NITROGEN METABOLISM IN *BOS INDICUS* AND *BOS TAURUS* CATTLE CONSUMING LOW-QUALITY FORAGES

A Thesis

by

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ABSTRACT

Five Angus (Bt) and 5 Brahman steers (Bi) fitted with ruminal and duodenal cannulas were used in concurrent 5 × 5 Latin squares to determine the effects of supplemental protein degradability and level of supplemental N on utilization of rice straw. Treatments consisted of a control (CON; no supplement) and two levels (50 and 100 mg N/kg BW) of an isonitrogenous supplement (27% CP), either high (H; 72%) or low (L; 28%) in DIP.

Forage OM intake (FOMI) was greater for Bt than Bi ($P = 0.05$). Supplementation increased FOMI in both Bt and Bi ($P < 0.05$). Organic matter digestibility (OMD) was greater in Bi than Bt ($P < 0.01$). Supplementation increased OMD for Bi ($P = 0.02$) but not Bt. Total digestible OM intake (TDOMI) was similar between subspecies ($P = 0.12$). *Bos indicus* had greater ruminal NH_3-N than Bt ($P < 0.01$). Plasma urea nitrogen (PUN) was greater for Bi than Bt ($P < 0.01$) for all treatments and at both 0 and 4 h after feeding. Supplementation tended ($P = 0.06$) to increase PUN versus CON in Bt, but not Bi ($P = 0.82$). *Bos taurus* had numerically ($P = 0.19$) greater total volatile fatty acids (VFA) across treatments than Bi. Total N intake increased versus CON ($P < 0.01$), and greater amounts of supplemental N increased total N intake within both subspecies ($P < 0.01$). Fecal N excretion was greater in Bt than Bi ($P = 0.01$). Supplementation increased ($P < 0.01$) fecal N versus CON for both subspecies. Urinary N tended to be higher for Bi than Bt ($P = 0.10$). Supplementation increased ($P < 0.05$) urinary N for both subspecies. Retained N was greater for Bt over Bi ($P = 0.07$).

While Bt had greater FOMI, increased OMD for Bi with supplementation resulted in similar TDOMI between subspecies. Overall, Bi had higher NH_3-N and PUN than Bt; which increased as level and degradability of supplements increased in both subspecies. Increased supplementation raised N excreted and N balance for both subspecies; fecal N was greater in Bt, while urinary N was greater in Bi.

NOMENCLATURE

ADF	Acid detergent fiber
ADG	Average daily gain
Bi	*Bos indicus*
Bt	*Bos taurus*
BW	Body weight
CP	Crude protein
d	Day
DDG	Dried distillers' grains
DIP	Degradable intake protein
DM	Dry matter
DMI	Dry matter intake
h	Hour
FOMI	Forage organic matter intake
MCP	Microbial crude protein
N	Nitrogen
NE_m	Net energy for maintenance
NDF	Neutral detergent fiber
OM	Organic matter
PUN	Plasma urea nitrogen

SEM	Standard error of the mean
TDOMI	Total digestible organic matter intake
TDN	Total digestible nutrients
UIP	Undegradable intake protein
VFA	Volatile fatty acids
Wk	Week

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Literature comparing digestion and metabolism in *Bos indicus* (Bi) and *Bos taurus* (Bt) cattle can be traced back nearly a century. Intuitively, we may expect Bi cattle to perform better than Bt when consuming LQF (LQF; less than 7% CP), as the genus was selected in tropical southeastern Asia, particularly the Indian subcontinent, utilizing low-quality, low-digestibility, high roughage diets. Accordingly, Bi are commonly used in tropical production systems as they are well adapted to high environmental temperatures and the associated nutritional stresses. Specifically, they can withstand hot and humid weather, tolerate intense sunshine, resist parasites, and utilize poor-quality forages (Chizzotti et al., 2008). In contrast, Bt were selected in Europe's more temperate climate, where they produced high quality carcasses on diets typically consisting of better quality forage (Howes et al., 1963; Moore et al., 1975). They are not as well adapted to high environmental temperatures and poor feed quality as Bi, but they do have the advantages of providing superior carcass quality, and being earlier maturing (Forbes et al., 1998).

A large portion of beef cattle production occurs in the southern US. Cattle raised in this region need to be physiologically and behaviorally adapted to high ambient temperatures, high humidity, and low-quality roughages. However, LQFs are need in most beef cattle operations at some point in the year. In addition to native ranges and managed pastures, crop residues are a major component of ruminant diets for much of the year in many systems (Leng and Nolan, 1984). These cellulolytic materials are

important as they provide nutrients to ruminant animals that are otherwise unusable by humans. However, LQFs are deficient in protein, which may decrease animal performance.

Before investigating the differences between Bi and Bt genotypes and their ability to utilize LQFs, it is important to have a general understanding of protein supplementation and its role in beef production. Protein supplementation of LQFs is an effective option for producers to address deficiencies in the available forages and increase or maintain animal productivity (Köster et al., 1996; Mathis et al., 2000; Bandyk et al., 2001). When LQF makes up the majority of the diet, N is normally the first limiting nutrient for microbial growth in the rumen. Protein supplementation improves animal performance by providing ruminally available N (RAN) to meet microbial N requirements (Köster et al., 1996). Addressing microbial N requirements increases microbial growth and fermentative activity (g of OM fermented ruminally). Increased fermentation results in increased forage intake, digestion, VFA production, and flow of MCP to the small intestine (Wickersham et al., 2008). Ultimately, this improves the protein and energy status of the animal by increasing the MP supplied to the small intestine (Wickersham et al., 2008), which improves the protein and energy status of the animal as well as its overall performance.

Protein is fractionated into degradable intake protein (DIP) and undegradable intake protein (UIP), based on its availability in the rumen. Supplying DIP directly provides N to microbes, and is regarded as the protein fraction that limits LQF utilization (Köster et al., 1996; Mathis et al., 2000; Bandyk et al., 2001). It is considered limiting

as it stimulates intake by providing a direct supply of RAN, improving cellulose fermentation, and increasing MCP flow to the duodenum. Supplemental UIP escapes rumen degradation and is digested and absorbed in the small intestine. Undegradable intake protein and MCP are digested into AA, di and tri-peptides and absorbed in the small intestine to meet MP requirements of the GIT. Absorbed amino acids can be deposited into proteins or degraded to meet energy requirements, with resultant ammonia being detoxified to urea. Urea can subsequently be recycled to the gastrointestinal tract or excreted in the urine. Wickersham et al. (2008) and Wickersham et al. (2009)reported high fractional transfer rates of urea to the GIT. Provision of UIP supplies RAN via N recycling (Wickersham et al., 2009),and increases protein flow to the duodenum, both resulting in improved forage utilization (Bandyk et al., 2001; Wickersham et al., 2009). The NRC (2000) recommends feeding 13% fermentable OM as DIP to supply an adequate proportion of RAN. This recommendation fails to account for all feeding situations and to account for N recycling. Therefore a better recommendation is to supplement 9.0 – 11.6% of fermentable OM as DIP when a ruminant's diet is LQF in order to meet RAN requirements of the microbes (Köster et al., 1996; Klevesahl et al., 2003; Wickersham et al., 2008).

Ruminal ammonia is a vital source of N for a number of rumen bacteria including cellulolytic bacteria (Bryant and Robinson, 1962). There is a positive relationship between the level of supplementation and ruminal ammonia concentrations (Köster et al., 1996; Mathis et al., 2000; Wickersham et al., 2008).

Ruminants can recycle large quantities of urea to the rumen rather than excreting it in the urine. Urea recycling saves N and supplies microbes with needed ammonia, improving the nitrogen economy of ruminants. Modern beef production system does not fully take advantage of the ability of ruminants to recycle N, as protein is usually fed in excess due to the high cost of energy feedstuffs (Marini and Van Amburgh, 2005). Excretion of excess N has increased public concern and interest in governmental oversight of pollution and environmental impact of production systems. Nitrogen runoff can affect ground and surface water quality, and ammonia emissions can affect air quality (Cole et al., 2005). These concerns have lead researchers to try and find ways to reduce N excretion and more effectively supplement protein, as a better understanding of whole-animal N metabolism will allow nutritionists to optimize growth performance while minimizing N excretion (Marini and Van Amburgh, 2005; Todd et al., 2006).

Subspecies differences: Intake

Protein deficiency reduces forage intake in ruminants by limiting microbial growth and OM fermentation in the rumen, which limits digesta passage (Hunter and Siebert, 1986b). Microbial protein synthesis is also limited on LQFs, resulting in less MCP for digestion and absorption in the small intestine to meet the tissue demand for protein (Hunter and Siebert, 1987). Numerous factors contribute to the complex regulation of feed intake in ruminants (Hunter and Siebert, 1985, 1986b). These include but are not limited to the physiological state, age, and genotype, as well as dietary characteristics such as fiber and protein concentration. For a specific animal, roughage

intake is largely dependent on OM removal from the rumen, which in turn is a function of fermentation rate and passage rate (Leng and Nolan, 1984).

Differences in intake between Bt and Bi steers depends on the quality of the diet, as LQF differences in intake between the two subspecies do not tend to be as significant as they are on higher quality forages (Hunter and Siebert, 1986b, 1987).

When consuming a LQF diet, Bt cattle have been shown to have greater intakes compared to Bi (Frisch and Vercoe, 1977; Hunter and Siebert, 1985; Hennessy et al., 2000). Frisch and Vercoe (1977) reported that Bt steers had 7% higher voluntary intakes than crossbred Bi steers on both alfalfa (28.5 versus 26.6 g/kg) and a low-quality pasture hay (25.4 versus 23.4 g/kg). Fasting metabolic rate was 3% greater for Bt than Bi × Bt cross after being fed both an alfalfa diet and a pasture hay diet. Similarly, Hennessy et al. (2000) observed that Bt steers had greater intakes than Bi steers when fed subtropical carpet grass, eating 10.9% more than Bi steers, and 5.1% more than Bt × Bi cross steers. Steers of all compositions increased intake of low-quality hay with protein supplementation; however, Bi steers had the lowest hay intakes and were the least responsive of the breed types to protein supplementation (Bt average intake = 6 kg DM/d versus Bi average intake = 5 kg DM/d). Hunter and Siebert (1985) evaluated the effect of DIP on intake of low-quality subtropical hays (Pangola grass and Spear grass) in Bi and Bt steers. When unsupplemented hay was fed, there was no significant difference between breeds in intake (Bt intake ranged from 11.3 to 17.8 g/kg BW; Bi ranged from 11.8 to 16.1 g/kg BW). However, when the forages were supplemented

with DIP, intake was significantly ($P < 0.05$) greater for Bt than Bi steers, as the Bt steers increased intake by 42% and the Bi intakes increased by 15%.

Moran et al. (1979) studied intake, digestion, and utilization of low-quality sorghum hay in Bi × Bt cross and Bt steers. *Bos taurus* steers had numerically greater DM intakes than the Bi × Bt cross (88.1 versus 81.2 g/kg BW) the differences were not significant. They suggested that the differences in cattle performance under field conditions are likely due to environmental factors such as diet selectivity, length of grazing period, and tolerance to parasites, than to inherent factors such as digestibility.

It has been reported that Bt have significantly greater intakes versus Bi when consuming higher-quality forages (Moran, 1976; Hunter and Siebert, 1986b). Hunter and Siebert (1986b) studied the intake of various roughage diets in Bi and Bt steers and concluded that intake of both breeds increased as forage protein concentration increased and fiber decreased. Differences in intake between the Bt and Bi steers were dependent on the quality of diet. They observed that Bi steers consumed 4.2% more Speargrass than Bt, but Bt had 10% greater intakes when Pangolagrass was fed. However, higher quality forages (pasture hay + alfalfa, and alfalfa), Bt ate significantly more than Bi (15% and 20% more). Similarly, in a review of previous literature, Varel et al. (1999) surmised that when higher-quality forage is fed (21% CP) or forage plus concentrate diet is fed, Bt cattle consume more feed relative to their maintenance requirements, therefore they gain more efficiently and faster than Bi cattle. Frisch and Vercoe (1969) observed similar results using Bi and Bt × Bt cross steers fed alfalfa hay. *Bos indicus* ate significantly less (9 versus 11 kg/d) than the Bt cross on a higher quality forage ration.

They hypothesized that Bi steers did not differ in weight change from Bt × Bt because of lower maintenance requirements of Bi animals. The NRC (2000) supports this suggestion by reducing the NEm requirement by 10% versus Angus, Hereford, Shorthorn, Charolais, and Limousin. Similarly, Vercoe (1970) found that, despite similar fasting heat productions, Bi cross steers maintained live-weight at lower feed intakes than did Bt steers. These results are similar to that of Moran (1976) and might suggest that the Bi steers may have a lower maintenance feed requirement and/or a higher efficiency of feed utilization above maintenance.

Frisch and Vercoe (1984) observed higher intakes by Bt crossbred steers when compared to Bi fed alfalfa. They concluded that Bt × Bt crossbred cattle had approximately 20% greater maintenance energy requirements than Bi (fasting metabolism values were as follows: Bt = 102, and Bi = 88 MJ/kg/d). They concluded that a greater energy requirement, Bt eat more to meet maintenance requirements, provided a diet is digested to the same extent in both subspecies. This is a reasonable assumption, as differences in apparent digestibility between breeds have seldom observed on diets that contained higher quality forages such as alfalfa (Moran and Vercoe, 1972; Warwick and Cobb, 1976).

Lower maintenance requirement in Bi have been suggested to reflect lower internal organ mass than Bt (Butler et al., 1956; Schneider and Flatt, 1975). Grimaud et al. (1998) observed no differences in either intake or total tract retention time between the two subspecies, but they reported that Bi cattle have a smaller total digestive tract

than Bt cattle. They conclude that this is in part responsible for the apparent higher degree of heat tolerance exhibited by the Bi genus.

Contrary to the previous studies, there is some research that has observed higher DMI in Bi than Bt animals when fed LQFs (Howes et al., 1963; Karue et al., 1972). Howes et al. (1963) reported greater intakes for Bi than Bt heifers fed a 6% CP hay diet (4.4 versus 3.8 kg/d). Karue et al. (1972) similarly observed that Bi had 23% greater DMI than Bt × Bt steers of similar weight on low-quality hay. When the same steers were supplemented with urea and cottonseed meal, DM intake was greater in the Bi steers than the Bt × Bt steers (105 versus 89 g/kg BW). They suggested higher intakes in Bi are a necessary survival mechanism for survival on fibrous low protein pastures the subspecies has gained from selection in subtropical and tropical regions.

Subspecies differences: Digestion

The reticulo-rumen is the primary site of fermentation in the ruminant animal, with a diverse microbial population degrading cellulolytic material to produce microbial crude protein and volatile fatty acids for the animal. Differences in fermentation rate and digestion have been observed between the Bi and Bt subspecies consuming LQFs (Hunter and Siebert, 1985). French (1940) reported greater fiber digestion in Bi than Bt. Accordingly, researchers have been trying to describe the mechanisms behind the digestive differences between the two subspecies. Hungate et al. (1960) and Phillips et al. (1960) were the first to suggest that Bi had faster rates of fermentation than Bt when LQFs were fed. When a 6% CP hay diet was fed to steers, Hungate et al. (1960) noted that Bi had a 17% faster fermentation rate, while Hungate et al. (1960) observed an 8%

faster fermentation rate in Bi versus Bt when fed a low-quality grass hay. Phillips et al. (1960) also concluded that Bi cattle fed an all low-quality diet (6% CP) had greater total tract DM digestion than Bt (67 versus 65%). Neither study, was able to demonstrate significant differences between the breeds, which may have been due to the number of animals that were used for the trials (Hegarty, 2004). Phillips et al. (1960) reported that Bi cattle had 3% greater ($P < 0.01$) OM digestion when fed low-quality grass than Bt. Howes et al. (1963) reported greater digestion coefficients for Bi heifers (63%) than for Bt (61%) when fed a low-quality roughage (6% CP hay) with protein supplementation. Moore et al. (1975) used *in vitro* dry matter disappearance (IVDMD) to compare the rumen inoculums from Bt and Bi steers. Results indicated that fermentation with inoculums from Bi were greater (71%) than inoculums from the Bt (67.4%). This data agrees with the aforementioned *in vivo* research which shows greater digestion of dry matter (DDM) in Bi animals over Bt fed a LQF.

Differences in protozoa populations have been distinguished between Bi and Bt, as O'Kelly and Spiers (1992) showed that at the same level of intake of LQF, there is a much greater ($P < 0.01$) protozoal population density in the rumen fluid of Bi compared to Bt (44.5×10^6 versus 19.7×10^6 /L). They also conclude that Bi achieve and maintain a higher body weight when consuming LQFs because more metabolizable energy and essential nutrients are supplied from the rumen to their body tissues, and that after the same period of feed deprivation, Bi cattle have fasted for a longer period of time because the residual feed in their rumens is fermented at a more rapid rate.

Grimaud et al. (1998) reported negligible differences in apparent digestion (64.3 versus 64.8%) between Bi and Bt fed a high fiber diet (62% NDF; 38% ADF). Kennedy (1982) observed that with LQF diets, apparent OM digestion did not differ significantly between Bi × Bt and Bt steers; however, the Bi × Bt digested numerically more OM in the reticulo-rumen than Bt (90 versus 83%). They did conclude that the greater apparent ruminal OM digestion for Bi × Bt compared to Bt (1.68 versus 1.53 kg/d), was balanced by less OM digestion post ruminally in Bi × Bt versus Bt (0.19 versus 0.31 kg/d), accounting for insignificant differences in total tract digestion. Hunter and Siebert (1986a) concluded that there was no advantage in rumen digestive efficiency in favor of Bi when the diet was deficient in protein (59 versus 59%). They postulate that studies in which no such advantage in which apparent digestibility was noticeable, that there was either compensatory post-ruminal digestion in the Bt animal, or that digesta was retained longer in the reticulo-rumen of Bi to enable the same degree of digestion to occur. However, Hunter and Siebert (1985) observed a shorter mean retention time of digesta in the reticulo-rumen of Bi (14.5 h) compared to Bt (26.2 h) fed low-quality diets which tends to support the first conclusion.

Similar digestion in both genotypes when adequate protein and energy is supplied to the rumen is likely the reason that differences in digestive efficiency between breeds have been rarely found on good quality diets. Hennessy et al. (2000) used Bi, Bt, and Bi × Bt steers to evaluate responses to supplemental protein when fed low-quality carpet grasses. Diet digestion did not differ between the breed types and N supplementation did not increase digestion, which is in agreement with Hunter and

Siebert (1985). Conversely, Moore et al. (1975) found that on a higher plane of nutrition with more than adequate energy and protein supplied (14% CP), Bt bulls had greater digestion than Bi bulls (71 versus 63%). However, when the diet contained less protein and energy (11% CP) Bi bulls had greater digestion than Bt bulls (54 versus 61%). While these differences may be due to a combination of the energy and protein supplied to the rumen, the impact of quality of diet on the digestibility of the animal is related to subspecies.

