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JAMES COOK
UNIVERSITY
AUSTRALIA

The physiological and metabolic effects of stressors associated with long duration transportation on male *Bos indicus* cattle

Thesis submitted

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For the degree of Master of Tropical Animal Science in the

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James Cook University

ABSTRACT

A series of experiments were conducted to examine the effect of stressors associated with long duration transportation, particularly feed and water deprivation, on male *Bos indicus* cattle.

In experiment 1, the effects of feed and water deprivation on microbial counts and rumen kinetics *of Bos indicus* steers were examined. Cannulated steers (n=4) were either deprived (deprived steers) for 72 hours or *had ad* libitum access (control steers) to feed and water in a 2 x 4 cross-over design. Rumen fluid was collected at day -6, 0, 4 and 9. Feed and water deprivation occurred from day -3 to day 0. Feed and water deprivation caused decreases in the numbers of cellulolytic bacteria (1.4 v 0.4 cfu x 10^6/mL; P = 0.001), live (23.7 v 0.8 x 10^9/mL; P = 0.001), dead (12.7 v 0.5 x 10^9/mL; P = 0.001) and total bacterial counts (36.4 v 1.4 x 10^9/mL; P = 0.001) at day 0, compared with the control steers. However, the deprived steers had greater numbers of cellulolytic bacteria (2.7 v 50.1 cfu x 10^6/mL; P = 0.001), live (18.3 v 42.2 x 10^9/mL; P = 0.001), dead (6. 5 v 19.1 x 10^9/mL; P = 0.001) and total bacterial counts (24.8 v 61.3 x 10^9/mL; P = 0.001) from rumen fluid on day 4, compared with the control steers. The numbers of total protozoa in rumen fluid from the deprived steers were less than (551.2 v 2.4 x 10^3/mL; P = 0.001) the control steers on day 0. However, the deprived steers had fewer protozoa in rumen fluid than the control treatment on day 4 (P = 0.001) and day 9 (P = 0.001). The concentrations of acetic, butyric, propionic and total VFA in rumen fluid from steers deprived of feed and water were less than the concentrations found in the control steers on day 0 (P = 0.001). The concentrations of VFA from deprived steers were not different to the concentrations of the same VFAs from the control steers at day 4 and 9. There were no differences between control and treatment groups for *in vitro* gas production (GP) on day -6. The cumulative *in vitro* GP from the control steers was greater than the deprived steers (P = 0.001) on day 0. However, the *in vitro* GP was not different for the following incubations on day 4 and 9. These results indicate that feed and water deprivation would have a negative but transient effect on the rumen kinetics of *Bos indicus* steers. Furthermore, these results would suggest that between one and four days after re-feeding the rumen environment returns to pre-deprivation functionality.

The results from experiment one led to the development of a second hypothesis; that treatment of *Bos indicus* bulls with fresh rumen fluid immediately after transportation would assist in returning the rumen to pre-stress functionality and therefore increase dry matter intake and glycogen concentration in the muscle. In this second experiment, twelve *Bos indicus* bulls were allocated to one of two treatment groups: rumen transfaunation (n = 6) given 10 kg of rumen fluid after transport or Control (n = 6), given 10 kg of deionised water after transport. Glycogen concentrations of the M semimembranosus, M. semitendinosis and M. Longisimus dorsi were measured before transport on day -7 and after transport on day 0, day 1, day 4 and day 9. Feed intake, liveweight gain, plasma metabolites and electrolytes were recorded on the sampling days. Rumen fluid transfaunation increased the dry matter intake of treated bulls compared to the bulls treated with deionised water. However, rumen transfaunation had no effect on glycogen concentrations of the *M. semimembranosus*, *M. semitendinosus* and *M. Longisimus dorsi* compared to the bulls treated with deionised water in this study. The *M. semimembranosus* and the *M. semitendinosus* decreased in glycogen concentration immediately after transport on day 0 but replete to pre–transportation concentrations within 1 day. However, the *M. Longisimus dorsi* demonstrated no change in glycogen concentration between the pre-transportation sample on day -7 and day 0 or day 1 after transportation. The *M. Longisimus dorsi* of the bulls increased in glycogen concentration between day 1 and day 4 after transportation. The bulls in this experiment demonstrated that muscle glycogen, as measured at the M. longissimus dorsi, cannot recover to sufficient concentrations (40 to 45 µmol of glycogen concentration per gram muscle) to ensure normal meat quality until between one and four days of rest when feeding Rhodes grass hay ad libitum.

DECLARATION

I declare that this thesis is my own work. This thesis has not been submitted in any form

for another degree or diploma at any university or other institution of tertiary education.

Information derived from published or unpublished work of others has been

acknowledged in this thesis.

Cardial Leverson Octovianus LEO-PENU

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List of Abbreviations

ACTH   -   Adrenocorticotropic hormone

ANS   -   Autonomic nervous system

CP   -   Crude protein

CRH   -   Corticotrophin-releasing hormones

DMI   -   Dry matter intake

F-6-P   -   Fructose 6-phosphate

GP   -   Gas production

G-6-P   -   Glucose 6-phosphate

HPA   -   Hypothalamic-pituitary-adrenal

NFC   -   Non-fiber carbohydrate

PFK   -   Phosphofructokinase

RFC   -   Rumen fermentative capacity

SCFA   -   Short-chain fatty acids

VFA   -   Volatile fatty acid

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ACKNOWLEDGEMENT

CHAPTER ONE

INTRODUCTION

Australia is well known as the largest exporter of live cattle in the world. During 2013-2014, Australian live cattle export reached 1.13 million head valued at AUS$1.05 million FOB (MLA, *http://www.mla.com.au/Prices-and-markets/Trends-and-analysis/Beef/Live-exports*). Most of these exported cattle are destined for feedlot in the Middle East and South-East Asia. To suply these market it is impossible to avoid periods of land or sea transportation. Activities such as handling, novel environment and mixing with unfamiliar cattle can induce stress and and alter homeostasis of transproted cattle. This response is to defend from all of the stressors to maintain homeostasis.

