFE-classifications, there were no differences ($P \geq 0.2$) in final BW, ADG, or DMI due to growing phase feed efficiency classification; however, HFE steers did have greater ($P = 0.04$) finishing phase G:F than LFE steers (Table 3).

Receiving and finishing phase diet digestibility

There were no differences ($P \geq 0.2$) in receiving phase DM or OM digestibility due to diet, FE classification, or the interaction between diet and FE classification (Table 4). There was an interaction ($P = 0.01$) wherein the steers grown on the high fiber, lesser starch roughage-based diet and classified as HFE had greater receiving phase NDF digestibility than the other groups, a digestibility advantage that exceeded 13%. Although there were no FE classification or diet × FE classification interaction effects ($P \geq 0.2$), ADF digestibility was greater ($P < 0.001$) in G-Rough versus G-Corn steers. Receiving phase CP digestibility tended to be greater ($P = 0.06$) for G-Rough versus G-Corn steers but CP digestibility was unaffected ($P \geq 0.3$) by FE classification, or the diet × FE classification interaction. There was also a difference ($P = 0.009$) in starch digestibility, as the G-Rough steers had more than a 6% advantage over the G-Corn steers. When finishing phase diet digestibility was evaluated, there were no three-way interactions ($P \geq 0.2$) for finishing phase digestibility measures between growing phase diet, finishing phase diet, and growing phase FE classification. There tended ($P \leq 0.1$; Table 5) to be an interaction between growing and finishing phase diets for finishing phase DM and OM digestibilities in which the roughage-grown/byproduct-finished steers had lesser DM and OM digestibilities than steers fed the other diet combinations. The F-Byp steers had greater ($P \leq 0.005$) NDF and ADF than the F-Corn steers . There was an interaction for finishing phase CP digestibility ($P = 0.05$) best explained by the main effect of finishing phase diet as the byproduct finished steers had greater finishing phase CP digestibility than the corn-finished steers and the difference was most pronounced between the corn-grown groups. Additionally, there was an interaction ($P = 0.03$) between growing and finishing phase diets, as the corn-grown/byproduct-finished

steers had greater ($P = 0.03$) starch digestibility than the roughage-grown/byproduct-finished steers while there were no differences ($P = 0.4$) in starch digestibility among the corn-finished steers.

Correlations across diet types

When examining phenotypic extremes for feed efficiency, there was a positive correlation for DM digestibility between feeding phases in the steers grown and finished on the corn-based diets (r = 0.49, $P = 0.02$; Table 6) as well as the steers grown on the roughage-based diet and finished on the byproduct-based diet (r = 0.68, $P < 0.01$). There was a negative correlation for G:F between feeding phases in the roughage-grown, corn-finished steers (r = -0.57, $P = 0.003$). No relationships between DM digestibility or G:F ($P \geq 0.3$) between feeding phases were detected when steers were grown on the corn-based diet and finished on the byproduct-based diet.

Discussion

Steers that were classified as highly feed efficient based on growing phase G:F continued to have greater G:F during the finishing phase regardless of diet changes from the growing to the finishing phase. Gain:feed is highly correlated to BW and maturity (Archer et al., 1999) and thus it was important to prevent any confounding effect of finishing phase initial BW on finishing phase G:F by accounting for initial BW in the statistical model for live performance data. It appears that numerically greater DMI in the steers classified as lowly feed efficient was a primary driver for the lesser finishing phase G:F noted in these steers versus the highly feed efficient steers, as there were minimal differences in ADG or

final BW between FE classifications. Thus, the relative classification of a steer as highly or lowly feed efficient based on G:F was repeatable from the growing phase to the finishing phase in this study. This repeatability is similar to results reported by Durunna et al. (2011) wherein G:F was positively correlated between growing and finishing phases whether steers changed from an oat-based growing diet to a barley-based finishing diet or continued consuming the same diet during both phases.

Interestingly, there were no positive correlations for G:F between the growing and finishing phases for any of the four diet combinations tested in the present study. Given the positive G:F correlation reported by Kelly et al. (2010) when heifers were fed high fiber diets during consecutive feeding phases, a similar positive G:F correlation was expected between phases for the G-Rough/F-Byp steers. There was, however, a negative G:F correlation for roughage-grown, corn-finished steers, suggesting that cattle that excel in G:F when grown with a high fiber diet may not excel in G:F after transitioning to a high concentrate diet. A review of the individual G:F data for the steers (not shown) reveals a pattern of greater losses in FE when steers switched from growing phase roughage to finishing phase corn-based diets rather than a FE improvement due to compensatory gain as may be expected when steers are fed high energy diets following the feeding of a moderate energy, high fiber diet (Drouillard et al., 1991). The negative G:F correlation between phases when steers were roughage-grown and corn-finished in the present study reinforces the idea that cattle should be FE tested using diets similar to the production environment of interest, such as finishing diets for feedlot applications (Durunna et al., 2011). If found to be repeatable, the results of the current study could also provide an opportunity to improve overall feed resource utilization, especially if cattle can be sorted to specific diet types based on genetic markers. Though not reported

here, the steers in the current study were genotyped with the Illumina BovineSNP50 assay (Matukumalli et al., 2009) as part of a larger FE investigation to evaluate the relationship between FE phenotype and genotype, with the goal of ultimately providing producers with a tool for identifying the ideal diet type for individual animals.

Despite the typical DM digestibility advantages of corn versus roughage-based diets, the roughage-grown HFE steers had numerically greater growing phase DM and OM digestibilities relative to the other FE classification and diet combinations, a pattern that was driven by more than a 13% advantage in NDF digestibility. The lack of DM digestibility advantage for the G-Corn steers relative to the G-Rough steers can be partially explained by the less digestible whole shell corn that comprised nearly 70% of the G-Corn diet. These results agree with previous digestibility investigations (Aikman, 2006) in which fiber digestibility linearly increased with increased fiber inclusion; however, Aikman (2006) also reported decreased DM and starch digestibility as fiber inclusion increased and wheat inclusion decreased; a contrast to the current results in which starch digestibility was greater in the higher fiber diet. Had the G-Corn diet been formulated with a processed corn, the DM digestibility may have been greater, a concept that is further evidenced by the decreased starch digestibility in the G-Corn relative to the G-Rough steers. Earlier work comparing whole shell and cracked corn (Turgeon et al., 1983) showed greater total tract starch digestibility for the cracked corn versus whole shell corn-based diets regardless of roughage inclusion. Conversely, Gorocica-Buenfil and Loerch (2005) found no effect of corn processing on nutrient digestibility when comparing whole shell and dry rolled corn with 8% hay inclusion, thus improving digestibility of the whole shell corn-based G-Corn diet may have required high moisture processing or steam-flaking to improve starch utilization

(Ramirez et al., 1985). Although the G-Rough diet contained cracked corn, approximately half of the corn in the diet was high moisture corn from the corn silage, which further helps to explain the starch digestibility advantage for the G-Rough steers. Additionally, the lack of differences in DM and OM digestibilities between the G-Corn and G-Rough diets may be attributed to the primary fiber sources in the G-Rough diet, namely the highly digestible soybean hulls and corn silage. Finally, using TiO_2 as an indigestible marker in the present study may be considered a limitation, though others (Hafez et al., 1988; Myers et al., 2004) have reported it to be an accurate way to estimate fecal output. Importantly, using TiO_2 as a marker instead of confining cattle to metabolism crates allowed for more animals to be tested, improving statistical power.

Although rumen kinetics were not measured in this study, a greater rate of passage for corn versus roughage in the respective G-Corn and G-Rough diets likely contributed to differences in starch digestibility (Eng et al., 1964). Additionally, differences in digestibility between LFE and HFE steers may have been influenced by differences in rumen bacterial composition that result from differences in ruminal pH, especially for pH-sensitive fibrolytic bacteria. Palmonari et al. (2010) suggested that rumen bacterial composition may vary between individuals due to differences in salivary buffer production as well as VFA absorption, thereby influencing bacterial populations due to mean pH as well as pH range. Thus, the genetic variation between individuals receiving the same diet could have influenced diet digestibility through differences in ruminal pH control or bacterial species present in the rumen as host genetics have been shown to influence microbial populations in the gastrointestinal tract between FE phenotypes (Guan et al., 2008; Zhou et al., 2009). Dry matter intake can also influence digestibility as it has been shown to have an inverse

relationship with the extent of nutrient digestibility (Colucci et al., 1982) and thus, titanium dioxide feeding period DMI was included as a covariate in the model for both receiving phase DM and OM digestibility. However, the greater DMI in LFE versus HFE steers (10.43 vs. 7.75 kg/d, data not shown) may help explain the differences in receiving phase digestibilities among the G-Rough groups. Similarly, Nkrumah et al. (2006) reported greater DMI in lowly versus highly efficient steers and DMI tended to be negatively correlated with DM digestibility. These authors also reported that highly efficient steers had greater DM and CP digestibility than lowly efficient steers as well as numerically greater fiber digestibility (Nkrumah et al., 2006). Work by Channon et al. (2004) suggested that total tract starch digestion ability in cattle could be genetically associated with RFI as greater starch utilization was noted in steers with greater FE. Lawrence et al. (2012) found no difference in total tract DM digestibility between highly and lowly feed efficient heifers fed fiber-based diets and Herd et al. (2002) found no difference in total tract DM digestibility between pasture-fed steers divergently selected for RFI. Based on the current study, fiber digestibility may be associated with FE when cattle are fed roughage-based diets while starch digestibility does not appear to play a major role in FE variation.

While differences in receiving phase fiber digestibility were influenced by growing period FE classification, finishing phase diet digestibility was unaffected by growing phase FE classification in the current study, suggesting that FE classification is only related to digestibility when measured during the same feeding period. However, when steers were reclassified by finishing phase G:F the highly efficient steers tended ($P = 0.13$, data not shown) to have greater finishing phase DM digestibility than the lowly efficient steers (72.8% vs. 70.3%). Continued work with additional animals is needed to determine the

influence of diet digestibility on FE classification of cattle and how this may be manipulated to improve cattle FE.

Dietary effects on finishing phase diet digestibility consistently agree with previous literature, as DM and OM digestibilities were greater in the steers fed the higher corn inclusion F-Corn diet whereas fiber digestibility was greater in steers fed the higher fiber F-Byp diet (Ludden et al., 1995; Aikman et al., 2006). The growing and finishing phase diet interaction for finishing phase starch digestibility may be explained by evaluating DMI; the corn-grown, byproduct- finished steers had 0.7 kg lesser DMI and 4.3% greater starch digestibility than the roughage-grown, byproduct-finished steers. Dry matter intake was applied as a covariate for starch digestibility; however, the relative DMI and starch digestibilities for the corn-grown, byproduct- finished steers and the roughage-grown, byproduct-finished steers still followed a pattern of greater digestibility in the group with lesser DMI as previously discussed (Colucci et al., 1982).

Further examining the relationship between digestibility in the growing and finishing phases, there were positive correlations for DM digestibility when steers were corn-grown and corn-finished or roughage-grown and byproduct-finished. It appears that steers grown and finished on similar diet types will exhibit comparable digestive capacity in both phases whether the diets are both higher in starch content or both higher in fiber content. However, this did not appear to be true if cattle were grown and finished on different diet types, as no relationship between DM digestibility across phases was found in steers grown and finished on different diets, such as the corn-grown, byproduct-finished steers or the roughage-grown, corn finished steers. The similarities in diet digestibility among cattle fed similar diets as well as the differences among cattle fed differing diets may arise, in part, from genetic variation

between individuals, or more specifically from differences in gene expression. Taniguchi et al. (2010) found that thousands of genes impacting epithelial function were differentially expressed in ruminal tissue when cattle were fed low or high concentrate diets, and thus it is conceivable that differences in gene expression may have contributed to the variation in gastrointestinal tract function from the growing to the finishing phase in the current study. Investigations comparing ruminal and intestinal tissue gene expression across multiple feeding phases would be beneficial for explaining total tract diet digestibility variation and would be further strengthened by an examination among the low and high FE phenotypic extremes.