Subspecies differences: Ruminal ammonia concentrations

Ruminal ammonia is a primary end product of ruminal protein degradation and therefore is often elevated with protein supplementation (especially DIP). A common observation in cattle fed LQF is low concentrations of ruminal ammonia (Hennessy and Nolan, 1988). Ruminal ammonia-N (NH_3-N) is a major source of N for rumen bacteria, and the minimum in vitro concentration where microbial cell synthesis is maximized has been established as 2.94- 4.7 *mM* (Slyter et al., 1979). *Bos taurus* cattle have been shown to have low NH_3-N concentrations and decreased in live weight gain when grazing subtropical pastures (Cohen and O'Brien, 1974) (Hennessy et al., 2000). Greater NH_3-N concentrations in Bi than Bt steers on LQF, are thought to result in improved digestion of the forages due to the greater availability of NH_3-N (Hennessy and Nolan, 1988). Conversely, the lower NH_3-N concentrations in Bt is related to lower rates of digestion of OM in the rumen, and longer mean retention times of digesta (Hunter and Siebert, 1985).

Hennessy et al. (2000) studied impact of supplementing rumen soluble N (urea) and undegradable intake protein (formaldehyde-treated casein; F-casein) on NH_3-N concentration of Bt and Bi steers consuming LQF. They observed that the when the forage was fed without supplementation Bi steers had greater NH_3-N concentrations (0.59 versus 2.28 *mM*). Interestingly, when urea was supplemented, the NH_3-N in Bt increased by 31% to 0.85 *mM*, but this did not occur in Bi steers, 2.47 *mM*. Similarly, when F-casein was supplemented, NH_3-N in Bt increased 4 fold (2.6 *mM*), but only 75% for Bi (3.15 *mM*). Nonetheless, the Bi steers still maintained greater NH_3-N concentrations than Bt steers with supplementation.

Hunter and Siebert (1985) reported less digestion of an unsupplemented LQF in Bt steers than Bi steers, which correlated with lower NH_3-N concentrations; 0.94 and 2.35 *mM*, respectively. Extent of digestion increased in both subspecies with supplementation and differences between Bi and Bt disappeared. Increased digestion was not achieved until NH_3-N concentrations of between 3.5 – 4.7 *mM*. In fact, it has been shown that in east Africa, the Boran breed of the Zebu (Bi) cattle are able to utilize LQF more efficiently than Bt animals (Karue et al., 1972), which is believed to result from faster rates of digestion due to maintenance of higher ruminal ammonia-N concentrations with less dietary N. Higher levels of ruminal ammonia-N suggest Bi cattle have a greater capacity to recycle urea-N (Frisch and Vercoe, 1977; Hunter and Siebert, 1985, 1987; Hennessy et al., 2000).

Not all research has shown that Bi have greater NH_3-N concentrations than Bt. Kennedy (1982) observed no differences in NH_3-N concentrations between Bt and Bi

crossbred steers fed alfalfa (14 versus 14 *mM*), and a slightly higher concentration for Bt when the diet was pasture hay (5.2 versus 4.3 *mM*). However, the pasture hay contained 12 g N/kg OM, thus ruminal N supply was not likely restricting rumen function (Milford and Minson, 1966). Hennessy and Nolan (1988) observed low NH_3-N in steers on an all low-quality pasture hay diet (Bt = 2.0 Bi = 2.3 *mM*). These values were significantly increased with supplementation of protein in both Bt and Bi (3.6 and 3.3 *mM*, respectively). Hunter and Siebert (1987) reported numerically greater NH_3-N concentrations in Bi steers versus Bt steers consuming 6% CP tropical pasture grass (3.47 versus 2.76 *mM*), however there was not a significant difference between the subspecies.

Hunter and Siebert (1985) suggested that the genotype differences in ruminal ammonia concentrations in favor of the Bi on LQFs may be explained by their superior ability to recycle endogenous N to the rumen. *Bos indicus* steers consistently had greater plasma urea N concentrations and greater rumen ammonia concentrations at the same rumen volume than Bt on unsupplemented diets, and higher concentrations of plasma urea, even when the forage was supplemented with protein.

Subspecies differences: Urea recycling

Ruminants are able to recycle large quantities of N the rumen, thus supplying bacteria with an additional source of ammonia. Microbial protein is synthesized in the rumen, and enters the small intestine along with dietary protein that escapes ruminal degradation and endogenous proteins where these N sources are degraded and absorbed. In the liver, most of the absorbed ammonia and a considerable part of AA-N are

converted to urea-N, which is released into the blood circulation, and then excreted into urine via the kidney or it re-enters the digestive tract via saliva or directly across the wall of the rumen.

When cattle graze LQF without adequate protein supplementation, a large proportion of urea that is produced within the animal is recycled, very little is excreted in the urine. At low dietary intakes of N recycling can by very efficient, with most of the urea coming from the endogenous metabolism of tissue and absorbed AA. Net flow of N within the body shifts from being recycled to the rumen toward being excreted in the urine as dietary N is increased. An increase in dietary N intake is associated with a greater urea output from the liver, while threshold blood plasma level is associated with greater urinary excretion rate (Hristov et al., 2004).

Urea production, excretion, and entry to the gut are linked to diet composition, intake, and the productive priorities of the animal (Obitsu and Taniguchi, 2009). Norton et al. (1979) reported that cross-breeds from Bi produced 30% more urea-N and transferred 60% more urea-N into the gut compared with the Shorthorn breed. Greater renal re-absorption of urea-N seemed to account for this higher gut entry in Bi (Norton et al., 1979).

Plasma concentrations of urea N (PUN) can be used as an indicator of protein balance, and therefore protein intake or protein catabolism (Obeidat et al., 2002). A positive relation between concentrations of PUN and NH_3-N (Vercoe, 1970; Hunter and Siebert, 1985) and PUN and urea entry rate into the rumen (Kennedy, 1982) has been established. In grazing studies in semi-desert rangeland, Bi cows maintained higher

body condition scores and had greater serum concentrations of urea-N in early lactation than Bt cows (Obeidat et al., 2002). Hunter and Siebert (1987) similarly noted that the PUN of Bi fed a LQF diet without protein supplementations were higher than Bt. They also observed that supplementation significantly increased PUN concentrations for both breed types. Hennessy et al. (2000) did not measure the extent of N cycling to the rumen in their study with Bi and Bt steers on subtropical hays, but concluded from higher NH_3-N in the Bi steers that Bi had a greater capacity to recycle N to the rumen.

Hunter and Siebert (1987) observed PUN values for both Bi and Bt steers on Pangola grass, and spear grass, a more fibrous and less digestible forage. Unsupplemented PUN concentrations were greater for Bi versus Bt for both grasses (Pangola: 1.06 versus 0.90; Spear: 0.51 versus 0.47). Supplementation increased PUN for both subspecies; however, but Bi continued to have greater concentrations of PUN (Pangola: 2.50 versus 2.27; Spear: 1.95 versus 1.16). *Bos indicus* steers cycled more body pool N to the rumen than Bt, which they described as a consequence of a greater pool of labile N in Bi. This ability may provide them with an advantage over Bt cattle when grazing LQF.

In contrast to the previous studies, Kennedy (1982) observed numerically greater PUN concentrations for Bt than Bi when alfalfa was fed (4.37 versus 4.17 *mM*), however when low-quality tropical pasture hay was fed, Bi had greater PUN than Bt (2.47 versus 1.68 *mM*). Similarly, Hennessy and Nolan (1988) observed low PUN in steers fed low-quality hay (1.07 versus 0.88 *mM*), and the PUN values were increased with protein supplementation in both Bt and Bi (2.64 versus 2.75 *mM*). This difference was also

noted in Nolan and Leng (1972) as they also observed greater PUN for Bi versus Bt when consuming low-quality stubble hay (0.42 versus 0.37mM).

Subspecies differences: Nitrogen balance

Karue et al. (1972) observed that when fed low-protein hay without supplementation, Bi steers lost more urinary N (31% more) and urinary urea N than Bt crossbred steers. In a follow up trial, steers fed the same LQF, but with isonitrogenous protein supplements of either urea or cottonseed meal. They found that the retention of N was influenced more by the source of N and level of N than by the subspecies of cattle; however, Bi steers lost more N in the urine than Bt × Bt steers did (36 versus 31%). They conclude that the reduction in urinary N loss in the Bt steers is indicative of the onset of an emergency N conserving mechanism, urea recycling, and apparently the low protein hay did not initiate this mechanism in the Bi steers. They also surmised that since the Bi steers continued a normal N metabolism as indicated by their urinary N excretion pattern that the emergency N conserving mechanisms were not initiated in Bt steers on the low-quality hay. Similarly, Kennedy (1982) concluded that Bi × Bt and Bt steers excreted urinary N differently fed alfalfa hay than low-quality pasture hay. When consuming alfalfa hay, Bt excreted more urinary N than Bi (55 versus 37 g/d). However, when the steers were on the pasture hay, the Bi crossbred steers excreted more urinary N than Bt (26 versus 24 g/d).

Likewise, Moore et al. (1975) found significant differences in N retention values among breeds fed a low-quality ration, as Bi had N retention values twice as large as those observed for Bt (30 versus 14%). They attribute this apparent low maintenance

requirement of this genus to low endogenous N loss and higher efficiency of N utilization when rations are fed low in protein.

Moran et al. (1979) observed Bi cross and Bt steers consuming low quality sorghum hay, and observed that Bt steers tended to excrete more fecal N than Bi. From this, they concluded that Bt have a lower efficiency of dietary N absorption than Bi. They were unable to detect significant differences in urinary N excretion between the subspecies. Similarly, Hunter and Siebert (1986b) observed slightly greater urinary N excretion (18 versus 15 g/d) favoring Bi versus Bt steers on low-quality tropical pasture hay. The N retention between subspecies slightly favored Bt, as they retained 2 g/d while Bi lost 1 g/d.

Conclusions

For beef cattle production to be profitable, producers must be able to utilize LQFs as an ingredient in cattle diets, while still meeting the animal's requirements for production. In order to improve animal efficiency on these LQFs, protein is often supplemented to meet the microbial requirements for N. Differences in how *Bos indicus* and *Bos taurus* cattle utilized these LQFs and respond to protein supplementation have been realized in previous research. Very few of these studies have occurred using cattle in the U.S.; however, and the number of animals and divergence of experimental methods that have been previously used leave room for improvement in terms of statistical confidence of results. Therefore, the objective of this research is to evaluate the effects of amount and source of protein supplement on intake, digestion, ruminal fermentation, and N retention in Bi and Bt steers consuming rice straw. The

environmental and economic sustainability of beef production is dependent on efficient utilization of nutrients such as protein, therefore we are confident that our research will help us better understand differences that exist between these two subspecies and improve the way in which we feed these two very different types of cattle.

CHAPTER II

MATERIALS AND METHODS

Study description

The experimental protocol was approved by the Institutional Animal Care and Use Committee at Texas A&M University, and included the use of anesthesia when surgical procedures were performed.

Five Angus steers (initial BW = 303 ± 10 kg) selected to represent the *Bos taurus* (Bt) subspecies and 5 Brahman steers (initial BW = 323 ± 28 kg) selected to represent the *Bos indicus* (Bi) subspecies were fitted with ruminal and proximal duodenal (double-L shaped; Streeter et al., 1991) cannulas were used in concurrent 5 × 5 Latin square experiments. Steers were housed in an enclosed, climate controlled barn with continuous lighting. Each steer received a subcutaneous vitamin injection (3 mL/animal; Vitamin AD Injection, Sparhawk Laboratories, Inc., Lenexa, KS) at the onset of the trial to safeguard against deficiency. Steers were provided ad libitum access to fresh water and a trace mineral-salt block (≥96.0% NaCl, 1.00% S, 0.15% Fe, 0.25% Zn, 0.30% Mn, 0.009% I, 0.015% Cu, 0.0025% Co, and 0.001% Se; United Salt Corporation, Houston, TX)). Rice straw (4.7% CP, 73% NDF; Table 1) was offered at 0730 h daily at 130% of the previous 3 d average consumption to ensure that access to forage was not restricting intake. Rice straw was chopped through a wire mesh screen (76mm × 76mm).

Treatments were arranged as a 2 × 2 factorial plus a control that received no supplementation. Two levels of supplemental N: 50 and 100 mg of N/kg BW were fed using 2 different protein supplements, a low DIP supplement (L): 100% DDG (28% DIP,

Table 1), and a high DIP supplement (H): a mixed protein supplement: 69.5% wheat middlings, 30% SBM, and 0.5% urea (72% DIP, Table 1). Treatments were fed in a feeder attached to the hay bunk at 0700 each morning.

Table 1. Chemical composition of forage and supplements

Item	Rice straw	L-DIP[1]	H-DIP[2]
		---------- % DM ----------	
OM	84.9	94.5	93.6
CP	4.7	26.7	26.9
NDF	72.8	41.8	35.0
ADF	52.3	19.0	12.4
Acid detergent insoluble ash	8.8	0.3	0.4

[1]L-DIP = low degradable intake protein supplement (100% dried distillers'grains).
[2]H-DIP = high degradable intake protein supplement (69.5% wheat middlings, 30% soybean meal, and 0.5% urea)

Sampling periods

Experimental periods were 15 d long with 9 d for adaptation to treatments, 5 d for measurements of intake and digestion, and 1 d for determination of duodenal flow, ruminal fermentation, and plasma urea N (PUN). Steers were housed in individual pens (2.1 m × 1.5 m) the initial 5 d of each period, then moved to individual metabolism crates the remaining 10 d of the period to allow for total collection of urine and feces, and facilitated ruminal infusion of $(^{15}NH_4)_2SO_4$. Metabolism crates were designed such that urine and feces would be collected in containers by gravity.

Steers were given a continuous intraruminal infusion of $(^{15}NH_4)_2SO_4$ (Cambridge Isotope Laboratories, Inc., Andover, MA) enriched at (10 atom percent excess (APE)) for 4 d starting at 0600 on d 12 through d 15. The marker was dissolved in a solution

(90% H_2O and 10% 1 N HCl) and infused via a syringe infusion pump (Harvard Apparatus, South Natick, MA) at a rate of 0.25 g/d per steer.

Intake, digestion, and N balance were measured from d 11 through d 14. Samples of hay (400g) were collected d 10 through d 13 and composited within each period to correspond with urine and feces collected from d 11 through 14. Orts were collected just before the daily feeding, and orts from 10 through d 13 were composited for each steer. Feces and urine collected over each 24-h period were thoroughly mixed and a portion of each (3% fecal material, 2% urine) was sampled and composited within animal for each period for measuring N retention, then frozen at -20°C. Urine pH was maintained below 3 to prevent NH_3 volatilization through the addition of 400 mL of 6 *M* HCl to urine containers prior to each collection day. Samples of H-DIP and L-DIP were collected once each period.

On d 15 of each period, ruminal fermentation, duodenal flow, and liquid passage rate were characterized. Rumen fluid was collected with the suction-strainer (Raun and Burroughs, 1962; 19 mm diameter, 1.5 mm mesh) at the following hours after feeding (0 h): 0, 2, 4, 8, 12, 16, and 20. A portable pH meter (Symphony, VWR, Radnor, PA) was used to measure the pH of each rumen fluid sample at the time of sampling. Subsamples of ruminal fluid were prepared and frozen at -20°C for later determinations of volatile fatty acid (VFA), and ruminal ammonia N (NH_3-N). Prior to freezing, 8 mL of rumen fluid was combined with 2 mL of 25% *m*-phosphoric acid for VFA analysis. Rumen fluid (8 mL) destined for ruminal NH_3-N analysis was combined with 1 mL of 1 *N* HCl. Whole ruminal contents (1 kg) and duodenal digesta (300 mL) were also collected at all

sampling times (except 24 h after feeding) to determine duodenal flows. The liquid fraction was frozen immediately at -20°C and the remaining material was returned to the rumen. Duodenal contents (300mL) were composited by steer across time within period, then immediately frozen at -20°C.

Immediately before (0655 h) and 4 h after feeding (1100 h), blood samples were collected from the jugular vein in each of the steers with 15 mL heparinized Vacutainer tubes (BD, Franklin Lakes, NJ). Samples were immediately placed on ice and then centrifuged at 5000 x *g* for 15 m. Plasma was retained and frozen (-20°C) for determination of urea-N concentration.

Laboratory analysis

Hay, ort, and fecal samples (used for fecal output determination) were partially dried in a forced-air oven (96 h, 55°C), allowed to air equilibrate, and then weighed to determine partial DM. Duodenal samples were lyophilized. All dried samples were ground (No. 4 Wiley Mill, Thomas Scientific, Swedesboro, NJ) to pass a 1-mm screen. Hay and supplement samples were composited on an equal weight basis across day. Ort and fecal samples were composited by steer across day within period. Hay, supplement, fecal, and duodenal samples were dried at 105°C for DM determination, and then incinerated for 8h at 450°C for determination of OM. Fecal and urine samples which were frozen following collection were thawed and analyzed for CP. Nitrogen was measured using the Elementar Rapid N Cube (Elementar, Hanua, Germany) and CP was calculated as N x 6.25. Analysis for NDF and ADF was performed using an Ankom Fiber Analyzer (Ankom Technology Corp., Macedon, NY) with sodium sulfite and

amylase omitted, and without correction for residual ash. Acid detergent insoluble ash was measured on hay, supplement, ort, fecal, and duodenal samples by combusting Ankom bags containing ADF residues for 8 h at 450°C in a muffle furnace. Rumen fluid samples were thawed and centrifuged at 20,000 x *g* for 5 m. Volatile fatty acid concentrations were measured using a gas chromatograph with methods described by Vanzant and Cochran (1994). Ammonia N and PUN concentrations were measured using a UV-vis (Sigma Diagnostics, St. Louis, MO) with colorimetric procedures as described by Broderick and Kang (1980). Chromium concentration was determined using an atomic absorption spectrometer with an air-acetylene flame.

Calculations

Duodenal flow was calculated by dividing intake ADIA output by the concentration of ADIA in duodenal digesta. Flow of undegradable intake protein to the duodenum was the difference between total N flow and measured microbial N flow, and thus would include endogenous secretions as part of undegradable intake protein. Digestibilities were calculated by the following formula: [1- (output of nutrient/ intake of nutrient)] x 100.