In northern Australia, *Bos indicus* cattle may be transported over long duration to finishing operations and as a consequence are deprived of feed and water for extended periods of time (Parker et al. 2003b). Managers of pasture and feedlot finishing operations that utilise *Bos indicus* cattle anecdotally report that when the animals are exposed to extended periods of feed and water deprivation of up to 72 hours, the rumen becomes dysfunctional and is unable to ferment and digest feedstuffs. Furthermore, the alleged rumen dysfunction is believed to contribute to a reduction in feed intake and body weight gain in newly received cattle and may also have a negative impact on meat quality such as dark cutting. In addition, there is a serious public concern about animal welfare to minimize stress of transported cattle during transportation for long duration. Moreover, efforts for minimizing the stresses have a positive correlation with animal production efficiency.

A review of the literature revealed that much of previous research investigating the physiological and metabolic effects of stressors associated with long duration transportation focused on sheep and *Bos taurus* cattle (Galyean et al., 1981, Fluharty et al., 1996, Gardner et al.,

2001, Earley and Murray, 2010). There are few studies about *Bos indicus* associated with long duration transportation especially the physiological and metabolic effects.

The objectives of the experiments presented within this thesis were:

- To enhance knowledge and understanding about the effects of feed and water deprivation on rumen kinetics of *Bos indicus* cattle.
- To investigate liveweight loss and glycogen concentration on *Bos indicus* bulls during and after long duration of transportation.

A literature review, three separate but related experiments, a general discussion and conclusion are all included in this thesis.

The literature review examines relevant research on the effect of long duration transportation on cattle. It addresses the impact of feed and water deprivation on live weight loss, rumen kinetics, and muscular glycogen concentrations. The focus of this review is to identify the opportunities of nutritional interventions for optimal recovery of *Bos indicus* cattle exposed to long distance transportation without feed and water.

The first two experiments conducted to study the negative but transient effect of feed and water deprivation of 72 hours on the rumen kinetics of *Bos indicus* cattle is presented in thesis. The greatest impact on rumen kinetics from feed and water deprivation on rumen bacteria and protozoa numbers including cellulolytic and total, live and dead bacteria in rumen fluid is discussed. Other measures of rumen functionality such as gas and VFA production due to feed and water deprivation for 72 hours are also discussed.

An experiment of nutritional interventions through rumen transfaunation for optimal recovery from long duration transportation without feed and water on *Bos indicus* cattle is detailed. The effects of the rumen fluid administration to *Bos indicus* cattle after a 12 h wet curfew and 24 h of transportation on dry matter intake, body weight, electrolyte and metabolite parameters in blood and muscular glycogen concentrations are also presented in this thesis.

CHAPTER TWO

LITERATURE REVIEW

2.1. Introduction

Transportation in relation to marketing of cattle is almost impossible to be avoided in northern Australia. Activities such as handling, novel environment, mixing with unfamiliar animals, and also short or long periods of feed and water deprivation lead to altered cattle homeostasis. Animal's stress response is to defend from all stressors that will maintain their homeostasis (Konturek et al., 2011).

This review examines the current literature on the effects of feed and water deprivation, transport and other accompanied stressors on live weight, rumen kinetics and muscle glycogen concentration of cattle. This review also examines some substrates fed to stressed animals for optimal recovery after exposure to stressors.

2.2. Stress and its impacts

Stress is commonly considered to be a response produced by diverse nocuous agents (Selye, 1936). Such nocuous agents are then defined as stressors that affect the body through activation of the hypothalamic-pituitary-adrenal axis and the central nervous system (Butcher and Lord, 2004). Stressors could be in the form of physical, psychological, or chemical stimuli from internal or external to disrupt homeostasis (Black, 2002). Adaptive responses however, would be evoked from stress to maintain the homeostasis of the cattle.

Regarding stress gastrointestinal responses, it is known that interactions between the gastrointestinal tract and brain occur in response to stress (Konturek et al., 2011). These interactions between the brain and the gut are through multiple parallel pathways including the autonomic nervous system (ANS), the hypothalamic pituitary-adrenal axis (HPA), enteric nervous

system as well as possible other connections (Black, 2002). The enteric nervous system plays an

important role in the physiological gut functions including secretion, motility, peptides, hormones

and immunological levels (Beaumont, 1977). In the condition of a stress response, the ANS

stimulates neurons in the hypothalamus to produce corticotrophin-releasing hormones (CRH) and

arginine vasopressin (Tache and Bonaz, 2007). CRH could affect the gut via modulation of

inflammation, an increase in gut permeability, a contribution to visceral hypersensitivity and

modulation of the gut motility (Tache and Bonaz, 2007). The pituitary gland then responds to

CRH by releasing adrenocorticotropin (ACTH) to stimulate adrenal glands to secrete

glucocorticoids such as cortisol (Marketon and Glaser, 2008). Another interaction is the

bidirectional interaction between brain, gut and intestinal micro flora. These interactions play a

vital role in the regulation of the stress response of the gut in the context of the development of

different gut disorders (Rhee et al., 2009). It has been reported that in the stress conditions, the

autonomic nervous system affects gut epithelium cells and mucus layers, thereby affecting the

enteric micro flora (Lyte et al., 2011). One study reported acute stress stimulated change in

colonic paracellular permeability of mast cell, overproduction of interferon-γ and morphology of

the colonic epithelium (Demaude et al., 2006). Moreover, stress also stimulated changes in the

micro flora diversity (Lyte et al., 2011).

2.3. Effects of feed and water deprivation and transportation on liveweight of cattle

Stressors such as handling, transportation, feed and water deprivation, extreme

temperature, novel environment, and mixing with unfamiliar cattle are common and moreover

lead to altered cattle homeostasis (Selye, 1936).