Like finishing phase diet digestibility, the interaction between growing phase diet and finishing phase diet impacted finishing phase steer performance, independent of any FE classification effect. Regardless of finishing phase diet, there were no differences among finishing phase performance in steers fed the G-Corn diet during the growing phase; however, within the G-Rough group, F-Byp steers had greater final BW, ADG, and DMI than the F-Corn steers. As there was no difference in G:F or initial BW between the roughage-grown groups, the greater final BW in the F-Byp versus F-Corn steers was driven by improved ADG that resulted from increased DMI. These results agree with previous work comparing a corn-based diet (75% dry-rolled corn) and a dry byproduct-based diet (40% dried distillers grain, 35% soybean hulls; Trejo et al., 2010); the authors reported greater final BW, DMI, and ADG in the byproduct-based diet as compared to the corn-based diet but did not note a difference in G:F. However, a meta-analysis by Klopfenstein et al. (2008) showed a cubic response in which G:F decreased as distillers grain inclusion increased from ~15% to 40% of the diet. Similar to the current study, the meta-analysis (Klopfenstein et al., 2008)

also reported a linear increase in DMI as dried distillers grain inclusion increased to 40% and a quadratic increase for ADG as distillers grain inclusion increased in the diet. Future investigations of growing and finishing phase diet interactions on growth may be worthwhile using greater grain inclusion and lesser byproduct inclusion as previous studies have found greater finishing performance when dried distillers grains were included at approximately 20-25% of the diet (Buckner et al, 2008; Klopfenstein et al., 2008).

Implications

Previous work has estimated that differences in digestive capacity are responsible for 10-14% of the FE variation between individual cattle. Additionally, although earlier research has evaluated the repeatability of FE across feeding phases in cattle, there has been limited evaluation of FE repeatability across changing diet types. In the current study growing phase FE classification had little effect on finishing phase performance; however, steers classified as highly feed efficient during the growing phase continued to maintain greater G:F than steers classified as poorly feed efficient during the growing phase. Furthermore, G:F was negatively correlated between feeding phases when steers were roughage-grown and corn-finished, indicating that FE evaluation may be most beneficial if conducted using diets similar to those fed in the production environment of interest. Effects of FE classification on nutrient digestibility were most substantial for the fiber digestibility advantages of the highly efficient roughage-fed steers as compared to other groups during the growing phase since there were no effects of growing phase FE classification on finishing phase nutrient digestibility. In this study, fiber digestibility appeared to contribute to FE variation while starch digestibility did not. The positive correlations for DM digestibility in the steers grown

and finished on similar diets suggests that digestibility measured during one feeding phase may be a reasonable indication of digestive capacity during a subsequent phase if the diets are similar in nutritional composition. Further research would be beneficial to define the underlying explanations for the differences in digestive capability of highly versus lowly feed efficient cattle.

Literature Cited

Aikman, P. C., D. E. Beever and D. J. Humphries. 2006. The effect of incremental replacement of wheat with soya hulls in diets for Jersey cows on lactational performance, diet digestibility and feeding behaviour. Livestock Sci. 104:23-32. doi:10.1016/j.livsci.2006.02.011.

AOAC. 1990. Official methods of analysis. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Archer, J. A., E. C. Richardson, R. M. Herd, and P. F. Arthur. 1999. Potential for selection to improve efficiency of feed use in beef cattle: a review. Aust. J. Agric. Res. 50:147-162. doi:10.1071/A98075.

Basarab, J. A., M. A. Price, J. L. Aalhus, E. K. Okine, W. M. Snelling, and K. L. Lyle. 2003. Residual feed intake and body composition in young growing steers. Can. J. Anim. Sci. 83:189.–204. doi:10.4141/A02-065.

Buckner, C. D., T. L. Mader, G. E. Erickson, S. L. Colgan, D. R. Mark, V. R. Bremer, K. K. Karges, and M. L. Gibson. 2008. Evaluation of dry distillers grains plus solubles inclusion on performance and economics of finishing beef steers. Prof. Anim. Sci. 24(5):404-410.

Channon, A. F., J. B. Rowe, and R. M. Herd. 2004. Genetic variation in starch digestion in feedlot cattle and its association with residual feed intake. Aust. J. Exp. Agric. 44(5):469-474. doi:10.1071/EA02065.

Colucci, P. E., L. E. Chase, and P. J. Van Soest. 1982. Feed intake, apparent diet digestibility, and rate of particulate passage in dairy cattle. J. Dairy Sci. 65(8):1445-1456. doi:10.3168/jds.S0022-0302(82)82367-9.

Drouillard, J. S., C. L. Ferrell, T. J. Klopfenstein, and R. A. Britton. 1991. Compensatory growth following metabolizable protein or energy restrictions in beef steers. J. Anim. Sci. 69:811-818. doi:/1991.692811x.

Durunna, O. N., F. D. N. Mujibi, L. Goonewardene, E. K. Okine, J. A. Basarab, Z. Wang, and S. S. Moore. 2011. Feed efficiency differences and reranking in beef steers fed grower and finisher diets. J. Anim. Sci. 89:158-167. doi: 10.2527/jas.2009-2514.

Eng, K. S., M. E. Riewe, J. H. Craig, and J. C. Smith. 1964. Rate of Passage of Concentrate and Roughage through the Digestive Tract of Sheep. J. Anim. Sci. 23(4): 1129-1132. doi:10.2134/jas1964.2341129x.

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analyses (apparatus, reagents, procedures, and applications). Agric. Handbook No. 379. ARS-USDA, Washington, DC.

Gorocica-Buenfil, M. A. and S. C. Loerch. 2005. Effect of cattle age, forage level, and corn processing on diet digestibility and feedlot performance. J. Anim. Sci. 83(3):705-714. doi:/2005.833705x

Guan, L. L., J. D. Nkrumah, J. A. Basarab, and S. S. Moore. 2008. Linkage of microbial ecology to phenotype: correlation of rumen microbial ecology to cattle's feed efficiency. FEMS Microbiol. Lett. 288:85-91. doi: 10.1111/j.1574-6968.2008.01343.x.

Hafez, S., W. Junge, and E. Kalm. 1988. Schatzung der verdaulichkeit mit einer indikator-methode bei milchkuhen im vergleich zum hohenheimer-futterwert-test. Arch. Tierernahr. 38:929–945. doi:10.1080/17450398809430920.

Hall, M. B. 2009. Determination of starch, including maltooligosaccharides, in animal feeds: Comparison of methods and a method recommended for AOAC collaborative study. J. AOAC Int. 92:42-49

Herd, R. M., R. S. Hegarty, R. W. Dicker, J. A. Archer, and P. F. Arthur. 2002. Selection for residual feed intake improves feed conversion in steers on pasture. Proc. Aust. Soc. Anim. Prod. 24:85-88.

Herd, R. M., V. H. Oddy, and E. C. Richardson. 2004. Biological basis for variation in residual feed intake in beef cattle. 1. Review of potential mechanisms. Aust. J. Exp. Agric. 44(5):423-430. doi:10.1071/EA02220.

Kelly, A. K., M. McGee, D. H. Crews, Jr., T. Sweeney, T. M. Boland, and D. A. Kenny. 2010. Repeatability of feed efficiency, carcass ultrasound, feeding behavior, and blood metabolic variables in finishing heifers divergently selected for residual feed intake. J. Anim. Sci. 88:3214-3225. doi: 10.2527/jas.2009-2700.

Klopfenstein, T. J., G. E. Erickson, and V. R. Bremer. 2008. BOARD-INVITED REVIEW: Use of distillers by-products in the beef cattle feeding industry. J. Anim. Sci. 86(5):1223-1231. doi:10.2527/jas.2007-0550.

Lawrence, P., D. A. Kenny, B. Earley, and M. McGee. 2012. Grazed grass herbage intake and performance of beef heifers with predetermined phenotypic residual feed intake classification. Animal. 6(10):1648-1661. doi:10.1017/S1751731112000559.

Ludden, P. A., M. J. Cecava and K. S. Hendrix.1995. The value of soybean hulls as a replacement for corn in beef cattle diets formulated with or without added fat. J. Anim. Sci. 73: 2706-2711.

Matukumalli, L. K., C. T. Lawley, R. D. Schnabel, J. F. Taylor, M. F. Allan, M. P. Heaton, J. O'Connell, S. S. Moore, T. P. L. Smith, T. S. Sonstegard, and C. P. Van Tassell. 2009. Development and characterization of a high density SNP genotyping assay for cattle. PLoS One 4: e5350.

Myers, W. D., P. A. Ludden, V. Nayigihugu and B. W. Hess. 2004. Technical Note: A procedure for the preparation and quantitative analysis of samples for titanium dioxide. J. Anim. Sci. 82:179-183. doi:/2004.821179x.

Nkrumah, J. D., E. K. Okine, G. W. Mathison, K. Schmid, C. Li, J. A. Basarab, M. A. Price, Z. Wang, and S. S. Moore. 2006. Relationships of feedlot feed efficiency, performance, and feeding behavior with metabolic rate, methane production, and energy partitioning in beef cattle. J. Anim. Sci. 84:145-153. doi:/2006.841145x.

NRC. 2000. Nutrient requirements of beef cattle. 7th rev. ed. Natl. Acad. Press, Washington, D.C.

Palmonari, A., D. M. Stevenson, D. R. Mertens, C. W. Cruywagen, and P. J. Weimer. 2010. pH dynamics and bacterial community composition in the rumen of lactating dairy cows. J. Dairy Sci. 93(1):279-287. doi: 10.3168/jds.2009-2207

Ramirez, R. G., H. E. Kiesling, M. L. Galyean, G. P. Lofgreen, and J. K. Elliott. 1985. Influence of Steam-Flaked, Steamed-Whole or Whole Shelled Corn on Performance and Digestion in Beef Steers. J. Anim. Sci. 61(1): 1-8. doi:10.2134/jas1985.6111.

Richardson, E. C. and R. M. Herd. 2004. Biological basis for variation in residual feed intake in beef cattle. 2. Synthesis of results following divergent selection. Aust. J. Exp. Agric. 44(5):431-440. doi:10.1071/EA02221.

Richardson, E. C., R. M. Herd, P. F. Arthur, J. Wright, G. Xu A, K. Dibley, and V. H. Oddy. 1996. Possible physiological indicators for net feed conversion efficiency in beef cattle. Proc. Aust. Soc. Anim. Prod. 21:103-106.

Taniguchi, M., G. B. Penner, K. A. Beauchemin, M. Oba, and L. L. Guan. 2010. Comparative analysis of gene expression profiles in ruminal tissue from Holstein dairy cows fed high or low concentrate diets. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics. 5(4):274-279. doi: 10.1016/j.cbd.2010.07.004.

Trejo, C. O., D. B. Faulkner, A. Shreck, J. W. Homm, T. G. Nash, S. L. Rodriguez-Zas, and L. L. Berger. 2010. Effects of co-products and breed of sire on the performance, carcass characteristics, and rates of ultrasound backfat and marbling deposition in feedlot cattle. Prof. Anim. Sci. 26(6):620-630.

Turgeon, O. A., D. R. Brink, and R. A. Britton. 1983. Corn particle size mixtures, roughage level and starch utilization in finishing steers diets. J. Anim. Sci. 57(3):739-749. doi:10.2134/jas1983.573739x.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597. doi:10.3168/jds.S0022-0302(91)78551-2.