Statistical analysis

Intake, digestion, N balance, duodenal flows, and plasma urea- N concentrations were analyzed using the MIXED procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC). Terms in the model included treatment and period, with steer as a random effect. Fermentation profile variables were analyzed using the MIXED procedure. Terms in the model were treatment, period, hour, and hour × treatment, with steer and treatment x

period x steer included as random terms. The repeated term was hour, with treatment x steer serving as the subject. Preplanned contrasts were used to separate the means. Contrasts were: 1) source by level interaction, 2) control versus supplementation, 3) 50 versus 100, and 4) H-DIP versus L-DIP. Treatment means were calculated using the LSMEANS option and the same contrasts mentioned above were used to partition the sum of squares.

CHAPTER III

RESULTS

Forage OM intake (**FOMI**) and total OMI (**TOMI**) were significantly greater for Bt than Bi (Table 2; $P < 0.05$), supplementation increased both measures of intake ($P \leq 0.05$) in both subspecies. For Bt, FOMI increased from 16.5 g/kg BW (CON) to 17.9 g/kg BW for H-50 and H-100. Similarly, FOMI was increased ($P = 0.04$) for Bi from the CON (13.5 g/kg BW) to 15.5 g/kg BW for L-50 and 15.4 g/kg BW for H-100. Total OMI increased an average of 2.9 g/kg BW versus the CON with supplementation for Bi, and 2.7 g/kg BW for Bt. There was not a source by level interaction for either FOMI or TOMI ($P \geq 0.15$), nor were there significant differences for either measure of intake due to level or source of supplemental protein for either subspecies ($P \geq 0.21$). Total digestible OM intake (**TDOMI**) was not statistically different between subspecies ($P = 0.12$), but increased ($P < 0.01$) with supplementation for both subspecies. A 14% increase in TDOMI was observed with supplementation for Bt, while TDOMI increased 29% with supplementation for Bi.

Similar to OMI responses, forage NDF intake (**NDFI**) and total NDFI were significantly greater for Bt than Bi ($P < 0.05$), and supplementation increased both measures of intake in both subspecies ($P \leq 0.06$). Total NDFI increased an average of 1.8 g/kg BW versus the CON with supplementation for Bi, and 1.5 g/kg BW for Bt. There was not a source by level interaction for forage NDFI or total NDFI ($P \geq 0.13$), nor were there significant differences in either measure of intake due to level or source of supplemental protein for either subspecies ($P \geq 0.18$).

Table 2. Effect of protein supplement source and level on intake in steers consuming rice straw

		Treatments[1]						Contrast *P*-value[2]			
		CON	L-50	L-100	H-50	H-100	SEM[3]	Bi vs Bt	CON vs Suppl	S × L	50 vs 100	H vs L
OMI, g/kg BW												
Forage	Bi	13.5	15.5	13.9	14.4	15.4	1.08	0.05	0.04	0.30	0.56	0.79
	Bt	16.5	17.7	16.8	17.9	17.9	1.01		0.05	0.15	0.33	0.21
Supplement	Bi	-	1.0	2.1	1.0	2.2	0.03	0.33	<0.01	0.92	<0.01	0.93
	Bt	-	1.0	2.1	1.0	2.1	0.01		<0.01	0.63	<0.01	0.65
Total	Bi	13.5	16.6	16.1	15.5	17.5	1.08		<0.01	0.30	0.15	0.79
	Bt	16.5	18.7	18.9	18.9	19.9	1.01		<0.01	0.15	0.22	0.21
Total digestible	Bi	7.2	9.3	9.1	8.9	9.9	0.60	0.12	<0.01	0.28	0.33	0.52
	Bt	9.0	10.1	10.0	10.2	10.7	0.54		<0.01	0.22	0.55	0.32
NDF, g/kg BW												
Forage	Bi	11.5	13.2	11.9	12.3	13.2	0.94	0.05	0.04	0.31	0.68	0.78
	Bt	14.1	15.1	14.3	15.3	15.3	0.87		0.06	0.13	0.32	0.18
Supplement	Bi	-	0.4	0.9	0.4	0.8	0.02	0.40	<0.01	<0.01	<0.01	<0.01
	Bt	-	0.4	0.9	0.4	0.8	0.01		<0.01	<0.01	<0.01	<0.01
Total	Bi	11.5	13.6	12.9	12.7	13.9	0.94	0.05	<0.01	0.44	0.58	0.96
	Bt	14.1	15.5	15.3	15.7	16.0	0.87		<0.01	0.20	0.92	0.26
Digestible	Bi	5.5	6.7	6.3	6.6	6.7	0.47	0.15	0.02	0.67	0.67	0.85
	Bt	6.6	7.5	6.8	7.1	7.4	0.40		0.04	0.26	0.34	0.67

[1]Control = 0 mg N/kg BW; L-50 = 50 mg N/kg BW L-DIP; L-100 = 100 mg N/kg BW L-DIP, H-50 = 50 mg N/kg BW H-DIP, H-100 = 100 mg N/kg BW H-DIP.

[2]Bi vs Bt = *Bos indicus* versus *Bos taurus*, CON vs Suppl = control versus supplementation, S × L = effect of increasing N source and increasing N level,

50 vs 100 = 50 mg N/kg BW versus 100 mg N/kg BW, H vs L = effect of all levels of L-DIP compared to all levels of H-DIP.

[3]SEM = standard error of the mean.

Organic matter digestibility (**OMD**) was greater in Bi than Bt steers (Table 3; $P < 0.01$), 56.3 versus 53.7%, respectively. Supplementation did not significantly affect OMD in Bt ($P = 0.53$), CON 54.4% and supplementation 53.6%. In contrast, OMD in Bi increased with supplementation ($P = 0.02$) from 53.4% for CON to 57.0% with supplementation. There was not a source by level interaction ($P \geq 0.84$) for either Bt or Bi. There were no significant differences ($P > 0.37$) between levels or sources for either Bi or Bt for OMD. *Bos indicus* had greater ($P < 0.01$) NDF digestion (**NDFD**) than Bt. Supplementation did not affect on NDFD for either subspecies ($P \geq 0.35$), and there were no significant differences in NDFD due to level or source of supplemental protein for either subspecies ($P \geq 0.19$).

Total nitrogen (N) intake was greater for Bt than for Bi (Table 4; $P = 0.06$) which was driven by the aforementioned differences in FOMI. For both subspecies and in accordance with our design, supplementation increased total N intake versus CON ($P < 0.01$). The protein source by level interaction for total N intake in Bt was significant ($P < 0.01$). This interaction resulted from a reduction in forage N intake as steers went from L-50 to L-100 (197 versus 236 mg/kg BW), which resulted in a smaller increase in total N intake than observed as steers moved from H-50 to H-100 (203 versus 249 mg/kg BW). As expected, greater amounts of supplemental N (50 versus 100) resulted in greater total N intake ($P < 0.01$). Supplementation with H resulted in greater total N intakes than L for Bt ($P < 0.01$); however, the magnitude of these differences was small (226 versus 217 mg/kg BW). Supplemental N source differences were not observed in Bi ($P = 0.19$).

Table 3. Effect of protein supplement source and level on digestion in steers consuming rice straw

		Treatments[1]						Contrast *P*-value[2]			
		CON	L-50	L-100	H-50	H-100	SEM[3]	Bi vs Bt	CON vs Suppl	S × L	50 vs 100	H vs L
Total tract digestion, %												
OM	Bi	53.4	56.1	58.3	56.7	56.9	1.1	<0.01	0.02	0.55	0.76	0.37
	Bt	54.3	54.2	53.7	52.7	53.7	1.2			0.67	0.55	0.82
NDF	Bi	48.3	49.6	51.8	48.9	48.1	1.6	<0.01	0.48	0.91	0.19	0.66
	Bt	47.5	48.5	45.1	44.1	46.2	1.4		0.35	0.84	0.27	0.67

[1]Control = 0 mg N/kg BW; L-50 = 50 mg N/kg BW L-DIP; L-100 = 100 mg N/kg BW L-DIP, H-50 = 50 mg N/kg BW H-DIP, H-100 = 100 mg N/kg BW H-DIP.

[2]Bi vs Bt = *Bos indicus* versus *Bos taurus*, CON vs Suppl = control versus supplementation, S × L = effect of increasing N source and increasing N level, 50 vs 100 = 50 mg N/kg BW versus 100 mg N/kg BW, H vs L = effect of all levels of L-DIP compared to all levels of H-DIP.

[3]SEM = standard error of the mean.

Table 4. Effect of protein supplement source and level on nitrogen retention in steers consuming rice straw

		Treatments[1]						Contrast *P*-value[2]				
		CON	L-50	L-100	H-50	H-100	SEM[3]	Bi vs Bt	CON vs Suppl	S × L	50 vs 100	H vs L
No. of observations	Bi	4	5	5	5	3						
	Bt	5	5	5	5	5						
N, mg/kg BW												
Forage intake	Bi	115	131	120	121	133	9.5	0.04	0.05	0.29	0.92	0.74
	Bt	140	150	142	155	153	8.2		<0.01	< 0.01	0.12	0.02
Supplement intake	Bi	-	48	96	49	98	1.8	0.41	<0.01	0.29	<0.01	0.33
	Bt	-	47	94	48	96	0.9		<0.01	0.12	<0.01	0.14
Total intake	Bi	115	179	215	169	231	9.8	0.06	<0.01	0.19	<0.01	0.55
	Bt	140	197	236	203	249	8.3		<0.01	<0.01	<0.01	<0.01
Fecal	Bi	88	118	114	103	128	1.2	0.01	<0.01	0.45	0.08	0.97
	Bt	113	145	151	134	154	1.4		<0.01	0.86	0.07	0.59
Urinary	Bi	61	88	109	96	117	8.8	0.10	<0.01	0.35	0.02	0.30
	Bt	59	85	87	74	102	7.5		<0.01	0.41	0.06	0.79
Apparent absorbed	Bi	27	61	102	66	103	9.2	0.23	<0.01	0.69	<0.01	0.60
	Bt	28	52	85	69	95	7.7		<0.01	0.07	<0.01	0.04
Balance	Bi	-15	-8	-3	-9	-11	15.4	0.18	0.12	0.14	0.66	0.21
	Bt	-32	-33	-2	-5	-7	10.5		<0.01	0.49	<0.01	0.26
Duodenal flow	Bi	138	194	229	152	196	15.7	0.42	<0.01	0.02	0.01	0.01
	Bt	152	187	232	193	201	14.8		<0.01	0.19	0.35	0.06
Ruminal balance	Bi	23	15	13	-18	-35	13.0	0.12	0.06	<0.01	0.46	<0.01
	Bt	12	-9	-4	-9	-48	9.8		0.02	0.02	0.12	0.05

[1]Control = 0 mg N/kg BW; L-50 = 50 mg N/kg BW L-DIP; L-100 = 100 mg N/kg BW L-DIP, H-50 = 50 mg N/kg BW H-DIP, H-100 = 100 mg N/kg BW H-DIP.

[2]Bi vs Bt = *Bos indicus* versus *Bos taurus*, CON vs Suppl = control versus supplementation, S × L = effect of increasing N source and increasing N level, 50 vs 100 = 50 mg N/kg BW versus 100 mg N/kg BW, H vs L = effect of all levels of L-DIP compared to all levels of H-DIP.

[3]SEM = standard error of the mean.

Fecal N excretion was greater in Bt than Bi ($P = 0.01$), 139 versus 110 mg/kg BW, respectively. Supplementation increased ($P < 0.01$) fecal N versus CON- 88 versus 115.8 mg/kg BW, in Bi and 113 versus 146 mg/kg BW in Bt. Both subspecies tended to increase ($P \leq 0.08$) fecal N excretion with greater N supplementation (50 versus 100 mg N/kg BW) across both sources.

Urinary N tended to be higher for Bi than Bt ($P = 0.10$). Supplementation significantly increased ($P < 0.05$) urinary N for both subspecies over the CON- 61 versus 103 mg/kg BW in Bi and 59 versus 87 mg/kg BW in Bt. Increasing supplemental N from 50 to 100 mg N/kg BW resulted in greater ($P \leq 0.06$) urinary N excretion in both subspecies (Bi: 92 versus 113 mg/kg BW; Bt: 80 versus 94 mg/kg BW).

Supplementation increased ($P < 0.01$) apparent absorbed N in both subspecies versus the CON- 27 versus 83 mg/kg BW in Bi, and 28 versus 75 mg/kg BW in Bt. There was a tendency for a source by level interaction for Bt ($P = 0.07$), but not Bi ($P = 0.69$). In Bt, an increase from L-50 to L-100 increased apparent absorbed N from 52 and 85 mg/kg BW versus 69 and 95 mg/kg BW from H-50 to H-100. Greater amounts of N supplementation (50 versus 100 mg N/kg BW) increased apparent absorbed N for both subspecies ($P < 0.01$).

An increase in N balance was observed with supplementation versus the CON ($P < 0.01$) in Bt (-32 versus -12 mg/kg BW), but was not significant in Bi ($P = 0.12$). There was not a source by level interaction ($P \geq 0.14$) for either Bt or Bi. Increasing N amount supplemented (50 versus 100 mg N/kg BW) increased ($P < 0.01$) N retained in Bt, but not in Bi ($P = 0.66$).

There was no difference in duodenal N flow between subspecies (Table 4; P = 0.42). Supplementation increased ($P < 0.01$) duodenal N flow versus the CON for both Bi (138 versus 193 mg/kg BW) and Bt (152 versus 203 mg/kg BW). There was a significant source by level interaction in Bi ($P = 0.02$). Increasing from H-50 to H-100 increased duodenal N flow from 152 to 196 mg/kg BW. In contrast, L supplements had greater duodenal N flows compared to H, yet the magnitude of the response to increased N with L was smaller- 194 versus 229 mg/kg BW. Greater duodenal N flow was observed for L versus H supplements in both subspecies ($P \leq 0.06$).

Measurements characterizing the effects of supplement source and level on ruminal fermentation included ruminal ammonia (**NH_3-N**), VFA, and pH (Tables 5 and 6). The treatment × time interaction was significant for ruminal NH_3-N and VFA, but this interaction was largely due to the magnitude of the difference that existed between treatments at different times rather than to changes in treatment rankings. Therefore to facilitate discussion, only treatment means are presented in the tables.

Ruminal NH_3 concentrations across time are presented for both Bi (Figure 1) and Bt (Figure 2). *Bos indicus* steers had significantly greater ruminal NH_3-N concentrations than Bt ($P < 0.01$). Supplementation increased ($P < 0.01$) NH_3-N concentrations versus the CON in both subspecies (Bi: 1.08 versus 2.35 m*M*; Bt: 0.58 versus 1.32m*M*). There was a significant supplement source by level interaction in Bi ($P < 0.01$) and a tendency for one in Bt ($P = 0.10$). Both interactions resulted from smaller increases in ruminal ammonia as steers went from L-50 to L-100 (1.48 to 2.15 m*M*, Bi; 0.84 to 0.96 m*M*, Bt) versus going from H-50 to H-100 (2.56 to 3.22 m*M*, Bi; 1.35 to 2.14 m*M*, Bt).

Table 5. Effect of protein supplement source and level on ruminal fermentation parameters in steers consuming rice straw

		Treatments[1]						Contrast *P*-value[2]				
		CON	L-50	L-100	H-50	H-100	SEM[3]	Bi vs Bt	CON vs Suppl	S × L	50 vs 100	H vs L
Ruminal ammonia, m*M*	Bi	1.08	1.48	2.15	2.56	3.22	0.3	<0.01	<0.01	<0.01	<0.01	<0.01
	Bt	0.58	0.84	0.96	1.35	2.14	0.2		<0.01	<0.01	<0.01	<0.01
Plasma urea N, m*M*												
Hour 0	Bi	2.24	2.54	3.40	2.83	3.16	0.3	<0.01	0.08	0.86	0.95	0.12
	Bt	1.67	1.71	2.12	1.98	2.42	0.2		0.05	0.11	0.10	0.02
Hour 4	Bi	2.87	2.63	3.43	3.02	4.37	0.3	<0.01	0.22	0.04	0.06	<0.01
	Bt	2.02	1.90	2.59	2.05	3.09	0.3		0.19	0.17	0.22	<0.01
pH	Bi	6.55	6.63	6.53	6.64	6.54	0.4	0.43	0.62	0.49	0.28	0.28
	Bt	6.71	6.63	6.59	6.63	6.63	0.4		<0.01	0.42	0.42	0.51

[1]Control = 0 mg N/kg BW; L-50 = 50 mg N/kg BW L-DIP; L-100 = 100 mg N/kg BW L-DIP, H-50 = 50 mg N/kg BW H-DIP, H-100 = 100 mg N/kg BW H-DIP.

[2]Bi vs Bt = *Bos indicus* versus *Bos taurus*, CON vs Suppl = control versus supplementation, S × L = effect of increasing N source and increasing N level, 50 vs 100 = 50 mg N/kg BW versus 100 mg N/kg BW, H vs L = effect of all levels of L-DIP compared to all levels of H-DIP.

[3]SEM = standard error of the mean.

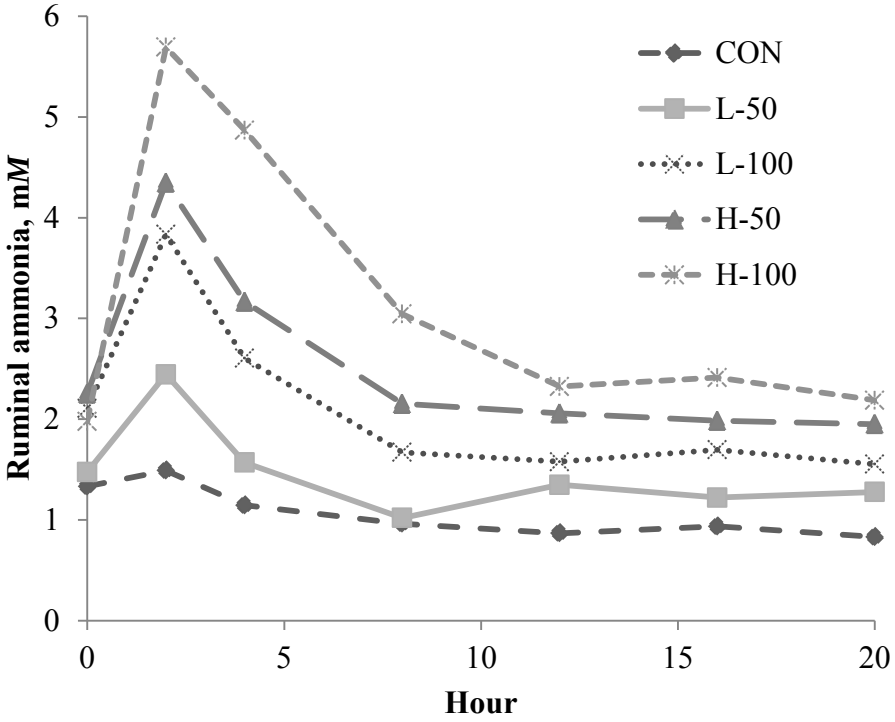

Figure 1. Effect of protein supplement amount and degradability of ruminal NH_3 concentrations in *Bos indicus* steers. CON = 0 mg N/kg BW; L-50 = 50 mg N/kg BW L-DIP; L-100 = 100 mg N/kg BW L-DIP, H-50 = 50 mg N/kg BW H-DIP, H-100 = 100 mg N/kg BW H-DIP. There is a significant difference ($P < 0.01$) between CON and supplemented treatments, H versus L, and 50 versus 100 mg N/kg BW. There is a source by level interaction ($P < 0.01$). There was an effect of hour and treatment × hour ($P < 0.01$).