During transportation, cattle generally experience feed and water deprivation for varying

periods, as well as rapid changes in feeding. These changes lead to at least two effects: loss of

appetite and liveweight (Hutcheson and Cole, 1986). Salerno (1949) evaluated the weight loss of

Bos taurus bulls transported for 24, 48, 72 and 96 hours and reported losses of 8.8, 10.8, 12.3 and 13.5%, respectively. Self and Gay (1972) reported a 7.2 to 9.2% shrinkage in Angus×Hereford crossbred feeder cattle purchased from a ranch or a sale yard and transpoted for 1,023 km. This result is similar to that of Earley *et al.* (2010) who reported a 4.6 to 7.5% weight loss in Charolais bulls having free access to water and travelled 280 to 1192 km during 6 to 24 hours. Moreover, Smith *et al.* (1982) reported that liveweight declined by 2.57 kg/hour during the first 5.3 hours of fasting. Across a number of studies it appears that liveweight loss is greater in the longer duration of transportation.
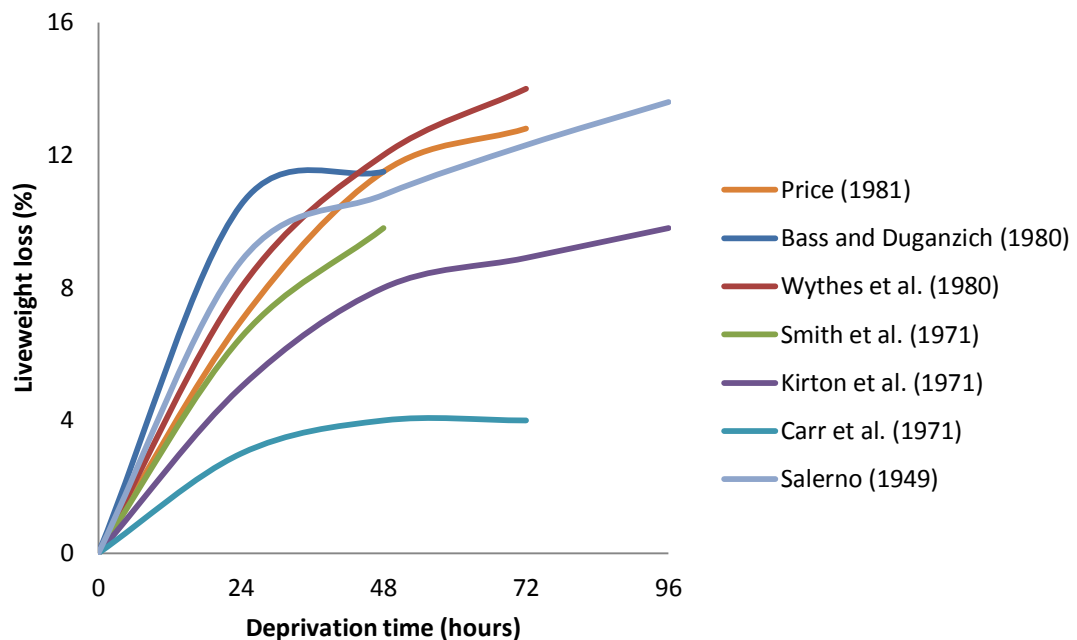
Figure 2.1. The loss in liveweight in fasted and transported cattle. Adapted from: Warriss (1990); Parker(2004)

Transportation combined with type of feed, rapid changes in feeding and feed and water deprivation lead to liveweight loss. Wythes *et al.* (1980) reported a 10.4% weight loss on 216 Zebu cross and shorthorn bullocks transported 1420 km- a journey of 10 hour by road in northern Australia after being held overnight in yards without water and feed. Research on *Bos sondaicus*

bulls showed that liveweight loss during five days' transportation, with stressors including feed and water deprivation, varied from 8.5 to 17.3% of initial liveweight (Leo-Penu et al., 2010). Cattle raised on legumes experienced greater liveweight loss during shipping than those raised on grass. Cattle fattened under a feedlot system lost significantly more liveweight than those fattened under the grazing system, 14.4% versus 9.6% respectively (Leo-Penu et al., 2010). Prefast diet influenced the cattle recovery from feed and water deprivation (Cole and Hutcheson, 1985a).

The proportion of liveweight loss is highest in the first 12 to 24 hour fasting, declining thereafter (Figure 2.1). The weight loss is attributed to the loss of gut fill by defecation and urination (Wythes et al., 1980). This gut contents of adult cattle account for more than 20% of the liveweight (Hughes, 1976 in Warriss, 1990) and faeces is produced during fasts of up to 5 days, although, the rates are reduced to 15-20% of those pre-fast (Blaxter and Wainman, 1966). Muscle glycogen also significantly decreased during long transportation and fasting periods (McVeigh and Tarrant, 1982, McVeigh et al., 1982). It is reported that the proportion glycogen in the liver and muscle accounts for 5 to 8% of its weight respectively (Guyton and Hall, 2001).

Supplementation with electrolyte solutions has been used to minimise the negative effects of transportation in *Bos indicus* cattle (Parker, 2004). Electrolyte supplementation for 18 days after long transportation of live export cattle resulted in a 2.9% liveweight advantages compared to control steers (Beatty et al., 2007). However, this weight advantage can be attributed to an increased water proportion due to an increase of isotonic fluid intake. Several attempts also have been made to improve the glucose supply to cattle during the marketing period (Hutcheson and Cole, 1986, Schaefer et al., 1990). It was reported that either glucose and electrolyte treatments on crossbred yearling bulls having a minimal and moderate stress appear to improve meat colour and reduce the amount of carcass loss by up to 3% (Schaefer et al., 1990).

The suppressed appetite of cattle exposed to extended periods of feed and water deprivation may take up to 14 days to equalise to pre-deprived levels (Cole and Hutcheson, 1985b, Galyean et al., 1981). This means that the rumen, which is involved with and also

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influenced dry matter intake (DMI), is affected detrimentally by extended feed and water deprivation. However, research conducted using Hereford vs. Friesian steers transported for 5, 10 and 15 hours losing 4.6, 6.5 and 7.0% of their liveweight, respectively, only needed 5 days to recover to pre-transport DMI levels (Warriss et al., 1995). Although different breeds of cattle present different responses to stressors, opportunities exist to intervene by providing treatments that reduce the effect of feed and water deprivation during transportation.