Zhou, M., E. Hernandez-Sanabria, and L. L. Guan. 2009. Assessment of the microbial ecology of ruminal methanogens in cattle with different feed efficiencies. Appl. Environ. Microbiol. 75:6524–6533. doi: 10.1128/AEM.02815-08.

Table 1. Composition and analysis of diets fed to steers during receiving and finishing phases.

	Receiving phase diet[1]		Finishing phase diet[2]	
Ingredient, % DM	G-Corn	G-Rough	F-Corn	F-Byp
Whole shell corn	69	-	-	-
Soybean hull pellets	11	40	-	20
Corn silage	-	21	-	-
Grass hay	10	14	8	8
Dried distillers grains	7.75	15.02	14.99	39.99
Cracked corn	-	8	75	30
Limestone	1.54	1.54	1.54	1.54
Salt	0.31	0.31	0.31	0.31
Urea	0.27	-	-	-
Vitamin A premix[3]	0.11	0.11	0.11	0.11
Trace mineral premix[4]	0.024	0.024	0.035	0.035
Rumensin 90[5]	0.014	0.014	0.013	0.013
Nutritional analysis, % DM				
DM, % as-fed basis	85.3	76.2	84.5	84.1
OM	95.2	93.3	95.6	94.1
NDF	29.4	51.7	24.4	42.7
ADF	12.1	29.6	8.0	18.7
CP	11.6	13.2	11.2	18.4
Starch	49.6	14.6	54.1	22.7

[1] Receiving phase diets: G-Corn = whole shell corn-based; G-Rough = forage and soybean hull-based

[2] Finishing phase diets: F-Corn = cracked corn-based; F-Byp = dried distillers grains and soybean hull-based

[3] Vitamin A premix contained 4,400,000 IU/kg

[4] Provided per kilogram of diet (from inorganic sources): 30 mg Zn, 20 mg Mn, 0.5 mg I, 0.1 mg Se, 10 mg Cu, 0.1 mg Co

[5] Provided Monensin at 200 $mg \cdot steer^{-1} \cdot d^{-1}$, Elanco Animal Health, Indianapolis, IN

Table 2. Finishing phase performance as affected by growing phase and finishing phase diets.

	G-Corn[1]		G-Rough[1]			*P*-values[3, 4]		
	F-Corn[2]	F-Byp[2]	F-Corn[2]	F-Byp[2]	SEM	G Diet	F Diet	G*F Diet
Initial BW[5], kg	472	489	495	496	-	-	-	-
Final BW, kg	619[ab]	617[ab]	611[b]	627[a]	4.0	0.7	0.07	0.03
ADG, kg/d	1.77[ab]	1.74[ab]	1.68[b]	1.87[a]	0.052	0.7	0.13	0.04
DMI, kg/d	11.5[ab]	11.4[ab]	11.2[b]	12.1[a]	0.26	0.5	0.14	0.05
G:F	0.154	0.153	0.150	0.156	0.0037	0.8	0.5	0.4

[a, b] Least squares means in a row without common superscript differ ($P < 0.05$)

[1] Growing phase diets: G-Corn = whole shell corn-based; G-Rough = forage and soybean hull-based

[2] Finishing phase diets: F-Corn = cracked corn-based; F-Byp = dried distillers grains and soybean hull-based

[3] *P*-values: G Diet = main effect of growing phase diet; F Diet = main effect of finishing phase diet; G*F Diet = interaction effect of growing and finishing phase diets

[4] Three way interaction between growing phase diet, finishing phase diet, and growing phase feed efficiency classification was not significant ($P > 0.2$)

[5] Finishing phase initial BW applied as a covariate for final BW, ADG, DMI, and G:F

Table 3. Finishing phase performance as affected by growing phase feed efficiency classification.

	LFE[1]	HFE[1]	SEM	*P*-value
Initial BW[2], kg	495	481	-	-
Final BW, kg	618	619	3.2	0.8
ADG, kg/d	1.74	1.79	0.042	0.5
DMI, kg/d	11.7	11.3	0.21	0.2
G:F	0.149	0.158	0.0029	0.04

[1] Growing phase feed efficiency classifications: LFE = least feed efficient; HFE = most feed efficient

[2] Finishing phase initial BW applied as a covariate for final BW, ADG, DMI, and G:F

Table 4. Receiving phase digestibility as affected by growing phase feed efficiency classification and diets.

	G-Corn[1]		G-Rough[1]			*P*-values[3]		
Digestibility, %	LFE[2]	HFE[2]	LFE[2]	HFE[2]	SEM	Diet	FE	Diet*FE
DM[4]	66.2	65.7	66.7	71.1	2.63	0.2	0.5	0.3
OM[4]	68.1	67.4	69.0	73.5	2.60	0.2	0.5	0.3
NDF	58.1[b]	57.1[b]	59.2[b]	73.0[a]	3.03	0.003	0.08	0.01
ADF	46.8	46.6	60.2	69.4	3.84	<0.001	0.3	0.2
CP	59.4	56.9	61.3	64.5	2.81	0.06	0.9	0.3
Starch	86.0	85.9	91.4	92.5	2.35	0.009	0.9	0.8

[a, b] Least squares means in a row without common superscript differ ($P < 0.05$)

[x, y, z] Least squares means in a row without common superscript tend to differ ($P < 0.1$)

[1] Growing phase diets: G-Corn = whole shell corn-based; G-Rough = forage and soybean hull-based

[2] Growing phase feed efficiency classifications: LFE = least feed efficient; HFE = most feed efficient

[3] *P*-values: Diet = main effect of growing phase diet; FE = main effect of growing phase feed efficiency classification; Diet*FE = interaction effect of growing phase diet and feed efficiency classification

[4] Dry matter intake applied as a covariate

Table 5. Finishing phase digestibility as affected by growing phase and finishing phase diets.

	G-Corn[1]		G-Rough[1]				*P*-values[3]	
Digestibility, %	F-Corn[2]	F-Byp[2]	F-Corn[2]	F-Byp[2]	SEM	G Diet	F Diet	G*F Diet
DM	73.0[y]	71.9[y]	73.4[y]	67.2[z]	1.48	0.2	0.01	0.1
OM	74.5[y]	73.9[y]	74.7[y]	69.1[z]	1.50	0.14	0.04	0.1
NDF	60.5	67.9	57.4	63.2	2.05	0.06	0.002	0.7
ADF	52.2	63.1	49.8	57.4	2.54	0.11	0.005	0.5
CP	65.7[b]	73.9[a]	67.9[b]	71.5[a]	1.16	0.9	<0.001	0.05
Starch	90.6[ab]	94.0[a]	92.1[ab]	89.7[b]	1.95	0.3	0.7	0.03

[a, b] Least squares means in a row without common superscript differ ($P < 0.05$)

[y, z] Least squares means in a row without common superscript tend to differ ($P < 0.1$)

[1] Growing phase diets: G-Corn = whole shell corn-based; G-Rough = forage and soybean hull-based

[2] Finishing phase diets: F-Corn = cracked corn-based; F-Byp = dried distillers grains and soybean hull-based

[3] *P*-values: G Diet = main effect of growing phase diet; F Diet = main effect of finishing phase diet; G*F Diet = interaction effect of growing and finishing phase diets

Table 6. Dry matter digestibility and gain:feed correlations across growing and finishing phase diets.

		Dry matter digestibility[1]		Gain:feed	
Growing phase diet[2]	Finishing phase diet[3]	Corr[4]	*P*-value	Corr[4]	*P*-value
Corn	Corn	0.49	0.02	0.07	0.7
Corn	Byproduct	0.25	0.3	0.13	0.6
Roughage	Corn	0.21	0.4	-0.57	0.003
Roughage	Byproduct	0.68	<0.001	-0.14	0.5

[1] Dry matter digestibility correlations based on receiving phase and finishing phase diet digestibilities; receiving phase diets similar to growing phase diets

[2] Growing phase diets: whole shell corn-based (Corn), forage and soybean hull-based (Roughage)

[3] Finishing phase diets: cracked corn-based (Corn), dried distillers grains and soybean hull-based (Byproduct)

[4] Corr: r, Pearson's correlation coefficient

CHAPTER 5.

RELATIONSHIP BETWEEN ANTIOXIDANT CAPACITY, OXIDATIVE STRESS, AND FEED EFFICIENCY IN BEEF STEERS

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Abstract

Feed efficiency (FE) can vary between individuals but sources of variation are not well characterized; oxidative stress is believed to contribute to variation. The objective of this study was to evaluate the relationship between FE, antioxidant activity and oxidative stress in feedlot steers representing phenotypic extremes for FE. Crossbred beef steers (n = 181) fed a 70 d growing phase (GP) diet of whole shell corn (G-Corn) or roughage (G-Rough) in GrowSafe bunks at University of Missouri were shipped to Iowa State University where the 12 most (HFE) and 12 least (LFE) efficient steers from each diet (n = 48; 467 ± 51 kg, SD) were selected for evaluation. Steers received diets similar to GP diets and 3 d after arrival, blood was sampled to evaluate antioxidant activity and oxidative stress markers for GP. Steers were transitioned to finishing phase (FP) corn (F-Corn) or byproduct-based diets (F-Byp) and on FP d 97, blood samples for FP were collected. Data for GP were analyzed as a 2×2 factorial, and data for FP as a 2×2×2 factorial using PROC MIXED of SAS. No GP diet × FP diet, FP diet × FE group, or three-way interactions were noted ($P \geq 0.11$) for FP

measures. Steers fed G-Rough had greater ($P = 0.04$) GP plasma protein carbonyl concentrations. During the GP, HFE had greater ($P \leq 0.04$) protein carbonyl and ratio of oxidized:reduced blood lysate glutathione concentrations than LFE. There were GP die × FE group interactions ($P \leq 0.03$) during GP and FP. During the GP, total blood lysate superoxide dismutase activity (SOD) was greater ($P \leq 0.03$) in G-Rough/LFE versus G-Rough/HFE and G-Corn/LFE; G-Corn/HFE was intermediate. The G-Rough/LFE had greater ($P < 0.04$) glutathione peroxidase activity (GPX) than other groups and greater ($P = 0.03$) plasma malondialdehyde concentrations than G-Corn/LFE. During the FP, the G-Rough/LFE had greater ($P \leq 0.04$) GPX than G-Rough/HFE and G-Corn/LFE; G-Corn/HFE was intermediate. The F-Byp had greater ($P < 0.01$) protein carbonyl than F-Corn and no other FP diet effects were noted ($P \geq 0.3$) for any FP measures. The GP diet and FE groups had stronger relationships with antioxidant activity and oxidative stress markers measured during the GP than the FP. Overall, antioxidant activity may play a role in FE as LFE, driven largely by G-Rough/LFE, had greater SOD and GPX than HFE, potentially using a greater proportion of energy otherwise available for tissue accretion.

Introduction

The increased cost of beef production, due largely to feed cost (USDA ERS, 2013), makes feed efficiency (FE) improvement paramount for the sustainability of the industry. Although FE can vary between individuals, the sources of variation are not well understood. Mitochondria consume 90% of cells' oxygen (Mao et al., 2011) and electron leakage from the electron transport chain (ETC) may reduce up to 2-4% of this oxygen to reactive oxygen species (ROS; Bottje et al., 2006). Oxidative stress occurs once ROS and other free radicals

exceed the detoxification or antioxidant capacity of a system (Chirase et al., 2004). Mitochondrial inefficiency and oxidative stress are among the physiological mechanisms believed to contribute to FE variation between individuals (Bottje and Carstens, 2009). Oxidative stress can decrease energetic efficiency as oxidation end-products like malondialdehyde (MDA) from lipid peroxidation (Del Rio et al., 2005) and protein carbonyls from protein oxidation (Dalle-Donne et al., 2003) must be degraded by processes such as the ATP-dependent ubiquitin system (Mehlhase and Grune, 2002). Previous investigations into the FE and oxidative stress relationship have focused primarily on ETC inefficiency and proton leak, noting greater hydrogen peroxide (H_2O_2) production in inefficient individuals (Bottje et al., 2002; Grubbs et al., 2013). Superoxide dismutases (SOD) are responsible for reducing ROS to H_2O_2 (Weisiger and Fridovich, 1973) and glutathione peroxidase (GPX) serves to further reduce H_2O_2 to H_2O via the oxidation of glutathione (Nordberg and Arner, 2001). Yet, limited work has evaluated the relationship between antioxidant activity and FE. Thus, the objective was to evaluate the relationship between FE, antioxidant activity, and oxidative stress in feedlot steers representing phenotypic extremes for FE. The hypothesis was that oxidative stress markers and thus antioxidant activity would be greater in steers with greater FE.