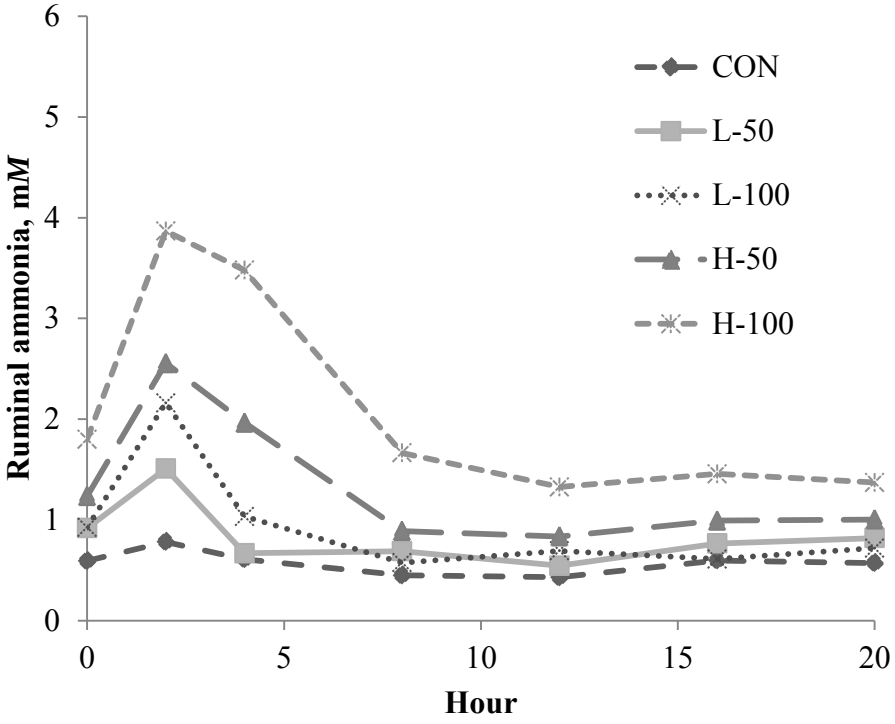

Figure 2. Effect of protein supplement amount and degradability of ruminal NH_3 concentrations in *Bos taurus* steers. CON = 0 mg N/kg BW; L-50 = 50 mg N/kg BW L-DIP; L-100 = 100 mg N/kg BW L-DIP, H-50 = 50 mg N/kg BW H-DIP, H-100 = 100 mg N/kg BW H-DIP. There is a significant difference ($P < 0.01$) between CON and supplemented treatments, H versus L, and 50 versus 100 mg N/kg BW. There is a source by level interaction ($P < 0.01$). There was an effect of hour and treatment × hour ($P < 0.01$).

Ruminal ammonia for both subspecies and all treatments peaked 4h after feeding and to baseline around 12 h after feeding.

Plasma urea nitrogen (**PUN**) concentrations are presented in Table 5. They were greater for Bi than Bt ($P < 0.01$) for all treatments and at both 0 and 4 h after feeding. Supplementation did tended ($P \leq 0.08$) to increase PUN versus CON at h0 for both Bt (1.67 versus 2.06 m*M*) and Bi (2.24 versus 2.98 m*M*). There was a source by level interaction for PUN ($P = 0.04$) in Bi at h4. Increasing the amount of supplement from L-50 to L-100 increased PUN from 2.63 to 3.43 m*M*, or 30%. However, going from H-50 to H-100 resulted in a 45% increase in PUN concentration at h 4 (3.02 versus 4.37 m*M*). The more degradable supplement, H, resulted in greater ($P < 0.02$) PUN concentrations for Bt versus L at both h 0 (2.2 versus 1.92 *mM*) and h 4 (2.57 versus 2.25 *mM*), but only at h 4 for Bi (2.57 versus 2.25 *mM*).

There was not a significant difference between the ruminal pH between the two subspecies ($P = 0.43$). Protein supplementation significantly decreased pH values ($P < 0.01$) for Bt steers compared to the CON (6.62 versus 6.71), but did not significantly affect Bi steers ($P = 0.62$).

Bos taurus steers had numerically (Table 6; $P = 0.19$) greater total VFA concentrations across all treatments than Bi steers except L-50. There was a tendency for a supplement source by level interaction in Bt ($P = 0.08$) which resulted from a relatively small increase in total VFA, 0.96 m*M*, going from L-50 to L-100 versus a 3.45 m*M* increase going from H-50 to H-100.

Table 6. Effect of protein supplement source and level on volatile fatty acid (VFA) composition in steers consuming rice straw

		Treatments[1]						Contrast *P*-value[2]				
	Subspecies	CON	L-50	L-100	H-50	H-100	SEM[3]	Bi vs Bt	CON vs Suppl	S × L	50 vs 100	H vs L
Total VFA, m*M*	Bi	68.38	73.94	70.83	68.73	69.78	1.90	0.19	0.32	0.29	0.16	0.16
	Bt	69.56	73.19	74.15	74.97	78.42	1.96		<0.01	0.08	0.23	0.10
Acetate: Propionate	Bi	4.43	4.30	4.07	4.46	4.30	0.66	0.66	0.05	<0.01	<0.01	<0.01
	Bt	4.37	4.29	4.10	4.41	4.22	0.70		0.15	0.10	<0.01	0.09
Molar percentage, %												
Acetate	Bi	75.37	74.05	73.09	74.55	73.88	3.80	0.20	<0.01	<0.01	<0.01	<0.01
	Bt	75.77	74.28	73.54	74.96	74.30	2.50		<0.01	0.03	<0.01	0.04
Propionate	Bi	17.12	17.30	18.07	16.81	17.25	3.20	0.46	0.37	<0.01	0.02	<0.01
	Bt	17.40	17.42	18.06	17.62	17.62	2.10		0.51	0.08	<0.01	0.08
Butyrate	Bi	6.23	7.06	7.23	6.89	7.13	1.40	0.10	<0.01	0.58	0.37	0.53
	Bt	5.52	6.47	6.84	6.42	6.37	1.40		<0.01	0.04	0.27	0.09
Isobutyrate	Bi	0.52	0.62	0.59	0.68	0.65	0.40	0.66	<0.01	0.02	0.23	0.02
	Bt	0.56	0.60	0.59	0.59	0.64	0.20		<0.01	0.02	0.15	0.07
Valerate	Bi	0.40	0.52	0.51	0.58	0.59	0.50	0.28	<0.01	0.82	0.11	0.51
	Bt	0.40	0.48	0.49	0.46	0.55	0.30		<0.01	0.06	<0.01	0.13
Isovalerate	Bi	0.35	0.46	0.52	0.49	0.50	0.30	0.84	<0.01	0.09	0.95	0.09
	Bt	0.36	0.45	0.49	0.46	0.53	0.10		<0.01	0.04	<0.01	0.12

[1]Control = 0 mg N/kg BW; L-50 = 50 mg N/kg BW L-DIP; L-100 = 100 mg N/kg BW L-DIP, H-50 = 50 mg N/kg BW H-DIP, H-100 = 100 mg N/kg BW H-DIP.

[2]Bi vs Bt = *Bos indicus* versus *Bos taurus*, CON vs Suppl = control versus supplementation, S × L = effect of increasing N source and increasing N level, 50 vs 100 = 50 mg N/kg BW versus 100 mg N/kg BW, H vs L = effect of all levels of L-DIP compared to all levels of H-DIP.

[3]SEM = standard error of the mean.

There was not a significant difference between the Acetate: Propionate (**A:P**) ratio between the two subspecies ($P = 0.66$). Supplementation decreased the A:P compared to the CON for Bt ($P = 0.05$), but this was not observed in Bi ($P = 0.15$). There was a significant supplement source by level interaction that decreased the A:P in Bi ($P < 0.01$), as going from L-50 to L-100 decreased the A:P from 4.30 to 4.07, versus 4.46 to 4.30 from H-50 to H-100. Similarly, in Bt there tended to be a source by level interaction that decreased A:P ($P = 0.10$). Increasing from L-50 to L-100 decreased 4.29 to 4.10. In contrast, H supplements decreased A:P from 4.41 to 4.22.

The molar proportions of butyrate, isobutyrate, valerate, and isovalerate increased ($P < 0.01$) with supplementation of both H and L versus the CON. In contrast, molar proportions of acetate were decreased ($P < 0.01$), and propionate was unchanged ($P \geq 0.37$) with supplementation. The only VFA that was significantly different between subspecies was butyrate, as it tended ($P = 0.10$) to be greater for Bi than Bt.

CHAPTER IV

DISCUSSION

This study was designed to evaluate differences in the ability of *Bos indicus* and *Bos taurus* cattle to utilize LQF, and their response to different sources and levels of supplemental protein. In accordance with previous work (Frisch and Vercoe, 1977; Hunter and Siebert, 1985; Hennessy et al., 2000), Bt had greater intake of LQF than Bi ($P < 0.05$) on all treatments. Initially, we hypothesized that Bi would have greater intake without supplementation (CON) as Bi have consistently been shown to maintain greater ruminal NH_3-N concentrations than Bt when consuming LQF (Hunter and Siebert, 1985; Hennessy and Nolan, 1988; Hennessy et al., 2000), and because ruminally available N is a primary limiter of LQF utilization (Beaty et al., 1994; Köster et al., 1996). Ruminal NH_3-N was greater in our study for Bi than Bt on the CON diet (1.08 and 0.58, respectively). However, Bt willingness to consume more LQF may reflect the demand to address greater maintenance requirements (Vercoe, 1970; Moran, 1976; Frisch and Vercoe, 1984), and suggests that ruminal ammonia may not be a good indicator of potential response to supplements across subspecies. An increase ($P \leq 0.05$) in FOMI with supplementation was observed for both subspecies, which we attribute mainly to the increased RAN supply from supplementation. Supplementation of N has been shown to stimulate forage OM intake (Köster et al., 1996; Bandyk et al., 2001; Wickersham et al., 2004). Hennessy et al. (2000) and Hunter and Siebert (1985) both reported that Bi intakes were less responsive to protein supplementation than Bt when fed LQF. In contrast to their findings, Bi increased their intake more, 1.3 versus 1.1 g/kg

BW than Bt; however, the magnitude of this difference was small. Intake response to source of supplement protein L (28% DIP) or H (72% DIP) did not differ. In contrast, Bandyk et al. (2001) and Wickersham et al. (2004) both observed greater FOMI in response to DIP supplementation than UIP though both elicited significant increases in intake over control. However, their studies were conducted with pure sources of UIP and DIP, whereas we used conventional sources of protein that contained a mixture UIP and DIP. Wickersham et al. (2004) observed similar intakes when the same amount of protein was provided as 100% DIP versus 25% DIP and 75% UIP, which was similar to the L supplement. Furthermore, the L supplement provided 28% DIP and the response to supplemental protein has been shown to be most dramatic at the first level of supplementation (Köster et al., 1996; Wickersham et al., 2004). Additionally, the absence of significant differences between N source in our study may have result from the apparently small response surface to supplemental protein.

Our observation of greater total tract digestion of OM and NDF in Bi versus Bt is in accordance with previous research (Hungate et al., 1960; Howes et al., 1963; Moore et al., 1975) The greatest OMD observed for Bt was CON, 54.4% followed by a small decrease to 53.6 % with supplementation. Decreased digestion with supplementation may reflect increased rates of passage associated with increased intake in response RAN (Köster et al., 1996; Wickersham et al., 2008). In contrast, OMD in Bi increased with supplementation ($P = 0.02$) from 53.4 % for CON to 57% with supplementation (Figure 3). Observed increases in OMD for Bi with protein supplementation are consistent with research using Bt (Beaty et al., 1994; Bandyk et al., 2001) and were presumably due to

improved N availability to the ruminal microbes (Figure 1). Type of protein supplement did not significantly affect OMD. However, greater observed digestion with L agrees with Bandyk et al. (2001) where slightly greater total tract digestion was recorded when UIP was supplied versus DIP (47.1 versus 44.7%). Total digestible OMI was not different between Bi and Bt ($P = 0.12$). For all treatments, Bt was numerically greater than Bi; however, greater FOMI and TOMI in Bt were offset by improvements in OMD observed in Bi. Total DOMI increased with supplementation for both subspecies ($P < 0.01$). Following the trend of greater FOMI increases in Bi compared with Bt, supplementation resulted in a 14% increase in TDOMI in Bt, while TDOMI increased 29% with supplementation in Bi.

Bach et al. (2005) suggested that approximately 80% of bacterial N is derived from ruminal NH_3-N, demonstrating the importance of ruminal NH_3-N in microbial protein synthesis. As expected, and in accordance with previous work (Hunter and Siebert, 1985; Hennessy and Nolan, 1988; Hennessy et al., 2000), we observed that Bi had significantly greater ruminal NH_3-N concentrations than Bt ($P < 0.01$). Greater ruminal NH_3-N concentrations resulting from N supplementation have been associated with improved extent of forage digestion due to greater microbial activity (Beaty et al., 1994; Bandyk et al., 2001).

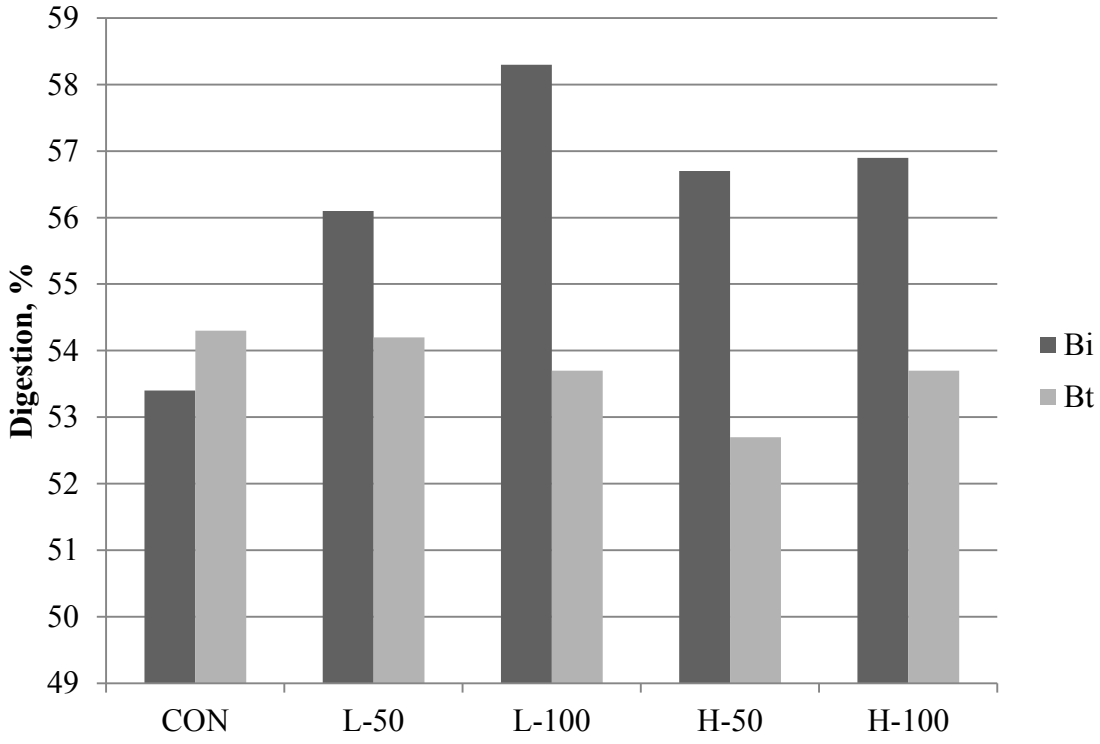

Figure 3. Effect of protein supplement amount and degradability on organic matter digestion in *Bos indicus* (Bi) and *Bos taurus* (Bt) steers. CON = 0 mg N/kg BW; L-50 = 50 mg N/kg BW L-DIP; L-100 = 100 mg N/kg BW L-DIP, H-50 = 50 mg N/kg BW H-DIP, H-100 = 100 mg N/kg BW H-DIP. There is a significant difference ($P < 0.01$) between Bi and Bt. There is a significant difference ($P = 0.02$) between CON and supplemented treatments for Bi.

Ruminal NH_3-N increased for both subspecies with increased supplementation ($P < 0.01$); however, digestion was only improved in Bi. Satter and Slyter (1974) concluded from an *in vitro* study that the optimal NH_3-N concentration for ruminal fermentation is 3.6 m*M*. They also reported that microbial limitations were removed when the NH_3-N concentration was1.4 m*M* or higher. Ruminal NH_3-N values in Bi exceeded this value with supplementation, which supports the increase in OM digestion observed compared to the CON. Greater ruminal NH_3-N concentrations observed for H

than L is supported by previous work (Bandyk et al., 2001), as they observed three fold higher NH_3-N values for steers that were supplemented ruminally (DIP) versus post-ruminally (UIP). The source by level interaction observed in Bi is due to the greater NH_3-N concentrations at H versus L, as both similar increases are observed for both L and H going from 50 to 100 (0.67 and 0.66 m*M*), but the magnitude of increase is much greater for L. Increased NH_3-N with L agrees with the ruminal N balance values for Bi which indicated large amounts of urea being recycled back to the rumen, correlating with the greater ruminal NH_3-N concentrations.