2.4. Effects of feed and water deprivation and transportation on rumen kinetics of cattle

Ruminant animals obtain their protein and energy from the fermentation of substrates in the rumen. The fermentative activity and capacity of the rumen, as measured by *in vitro* gas production, is reported to have the greatest impact on dry matter intake in post-feed and water deprived cattle (Cole and Hutcheson, 1985b). Therefore, increasing rumen fermentative capacity after feed and water deprivation would assist water and feed fasted cattle to increase their appetite in the post deprivation period, and provide a source of readily available protein and energy for the animal. DMI returned to normal after the reintroduction of feed and water, while in other studies, several days were required for DMI to return to normal (Cole and Hutcheson, 1985a, Bond et al., 1975). However, other studies reported that there was a consistent reduction in dry matter intake as a result of water restriction (Parker et al., 2003a, Wilson, 1970, Parker, 2004). Although the main factor causing these discrepancies is not clear, the quantity of feed or type of diet fed before fasting could be responsible. Few substrates, however, have been tested to determine if fermentative activity and capacity can be improved to shorten the duration of the recovery period after long-term feed and water deprivation associated with long transportation periods.

Although, several attempts also have been made to reduce the amount of carcass weight loss and improve meat colour through glucose and electrolyte treatments (Schaefer et al., 1990), this weight advantage may be contributed to an increased water proportion due to an increase of

isotonic fluid intake. Also the effectiveness of oral glucose administration to ruminant animals is still in doubt due to rumen degradation.

The rumen microorganisms respond strongly to the hormonal changes in respose to stress (Freestone and Lyte, 2010). It is speculated that multiple stressors during transportation of cattle could enhance enteropathogen growth and lead to dissemination onto meat (Freestone and Lyte, 2010). As previously mentioned (section 2.2), acute stress has stimulated changes in the colonic paracellular permeability of mast cells, overproduction of interferon-γ, morphology of the colonic epithelium (Demaude et al., 2006), and the micro flora diversity (Lyte et al., 2011). A previous study using sheep exposed to three days fasting, Tamate *et al.* (1974) reported a significant changes in the ultrastructure of the proximal region of the basal cell cytoplasm of rumen epithelium. This change was believed to be led by bidirectional interactions between the nervous system and the micro flora (Rhee et al., 2009, Konturek et al., 2011).

Prolonged feed and water deprivation and transportation of cattle decreased the saliva flow, increased the rumen pH, decreased the number of rumen microorganism and decreased the production and absorption of VFA in the rumen (Fluharty et al., 1996, Gabel et al., 1993, Galyean et al., 1981, Silanikove and Tadmor, 1989). A lower VFA absorption has also been found in beef cattle exposed to short term feed restriction (Zhang et al., 2013). This deprivation strongly influenced the rumen pH (Silanikove, 1989, Thorlasius and Lodge, 1973), significantly increasing from 6.73 to 8.00 during fasting (Galyean et al., 1981). The alkaline condition might be contributed to by the rumen digesta flow in and out, VFA production, absorption and the transportation mechanism in the rumen. Elevated rumen pH values have been significantly reduce VFA absorption (Thorlasius and Lodge, 1973).

2.4.1. Volatile fatty acids (VFA)

In ruminants, VFA or short-chain fatty acids (SCFA) contribute 60 to 80% of the metabolizable energy available (Bergman, 1990). The VFA and their dissociated anions are

produced in several parts of the gastrointestinal tract through microbial activity (Bergman, 1990).

The VFAs consist of formic, acetic, propionic, butyric, isobutyric, valeric, isovaleric, 2-methylbutyric, hexanoic and heptanoic acid (Bergman, 1990). The predominant forms of VFA are acetic, propionic and butyric acids, which are produced from the fermentation of cellulose, fiber, starches and sugar (Bergman, 1990). Ruminant animals produce the greatest amounts of VFA of any animals. In ruminants, the predominant VFA is acetic acid or acetate which is present in higher concentrations than all other organic anions combined (Bergman, 1990). In general, concentration ratios of acetate to propionate to butyrate are reported to highly vary (Table 2.1), and the differences depend on the composition of diet and the time after feeding.

Table 2.1. Concentrations of Volatile Fatty Acids in rumen

Diet	Species	Total VFA (mmol/L)	Molar proportions of VFA (%)		
			Acetate	Propionate	Butyrate
Hay	Sheep	106	69	20	11
Grain	Sheep	76	53	34	13
Grass	Cattle	148	70	19	11
Grain	Cattle	122	46	42	12

Adapted from: Bergman (1990), Wheaton *et al.* (1970), Bergman *et al* (1965), Balch and Rowland (1957), and Phillipson (1952).

Different proportions of individual VFA production may also be found when fiber is fermented rather than readily fermented carbohydrate (Bergman, 1990, Barcroft et al., 1943). This difference, however, is more likely due to different rumen environments created by different rates and extent of digestion (Caple et al., 2007, Perry and Cecava, 1995). A higher proportion of roughage in the diet produces a rumen environment that favours acetic acid producing microorganisms (Bergman, 1990). On the other hand, a high concentrate diet tends to provide a conducive environment for propionic acid producing micro-organisms (Bergman, 1990).

Therefore, the acetic-propionic acid ratio will be higher in fiber-rich rather than a concentrate diet. This different proportion of individual VFA may explain different efficiencies of energy use between high fiber and concentrate diets; however, this may only be true when such energy is to be retained as body weight gain.

In terms of feed and water deprivation and transportion of cattle, the reduced feed intake seems the major cause of ruminal and physiological changes (Cole, 1991, Cole and Hutcheson, 1988, Fluharty et al., 1996, Parker et al., 2003b, Cole and Hutcheson, 1981). Further, fasting adversely affects rumen fermentation (Cole and Hutcheson, 1981; Fluharty *et al.*, 1996). It has been reported that rumen substrates and rumen VFA production and absorption significantly decreased after being exposed to feed and water restriction or deprivation (Fluharty et al., 1996, Zhang et al., 2013, Galyean et al., 1981).