Materials and Methods

Growing Phase

All procedures and protocols were approved by the Iowa State University animal care and use committee (1-11-7059-B). One hundred eighty-one steers (288 ± 37 kg, SD) were fed growing phase diets and DMI, ADG and feed efficiency were determined at the

University of Missouri (Columbia, MO). Each steer received an electronic ID tag (Allflex US Inc., Dallas-Fort Worth Airport, TX) to facilitate individual intake measurement using a Growsafe System (GrowSafe Systems Ltd., Airdrie, AB, Canada). Steers were treated with Excede (Zoetis, Florham Park, NJ) on arrival, vaccinated against viral and clostridial infections (Bovi Shield Gold 5 and Ultrabac 7, Zoetis, Florham Park, NJ) and poured with Cydectin (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) to control internal and external parasites. All steers were implanted with Revalor IS (Merck Animal Health, Summit, NJ). The steers were housed in earthen lots with *ad libitum* water access, shelter access, and were fed once daily in GrowSafe bunks using a truck-mounted mixer to blend feed ingredients. Following a 21 d receiving phase, steers were weighed prior to feeding on two consecutive days to establish an initial BW and steers were stratified by weight across one of two growing phase diets. Growing phase diets (Table 1) were composed primarily of whole-shell corn (G-Corn; 88 steers) or rye baleage and soybean hulls (G-Rough; 93 steers). Total mixed ration (TMR) samples were collected weekly, dried at 55°C, and ground to 2mm for mineral analysis. Intermediate BW measurements were taken every 21-28 d and steers were fed growing phase diets for 70 d total. At the completion of the growing phase, steers were weighed on two consecutive days to determine growing phase final BW. Individual growth and residual feed intake (RFI) values within diet were calculated, and steers were ranked by RFI within diet. Steers were then transported 445 km to Iowa State University (ISU) for the finishing phase.

Finishing Phase

The 12 least (LFE; Table 2) and 12 most (HFE) feed efficient steers from each growing phase diet were selected (n = 48 total, 24/growing phase diet, 467 ± 51 kg, SD) for determination of growing and finishing phase antioxidant capacity and oxidative stress marker measurements. At ISU, steers were housed in pens of 6 steers with concrete flooring, equipped with GrowSafe feed bunks to facilitate individual feed intake measurements, and a partial roof. Three d after arrival at ISU, liver and jugular blood samples were collected two hours after feeding on the 48 steers to determine growing phase trace mineral status and to evaluate antioxidant activity and oxidative stress markers. Liver biopsy samples were collected using methods described by Engle and Spears (2000), transported to the laboratory on ice, dried at 70°C in a forced-air oven, digested (CEMS MARSXpress, Matthews, NC) in trace metal grade nitric acid, and prepared for mineral analysis using methods described by Richter et al. (2012). Steers were initially fed receiving diets nutritionally similar to their respective growing phase diets for 15 d and were then transitioned over 18 days to finishing diets (Table 3) composed primarily of grain byproducts (F-Byp) or cracked corn (F-Corn; 24 steers/finishing diet; 12 steers per growing diet × finishing diet; 6 steers per growing diet × finishing diet × FE classification). The TMR samples were collected weekly, dried at 70°C, and ground to 2mm for mineral analysis. At the beginning of the finishing phase, steers were weighed prior to feeding on two consecutive days to determine finishing phase initial BW. On d 28 of the finishing phase steers were implanted with Component TE-S (Elanco Animal Health, Greenfield, IN). Steers were fed finishing diets until an estimated average 1.27 cm backfat depth was reached, receiving Optaflexx (200 mg·steer^{-1}·day^{-1}, ractopamine hydrochloride, Elanco Animal Health, Greenfield, IN) for the final 28 d of the finishing test

period prior to harvest; the total finishing phase was 97 d. Jugular blood was also collected prior to feeding on d 97 to evaluate finishing phase antioxidant activity and oxidative stress markers. Intermediate weights were measured every 28 d during the finishing phase and steers were weighed prior to feeding for two consecutive days at the conclusion of the finishing phase to determine finishing phase final BW. All diets were formulated to meet or exceed NRC recommendations for growing cattle (NRC, 2000).

Sample Analysis

Total mixed ration samples were analyzed for CP via combustion (AOAC, 1990; LECO Tru-Mac, LECO Corporation, St. Joseph, MI) and NDF (α-amylase included; Van Soest et al., 1991) and ADF (Goering and Van Soest, 1970) content using an ANKOM200 Fiber Analyzer (ANKOM Technology, Macedon, NY). Acid digested liver samples were analyzed at the ISU Veterinary Diagnostic Laboratory (Ames, IA) by technicians masked to treatments using inductively coupled plasma mass spectroscopy (PerkinElmer, Waltham, MA). Dried and ground TMR samples were prepared and analyzed for Cu, Mn, and Zn content using methods described by Richter et al. (2012). Total mixed ration Se content was calculated from NRC (2000) ingredient and supplement Se concentrations. Jugular blood from each steer was collected into 10 mL potassium EDTA-treated vacuum tubes (Becton, Dickinson and Co., Franklin Lakes, NJ) and 10 mL heparinized vacuum tubes (Becton, Dickenson and Co). Blood tubes were transported on ice back to the laboratory and centrifuged at 1,000 × *g* for 10 minutes at 4°C. Plasma from the potassium EDTA-treated tubes was removed and stored at -80°C until malondialdehyde (MDA, μM) determination using a commercially available thiobarbituric acid reactive substances kit (Item no.

10009055, Cayman Chemical Co., Ann Arbor, MI). Plasma from the potassium EDTA-treated tubes was also used for ascorbate (ASC, μM) analysis using a commercially available kit (Item no. 700420, Cayman Chemical Co.) after preparation and storage using methods described by Pogge and Hansen (2013).

After removal of plasma, packed red blood cells in the potassium EDTA-treated tubes were prepared using methods described by Pogge et al. (2012) and frozen at -80°C until analysis for total SOD, Mn-dependent SOD (MnSOD), Cu-Zn-dependent SOD (CuZnSOD), and GPX activities. Total SOD activity was determined using a commercially available kit (Item no. 706002, Cayman Chemical Co.), as was MnSOD by inhibiting CuZnSOD using 3mM potassium cyanide; CuZnSOD was subsequently determined by subtracting MnSOD activity from total SOD activity. A unit of SOD activity (U) was defined as the amount of the enzyme required to dismutate 50% of the superoxide radical and the activity was expressed per g of hemoglobin (1,000 U·g hemoglobin^{-1}). Glutathione peroxidase activity was determined using a commercially available kit (Item no. 703102, Cayman Chemical Co.), expressed per g of hemoglobin, and defined such that a unit of GPX activity was that required to oxidize 1.0 nmol of reduced nicotinamide adenine dinucleotide phosphate (NADPH) to the oxidized form (NADP+) per minute at 25°C (mmol·minute^{-1}·g hemoglobin^{-1}). Hemoglobin concentration of the red blood cell lysate was determined using methods described by Hansen et al. (2010) and a hemoglobin standard (Pointe Scientific, Inc., Canton, MI).

Plasma from the heparinized tubes was collected and frozen at -80°C until preparation and analysis for protein carbonyls using a commercially available kit (Item no. 10005020, Cayman Chemical). Plasma protein carbonyl was reflected as nmol protein carbonyl/mg

plasma protein with plasma protein concentration determined by comparison with a known concentration of bovine serum albumin (Fisher Scientific, Fair Lawn, NJ) in guanidine hydrochloride. Packed red blood cells from the heparinized tubes were lysed and prepared for total glutathione (tGSH, μM), oxidized glutathione (GSSG, μM), and reduced glutathione (GSH) determination using methods described by Pogge et al. (2015) and a commercially available kit (Item no. 703002, Cayman Chemical Co.). The oxidized:reduced glutathione ratio (GSSG:GSH) was calculated by dividing GSSG by GSH. Plasma ASC concentrations were determined fluorimetrically (Synergy 4 microplate reader, BioTek, Winooski, VT) whereas plasma MDA concentrations, lysate SOD activity, lysate GPX activity, plasma protein carbonyl concentrations, deproteinated lysate glutathione concentrations, lysate hemoglobin concentrations, and plasma protein concentrations were determined colorimetrically (BioTek EONC plate reader, BioTek).

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). For growing phase antioxidant activities, oxidative stress markers, and liver trace mineral concentrations determined shortly after arrival at ISU, growing phase diet, growing phase FE classification (HFE, LFE) and the interaction were included in the model as fixed effects. For antioxidant activities and oxidative stress markers measured in blood collected at the conclusion of the finishing phase (d 97), growing phase diet, growing phase FE classification (HFE, LFE), finishing phase diet, and all interactions were included in the model as fixed effects. Interactions were removed from the model if the *P*-value exceeded 0.20. No three-way interactions were found to be significant during the finishing phase.

Assay plate was applied as a random effect for the oxidative marker and antioxidant activity analyses. Steer was the experimental unit (24 steers/growing phase diet; 12 steers/growing phase diet × FE classification; 6 steers/ growing phase diet × FE classification × finishing phase diet). Significance was declared when $P \leq 0.05$ and tendencies were declared when $0.06 \leq P \leq 0.10$. Means were separated using the pdiff command in SAS and data reported are least square means and standard errors of the means generated using the lsmeans command in SAS.

Results

Growing phase

At the conclusion of the growing phase, no interaction between growing phase diet and growing phase FE classification was noted ($P \geq 0.13$; Table 3) on tGSH, GSSG, or GSH concentrations in red blood cell lysate, or GSSG:GSH. Neither growing phase diet nor FE classification had an effect ($P \geq 0.11$) on tGSH or GSH concentrations. However, growing phase GSSG concentrations were greater ($P = 0.03$) in roughage-grown steers than corn-grown steers (43 and 32 μM for G-Rough and G-Corn, respectively) but were unaffected ($P = 0.3$) by growing phase FE classification. The oxidized:reduced glutathione ratio, an indicator of oxidative stress, was greater ($P = 0.04$) in HFE versus LFE steers (0.23 vs. 0.17) and tended to be greater ($P = 0.07$) in roughage-grown steers as compared to corn-grown steers. Growing phase plasma protein carbonyl concentrations, a protein oxidation marker, were not affected by the growing phase diet × FE classification interaction ($P = 0.8$); however, protein carbonyl concentrations were greater in roughage-grown steers compared to corn-grown steers ($P = 0.04$; 0.32 vs. 0.29 nmol/mg protein for G-Rough and G-Corn,

respectively), and were greater in HFE versus LFE steers ($P = 0.03$; 0.32 vs. 0.29 nmol/mg protein for HFE and LFE, respectively). There was an interaction ($P = 0.03$) between growing phase diet and FE classification for plasma MDA, a product of lipid peroxidation, as the roughage-grown LFE steers had greater ($P = 0.03$) concentrations than corn-grown LFE steers while there were no differences ($P = 0.4$) in growing phase MDA concentrations between the HFE steers.