A positive relationship has been identified between concentrations of ruminal NH_3-N and plasma urea-N; (Hunter and Siebert, 1985; Hennessy and Nolan, 1988; Wickersham et al., 2008), and was similarly observed in our study (Figure 4) for both subspecies. Greater PUN concentrations observed in our trial for Bi than Bt agrees with previous research (Vercoe, 1970; Hunter and Siebert, 1985; Coleman and Frahm, 1987), and is positively correlated with the greater ruminal NH_3-N. When there is a ruminal deficiency in available N, as was observed in our trial, ruminal NH_3-N and PUN concentrations are relatively low, and the proportion of N recycled back to the rumen as urea is high (Hammond, 1997; Wickersham et al., 2008). While we did not directly measure N recycling, we calculated ruminal N balance (RNB), as it is generally considered to be an indicator of N recycling (Lebzien et al., 2006). A shortage of N in the rumen (positive RNB) suggests greater capture of endogenous urea-N, and it was evident in our study that low ruminal NH_3-N resulted in increased N capture. *Bos indicus* tended to have a greater RNB ($P = 0.12$) compared to Bt, indicating that Bi were

more efficient at capturing urea-N in microbial protein than Bt. Observing the RNB of both subspecies at both L-50 and L-100, the positive values for Bi (15 and 13 mg/kg BW) compared to the negative values for Bt (-9 and -4 mg/kg BW) demonstrate that more recycled N is being captured ruminally when L is supplemented to Bi than Bt. Available energy did not increase as rapidly as metabolizable protein supply. This may have resulted in the catabolism of the excess protein to increase urea production and subsequent recycling to the gut (Wickersham et al., 2008). Wickersham et al. observed greater urea production as a fraction of total N intake with UIP supplemental protein than when supplemental DIP was provided. Greater PUN and ruminal NH_3-N concentrations observed in our study with more N and higher protein degradability were similarly reported by Bandyk et al. (2001) and Wickersham et al. (2008). High rumen NH_3-N concentrations have been reported to be negatively correlated to urea transfer across the rumen wall (Kennedy and Milligan, 1980). Previous literature has set the limit of urea transfer to the rumen in cattle on LQF at ruminal NH_3-N concentrations of 3.1 - 3.7 m*M* (Vercoe, 1969) and 3.5 - 4.7 m*M* (Thornton, 1970). Ruminal NH_3-N concentrations were in the range of 0.58 – 3.22 m*M* in our present study which is similar to Hennessy and Nolan's (1988) values of 0.4 – 3.2 m*M*. These values lead them to conclude that it is unlikely these NH_3-N concentrations were limiting the transfer of urea N from the blood to the rumen.

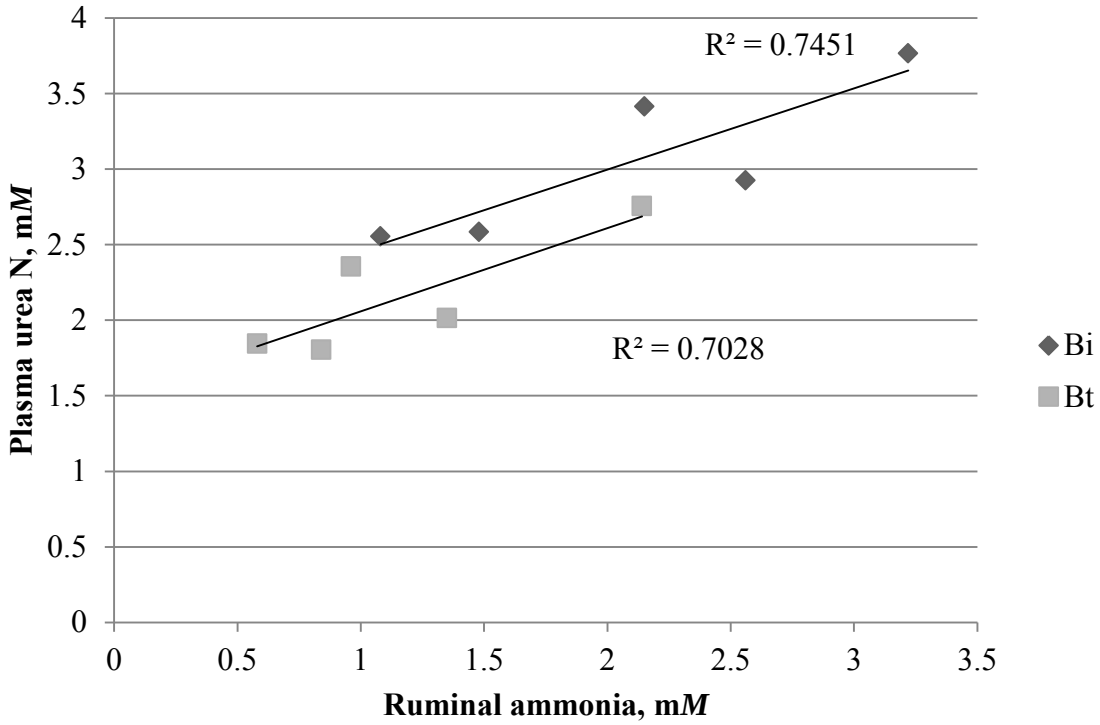

Figure 4. Effect of protein supplement amount and degradability on ruminal ammonia and plasma urea N in *Bos indicus* (Bi) and *Bos taurus* (Bt) steers.

Total N intake was greater for Bt, a result of greater FOMI. While Bt consumed more total N than Bi, this did not result in greater N balance when compared with Bi (P = 0.18). Previous work has shown that as N intake increases, the majority of excess N is excreted in the urine, with fecal N excretion increasing proportionately less (Marini and Van Amburgh, 2005). As total N intake increased, the percentage of N excreted in feces decreased from 59 to 52% in Bi and from 66 to 60% in Bt, while urine increased from 41 to 48% in Bi and from 34 to 40% in Bt. When LQF makes up the majority of a diet, N is typically the first-limiting factor to the growth of the microbial population in the rumen (Köster et al., 1996). Greater fecal N excretion in Bt than Bi (P = 0.01; 139

versus 110 mg/kg), was observed in our study and is in agreement with previous work (Moran et al., 1979). Fecal excretion of N compared with total N intake is highly correlated ($R^2 \geq 0.85$; Figure 5), and demonstrates that Bt have greater metabolic fecal N loss than Bi. Greater observed fecal N values in Bt compared to Bi would makes sense as they also have greater total N intake.

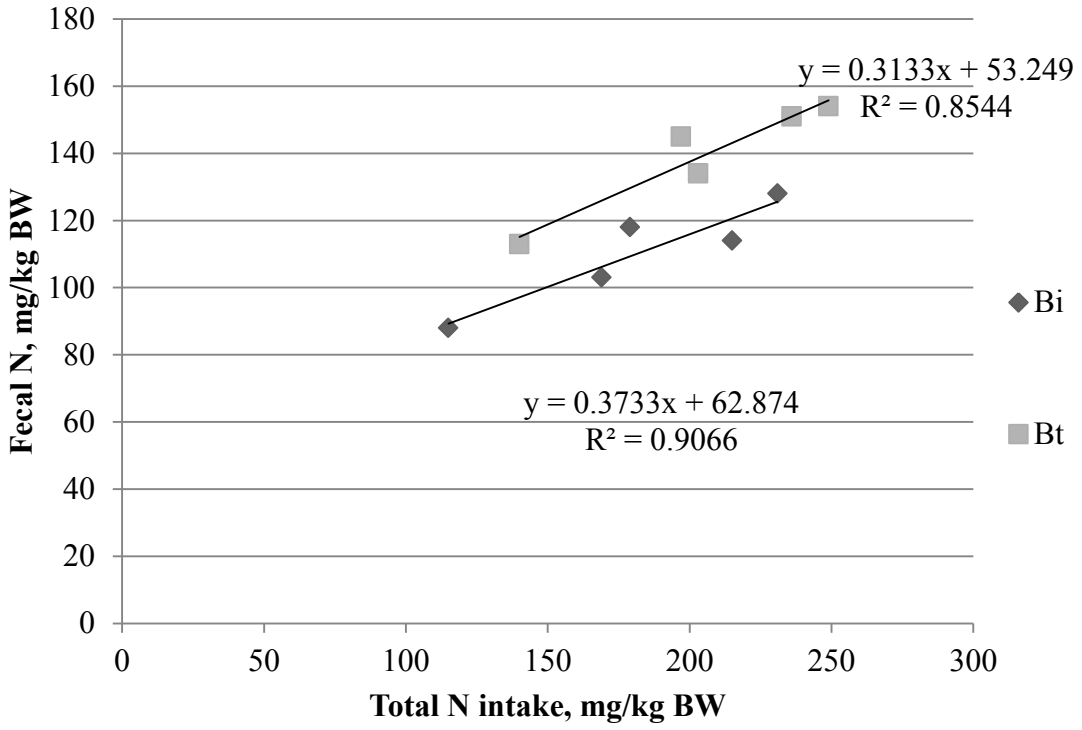

Figure 5. Effect of protein supplement amount and degradability on fecal N excretion and total N intake in *Bos indicus* (Bi) and *Bos taurus* (Bt) steers.

Urinary N tended to be greater for Bi than Bt ($P = 0.10$) which agrees with previous literature (Karue et al., 1972; Kennedy, 1982; Hunter and Siebert, 1986b). In our study, supplementation increased urinary N in both subspecies over the CON an average of 68% in Bi (61 versus 103 mg/kg BW) and 48% in Bt (59 versus 87 mg/kg

BW). Greater ruminal NH_3-N and PUN concentrations in both subspecies with N supplementation versus the CON suggests that increases in N without a matching increase in energy creates surplus N, which will cannot be utilized by the microbes, and thus increases N excreted in the urine. Greater urinary N excreted by Bi compared to Bt would be expected, as Bi have significantly greater concentrations of ruminal NH_3-N and PUN, which might lead to an excess N on the LQF, thus greater excretion of N in the urine would be observed. Increasing supplemental provision of N from 50 to 100 mg N/kg BW resulted in greater urinary N excretion on average for both subspecies. This agrees with previous research that concludes that increased loss of N in the urine is positively correlated with greater amounts of N are fed in the diet (Hristov et al., 2004; Marini and Van Amburgh, 2005). Like fecal N excretion, Bt have greater metabolic N loss in urine than Bi (Figure 6), as at a 0 N intake, Bt have greater N excretion (9.5 versus 8.9 mg N/kg BW).

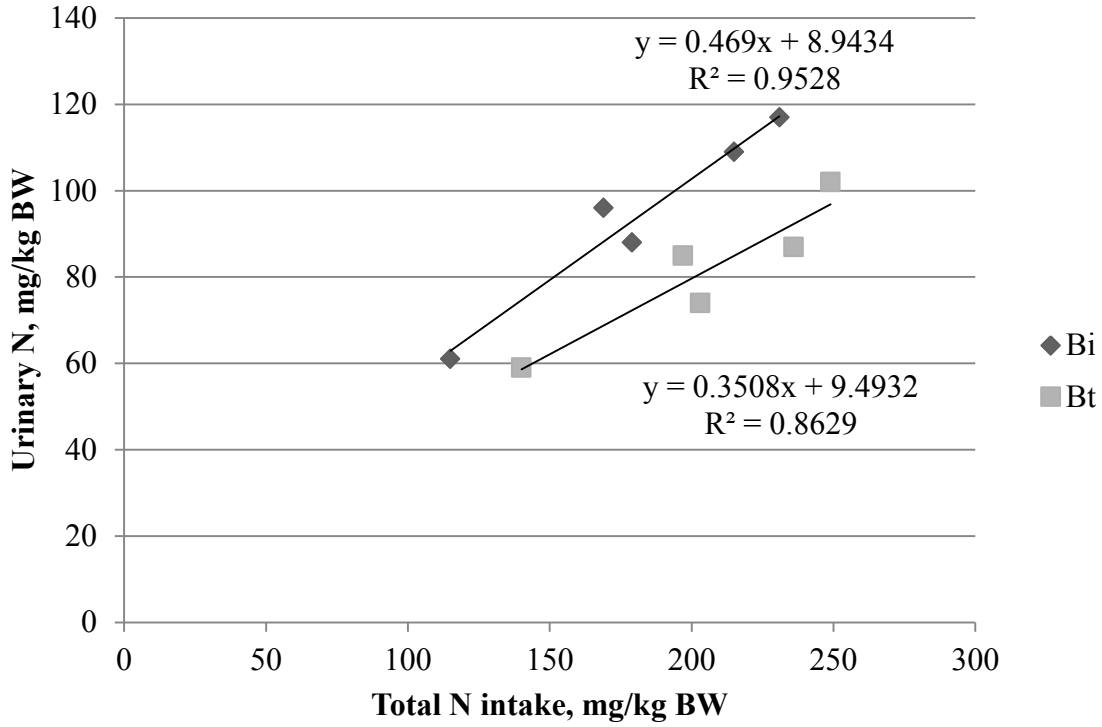

Figure 6. Effect of protein supplement amount and degradability on urinary N excretion and total N intake in *Bos indicus* (Bi) and *Bos taurus* (Bt) steers.

While not part of our initial hypothesis or premise when engaging in this research, after observing the results from our research we believe that protozoal population differences potentially played a large part in our results. O'Kelly and Spiers (1992) reported that at equal intakes of LQF, there was a much greater ($P < 0.01$) protozoal population density in the rumen fluid of Bi compared to Bt (44.5×10^6 versus 19.7×10^6 /L). The impact of protozoa on rumen fermentation and ruminal metabolism of N that has been documented in previous literature (Jouany, 1996; Eugène et al., 2004) lends tremendous evidence towards helping us answer the differences between subspecies in our trial. Eugène et al. (2004)'s meta-analysis of the 52 trials concluded

that there is a significant depression in total tract OMD when animals are defaunated (67.5 versus 65.9%), which supports the hypothesis that Bi animals may possess a greater ruminal protozoa population compared with Bt. They similarly concluded that defuanation reduces urinary N and increases fecal N, effectively shifting N excretion patterns. The authors attribute this change in excretion to the reduced ruminal NH_3 concentrations which leads to less urea synthesis and urinary excretion. This agrees with our data, as lower urinary N (81 versus 94 mg/kg BW) and greater fecal N values (139 versus 110 mg/kg BW) are also observed in Bt compared to Bi. Greater ruminal NH_3-N concentrations found in Bi when compared to Bt steers on low-quality subtropical hay, are thought to result in improved rates of digestion of the forages due to the greater availability of N, and possibly greater protozoal populations. Predation of bacteria by protozoa within the rumen can cause N recycling in the rumen, as they can 'graze' and digest the rumen bacteria, reducing outflow and increasing ammonia release within the rumen (Lapierre and Lobley, 2001). Eugène et al. (2004) showed that there is a significant decrease in ruminal NH_3-N in rumen fluid by 2.95 *mM* after defaunation, therefore lower ruminal NH_3-N in Bt may as well be attributed to lower microbial synthesis, less bacterial recycling and less bacteria proteolysis with a lower protozoal population in the rumen (Jouany, 1996). Better methods such as the 16S rDNA pyrosequencing can describe the species and proportions of protozoa in the rumen much more quantitatively and accurately, thus, future research is needed to help explain differences we observed in our study and if they might be due to cattle subspecies differences in rumen microbial populations.

CHAPTER V

CONCLUSIONS

Bos taurus cattle have greater intakes than Bi when consuming LQF to compensate for lower ruminal digestive ability, and greater requirements for maintenance. Provision of both low and high degradable protein supplements increased intake versus the CON for both subspecies, but only improved total tract digestion Bi. The greater ruminal digestion observed in Bi than Bt may be attributed to greater ruminal NH_3-N concentrations, which results from apparently greater urea recycling, especially when L-DIP supplements are fed. Nitrogen balance did not differ between subspecies; however, fecal N excretion is greater in Bt compared to Bi, mainly due to reduced digestive capabilities of Bt, while urinary N excretion is greater in Bi than Bt which may be a reflection of greater ruminal NH_3-N and plasma urea-N concentrations observed in Bi. Future research to help better understand possible microbial differences between subspecies will be beneficial in helping to fully understand our results.

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APPENDIX

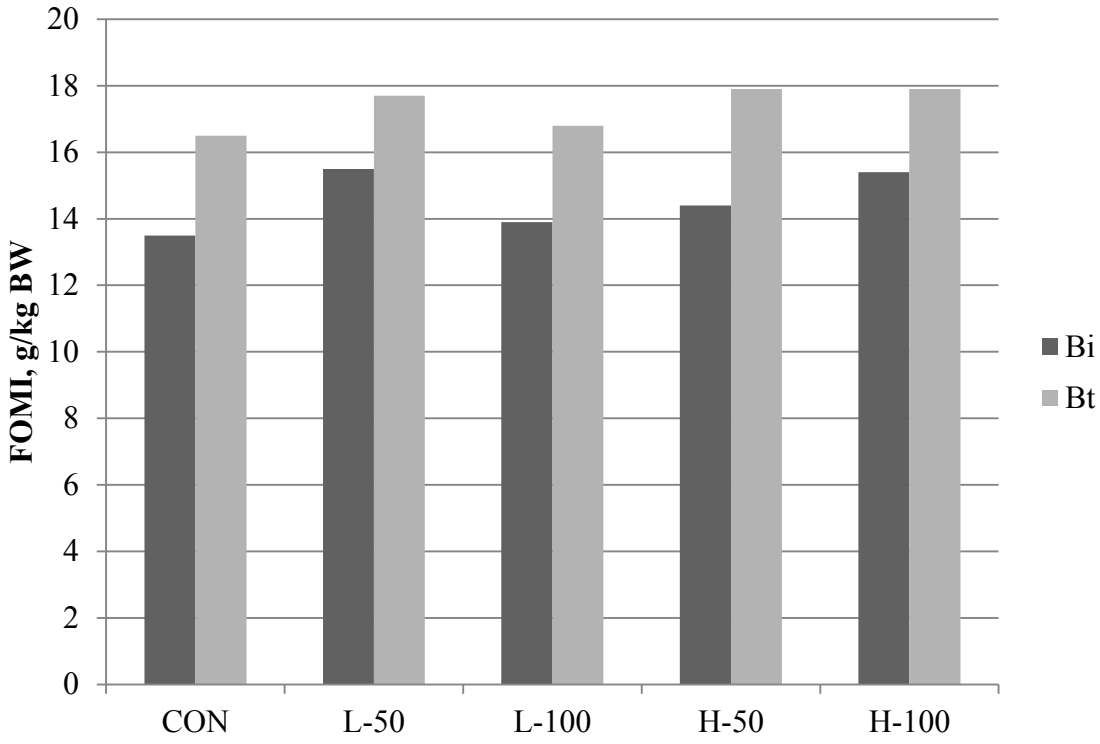

Figure A-1. Effect of protein supplement amount and degradability on forage organic matter intake in *Bos indicus* (Bi) and *Bos taurus* (Bt) steers. CON = 0 mg N/kg BW; L-50 = 50 mg N/kg BW L-DIP; L-100 = 100 mg N/kg BW L-DIP, H-50 = 50 mg N/kg BW H-DIP, H-100 = 100 mg N/kg BW H-DIP. There is a significant difference ($P = 0.05$) between Bi and Bt. There is a significant difference ($P < 0.01$) between CON and supplemented treatments for both subspecies.