During deprivation where the VFA absorption rate is low, the absorption rate of acetate however, would have been more rapid than absorption of other VFAs (see Figure 2.2). This absorption rate appears to be inverse while the acidic condition where the order of rumen VFA absorption is proportional to chain length (Barcroft et al., 1943, Cole and Hutcheson, 1981, Thorlasius and Lodge, 1973). In addition, acetate is a metabolic precursor of acetyl CoA, which would be metabolised via tricarboxylic acid cycle for generating CO_2, H_2O and ATP (Guyton and Hall, 2001). This might be an opportunity to manipulate the "dead rumen" during and after fasting periods, to speed up the return to pre-fast condition by providing acetic acid sources into the rumen. However, acetate transportation is influenced by the presence of other fatty acids. The acetate appears to be rapidly absorbed with the presence of propionate and also is produced by conversion from butyrate (Stevens and Stettler, 1966). Conversely, the presence of acetate and butyrate is required for a greater transportation of propionate (Stevens and Stettler, 1966, Preston and Leng, 1987). Therefore, an effort to manipulate the rumen for minimising stress might need to consider the presence of the fatty acids.
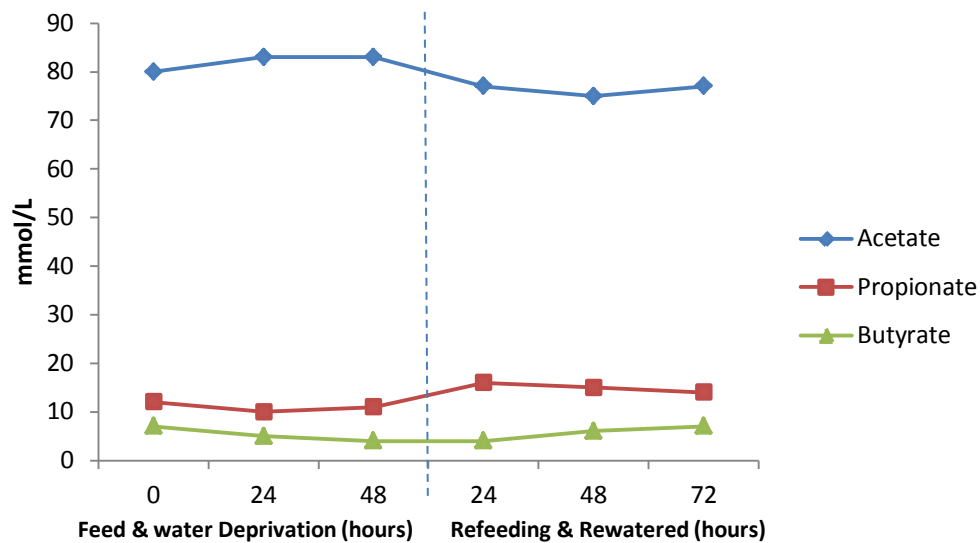
Figure 2.2. Rumen molar proportions of acetate, propionate, and butyrate in steers deprived of feed and water. Adapted from: Cole and Hutcheson (1981).

In practical applications, transferring rumen fluid from a healthy donor animal to a stressed animal is advocated in the dairy industry to improve dry matter intake and milk yield of the stressed cow (Rager et al. 2004). Rumen transfaunation has been recommended for cows with acidosis-related rumen stasis and also after surgical correction of left sided displacement of abomasums (Rager et al., 2004). The rumen transfaunation procedure may enhance energy production with VFA contained in the rumen fluid; therefore, shortening the recovery time (Rager et al., 2004). Previous authors have also reported a successful acetate supplementation to enhance the rate of muscle glycogen repletion on stressed horses and rats (Waller et al., 2009, Fushimi and Sato, 2005, Fushimi et al., 2001). In addition, acetate is a predominant VFA in the rumen fluid, while the presence of other fatty acids could enhance the absorption and utilization of acetate (Stevens and Stettler, 1966, Preston and Leng, 1987).

2.4.2. Rumen micro-organisms

Rumen micro-organisms have an important role in feed digestion in the rumen producing VFA, CO_2, CH_4, NH_3 and ATP. In general, normal rumen contains $10^{9 \text{ to } 10}$ bacteria per millilitre of rumen content (McDonald et al., 2002). More than two hundred species of rumen bacteria has been identified but not their entire role has been well known. The population of each bacterium varies between species depending on the diets (McDonald et al., 2002). Polysaccaride fibre diets promote a high total count of gram-positive cocci such as *Ruminococcus albus* and *Ruminococcus flavefaciens* and gram-negative rods such as *Bacteroides succinogenes* (Demeyer, 1981). Additionally, concentrate diets encourage the proliferation of lactobacilli (McDonald et al., 2002).

Table 2.2. Rumen bacteria with their *in vitro* energy source and fermentation products

Species	Energy sources	Ferementation products (excluding gases)						Alternative energy sources
		Acetic	Propionic	Butyric	Lactic	Succinic	Formic	
Fibrobacter succinogenes	Cellulose	+				+	+	Glucose, starch
Ruminococus Flavefaciens	Cellulose	+			+	+	+	Xylan
Ruminococus Albus	Cellobiose	+					+	Xylan
Streptococus bovis	Starch				+			Glucose
Prevotella ruminicola	Glucose	+				+	+	Xylan, starch
Megasphaera elsdenii	Lactate	+	+	+				Glucose, glycerol
Lachnospira multipara	Pectins	+				+		Glucose, fructose

Source: McDonald *et al.* (2002)

Normal rumen contains 10^6 protozoa per millilitre of rumen content and more than a hundred species of rumen protozoa has been identified (McDonald et al., 2002). Protozoa in the rumen

are predominantly ciliates while flagellates are less numerous. The ciliates belong to two families:

1) Isotrichidae (holotrichs) which include the genera *Isotricha* and *Dasytricha*, 2)

Ophryoscolecidae (oligotrichs) which include the genera *Entodinium, Diplodinium, Epidinium,* and

Ophryoscolex (McDonald et al., 2002). Further, Ozutsumi *et al.* (2005) reported that the rumen

bacteria diversity is affected by the presence and absence of protozoa. They reported that in the

absence of protozoa, *Ruminococcus albus, Ruminococcus flavefaciens, Prevotella ruminicola*, and

the CUR-E cluster were the major group in the rumen, while in the presence of protozoa,

Fibrobacter succinogenes was the majority (Ozutsumi et al., 2006). The number of 16S rRNAgene

copies of F. succinogenes was 8.1 6.4 (copies/mL) wich was higher in the rumen having protozoa

than in the rumen without protozoa (Ozutsumi et al., 2006). Ciliate protozoa as a predator for

bacteria can digest large quantities of bacteria in the rumen as a source of nitrogen and nucleic

acid (Coleman, 1979, Jouany et al., 1988). However, if the ciliate protozoa is absent, the numbers

of rumen bacteria is always greater (Mathieu et al., 1996, Hegarty, 1999, Hegarty et al., 1994,

Jouany et al., 1988, Coleman, 1979).