Evaluating growing phase antioxidant activities in red blood cell lysate, Cu-Zn SOD activity was unaltered ($P \geq 0.14$; Table 4) by growing phase diet, FE classification, or the interaction. However, there was an interaction ($P = 0.01$) between growing phase diet and FE classification for total SOD activity, driven by differences in MnSOD activity where roughage-grown LFE had greater ($P \leq 0.05$) Mn and total SOD compared with roughage grown HFE and corn-grown LFE steers, while corn-grown HFE steers were intermediate. There was also an interaction ($P = 0.002$) in which roughage-grown LFE steers had greater ($P \leq 0.04$) growing phase GPX activity than the other growing phase diet × FE classification combinations. In plasma, concentration of the antioxidant ascorbate was unchanged ($P \geq 0.4$) due to growing phase diet, FE classification, or the interaction.

Based on liver samples collected at the end of the growing phase, there was no effect ($P \geq 0.3$; Table 5) of the growing phase diet × FE classification on concentrations of any measured trace mineral. Liver Cu and Se concentrations were greater ($P \leq 0.05$) in roughage-grown steers versus corn-grown steers. However, liver Fe, Mn, and Zn concentrations were unaffected ($P \geq 0.5$) by growing phase diet. Feed efficiency classification affected liver Fe and Se concentrations, as both minerals were greater ($P \leq 0.04$) in LFE versus HFE steers.

Manganese tended to be greater ($P = 0.08$) in LFE steers than HFE steers but there was no difference ($P \geq 0.6$) in liver Cu or Zn concentrations due to growing phase FE classification.

Finishing phase

At the conclusion of the finishing phase, there were no interactions ($P \geq 0.11$) between growing phase diet × finishing phase diet, growing phase FE classification × finishing phase diet, or growing phase diet × growing phase FE classification × finishing phase diet on any measured oxidative stress markers or antioxidant activities. There was also no effect ($P \geq 0.3$; Table 6) of growing phase diet or FE classification on any oxidative stress markers measured in red blood cell lysate or plasma. Finishing phase tGSH and GSH concentrations in red blood cell lysate were unaffected ($P \geq 0.6$) by the growing phase diet × FE classification interaction. However, there was an interaction ($P = 0.04$) in which HFE steers tended to have greater ($P = 0.07$) GSSG concentrations than LFE steers in the roughage-grown group, but there were no differences ($P = 0.13$) due to FE classification within the corn-grown group. There tended to be an interaction between growing phase diet and FE classification ($P = 0.07$) on the GSSG:GSH ratios as the HFE steers had numerically greater ratios than LFE steers among the roughage-grown steers whereas LFE steers had numerically greater ratios than HFE steers within the corn-grown group. Similar to the growing phase measures there was no growing phase diet × FE classification interaction ($P \geq 0.6$) on plasma protein carbonyl or MDA concentrations at the conclusion of the finishing phase. Finishing phase diet had no effect ($P \geq 0.3$; Table 7) on any finishing phase glutathione or MDA measures; however, byproduct-finished steers had greater ($P = 0.001$) finishing phase plasma protein carbonyl concentrations than corn-finished steers.

Finishing phase red blood cell lysate SOD, GPX, and plasma ascorbate activities were unaffected ($P \geq 0.2$) by finishing phase diet or any finishing phase diet interaction. Total SOD activity in red blood cell lysate during the finishing phase tended to be greater in roughage-grown steers compared to corn-grown steers ($P = 0.06$; Table 8) but was unaltered ($P = 0.6$) by growing phase FE classification or the growing phase diet × FE classification interaction. Manganese SOD activity did not differ ($P \geq 0.2$) due to growing phase diet, FE classification, or the interaction. Although Cu-Zn SOD activity was unaffected ($P \geq 0.14$) by growing phase diet or the growing phase diet × FE classification interaction, there was a FE classification effect in which LFE steers tended to have greater ($P = 0.06$) Cu-Zn SOD activity than HFE steers. Finishing phase GPX activity in red blood cell lysate was impacted ($P = 0.02$) by a growing phase diet × FE classification interaction in which the roughage-grown LFE steers had greater ($P \leq 0.04$) GPX activity than the corn-grown LFE steers and the roughage-grown HFE steers; the corn-grown HFE steers were intermediate and did not differ ($P \geq 0.2$) from the other groups. At the conclusion of the finishing phase, plasma ascorbate concentration was not affected ($P \geq 0.4$) by growing phase diet, FE classification, or the interaction.

Discussion

Steers in the current investigation were genotyped as part of a larger FE study to evaluate the relationship between FE phenotype and genotype; live performance and carcass traits for the current steers are evaluated and discussed elsewhere (Russell, 2015). Protein oxidation in a system can be used as an indicator of oxidative stress, and can be determined by measuring protein carbonyl which are relatively stable and easily detected (Dalle-Donne

et al., 2003). Likewise, GSSG:GSH is an indicator of oxidative stress as a greater ratio indicates a greater degree of oxidative stress (Ojano-Dirain et al., 2005). The main effects of growing phase diet and FE classification on protein oxidation and GSSG:GSH appear largely driven by the highly efficient roughage-grown steers, suggesting greater measurable oxidative stress in these steers at the conclusion of the growing phase. Conversely, greater protein carbonyl concentrations have been consistently reported in lowly efficient versus highly efficient individuals; in post-harvest muscle in steers (Sandelin et al., 2005), as well as in muscle (Iqbal et al., 2004), liver (Iqbal et al., 2005), and duodenal tissue (Ojano-Dirain et al., 2007) from broilers. Measuring plasma versus muscle or organ protein carbonyl concentrations may be a source of variation but previous plasma protein carbonyl comparisons in FE-phenotyped livestock are limited.

Also dissimilar to the current findings, Ojano-Dirain et al. (2005) reported an increase in duodenal mucosa GSSG:GSH in the least efficient broilers compared to the most efficient broilers (0.093 vs. 0.070 GSSG:GSH, 0.008 SE) selected from an original group of 100. Comparing results between species may be especially difficult as the poultry FE investigations (Iqbal et al., 2004, 2005; Ojano-Dirain et al. 2005, 2007) utilized broilers divergently bred for FE over multiple generations versus steers identified as FE extremes among a tested population, as was the current study design. Since GSSG:GSH values exceeding 0.1 are indicative of oxidative stress (Ithayaraja, 2011), it appears all steers in the current study were suffering from some degree of oxidative stress, regardless of FE phenotype. The increased protein carbonyl and GSSG:GSH in the highly-efficient roughage-grown steers may be indicative of a greater tolerance for oxidative stress as compared to the lowly-efficient steers. The regeneration of GSH from GSSG is accomplished by glutathione

reductase and is an energy-dependent process (Nordberg and Arner, 2001). Therefore, decreased GSH regeneration may explain the increased GSSG:GSH ratio in the highly-efficient roughage-grown steers and would contribute to the improved energetic efficiency in the highly efficient phenotype. Decreased GSH in the highly-efficient roughage-grown steers would result in decreased antioxidant capacity, and thus a greater possibility of protein oxidation, leading to increased protein carbonyl concentrations. Future work evaluating GSH, GSSG, and glutathione reductase activity may help determine the cause of GSSG:GSH differences between individuals.

Considering the role of antioxidants to neutralize ROS and delay or inhibit oxidation (Sies, 1997), evaluating antioxidant activity was important to more clearly evaluate the relationship between FE and oxidative stress. Total SOD activity was increased in the lowly-efficient roughage-grown steers due to increased MnSOD activity. Differences in antioxidant activity were likely not due to trace mineral status as animals were adequate (Kincaid, 2000) and trace mineral dependent enzyme activities were not correlated with respective trace mineral concentrations ($P \geq 0.4$, data not shown). However, trace mineral-dependent antioxidant enzyme activity may have been affected if steers had been deficient. In addition to greater growing phase red blood cell lysate MnSOD and GPX activity, the less efficient roughage-grown steers had numerically greater plasma MDA concentrations, an indicator of lipid peroxidation and an oxidative stress marker. These results suggest an increased oxidative stress load in the less efficient roughage-grown steers, especially as previous investigators have reported MnSOD activity to be induced in response to oxidative stress (Shull et al., 1991). Since MDA acts in a toxic manner, causing further oxidative damage (Marnett, 1999), increased MDA concentrations are of greater concern than increased protein

carbonyl concentrations. Growing phase plasma ascorbate concentrations did not differ due to diet or FE classification, though the range of values (4.51 to 5.32 mg ascorbate/L) met or exceeded the expected range of plasma ascorbate concentrations reported in healthy beef cattle (2.4 to 4.7 mg ascorbate/L; Smith et al., 2009). It is possible that the poorly efficient steers had a less graded response to oxidative stress, generating greater quantities of antioxidant enzymes in response to stress, potentially utilizing energy for the antioxidant system, such as GSH regeneration (Nordberg and Arner, 2001), rather than tissue accretion.

The relationships between growing phase FE classification, diet, and oxidative stress were further evaluated at the conclusion of the finishing phase to determine whether antioxidant activity and oxidative stress markers were consistent in steers across feeding phases. Effects of finishing phase diet or interactions with the finishing phase diet were limited. Steers finished with the byproduct-based diet had greater plasma protein carbonyl concentrations. It is unlikely increased plasma protein carbonyl concentrations in the byproduct-fed steers are due to increased dietary protein (Petzke et al., 1999) but could be a result of increased oxidative stress due to greater dietary S in the byproduct diet (0.28% S, DM basis; data not reported) versus the corn diet (0.17% S, DM basis). Though previous research showed no difference in oxidative stress markers in steers fed diets containing 0.22% versus 0.34% dietary S, increased oxidative stress was detected in steers fed diets containing 0.55% dietary S (Pogge and Hansen, 2013). Further analysis of oxidative stress markers in cattle fed differing diets and nutrient concentrations may help explain the current finishing phase plasma protein carbonyl results. Consistent with the growing phase, the more efficient roughage-grown steers maintained numerically greater GSSG:GSH. Though GPX decreased substantially from the growing phase to the finishing phase across all groups, the

increased GPX activity in the less efficient roughage-grown steers was also consistent between feeding phases. The GPX and GSSG have a nearly inverse relationship in this study, a result that is unexpected because GPX converts GSH to GSSG to detoxify H_2O_2, thus increased GPX and GSSG would be expected. However, the pattern is consistent between phases. As previously discussed, the LFE steers may have been less energetically efficient, in part, because of greater energy-dependent GSH regeneration (Nordberg and Arner, 2001), thereby decreasing the GSSG:GSH ratio to a greater extent in those LFE steers. Further work is needed to evaluate the glutathione system in beef cattle, especially during different growth phases.

Both MnSOD and GPX activities decreased in all groups from the growing phase to the finishing phase. A positive correlation between GPX activity and whole blood Se concentrations has been previously reported in ruminants (Anderson et al., 1978) but Masters et al. (1988) found limited correlations between dietary Mn concentrations and MnSOD activity in rams. Although finishing phase tissue mineral concentrations were not analyzed in the current study, finishing phase diets exceeded NRC (2000) recommendations for Se and Mn content, making finishing phase deficiencies unlikely. It is unclear whether decreases in MnSOD and GPX activity from the growing phase to the finishing phase were a consequence of aging as Cand and Verdetti (1989) reported decreased total SOD and GPX activity in liver and kidney of rats due to age. Conversely, Lammi-Keefe, et al. (1984) reported greater MnSOD activity in adult versus young rats and Zhang et al. (1989) reported increased GPX activity in rats due to age as well. Based on the previous discussion regarding increased MnSOD expression induced by increased oxidative stress (Shull et al., 1991), it is conceivable that blood samples collected shortly after arrival at ISU reflect the effect of

transit stress on antioxidant expression and activity. Transit stress has been reported to increase oxidative stress markers in steers (Chirase et al., 2004) and oxidative stress has been shown to cause an SOD upregulation (Huang et al., 2015), a concept that also helps explain greater growing phase MnSOD activity in the current study compared to previously reported MnSOD activity values in feedlot steers (Genther and Hansen, 2014). It is unclear why finishing phase MnSOD activity in the current study was markedly lesser than values reported by Genther and Hansen (2014). Expectedly, plasma ascorbate also decreased from the growing to the finishing phase; an observation previously documented in fattening cattle (Matsui, 2012).