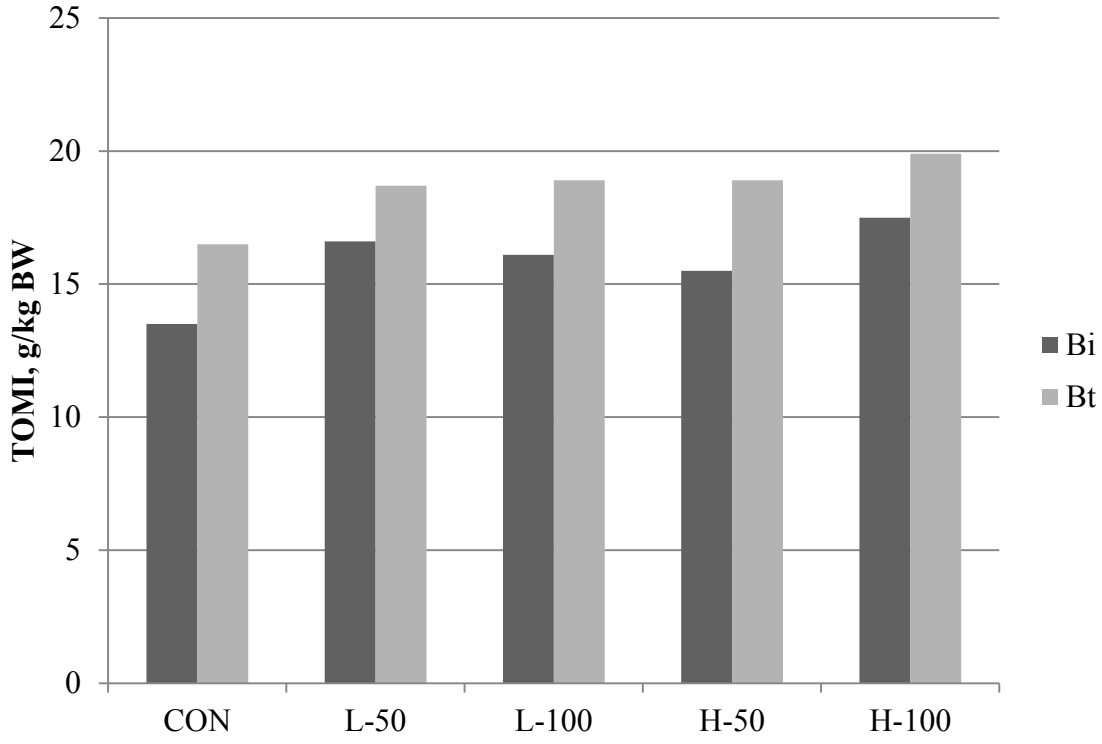

Figure A-2. Effect of protein supplement amount and degradability on total organic matter intake in *Bos indicus* (Bi) and *Bos taurus* (Bt) steers. CON = 0 mg N/kg BW; L-50 = 50 mg N/kg BW L-DIP; L-100 = 100 mg N/kg BW L-DIP, H-50 = 50 mg N/kg BW H-DIP, H-100 = 100 mg N/kg BW H-DIP. There is a significant difference ($P = 0.05$) between Bi and Bt. There is a significant difference ($P < 0.01$) between CON and supplemented treatments for both subspecies.

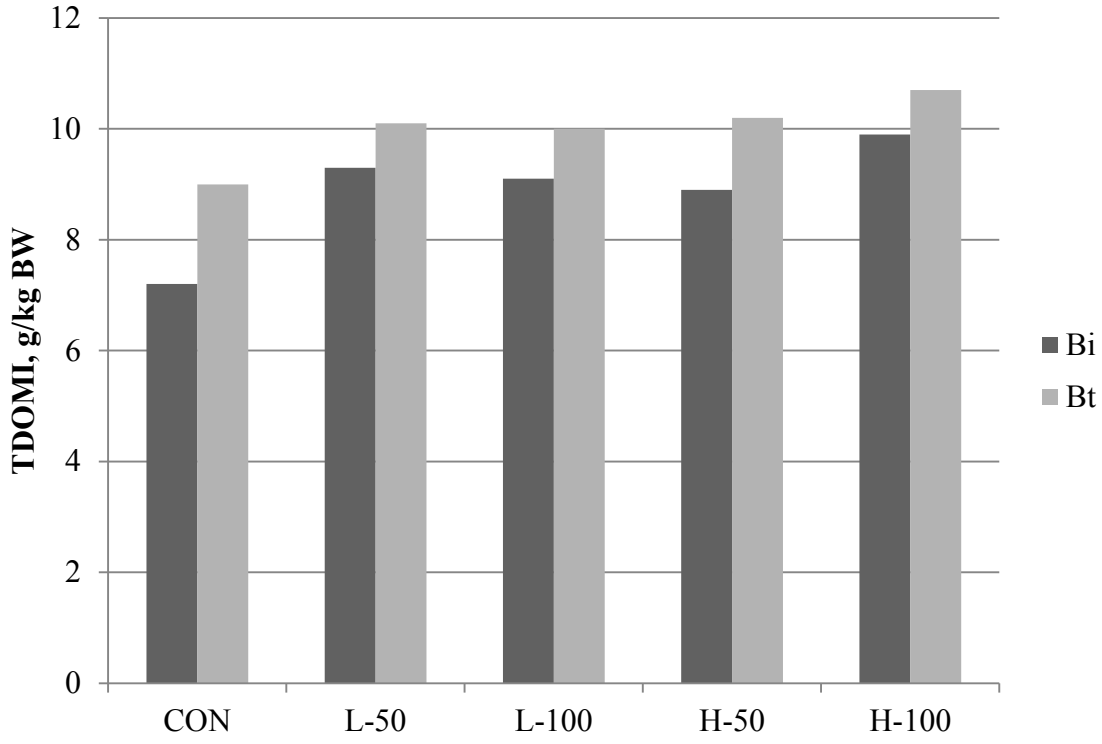

Figure A-3. Effect of protein supplement amount and degradability on total digestible organic matter intake in *Bos indicus* (Bi) and *Bos taurus* (Bt) steers. CON = 0 mg N/kg BW; L-50 = 50 mg N/kg BW L-DIP; L-100 = 100 mg N/kg BW L-DIP, H-50 = 50 mg N/kg BW H-DIP, H-100 = 100 mg N/kg BW H-DIP. There is no significant difference ($P = 0.12$) between Bi and Bt. There is a significant difference ($P < 0.01$) between CON and supplemented treatments for both subspecies.

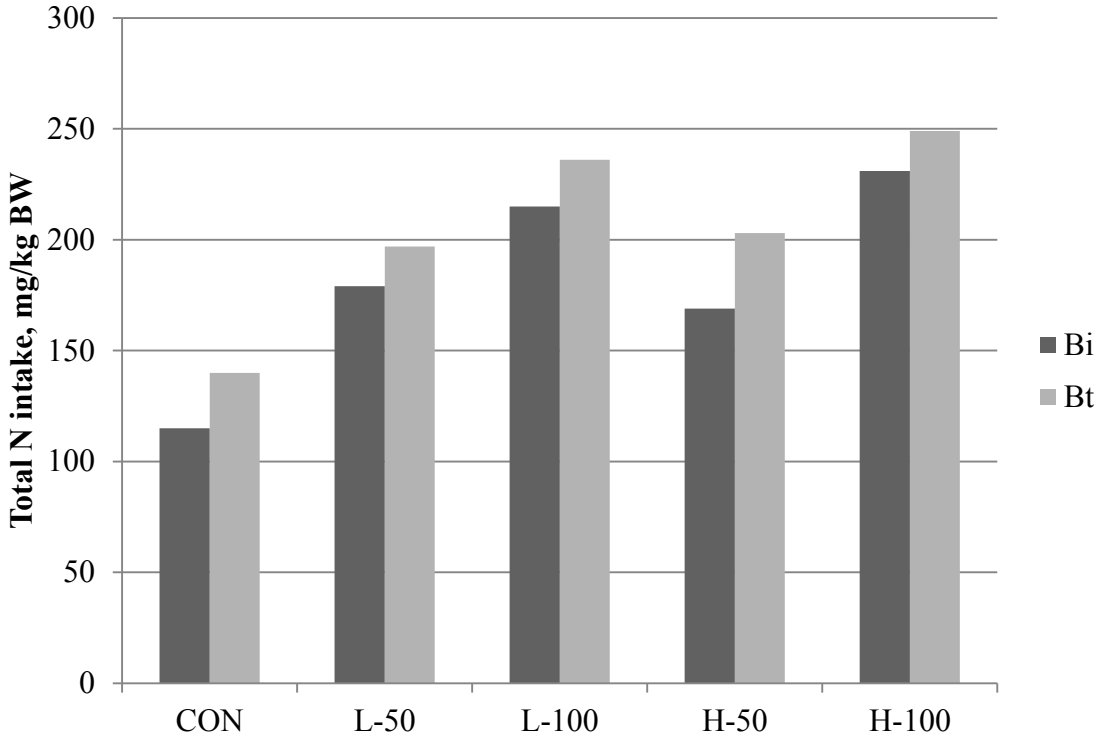

Figure A-4. Effect of protein supplement amount and degradability on total N intake in *Bos indicus* (Bi) and *Bos taurus* (Bt) steers. CON = 0 mg N/kg BW; L-50 = 50 mg N/kg BW L-DIP; L-100 = 100 mg N/kg BW L-DIP, H-50 = 50 mg N/kg BW H-DIP, H-100 = 100 mg N/kg BW H-DIP. There is a significant difference ($P = 0.06$) between Bi and Bt. There is a significant difference ($P < 0.01$) between CON and supplemented treatments and 50 versus 100 mg N/kg BW for both subspecies. There is a source by level interaction ($P < 0.01$) for Bt but not for Bi ($P = 0.19$).

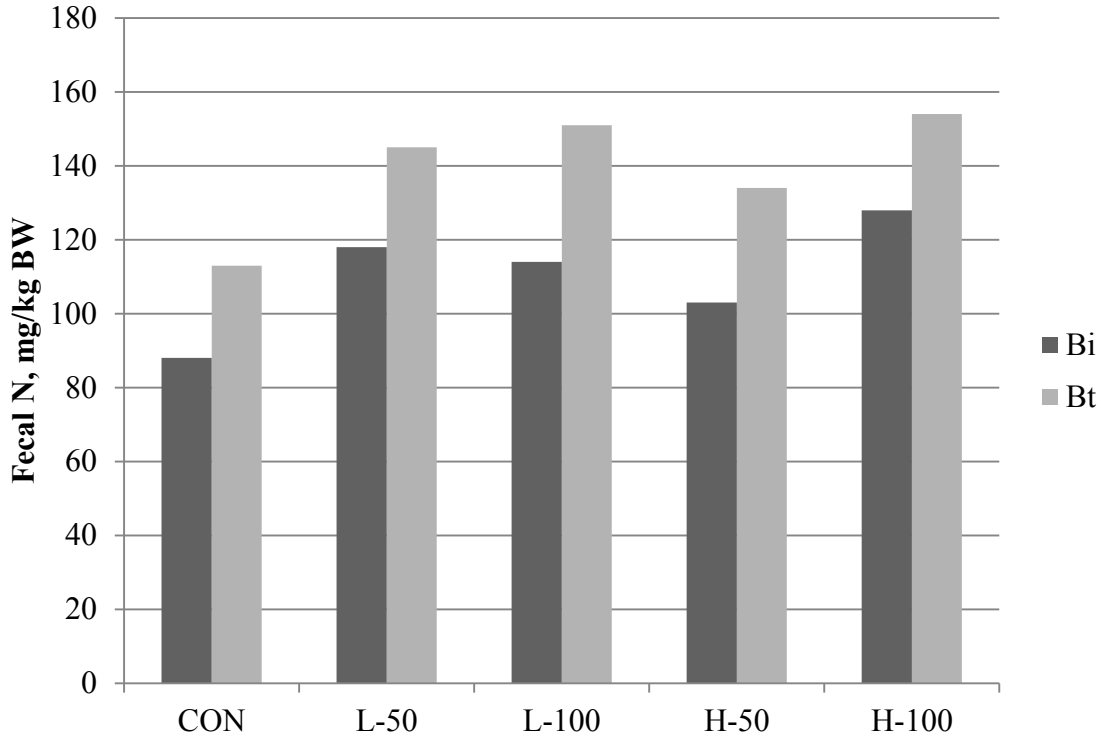

Figure A-5. Effect of protein supplement amount and degradability on fecal N excretion in *Bos indicus* (Bi) and *Bos taurus* (Bt) steers. CON = 0 mg N/kg BW; L-50 = 50 mg N/kg BW L-DIP; L-100 = 100 mg N/kg BW L-DIP, H-50 = 50 mg N/kg BW H-DIP, H-100 = 100 mg N/kg BW H-DIP. There is a significant difference ($P = 0.01$) between Bi and Bt. There is a significant difference ($P < 0.01$) between CON and supplemented treatments, and there tended to be a difference ($P \leq 0.08$) between 50 versus 100 mg N/kg BW for both subspecies.

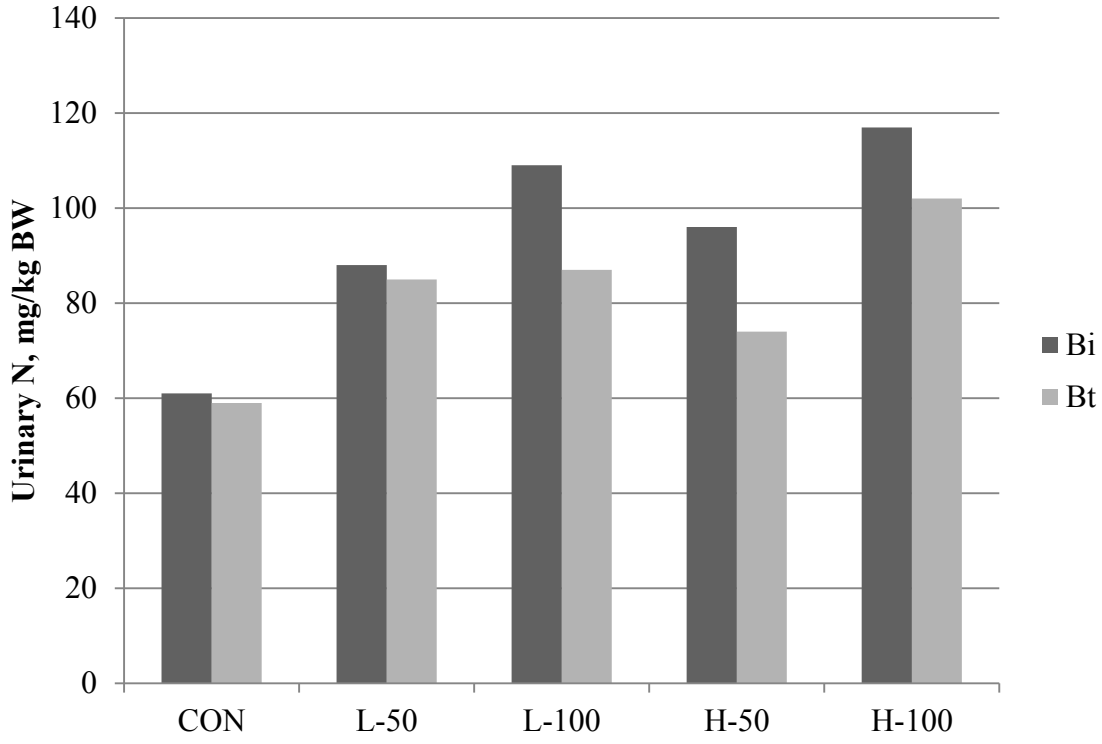

Figure A-6. Effect of protein supplement amount and degradability on urinary N excretion in *Bos indicus* (Bi) and *Bos taurus* (Bt) steers. CON = 0 mg N/kg BW; L-50 = 50 mg N/kg BW L-DIP; L-100 = 100 mg N/kg BW L-DIP, H-50 = 50 mg N/kg BW H-DIP, H-100 = 100 mg N/kg BW H-DIP. There tended to be a difference ($P = 0.10$) between Bi and Bt. There is a significant difference ($P < 0.01$) between CON and supplemented treatments, and there tended to be a difference ($P \leq 0.06$) between 50 versus 100 mg N/kg BW for both subspecies.

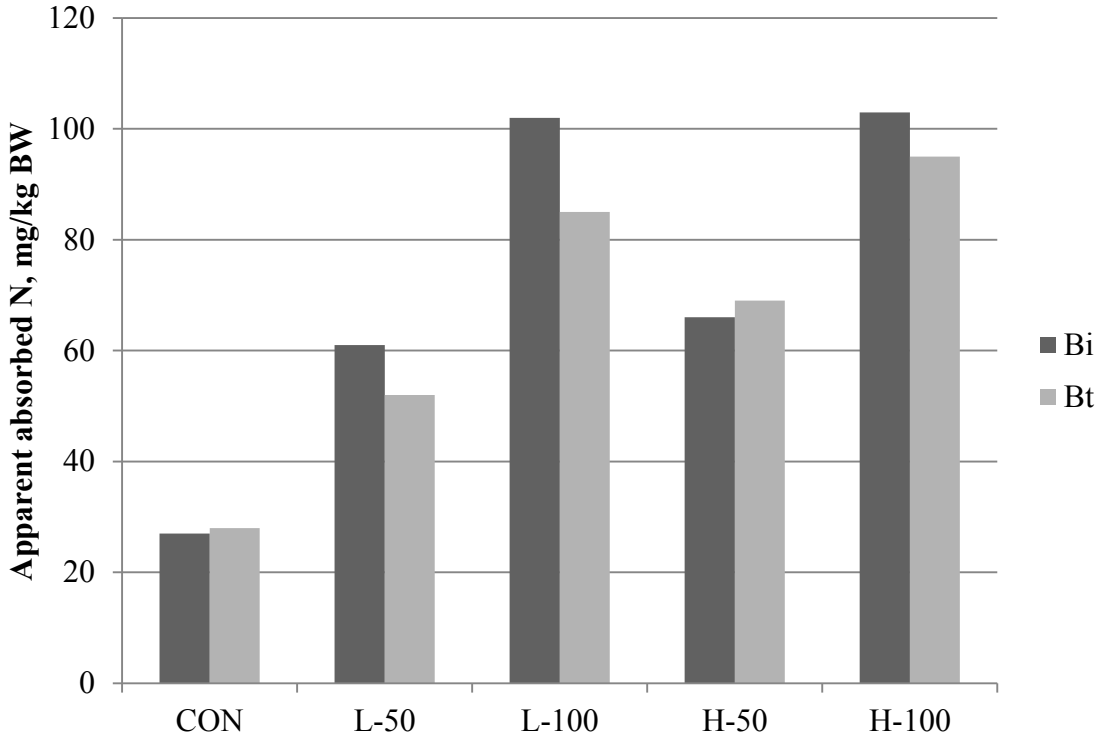

Figure A-7. Effect of protein supplement amount and degradability on apparent absorbed N in *Bos indicus* (Bi) and *Bos taurus* (Bt) steers. CON = 0 mg N/kg BW; L-50 = 50 mg N/kg BW L-DIP; L-100 = 100 mg N/kg BW L-DIP, H-50 = 50 mg N/kg BW H-DIP, H-100 = 100 mg N/kg BW H-DIP. There is no difference ($P = 0.23$) between Bi and Bt. There is a significant difference ($P < 0.01$) between CON and supplemented treatments, and there tended to be a difference ($P \leq 0.01$) between 50 versus 100 mg N/kg BW for both subspecies.