Another rumen microorganism is fungi. More than 12 species has been identified, with

most belonging to the genera *Neocallimastix* (McDonald et al., 2002). Most polysaccharide and

soluble sugars can be utilized by the rumen fungi, with the exception of pectin, polygalacturonic

acid, arabinose, fucose, mannose and galactose (McDonald et al., 2002). However, there is lack of

data about fungi in relation to stressors of feed and water deprivation and transportation

exposure on cattle.

Stressors of feed and water deprivation and transportation strongly influence the rumen

microorganisms of the animals; total counts of both rumen protozoa and bacteria have been

reported to significantly decline in cattle deprived for 32h (Galyean et al., 1981), but it was found

the population gradually returned to pre-fast levels by more than four days (Galyean et al., 1981).

In contrast, Fluharty *et al.* (1996) reported that the total number of rumen bacteria per millilitre

of rumen content did not significantly change after feed and water deprivation for 48h to 72h.

Fluharty *et al.* (1996) argued that the rumen bacteria were able to rapidly regrow to pre-fast levels soon after re-feeding. A number of factors may have contributed to the contrasting reports regarding the effects of feed and water deprivation on rumen bacterial populations, including the age of the cattle, their genotype, the time off feed and water, and dietary regimen (Galyean et al., 1981). However, the different sampling and enumeration procedures between the reported studies are notable. Studies utilising the most probable number method to measure the bacterial populations have been associated with large variances from the mean bacterial counts and hence, a lack of significance in results (Fluharty *et al.* 1996).   Whereas studies involving actual counts of bacteria (Galyean et al., 1981) demonstrate a difference between treatments for feed and water deprived or control animals. Therefore, the consideration of an appropriate method should be given in rumen kinetics studies, in particular when looking at rumen bacteria numbers.

2.4.3.   Rumen fermentative capacity and *in vitro* gas production

Rumen fermentative capacity (RFC) usually is determined by measuring *in vitro* GP, which reflects feed conversion into VFA and gases (Cole and Hutcheson, 1985b). RFC was determined by measuring *in vitro* gas production (Cole and Hutcheson, 1985b). Many researchers have been using *in vitro* GP to evaluate feedstuffs (Theodorou et al., 1994, Tagliapietra et al., 2011). For instance, *in vitro* GP can be used to detect the variations in efficiency of microbial production (Menke et al., 1979) using a associated measure of true substrate degradability (TSD) (Goering and Van Soest, 1970, Blummel and Lebzien, 2001). The difference between degradability measurement and gas volume measurement is assumed to be microbial biomass.

Therefore, some *in vitro* techniques measure GP with low cost and accuracy. The Hohenheim technique uses an equation based on the amount of gas produced in syringes to estimated feed metabolizable energy content (ME_{Menke}) after 24 hour of incubation (GP24) using some chemical components (Menke and Steingass, 1988, Tagliapietra et al., 2011). Another technique is the UC Davis approach, which uses a different equation but is still based on GP24

(Robinson et al., 2004). Another technique is a semi-automated system described by Theodorou *et al.* (1994) and Mauricio *et al.* (1999). The latest technique is an automated GP system such as product of ANKOM, RFS Gas Production (Pell and Schofield, 1993, Cone et al., 1996, Tagliapietra et al., 2010).

During a long duration transport to market, cattle may encounter short or long periods of feed and water deprivation. This prolonged deprivation leads to negatively influencing the normal rumen kinetics of the animals. Cole and Hutcheson (1981), by measuring *in vitro* GP, reported that rumen fermentative capacity (RFC) was still below pre-deprived levels at 168 hour after two sequential short periods of feed and water deprivation (Cole and Hutcheson, 1985b). Also, they reported that RFC seemed to have the greatest impact on dry matter intake in post-feed and water deprived cattle for 14 days following a period of feed and water deprivation (Cole and Hutcheson, 1985a, Cole and Hutcheson, 1985b). RFC is actually the reflection of microorganism activity in the rumen (Nyak and Yusdja, 2007). Getachew *et al.* (2004) reported that *in vitro* GP is correlated with VFA production, non-fiber carbohydrate (NFC), and also with crude protein (CP) of a feed. Therefore, increasing rumen fermentative capacity after feed and water deprivation would assist the cattle to increase their appetite in the post deprivation period, and provide a source of readily available protein and energy for the animal.

2.5. Effects of feed and water deprivation and transportation on muscle glycogen concentration of cattle

Glucose is the main energy source for an animal. In ruminant animals, glucose would be generally produced from VFA as the main substrate (Perry and Cecava, 1995, Rennie and Holloszy, 1977). In ruminants, only a small amount of glucose is absorbed from the gastrointestinal tract, even on a high carbohydrate diet, so these animals normally rely on gluconeogenesis (McVeigh and Tarrant, 1982). Gluconeogenesis is the process to form glucose in moderate quantities from

amino acids and the glycerol proportion of fats when the store of carbohydrates is decreasing in the body (Guyton and Hall, 2001). This process is extremely important in preventing an excessive glucose reduction in the blood due to cellular inactive during fasting period (Guyton and Hall, 2001). Therefore, low glucose availability can lower the glycogen repletion rate in cattle muscle (McVeigh and Tarrant, 1982, McVeigh et al., 1982). Glycogen is a key role for the homeostatic control of blood glucose and energy balance of animals (Guyton and Hall, 2001).