Increased DMI among the less efficient steers was likely responsible for the greater growing phase liver Fe, Se, and tendency for greater liver Mn concentrations as compared to the more efficient steers, though Cu and Zn concentrations were only numerically greater in the less efficient steers. Despite greater Zn, Cu, and Mn concentrations in the G-Rough diets, only liver Cu was greater in the G-Rough steers. Interestingly, despite greater calculated Se concentration in the G-Corn diet, the G-Rough steers had greater liver Se, though ingredient variation may not be reflected in the diet Se calculation. The primary focus of the current experiment was an exploration of antioxidant activity and oxidative stress as contributors to biological variation in growing phase FE. However, finishing phase liver mineral analysis would have been beneficial not only for evaluating correlations with mineral-dependent finishing phase antioxidant activities but also to evaluate trace mineral status from the growing to the finishing phase.

Implications

Observations in the current study were largely driven by differences among the roughage-grown steers. In that group, it appears that steers with greater FE may have a greater tolerance for oxidative stress than less efficient steers, as evidenced by the increased growing phase GSSG:GSH and protein carbonyl concentrations. Based on the respective GSSG:GSH ratios, all groups were experiencing oxidative stress yet the less efficient roughage-grown steers generated the most antioxidant activity, possibly a greater reaction to oxidative stress than the more efficient steers. Increased antioxidant activities, namely MnSOD and GPX, were consistently detected among less efficient steers grown on the roughage-based diet, yet differences in corn-grown steers were not detected. Since conventional beef systems typically utilize roughage-based diets for the backgrounding or growing phases, further evaluation of the relationship between FE, oxidative stress, and antioxidant activity may be especially important for identifying opportunities for FE improvement in the growing phase. Liver mineral concentrations were adequate, and not correlated with the mineral-dependent antioxidants, suggesting that differences in antioxidant activity were not the result of mineral nutrition in the present study. As steers in the current study were purchased from sale barns and were of unknown origin, future investigations into antioxidant activity and oxidative stress markers may be beneficial using steers of similar genetic background identified by FE phenotype. Additional measurements pre- and post-transit would be beneficial for determining transit stress effects on antioxidants and oxidative stress markers.

Literature Cited

Anderson, P. H., S. Berrett and D. S. P. Patterson. 1978. Glutathione peroxidase activity in erythrocytes and muscle of cattle and sheep and its relationship to selenium. J. Comp. Path. 88(2):181-189.

AOAC. 1990. Official methods of analysis. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Bottje, W., Z. X. Tang, M. Iqbal, D. Cawthon, R. Okimoto, T. Wing and M. Cooper. 2002. Association of Mitochondrial Function with Feed Efficiency within a Single Genetic Line of Male Broilers. Poultry Sci. 81:546-555. doi:10.1093/ps/81.4.546.

Bottje, W., N. R. Pumford, C. Ojano-Dirain, M. Iqbal, and K. Lassiter. 2006. Feed Efficiency and Mitochondrial Function. Poultry Sci. 85(1):8-14. doi:10.1093/ps/85.1.8

Bottje, W. G. and G. E. Carstens. 2009. Association of mitochondrial function and feed efficiency in poultry and livestock. J. Anim. Sci. 87:E48-E63. doi:10.2527/jas.2008-1379.

Cand, F. and J. Verdetti. 1989. Superoxide dismutase, glutathione peroxidase, catalase, and lipid peroxidation in the major organs of the aging rats. Free Radical Biology and Medicine. 7(1):59-63. doi:10.1016/0891-5849(89)90101-9.

Chirase, N. K., L. W. Greene, C. W. Purdy, R. W. Loan, B. W. Auvermann, D. B. Parker, E. F. Walborg Jr, D. E. Stevenson, Y. Xu, and J. E. Klaunig. 2004. Effect of transport stress on respiratory disease, serum antioxidant status, and serum concentrations of lipid peroxidation biomarkers in beef cattle. Amer. J. Vet. Research. 65(6):860-864. doi:10.2460/ajvr.2004.65.860.

Dalle-Donne, I., R. Rossi, D. Giustarini, A. Milzani, and R. Colombo. 2003. Protein carbonyl groups as biomarkers of oxidative stress. Clinica chimica acta. 329(1):23-38. doi:10.1016/S0009-8981(03)00003-2.

Del Rio, D., A. J. Stewart, and N. Pellegrini. 2005. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutrition, Metabolism and Cardiovascular Diseases. 15(4):316-328. doi:10.1016/j.numecd.2005.05.003.

Engle, T. E., and J. W. Spears. 2000. Effects of dietary copper concentration and source on performance and copper status of growing and finishing steers. J. Anim. Sci. 78:2446–2451. doi:/2000.7892446x.

Genther, O. N., and S. L. Hansen. 2014. A multielement trace mineral injection improves liver copper and selenium concentrations and manganese superoxide dismutase activity in beef steers. J. Anim. Sci. 92(2):695-704. doi:10.2527/jas.2013-7066.

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analyses (apparatus, reagents, procedures, and applications). Agric. Handbook No. 379. ARS-USDA, Washington, DC.

Grubbs, J. K., A. N. Fritchen, E. Huff-Lonergan, J. C. M. Dekkers, N. K. Gabler, and S. M. Lonergan. 2013. Divergent genetic selection for residual feed intake impacts mitochondria reactive oxygen species production in pigs. J. Anim. Sci. 91(5):2133-2140. doi:10.2527/jas2012-5894.

Hansen, S. L., M. S Ashwell, A. J. Moeser, R. S. Fry, M. D. Knutson, and J. W. Spears. 2010. High dietary iron reduces transporters involved in iron and manganese metabolism and increased intestinal permeability in calves. J. Dairy Sci. 93:656–665. doi:10.3168/jds.2009-2341.

Huang, C., H. Jiao, Z. Song, J. Zhao, X. Wang, and H. Lin. 2015. Heat stress impairs mitochondria functions and induces oxidative injury in broiler chickens. J. Anim. Sci. 93(5):2144-2153. doi:10.2527/jas.2014-8739.

Iqbal, M., N. R. Pumford, Z. X. Tang, K. Lassiter, T. Wing, M. Cooper and W. Bottje. 2004. Low Feed Efficient Broilers Within a Single Genetic Line Exhibit Higher Oxidative Stress and Protein Expression in Breast Muscle with Lower Mitochondrial Complex Activity. Poultry Sci. 83:474-484. doi:10.1093/ps/83.3.474.

Iqbal, M., N. R. Pumford, Z. X. Tang, K. Lassiter, C. Ojano-Dirain, , T. Wing, M. Cooper, and W. Bottje. 2005. Compromised liver mitochondrial function and complex activity in low feed efficient broilers are associated with higher oxidative stress and differential protein expression. Poultry sci. 84(6):933-941. doi:10.1093/ps/84.6.933.

Ithayaraja, C. M. 2011. Mini-review: Metabolic functions and molecular structure of glutathione reductase. Int. J. Pharm. Sci. Rev. Res. 9:104-115.

Kincaid, R. L. 2000. Assessment of trace mineral status of ruminants: A review. J. Anim. Sci. 77:1–10. doi:10.2134/jas2000.77E-Suppl1x.

Lammi-Keefe, C. J., P. B. Swan, and P. V. J. Hegarty. "Copper-zinc and manganese superoxide dismutase activities in cardiac and skeletal muscles during aging in male rats." Gerontology 30, no. 3 (1984):153-158. doi:10.1159/000212623.

Mao, G., G. A. Kraus, I. Kim, M. E. Spurlock, T. B. Bailey, and D. C. Beitz. 2011. Effect of a mitochondria-targeted vitamin E derivative on mitochondrial alteration and systemic oxidative stress in mice. Brit. J. Nutr. 106:87-95. doi:10.1017/S0007114510005830.

Marnett, L. J. 1999. Lipid peroxidation—DNA damage by malondialdehyde. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 424(1):83-95. doi:10.1016/S0027-5107(99)00010-X.

Masters, D. G., D. I. Paynter, J. Briegel, S. K. Baker, and D. B. Purser. 1988. Influence of manganese intake on body, wool and testicular growth of young rams and on the concentration of manganese and the activity of manganese enzymes in tissues. Crop and Pasture Sci. 39(3):517-524. doi:10.1071/AR9880517.

Matsui, T. 2012. Vitamin C nutrition in cattle. Asian-Australasian J. Anim. Sci. 25(5):597-605. doi:10.5713/ajas.2012.r.01.

Mehlhase, J. and T. Grune. 2002. Proteolytic response to oxidative stress in mammalian cells. Biol. Chem. 383:559-567. doi:10.1515/BC.2002.057.

Nordberg, J. and E. S. J. Arner. 2001. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free radical biology and medicine. 31(11):1287-1312.

NRC. 2000. Nutrient requirements of beef cattle. 7th rev. ed. Natl. Acad. Press, Washington, D.C.

Ojano-Dirain, C., M. Iqbal, T. Wing, M. Cooper, and W. Bottje. 2005. Glutathione and respiratory chain complex activity in duodenal mitochondria of broilers with low and high feed efficiency. Poultry science. 84(5):782-788. doi:10.1093/ps/84.5.782.

Ojano-Dirain, C., M. Toyomizu, T. Wing, M. Cooper, and W. G. Bottje. 2007. Gene Expression in Breast Muscle and Duodenum from Low and High Feed Efficient Broilers. Poultry Sci. 86(2):372-381. doi:10.1093/ps/86.2.372.

Petzke, K. J., J. Proll, J. Brückner, and C. C. Metges. 1999. Plasma protein carbonyl concentration is not enhanced by chronic intake of high-protein diets in adult rats. J. Nutr. Biochem. 10(5):268-273. doi:10.1016/S0955-2863(99)00006-6.

Pogge, D. J., E. L. Richter, M. E. Drewnoski, and S. L. Hansen. 2012. Mineral concentrations of plasma and liver after injection with a trace mineral complex differ among Angus and Simmental cattle. J. Anim. Sci. 90:2692–2698. doi:10.2527/jas.2011-4482.

Pogge, D. J. and S. L. Hansen. 2013. Supplemental vitamin C improves marbling in feedlot cattle consuming high sulfur diets. J. Anim. Sci. 91:4303–4314. doi:10.2527/jas.2012-5638.

Pogge, D. J., S. M. Lonergan, and S. L. Hansen. 2015. Impact of supplementing vitamin C for 56, 90, or 127 days on growth performance and carcass characteristics of steers fed a 0.31 or 0.59% sulfur diet. J. Anim. Sci. 93(5):2297-2308. doi:10.2527/jas.2014-7913.

Richter, E. L., M. E. Drewnoski, and S. L. Hansen. 2012. Effects of increased dietary sulfur on beef steer mineral status, performance and meat fatty acid composition. J. Anim. Sci. 90:3945–3953. doi:10.2527/jas.2011-4512.

Russell, J. R. 2015. Feed efficiency in beef cattle: Relationship with digestibility, antioxidant activity, oxidative stress, growth performance, and carcass characteristics. PhD Diss. Iowa State Univ., Ames.