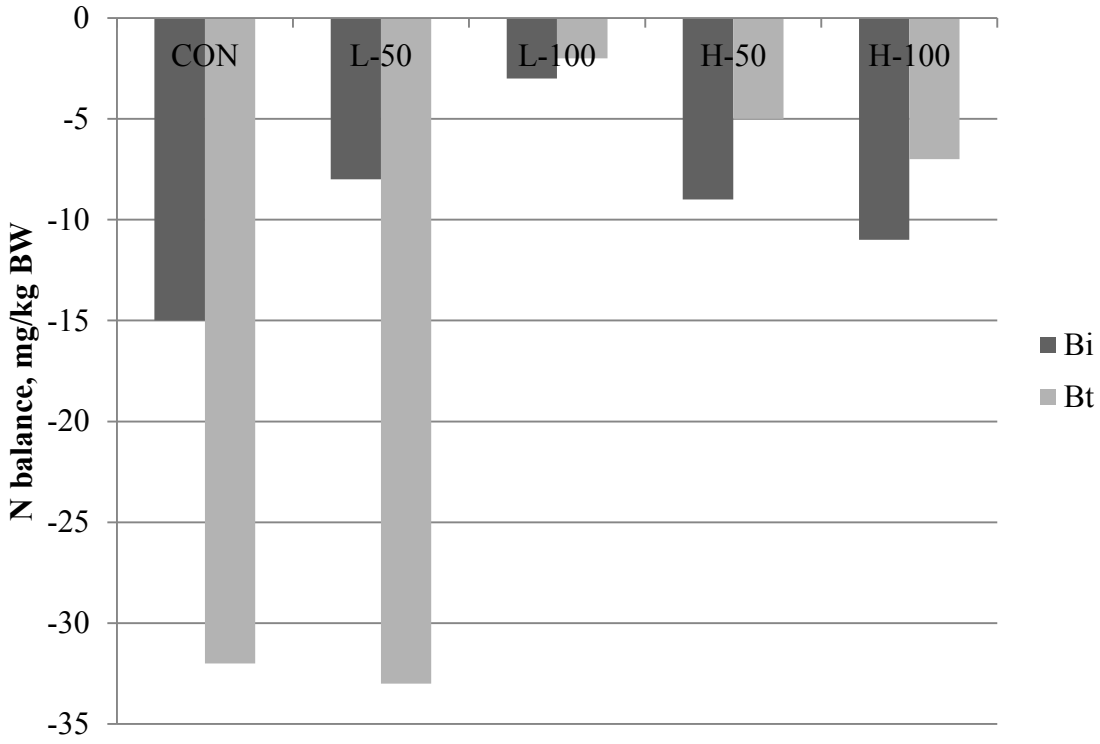

Figure A-8. Effect of protein supplement amount and degradability on N balance in *Bos indicus* (Bi) and *Bos taurus* (Bt) steers. CON = 0 mg N/kg BW; L-50 = 50 mg N/kg BW L-DIP; L-100 = 100 mg N/kg BW L-DIP, H-50 = 50 mg N/kg BW H-DIP, H-100 = 100 mg N/kg BW H-DIP. There is no difference ($P = 0.18$) between Bi and Bt. There is a significant difference ($P < 0.01$) between CON and supplemented treatments, and between 50 versus 100 mg N/kg BW for Bt.

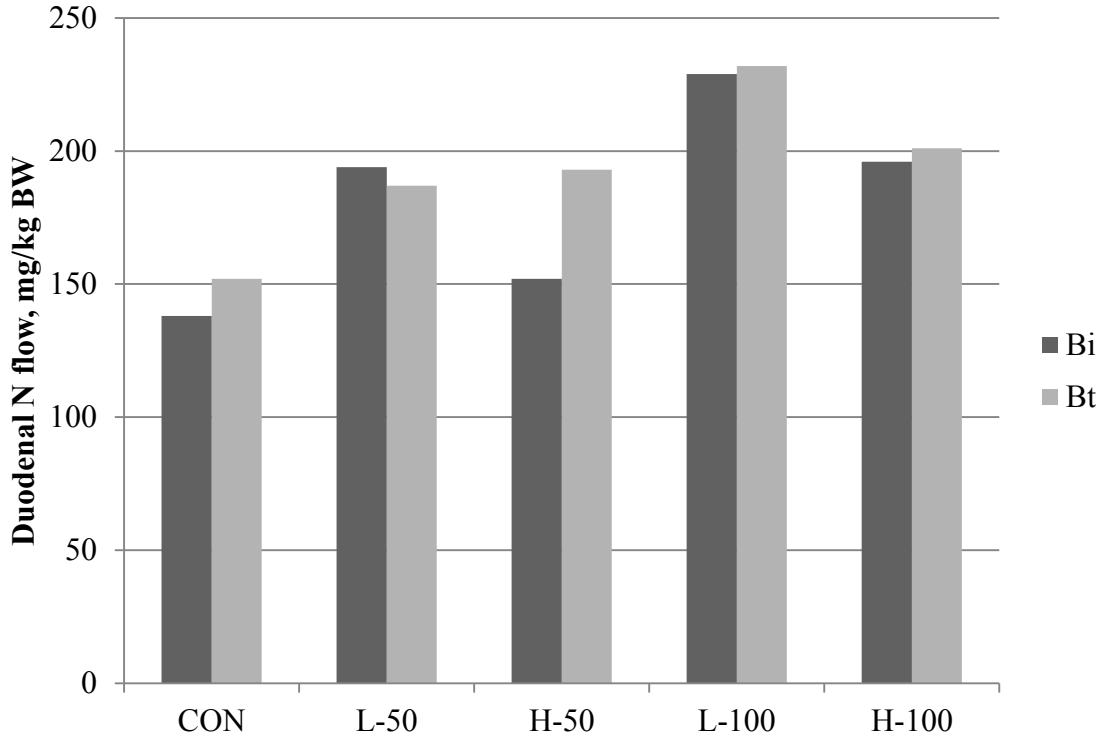

Figure A-9. Effect of protein supplement amount and degradability on duodenal N flow in *Bos indicus* (Bi) and *Bos taurus* (Bt) steers. CON = 0 mg N/kg BW; L-50 = 50 mg N/kg BW L-DIP; L-100 = 100 mg N/kg BW L-DIP, H-50 = 50 mg N/kg BW H-DIP, H-100 = 100 mg N/kg BW H-DIP. There is no difference ($P = 0.45$) between Bi and Bt. There is a significant difference ($P < 0.01$) between CON and supplemented treatments, and there tended to be a difference ($P \leq 0.01$) between 50 versus 100 mg N/kg BW for both subspecies.

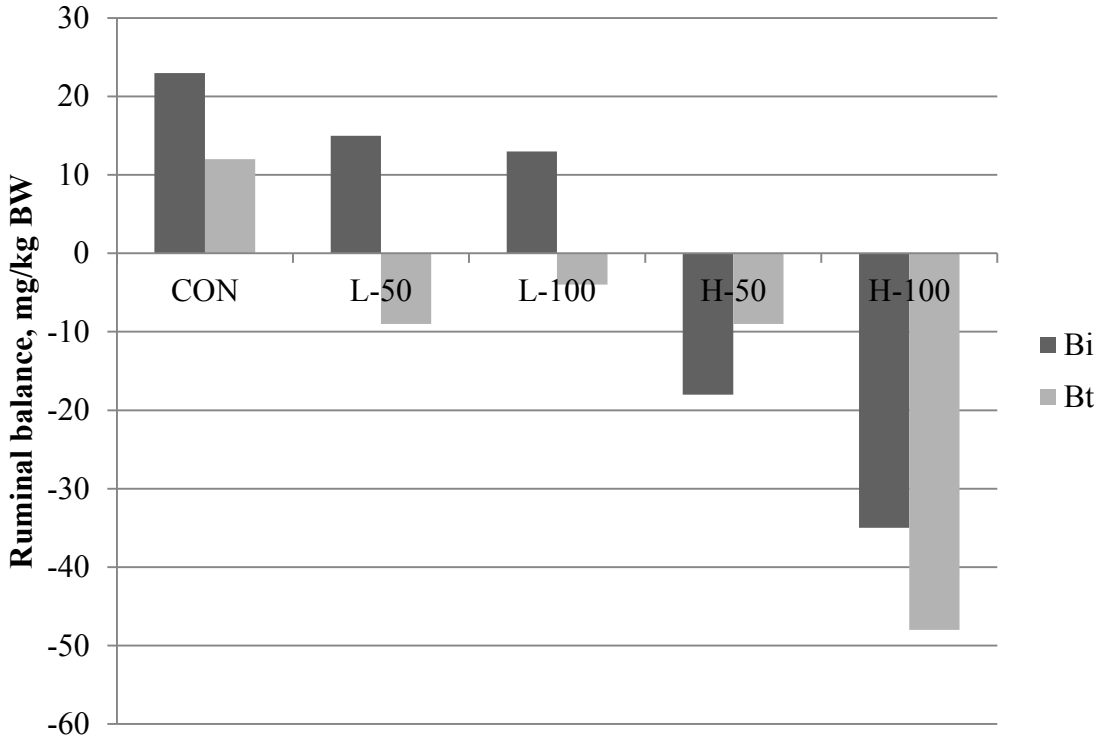

Figure A-10. Effect of protein supplement amount and degradability on ruminal balance in *Bos indicus* (Bi) and *Bos taurus* (Bt) steers. CON = 0 mg N/kg BW; L-50 = 50 mg N/kg BW L-DIP; L-100 = 100 mg N/kg BW L-DIP, H-50 = 50 mg N/kg BW H-DIP, H-100 = 100 mg N/kg BW H-DIP. There tended to be a difference ($P = 0.12$) between Bi and Bt. There is a significant difference ($P \leq 0.06$) between CON and supplemented treatments for both subspecies. There is a significant ($P \leq 0.02$) source by level interaction for both Bi and Bt., and there is a difference ($P \leq 0.05$) between H and L for both subspecies.

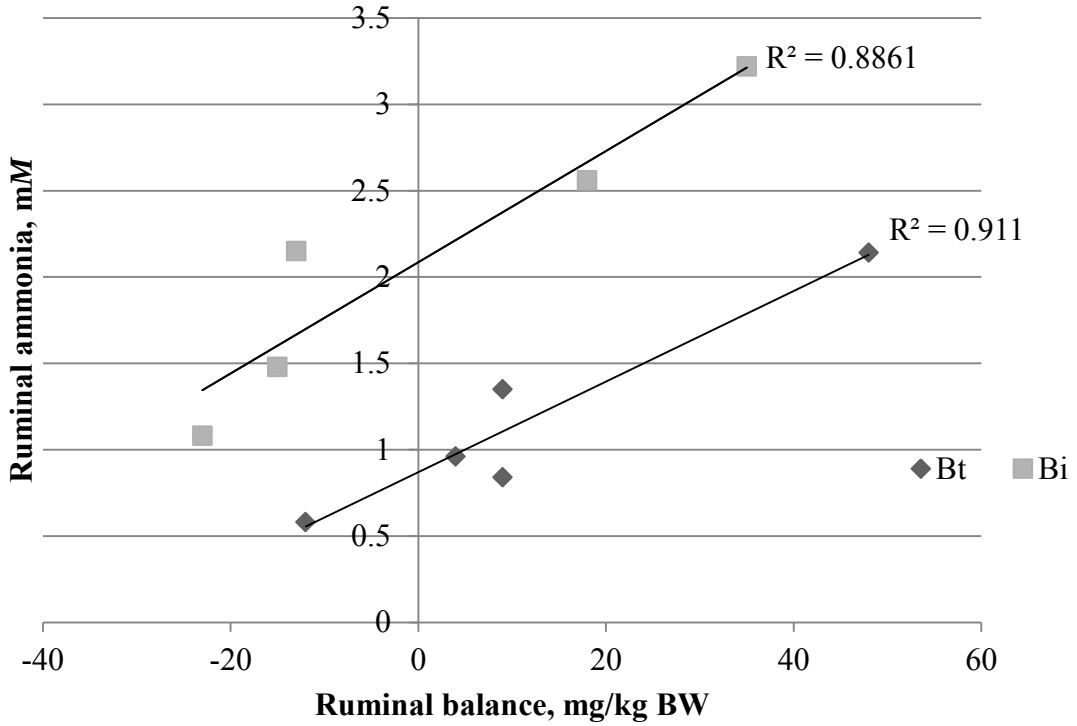

Figure A-11. Effect of protein supplement amount and degradability on ruminal balance and ruminal ammonia in *Bos indicus* (Bi) and *Bos taurus* (Bt) steers

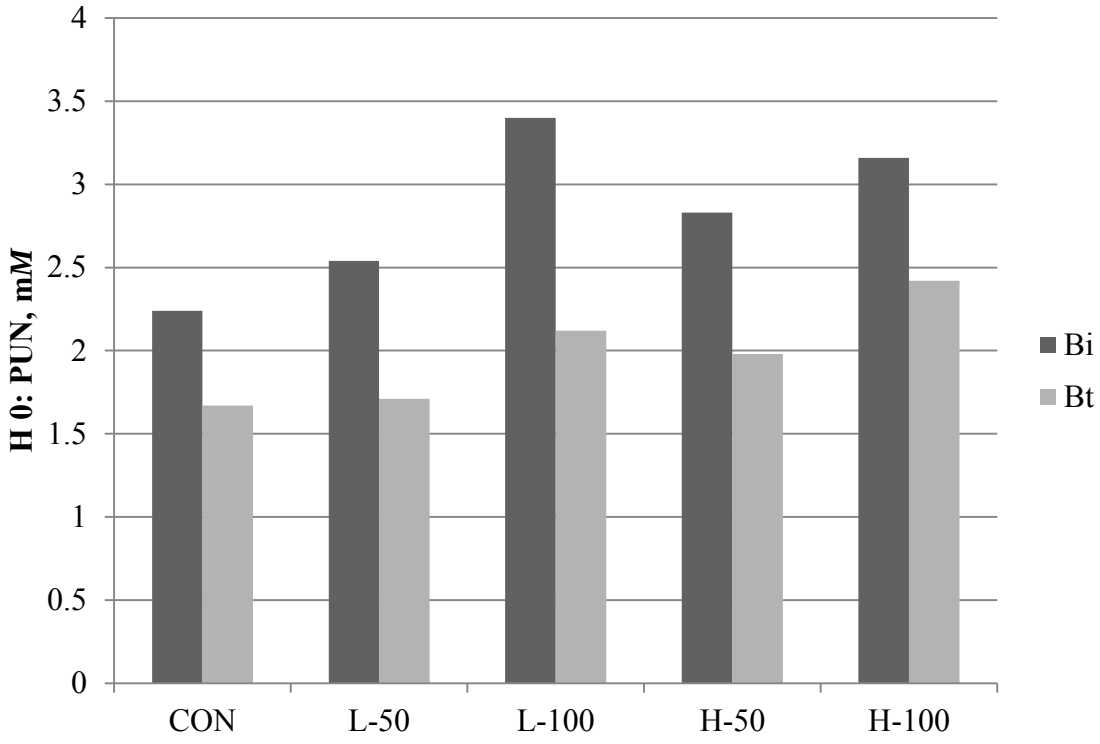

Figure A-12. Effect of protein supplement amount and degradability on hour 0 plasma urea N in *Bos indicus* (Bi) and *Bos taurus* (Bt) steers. CON = 0 mg N/kg BW; L-50 = 50 mg N/kg BW L-DIP; L-100 = 100 mg N/kg BW L-DIP, H-50 = 50 mg N/kg BW H-DIP, H-100 = 100 mg N/kg BW H-DIP. There is a significant difference ($P < 0.01$) between Bi and Bt. There is a significant difference ($P < 0.08$) between CON and supplemented treatments for both Bi and Bt. There tended to be a difference ($P \leq 0.10$) between H versus L and 50 versus 100 mg N/kg BW for Bt.

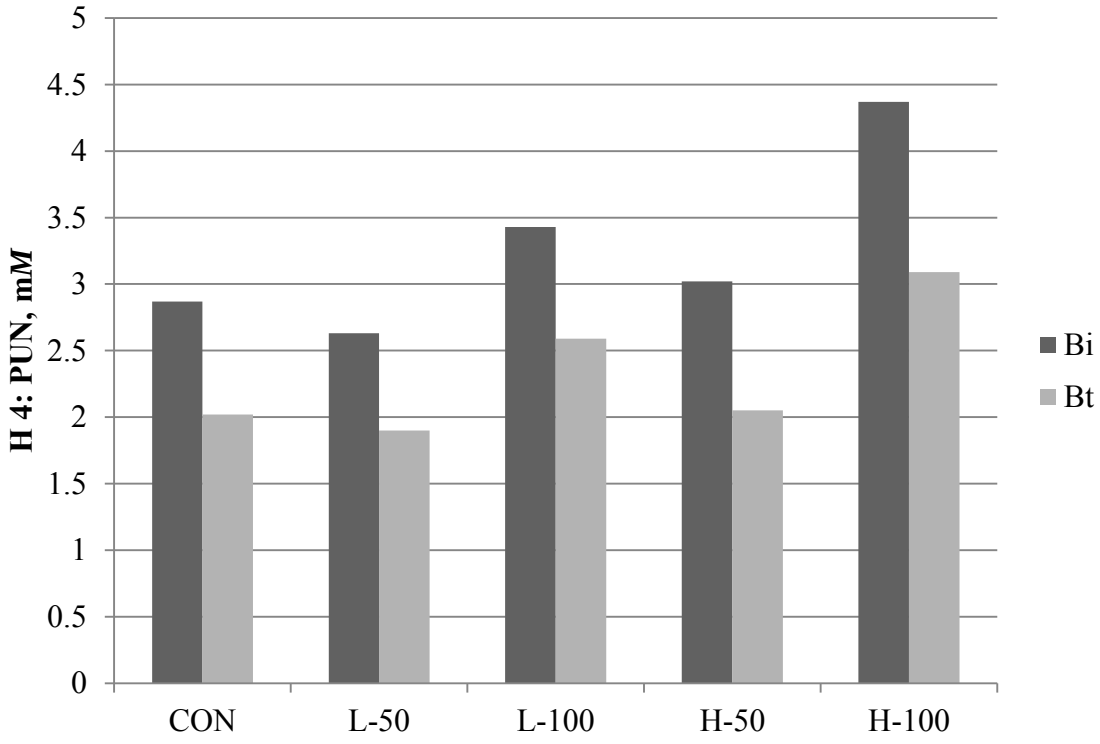

Figure A-13. Effect of protein supplement amount and degradability on hour 4 plasma urea N in *Bos indicus* (Bi) and *Bos taurus* (Bt) steers. CON = 0 mg N/kg BW; L-50 = 50 mg N/kg BW L-DIP; L-100 = 100 mg N/kg BW L-DIP, H-50 = 50 mg N/kg BW H-DIP, H-100 = 100 mg N/kg BW H-DIP. There is a significant difference ($P < 0.01$) between Bi and Bt. There is a significant ($P = 0.04$) source by level interaction for Bi. There is a significant difference ($P \leq 0.01$) for both subspecies.