During exercise or deprivation and the handling-transportation period, in the muscle metabolism, animals rely less on carbohydrate and more on fat utilization via operation of the glucose-fatty acid cycle (Randle et al., 1964, Bergman, 1990). In ruminants, metabolism of fats and lipids mainly occurs in adipose tissue rather than in the liver (Cook and Miller, 1965, Knowles et al., 1974, Bergman, 1990). An increasing beta-oxidation due to exercise results in an increase in intramitochondrial citrate (Coggan et al., 1993), some of which escapes into the cytosol and can be a potent inhibitor of phosphofructokinase (PFK) (Parmeggiani and Bowman, 1963). The inhibiting of PFK leads to the accumulation of fructose 6-phosphate (F-6-P) as well as glucose 6-phosephate (G-6-P). This mechanism has been shown to result in muscle glycogen sparing during treadmill running in rats by elevating plasma free fatty acid (Hickson et al., 1977, Rennie et al., 1976).

Glycogen repletion of muscles is influenced by the ME value of the total diet (Tudor *et al*. 1996; Pethick *et al*. 1999). Limited energy supply might cause glycogenolysis in the muscle of the animal to fulfil the energy requirement during long distance transportation and fasting periods. Gardner *et al.* (2001) found that the rate of muscle glycogen replition were different in some muscles. They reported that muscle *M. semimembranosis* was more responsive to repletion compared with the *M. semitendinosis* after steers were exercised (Gardner *et al.* 2001). In the study of Gardner *et al.* (2001) the glycogen concentration of the *M. semitendinosis* remained at a depleted level for 72 hours despite a dietary ME concentration of 11.3 MJ/kg DM. Other study reported that fasting for three days has been demonstrated to decrease the rate of repletion of

muscle glycogen in the *M. Longissimus dorsi* of beef heifers (McVeigh and Tarrant 1982).

However, the loss of glycogen would be replenished at a greater rate through giving acetate

supplementation (Waller et al., 2009). Fushimi, et al., (2001) reported that feeding a diet

containing acetic acid after fasting, enhanced glycogen repletion in the liver and skeletal muscles

of rats. Moreover, a rapid muscle glycogen resynthesis during 4 hours of acetate supplementation

post exercise has been demonstrated on the gluteus medius muscle of horses (Waller et al.,

2009). Acetate is a metabolic precursor for acetyl-CoA, which is metabolized to CO_2 and H_2O in

the tricarboxylic acid cycle, producing ATP within mitochondria (Waller et al., 2009). The acetate

is immediately absorbed once it is consumed and then occurs in liver and peripheral tissues

(Cummings et al., 1987, Pomare et al., 1985). The feed containing acetic acid might stimulate

glycogenesis by increasing the influx of glucose-6-phosphate into the glycogen synthesis pathway

through high activities of acetyl-CoA synthesis (Fushimi et al., 2001). The accumulation of acetyl-

CoA from acetate uptake, glycolysis or β-oxidation combines with oxaloacetate to form citrate

(Waller et al., 2009). Excess Ac-CoA with carnitine is then catalysed by carnitine acetyltransferase

to form Acetyl-Carn (Waller et al., 2009). The formation of Acetyl-Carn maintains the

mitochondrial CoA pool (Waller et al., 2009). The Acetyl-Carn also provide a store of available

'active acetate' for Acetyl-CoA into the citric acid cycle (Harris et al., 1987). In addition, pyruvate

dehydrogenase is decreased during periods of increased Acetyl-CoA accumulation secondary to

increased oxidation of fat fuels (Putman et al., 1993). Therefore, inhibitions of PFK and pyruvate

dehydrogenase may directly inhibit glycolysis, thereby preserving glycogen concentration.

Glycogen in the muscle also plays a pivotal role in post-mortem anaerobic reactions (Wulf

et al. 2002). After absorption into a cell, glucose can be used immediately for release of energy to

the cell, or it can be stored in the form of glycogen, which is a large polymer of glucose (Guyton

and Hall, 2001). All cells of the body are capable of storing at least some glycogen, but certain

cells can store large amounts, especially liver cells, which can store up to 5 to 8% of their weight

as glycogen, and muscle cells, which can store up to 1 to 3% glycogen (Guyton and Hall, 2001).

This substrate is crucial for producing lactic acid during post-mortem metabolism, which results in lowering the pH of muscle (Wulf *et al.* 2002). Thus, an insufficient concentration of muscle glycogen can lead to an inadequate concentration of lactic acid development, and may result in dark cutter (DFD) meat with an ultimate post-mortem pH > 6.0 (Kivikari, 1996, Immonen et al., 2000).

The pH often adversely affects flavour, colour and microbial acceptability (Warriss, 1990, Purchas et al., 1999). This deficiency in muscle glycogen is promoted by physiological stress and physical activities including feed and water deprivation (McVeigh et al., 1982, McVeigh and Tarrant, 1982). A research project has reported that 57 mmol glycogen per kg beef muscle was sufficient to lower the pH from 7.1 to 5.5 (McVeigh and Tarrant, 1982). Another research study reported that 40 to 45 mmol glycogen per kg was actually sufficient to lower the pH of beef from 7.2 to 5.5 (Kivikari, 1996, Immonen et al., 2000). After exercise or physical activities, a high degree of glycogen concentration also is depleted (Aguera et al., 2001).

2.6. Summary

This review suggests an evaluation of the rumen kinetics of Brahman cattle (*Bos indicus*) exposed to extreme feed and water deprivation to obtain adequate data for improving the optimal post long duration transportation recovery. It is further suggested that there is a potential application with acetate supplementation to improve the optimal recovery. However, little is known about the usage of rumen fluid administration as source of VFA especially acetate. We hypothesized that rumen transfaunation containing readily absorbed Volatile Fatty Acid (VFA) may improve rumen kinetics, especially VFA absorption, thereby leading to increased feed intake, body weight and muscle glycogen concentration preservation on cattle exposed to feed and water deprivation during long duration transportation.