Sandelin, B. A., A. H. Brown, Jr., M. A. Brown, C. Ojano-Dirain, and R. T. Baublits. 2005. Association of Mitochondrial Function with Feed Efficiency in Angus Cattle. Univ. Arkansas, Fayetteville. http://arkansasagnews.uark.edu/535-30.pdf (Accessed 20 September, 2015).

Shull, S., N. H. Heintz, M. Periasamy, M. Manohar, Y. M. Janssen, J. P. Marsh, and B. T. Mossman. 1991. Differential regulation of antioxidant enzymes in response to oxidants. J. Biol. Chem. 266(36):24398-24403. doi:10.1016/0304-4165(77)90284-7.

Sies, H. 1997. Oxidative stress: oxidants and antioxidants. Exp. Physiol. 82(2):291-295. doi:10.1113/expphysiol.1997.sp004024.

Smith, S. B., H. Kawachi, C. B. Choi, C. W. Choi, G. Wu, and J. E. Sawyer. 2009. Cellular regulation of bovine intramuscular adipose tissue development and composition. J. Anim. Sci. 87 (E. Suppl):72-82. doi:10.2527/jas.2008-1340.

USDA ERS. 2013. Farm production expenses, 2009-2013F.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597. doi:10.3168/jds.S0022-0302(91)78551-2.

Weisiger, R. A. and I. Fridovich. 1973. Superoxide dismutase organelle specificity. J. Biol. Chem. 248(10):3582-3592.

Zhang, L., M. Maiorino, A. Roveri, and F. Ursini. 1989. Phospholipid hydroperoxide glutathione peroxidase: specific activity in tissues of rats of different age and comparison with other glutathione peroxidases. Biochimica et Biophysica Acta. 1006(1):140-143. doi:10.1016/0005-2760(89)90336-6.

Table 1. Composition and analysis of diets fed to steers during the growing phase

Ingredient, % DM	G-Corn[1]	G-Rough[1]
Whole shell corn	65.1	-
Soybean hull pellets	-	38.2
Rye baleage	-	32.5
Dried distillers grains	24.5	22.2
Soyplus[2]	4.51	4.05
Porcine blood meal	3.50	2.02
Limestone	1.2	0.61
Urea	0.47	-
Vitamin premix[3]	0.25	0.2
Salt	0.13	0.11
Pellet binder	0.13	-
Choice white grease	0.10	-
MU TM premix[4]	0.09	0.07
MFP[5]	-	0.05
Rumensin 90[6]	0.01	0.01
Nutritional analysis		
DM, % as-fed basis	88.3	68.3
NDF[7], % DM	21.1	52.3
ADF[7], % DM	4.9	29.0
CP[7], % DM	23.1	22.3
Zinc[7], mg/kg DM	56.8	72.3
Copper[7], mg/kg DM	10.8	14.4
Manganese[7], mg/kg DM	23.7	47.4
Selenium[8], mg/kg DM	0.32	0.24

[1] Growing phase diets: G-Corn = whole shell corn-based; G-Rough = rye baleage and soybean hull-based.
[2] Soyplus (West Central Cooperative, Ralston, IA).
[3] Vitamin premix fulfills 2,200 IU vitamin A, 275 IU vitamin D, 100 IU vitamin E per kg of diet.
[4] Trace mineral premix fulfills 10 mg Cu, 50 mg Fe, 20 mg Mn, 30 mg Zn, 0.1 mg Co, 0.1 mg Se, 0.5 mg I per kg diet.
[5] DL-methionine hydroxy analogue calcium (84% methionine, Novus International, Saint Charles, MO).
[6] Provided Monensin at 150 $mg \cdot steer^{-1} \cdot d^{-1}$, Elanco Animal Health, Indianapolis, IN.
[7] Determined from analysis of total mixed rations.
[8] Calculated from ingredient concentrations (NRC, 2000).

Table 2. Descriptive statistics of most and least feed efficient steers during the growing phase[1]

	G-Corn[2]		G-Rough[2]	
Item	LFE[3]	HFE[3]	LFE[3]	HFE[3]
Steers, n	12	12	12	12
Average RFI	1.34	-1.37	1.13	-1.22
SD from mean[4]	1.57	1.61	1.54	1.67
Average G:F	0.186	0.274	0.193	0.254
Average ADG, kg/d	1.68	1.70	2.00	1.97
Average DMI, kg/d	8.89	6.33	10.30	7.93

[1] Steers identified as most and least feed efficient within total growing phase diet groups: G-Corn = 88 steers; G-Rough = 93 steers.

[2] Growing phase diets: G-Corn = whole shell corn-based; G-Rough = rye baleage and soybean hull-based.

[3] Growing phase feed efficiency classifications: LFE = least feed efficient; HFE = most feed efficient.

[4] Average SD from the RFI mean within growing phase diet.

Table 3. Composition and analysis of diets fed to steers during the finishing phase

Ingredient, % DM	F-Corn[1]	F-Byp[1]
Cracked corn	75	30
Dried distillers grains	14.99	39.99
Soybean hull pellets	-	20
Bromegrass hay	8	8
Limestone	1.54	1.54
Salt	0.31	0.31
Vitamin A premix[2]	0.11	0.11
Trace mineral premix[3]	0.035	0.035
Rumensin 90[4]	0.013	0.013
Nutritional analysis		
DM, % as-fed basis	87.8	89.0
NDF[5], % DM	24.4	42.7
ADF[5], % DM	8.0	18.7
CP[5], % DM	11.2	18.4
Zinc[5], mg/kg DM	43.8	50.0
Copper[5], mg/kg DM	10.5	11.2
Manganese[5], mg/kg DM	28.6	29.2
Selenium[6], mg/kg DM	0.18	0.24

[1] Finishing phase diets: F-Corn = cracked corn-based; F-Byp = dried distillers grains and soybean hull-based.

[2] Vitamin A premix contained 4,400,000 IU/kg.

[3] Provided per kilogram of diet (from inorganic sources): 30 mg Zn, 20 mg Mn, 0.5 mg I, 0.1 mg Se, 10 mg Cu, 0.1 mg Co.

[4] Provided Monensin at 200 $mg \cdot steer^{-1} \cdot d^{-1}$, Elanco Animal Health, Indianapolis, IN.

[5] Determined from analysis of total mixed rations.

[6] Calculated from ingredient concentrations (NRC, 2000).

Table 4. Growing phase oxidative stress markers in beef steers as affected by growing phase diet and feed efficiency classification[1]

	G-Corn[2]		G-Rough[2]			*P*-values[4]		
Item	LFE[3]	HFE[3]	LFE[3]	HFE[3]	SEM	Diet	FE	Diet*FE
Red blood cell lysate								
Glutathione, μM								
Total (tGSH)	215	204	256	223	19.3	0.13	0.3	0.6
Oxidized (GSSG)	31	34	39	47	6.3	0.03	0.3	0.5
Reduced (GSH)	185	173	219	177	16.7	0.3	0.11	0.4
Ratio[5]	0.16	0.18	0.17	0.28	0.031	0.07	0.04	0.13
Plasma								
Protein carbonyl[6]	0.27	0.30	0.30	0.34	0.016	0.04	0.03	0.8
Malondialdehyde[7]	8.1[b]	9.8[ab]	10.7[a]	8.9[ab]	0.79	0.3	0.9	0.03

[a, b] Least squares means in a row without common superscript differ ($P < 0.05$).

[1] Blood samples were collected 3 d after conclusion of growing phase.

[2] Growing phase diets: G-Corn = whole shell corn-based; G-Rough = rye baleage and soybean hull-based.

[3] Growing phase feed efficiency classifications: LFE = least feed efficient; HFE = most feed efficient.

[4] *P*-values: Diet = main effect of growing phase diet; FE = main effect of growing phase feed efficiency classification; Diet*FE = interaction effect of growing phase diet and feed efficiency classification.

[5] Ratio of oxidized:reduced glutathione (GSSG:GSH).

[6] Reported as nmol/mg protein.

[7] Reported as μM.

Table 5. Growing phase antioxidant enzyme activities in beef steers as affected by growing phase diet and feed efficiency classification[1]

	G-Corn[2]		G-Rough[2]			*P*-values[4]		
Item	LFE[3]	HFE[3]	LFE[3]	HFE[3]	SEM	Diet	FE	Diet*FE
Red blood cell lysate								
SOD[5]								
Total	28.4[b]	32.2[ab]	36.9[a]	26.4[b]	2.66	0.6	0.2	0.01
Manganese	21.7[b]	23.6[ab]	27.8[a]	20.0[b]	3.67	0.5	0.17	0.03
Copper-Zinc	8.1	9.1	8.9	6.6	2.54	0.5	0.6	0.14
GPX[6]	161.0[b]	174.3[b]	224.5[a]	129.6[b]	17.23	0.6	0.02	0.002
Plasma								
Ascorbate μM	29.4	28.4	30.2	25.6	3.45	0.8	0.4	0.6

[a, b] Least squares means in a row without common superscript differ ($P < 0.05$).

[1] Blood samples were collected 3 d after conclusion of growing phase.

[2] Growing phase diets: G-Corn = whole shell corn-based; G-Rough = rye baleage and soybean hull-based.

[3] Growing phase feed efficiency classifications: LFE = least feed efficient; HFE = most feed efficient.

[4] *P*-values: Diet = main effect of growing phase diet; FE = main effect of growing phase feed efficiency classification; Diet*FE = interaction effect of growing phase diet and feed efficiency classification.

[5] Superoxide dismutase activity; one unit of SOD activity (U) is defined as the enzyme required to dismutate 50% of the superoxide radical; reported as 1,000 U·g hemoglobin^{-1}.

[6] Glutathione peroxidase; one unit of GPX activity is defined as the enzyme required to oxidize 1.0 nmol of reduced nicotinamide adenine dinucleotide phosphate (NADPH) to the oxidized form (NADP+) per minute at 25°C; reported as mmol·minute^{-1}·g hemoglobin^{-1}.

Table 6. Growing phase liver mineral status of beef steers as affected by growing phase diet and growing phase feed efficiency classification[1]

	Growing phase diets[2]		FE classification[3]			*P*-values[4]	
Liver, mg/kg DM	G-Corn	G-Rough	LFE	HFE	SEM	Diet	FE
Copper	287.3	343.2	332.9	297.6	18.78	0.04	0.2
Iron	146.8	150.6	154.7	142.6	3.64	0.5	0.02
Manganese	8.77	9.05	9.32	8.50	0.320	0.5	0.08
Selenium	2.04	2.28	2.28	2.03	0.084	0.05	0.04
Zinc	114.7	114.8	116.6	112.9	5.47	1.0	0.6

[1] Liver samples were collected 3 d after conclusion of growing phase.
[2] Growing phase diets: G-Corn = whole shell corn-based; G-Rough = rye baleage and soybean hull-based.
[3] Growing phase feed efficiency classifications: LFE = least feed efficient; HFE = most feed efficient.
[4] *P*-values: Diet = main effect of growing phase diet; FE = main effect of growing phase feed efficiency classification; Growing phase diet × feed efficiency classification effect was not significant ($P \geq 0.3$).

Table 7. Finishing phase oxidative stress markers in beef steers as affected by growing phase diet and feed efficiency classification[1]

	G-Corn[2]		G-Rough[2]			*P*-values[4,5]		
Item	LFE[3]	HFE[3]	LFE[3]	HFE[3]	SEM	Diet	FE	Diet*FE
Red blood cell lysate								
Glutathione, μM								
Total (tGSH)	221	212	222	217	15.4	0.9	0.7	0.9
Oxidized (GSSG)	35[xy]	25[y]	29[xy]	37[x]	7.2	0.5	0.9	0.04
Reduced (GSH)	213	200	215	216	27.5	0.4	0.6	0.6
Ratio[6]	0.16	0.13	0.14	0.17	0.050	0.6	1.0	0.07
Plasma								
Protein carbonyl[7]	0.30	0.32	0.30	0.31	0.036	0.7	0.3	0.8
Malondialdehyde[8]	8.0	7.6	7.5	7.9	0.64	0.9	1.0	0.6

[x, y] Least square means in a row without common superscript tend to differ ($P < 0.1$).