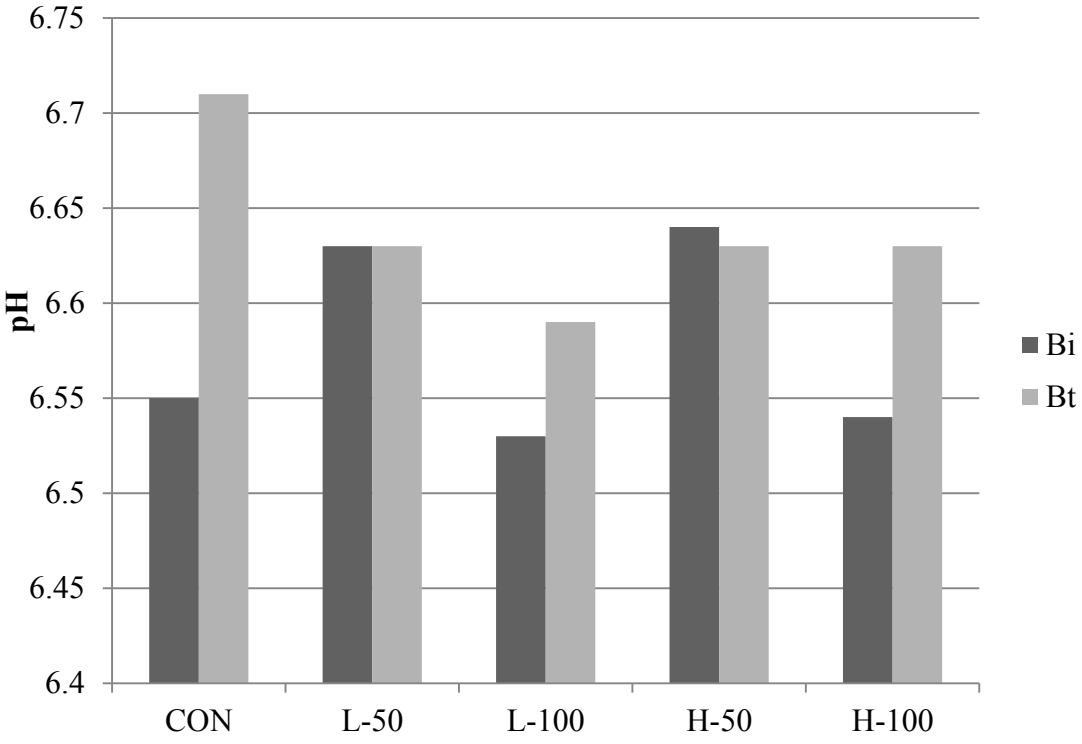

Figure A-14. Effect of protein supplement amount and degradability on pH in *Bos indicus* (Bi) and *Bos taurus* (Bt) steers. CON = 0 mg N/kg BW; L-50 = 50 mg N/kg BW L-DIP; L-100 = 100 mg N/kg BW L-DIP, H-50 = 50 mg N/kg BW H-DIP, H-100 = 100 mg N/kg BW H-DIP. There is no difference ($P = 0.43$) between Bi and Bt. There is a significant difference ($P < 0.01$) between CON and supplemented treatments in Bt.

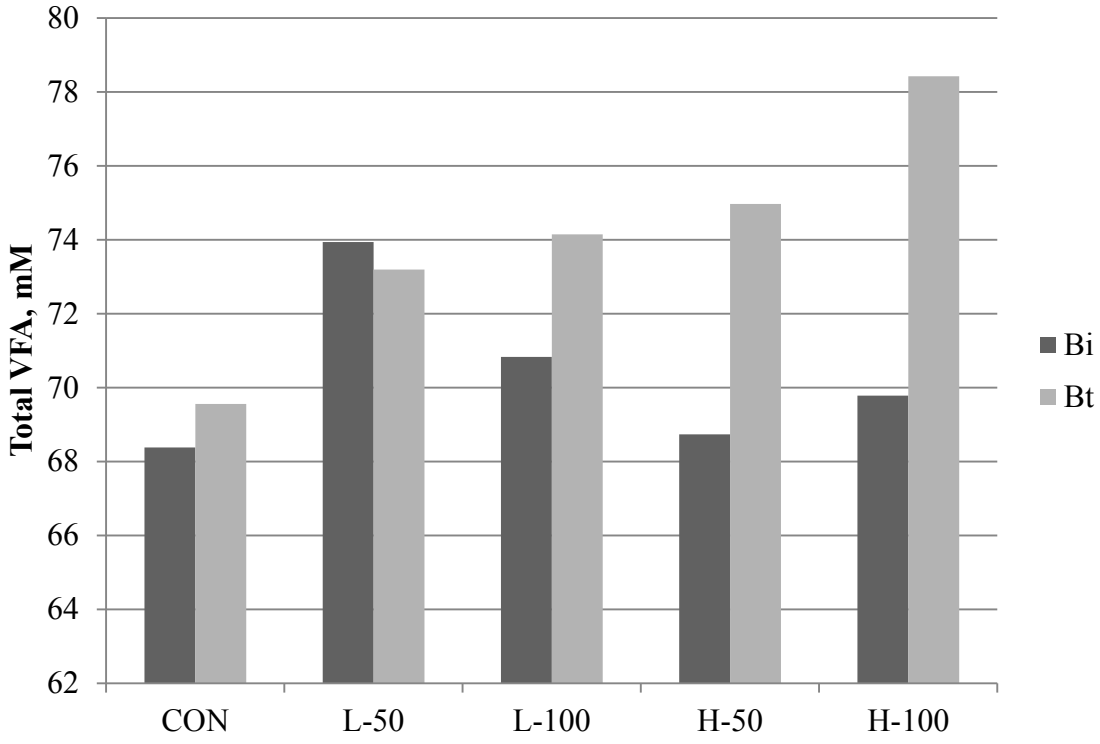

Figure A-15. Effect of protein supplement amount and degradability on total VFA in *Bos indicus* (Bi) and *Bos taurus* (Bt) steers. CON = 0 mg N/kg BW; L-50 = 50 mg N/kg BW L-DIP; L-100 = 100 mg N/kg BW L-DIP, H-50 = 50 mg N/kg BW H-DIP, H-100 = 100 mg N/kg BW H-DIP. There is no difference ($P = 0.19$) between Bi and Bt. There is a significant difference ($P < 0.01$) between CON and supplemented treatments, and there tended to be a source by level interaction ($P = 0.08$) in Bt.

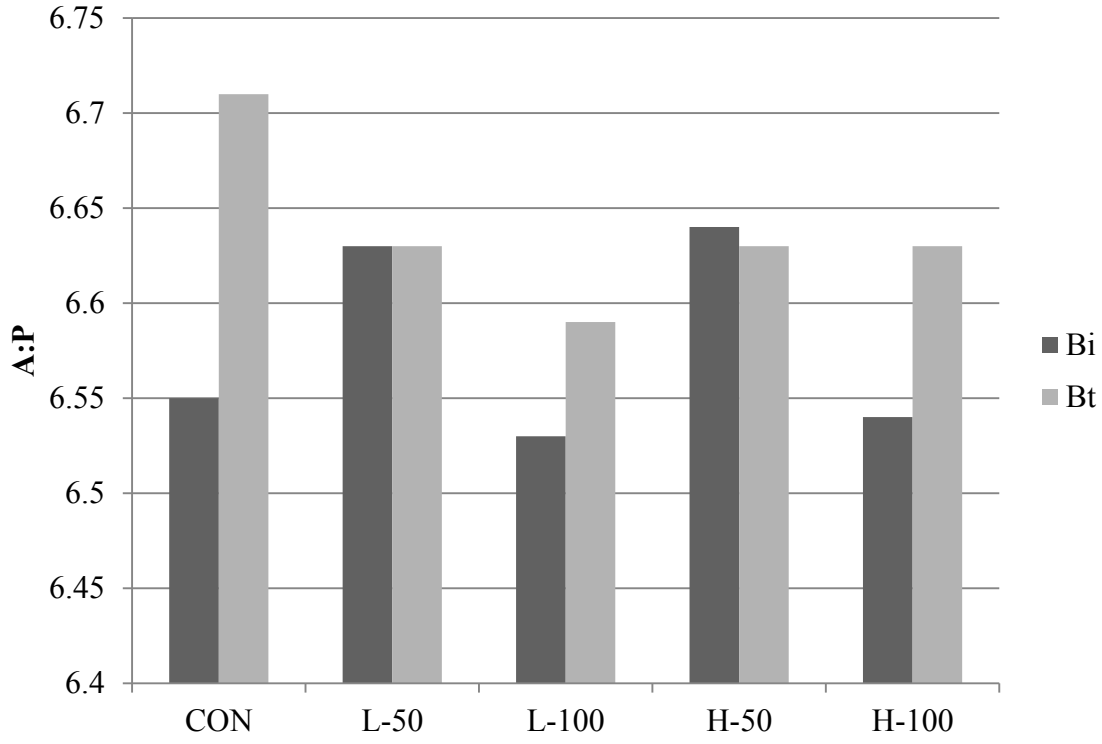

Figure A-16. Effect of protein supplement amount and degradability on acetate: propionate in *Bos indicus* (Bi) and *Bos taurus* (Bt) steers. CON = 0 mg N/kg BW; L-50 = 50 mg N/kg BW L-DIP; L-100 = 100 mg N/kg BW L-DIP, H-50 = 50 mg N/kg BW H-DIP, H-100 = 100 mg N/kg BW H-DIP. There is no difference ($P = 0.66$) between Bi and Bt. There is a significant difference ($P \leq 0.05$) between CON and supplemented treatments, 50 versus 100 mg N/kg BW, and H versus L, and there is a significant source by level interaction for Bi. There is a significant difference ($P < 0.01$) between 50 versus 100 mg N/kg BW for Bt.

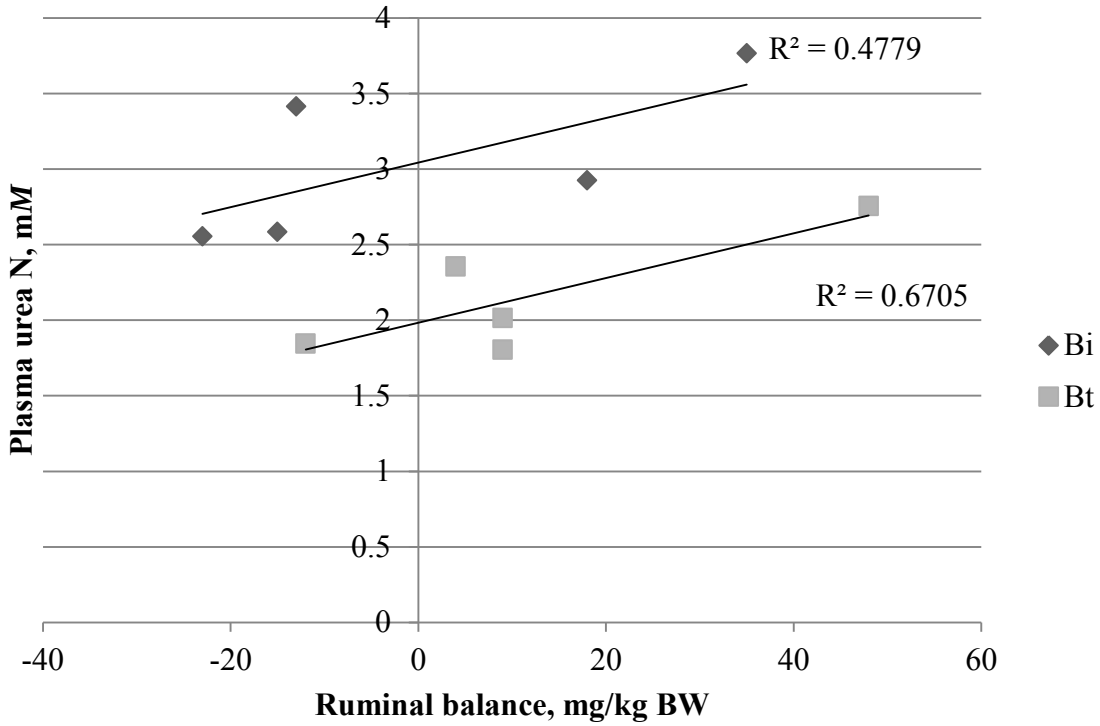

Figure A-17. Effect of protein supplement amount and degradability on ruminal balance and plasma urea N in *Bos indicus* (Bi) and *Bos taurus* (Bt) steers.

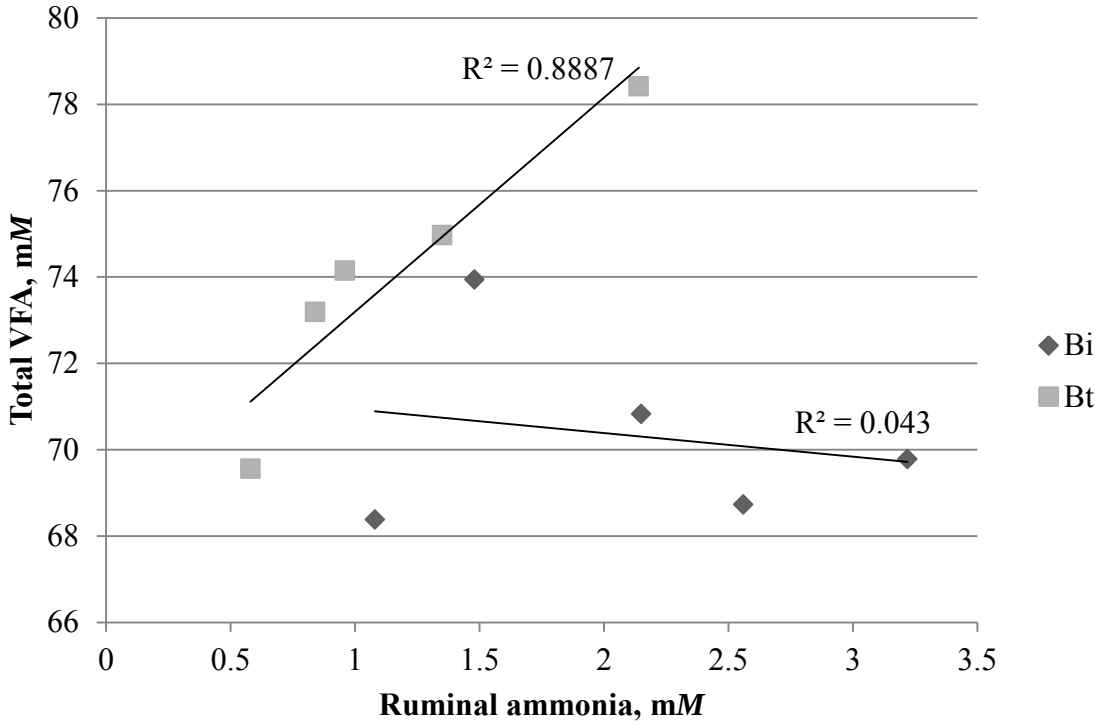

Figure A-18. Effect of protein supplement amount and degradability on total VFA concentration and ruminal ammonia in *Bos indicus* (Bi) and *Bos taurus* (Bt) steers.

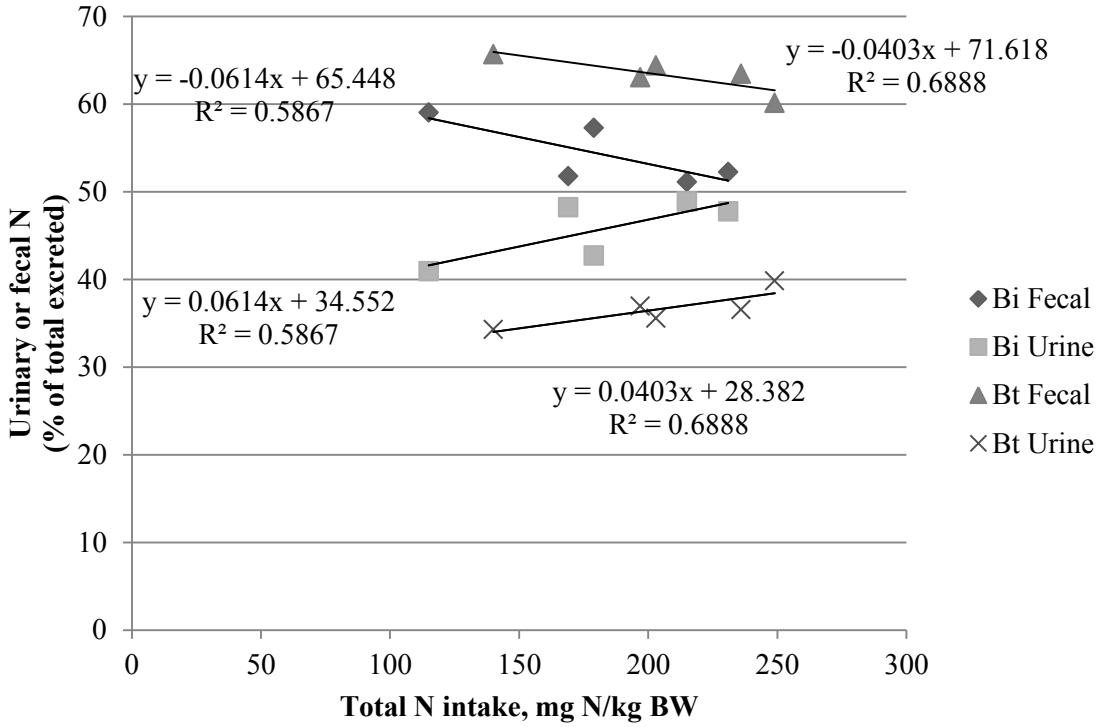

Figure A-19. Effect of total N intake (mg N/kg BW) on the route of N excretion in *Bos indicus* (Bi) and *Bos taurus* (Bt) steers.