[1] Blood samples were collected at conclusion of finishing phase.

[2] Growing phase diets: G-Corn = whole shell corn-based; G-Rough = rye baleage and soybean hull-based.

[3] Growing phase feed efficiency classifications: LFE = least feed efficient; HFE = most feed efficient.

[4] *P*-values: Diet = main effect of growing phase diet; FE = main effect of growing phase feed efficiency classification; Diet*FE = interaction effect of growing phase diet and feed efficiency classification.

[5] Effects of growing phase diet × finishing phase diet, growing phase feed efficiency classification × finishing phase diet, and growing phase diet × growing phase feed efficiency classification × finishing phase diet were not significant ($P \geq 0.11$).

[6] Ratio of oxidized:reduced glutathione (GSSG:GSH).

[7] Reported as nmol/mg protein.

[8] Reported as μM.

Table 8. Finishing phase oxidative stress markers in beef steers as affected by finishing phase diet[1]

Item	F-Corn[2]	F-Byp[2]	SEM	*P*-value[3]
Red blood cell lysate				
Glutathione, μM				
Total (tGSH)	209	227	10.9	0.3
Oxidized (GSSG)	32	31	6.5	0.8
Reduced (GSH)	209	213	26.1	0.8
Ratio[4]	0.15	0.15	0.048	1.0
Plasma				
Protein carbonyl[5]	0.28	0.34	0.034	0.001
Malondialdehyde[6]	7.8	7.8	0.46	1.0

[1] Blood samples were collected at conclusion of finishing phase.
[2] Finishing phase diets: F-Corn = cracked corn-based; F-Byp = dried distillers grains and soybean hull-based.
[3] Effects of growing phase diet × finishing phase diet, growing phase feed efficiency classification × finishing phase diet, and growing phase diet × growing phase feed efficiency classification × finishing phase diet were not significant ($P \geq 0.11$).
[4] Ratio of oxidized:reduced glutathione (GSSG:GSH).
[5] Reported as nmol/mg protein.
[6] Reported as μM.

Table 9. Finishing phase antioxidant enzyme activities in beef steers as affected by growing phase diet and feed efficiency classification[1]

	G-Corn[2]		G-Rough[2]			*P*-values[4,5]		
Item	LFE[3]	HFE[3]	LFE[3]	HFE[3]	SEM	Diet	FE	Diet*FE
Red blood cell lysate								
SOD[6]								
Total	22.8	21.1	24.7	24.6	2.02	0.06	0.6	0.6
Manganese	12.8	13.9	13.9	14.8	2.04	0.2	0.3	1.0
Copper-Zinc	10.2	7.1	11.1	9.7	1.69	0.14	0.09	0.5
GPX[7]	46.8[b]	54.5[ab]	60.7[a]	44.9[b]	4.84	0.7	0.4	0.02
Plasma								
Ascorbate, μM	13.2	14.3	13.6	14.7	3.73	0.9	0.4	1.0

[a, b] Least squares means in a row without common superscript differ ($P < 0.05$).

[1] Blood samples were collected at conclusion of finishing phase.

[2] Growing phase diets: G-Corn = whole shell corn-based; G-Rough = rye baleage and soybean hull-based.

[3] Growing phase feed efficiency classifications: LFE = least feed efficient; HFE = most feed efficient.

[4] *P*-values: Diet = main effect of growing phase diet; FE = main effect of growing phase feed efficiency classification; Diet*FE = interaction effect of growing phase diet and feed efficiency classification.

[5] Effects of finishing phase diet, growing phase diet × finishing phase diet, growing phase feed efficiency classification × finishing phase diet, and growing phase diet × growing phase feed efficiency classification × finishing phase diet were not significant ($P \geq 0.2$).

[6] Superoxide dismutase activity; one unit of SOD activity (U) is defined as the enzyme required to dismutate 50% of the superoxide radical; reported as 1,000 U·g hemoglobin^{-1}.

[7] Glutathione peroxidase; one unit of GPX activity is defined as the enzyme required to oxidize 1.0 nmol of reduced nicotinamide adenine dinucleotide phosphate (NADPH) to the oxidized form (NADP+) per minute at 25°C; reported as mmol·minute^{-1}·g hemoglobin^{-1}.

CHAPTER 6.

GENERAL CONCLUSIONS

Feed efficiency (FE) is a major contributor to cattle profitability but can vary greatly between individuals. The purpose of this research was to evaluate the relationship between FE and other production traits, assess the repeatability of FE across different feedlot growth phases and diet types, and explore some of the physiological mechanisms responsible for FE variation. As with all research, the studies in this dissertation provided answers to several inquiries while also generating additional questions.

Examining the relationship between FE across multiple growth phases and diet types is important for determining means by which to select and manage cattle based on FE phenotype. Measuring individual intake to calculate FE is expensive and laborious; therefore, if FE was repeatable across multiple growth phases FE could be measured once and cattle could be managed accordingly during subsequent phases based on the initial FE phenotype. However, conventional feedlot cattle production entails different feeding phases utilizing different diet types, often a high fiber diet fed during the growing phase followed by a high energy diet during the finishing phase when a quality-based market is targeted. Limited work has previously investigated the relationship between FE across multiple phases that provide differing diet types. As such, one of the central questions at the onset of this research was to determine whether FE measured with one growing phase diet could predict FE when cattle were subsequently fed a different finishing phase diet. Over the course of five years, six groups of crossbred steers, totaling 985, were fed corn or roughage-based growing phase diets and individual FE was measured at the University of Missouri. While roughage and

other high fiber diets are traditionally fed to steers during the growing phase, energy-dense grain-based diets are conceivable in conventional cattle production, necessitating the incorporation of that diet type in the comparison. Upon arrival at Iowa State University, steers were fed finishing phase diets that were corn or byproduct-based to evaluate conventional finishing phase diet types.

In chapter 3, it was determined that steers classified as highly feed efficient (HFE) based on growing phase G:F maintained greater G:F in the finishing phase, a relationship that was also congruent for mid (MFE) and low (LFE) feed efficient steers. Thus, growing phase FE appeared to be a reasonable predictor of finishing phase FE. Perhaps the most interesting revelation was that although finishing phase G:F was not directly affected by growing or finishing phase diets, an evaluation of other growth traits revealed differences in how G:F differences resulted from underlying sources of variation. Among steers grown on roughage, finishing phase ADG differed between FE classifications yet DMI was unaffected by FE classification. Dissimilarly, among the corn-grown steers there were no differences detected in finishing phase ADG between FE classifications but DMI differed between classifications. Thus, it appeared that ADG differences were responsible for finishing phase G:F variation among roughage-grown steers whereas differences in finishing phase G:F among the corn-grown steers resulted from differences in DMI. Though growth performance was affected by growing phase diet and FE classification, carcass differences were limited. Finishing phase G:F was unaffected by any growing or finishing phase diet combinations but other growth and carcass traits were impacted. Roughage-grown byproduct-finished steers had greater DMI and consequently had greater ADG than other diet combinations. Along with increased ADG, the addition of increased HCW and no loss in marbling score relative to other diet

combinations suggests that the roughage/byproduct diet combination may be the most economically advantageous. There were limited differences among corn-grown steers or corn-finished steers; hence, diet-driven differences were largely isolated to steers fed the high fiber diets.

The basis for chapter 4 was an investigation into the relationship between diet digestibility and FE as well as the repeatability of FE between feeding phases on the individual steer level. Two groups of steers were grown and FE phenotyped with corn or roughage-based diets and finished with corn or byproduct-based diets. Titanium dioxide was fed to estimate fecal output and determine diet digestibility, and steers were ranked by G:F to determine HFE and LFE classifications. There was a positive correlation for DM digestibility between feeding phases when steers were grown and finished on similar diets, specifically the roughage-grown byproduct-finished steers and the corn-grown corn-finished steers. Although there were no differences in DM digestibility due to FE classification, it did appear that digestibility measured during one feeding phase may help predict digestive capacity during a subsequent phase if similar diet types were fed. Interestingly, fiber digestibility appeared to contribute to FE variation while starch digestibility did not, indicating that there may be more opportunity for improving FE via selection or management for better fiber utilization. Feed efficiency classification effects were most pronounced for growing phase fiber digestibility advantages in the roughage-grown HFE steers. Overall, finishing phase G:F was greater in HFE versus LFE steers. However, a negative correlation was detected between growing and finishing phase G:F values, suggesting that FE may be most accurately predicted when cattle are FE tested using diets similar to the production environment of

interest. Though, considering there were no diet interactions detected in finishing phase G:F in chapter 3, the effect of differing diets on G:F is not yet conclusive.

Chapter 5 focused on variation in endogenous antioxidant activity and oxidative stress between steers representing phenotypic extremes for FE, a focus based on previous suggestions that differences in metabolism may be partially responsible for FE variation between individuals. The most and least feed efficient steers were identified during the growing phase by calculating residual feed intake and steers were classified as HFE and LFE. Antioxidant activity and oxidative stress markers were measured in blood samples gathered at the conclusion of the growing and finishing phase, along with liver samples gathered at the conclusion of the growing phase. The growing phase diet and FE classifications had stronger relationships with growing phase antioxidant activity and oxidative stress markers; hence, the relationship between diet, FE, and oxidative stress markers may be strongest when measured in the same period. Antioxidant activity appears to play a role in FE variation, most notably among the roughage-grown steers as both Mn superoxide dismutase and glutathione peroxidase activities were greater in the roughage-grown LFE steers compared to the HFE steers. The increased antioxidant activity may decrease FE due to the use of energy for increased antioxidant synthesis and activity; energy otherwise available for tissue accretion. Liver trace mineral concentrations were adequate among all steers and were not correlated with the respective mineral-dependent antioxidants; thus, differences in antioxidant activity were likely not a result of trace mineral nutrition. Though all steers appeared to be suffering from some degree of oxidative stress, the greatest differences were detected in roughage-grown steers as the roughage-grown HFE steers had increased protein oxidation markers and increased oxidized:reduced glutathione (GSSG:GSH), suggesting that these HFE steers may

be more tolerant of oxidative stress. Energetically, the ramifications of decreased GSSG:GSH in the LFE steers could be a result of increased GSH regeneration via glutathione reductase activity, an energy-dependent process that would further decrease energy available for tissue accretion.

Ultimately, FE was repeatable across feeding phases but growing phase FE may be a better predictor of subsequent FE when diet types between feeding phases are similar. Though starch digestibility had no relationship with FE, fiber digestibility contributes to FE variation between individuals. Antioxidant activity may influence FE as antioxidant activities were greater in LFE steers, conceivably utilizing energy otherwise utilized for tissue growth. Average daily gain influenced finishing phase G:F differences among roughage-grown steers but DMI was the underlying source of variation among corn-grown steers; a concept that undoubtedly warrants further investigation. Future research should evaluate cattle performance using multiple growing and finishing phase diet combinations but may consider particularly focusing on high fiber diets as roughage-grown steers were the predominant source of variation in the present studies. Understanding the digestive differences between highly and lowly feed efficient steers may be best accomplished by exploring differences in microbial populations/activities. Finally, the relationship between FE, antioxidant activities, and oxidative stress markers are only beginning to be understood. Future research should consider adding additional analyses, specifically a glutathione reductase assay to characterize GSH regeneration as well as additional timepoints pre- and post-transit to evaluate the impact of transit stress on antioxidant activity and oxidative stress. Collectively, these recommendations will help investigators continue to develop a greater understanding of FE in order to improve beef cattle production.