CHAPTER THREE

THE INFLUENCE OF INCUBATION MEDIA
ON THE FERMENTATIVE END PRODUCTS OF *IN VITRO* RUMEN FLUID CULTURES

ABSTRACT: The current experiment was conducted to compare the Goering-Van Soest and

Kansas State incubation media as commonly used in rumen *in vitro* studies to evaluate feedstuffs.

Incubation was carried out using a commercial wireless GP apparatus (Ankom[RF] Gas Production

System, Macedon, NY, USA) consisting of 19 bottles (250 mL Schott bottles) equipped with

pressure module sensors and a wireless receiver and computer was used. Treatments were

assigned into two different media groups: Kansas State media versus Goering-Van Soest media

including eight replicates and two controls for each treatment. Rhodes grass (*Chloris gayana*) hay

was used as substrates. Approximately 1 L of rumen content (pH = 6.8) was collected from two

fistulated *Bos indicus* steers fed chaffed Rhodes grass hay approximately 3 hours after morning

feeding. The rumen contents were strained and used as the inoculant for the *in vitro* study. The

in vitro incubation was undertaken for 72 hour. The Goering-Van Soest media allowed greater

gas production than the Kansas State media at 24, 48 and 72 hours incubation (P = 0.001). The

Goering-Van Soest media maintained inoculant pH at 6.80, whereas the Kansas State media

resulted in a decrease (P = 0.001) in pH from 6.80 to 6.39 over the incubation period. Similar

trends were shown in total VFA, acetic, butyric acids concentration (P = 0.004; P = 0.001; P = 0.03,

respectively) with the exception of propionic, iso-butyric, iso-valeric and valeric acids. The

Goering-Van Soest media demonstrated a greater buffering capacity for *in vitro* fermentation

studies of Rhodes grass hay as demonstrated by constant pH, higher GP and VFA concentrations.

Therefore, when undertaking or comparing *in vitro* studies using cellulolytic substrates

consideration needs to be given to the incubation media used.

3.1. Introduction

The incubation media is a factor that may influence the GP concentrations of *in vitro* rumen fermentation and degradability of feedstuffs (Mould et al., 2005). Although *in vitro* GP is associated with feedstuff degradation (Mould et al. 2005). Gas production alone does not provide direct information on either the extent of degradation or the quantity of fermentative end products. Some authors have questioned the use of complex buffer solutions such as the Goering Van Soest media, suggesting a simplified *in vitro* media would improve safety and reduce cost without negatively impacting on analytical precision (Mould et al., 2005, Rymer et al., 2005). The current experiment was conducted to compare the Goering-Van Soest buffer and the Kansas State buffer as incubation media commonly used in rumen *in vitro* studies to evaluate the fermentation of feedstuffs (Goering and Van Soest, 1970, Marten and Barnes, 1979). It was hypothesised that the Phosphate : Carbonate : Nitrogen proportion and requirement for microminerals in incubation media would have an effect on fermentative end products. The objective of this experiment was to determine which incubation media to be used in experiment two (chapter 4).

3.2. Materials and Methods

3.2.1. Experimental management

The incubation of rumen fluid and media was carried out using a commercial wireless GP apparatus (Ankom^{RF} Gas Production System, Macedon, NY, USA) consisting of 19 bottles (250 mL Schott bottles) equipped with pressure module sensors (pressure range: from −69 to +3447 kPa; resolution: 0.27 kPa; accuracy: ± 0.1% of measured value) a wireless receiver and a computer. The two media treatments (Kansas State media and Goering-Van Soest media) were allocated to bottles at random. There were eight replicated bottles and two control bottles for each treatment. The control bottles contained no hay substrate.

3.2.2.In vitro incubation

The Kansas state media was prepared without urea according to the method of Marten and Barnes (1979). The Goering Van Soest media was prepared according to the method of Goering and Van Soest (1970). The chemical composition of the Kansas state media and the Goering Van Soest media are described in Table 3.1.

Table 3.1. Chemical composition (g/L) of the Kansas State media and the Goering Van Soest media used in the *In vitro* incubation of rumen fluid with Rhodes grass hay.

Elements	Incubation Media	
	Kansas State	Goering-Van Soest
PO_4^{3-}	6.84	2.73
CO_3^{2-}	1.67	9.66
Na	1.51	4.05
K	2.82	0.61
CL	0.35	0.07
Ca	0.03	0.01
S	0.09	0.11
SO_4	0.19	0.08
Mg	0.05	0.02
N	-	0.27
[*]Mn	-	4.78
[*]Co	-	0.43
[*]Fe	-	2.85
[*]Resaruzine	-	0.69
PO_4^{3-} : CO_3^{2-} ratio	4.11	0.28
N:S ratio	-	2.38

SO_4 concentration are included in the S concentration 0.19
[*]mg/L

Approximately 1 L of rumen contents was collected from two fistulated *Bos indicus* steers fed chaffed Rhodes grass (*Chloris gayana*) hay *ad lib* (Table 3.2) approximately 3 hours after the morning feeding. The rumen contents were collected from the dorsal and ventral sac of the rumen of each steer according to the method of (Bueno et al., 2005), strained through a 2.5 mm sieve and pooled. The contents were transferred to sterile thermal flasks preheated to 39 ± 1 ^{o}C with approximately 10 g rumen digesta included in each flask. The rumen fluid was taken immediately to the laboratory to conduct *in vitro* studies.

Table 3.2. The chemical composition (based on %DM) of Rhodes grass (*Chloris gayana*) hay fed to *Bos indicus* steers and used as substrate for rumen fluid *in vitro* incubation

Dry matter (%)	86.0
Ash (%)	6.0
Organic matter (%)	80.1
Crude protein (%)[*]	12.0
NDF[**] (%)	69.6
ADF[**] (%)	38.6

The analysis is on a bulked sample collected throughout the experimental period

[*]Nitrogen content was determined by Kjeldahl analysis (AOAC, 1994).

[*]NDF and ADF were determined by the procedures of Goering and Van Soest (1970) as modified by Mertens *et al.* (2002)

Gas production was determined by measuring the *in vitro* GP of 25 mL of strained rumen fluid with 1 g dry matter of Rhodes grass hay in each 250 mL bottle; Rhode grass hay was ground to 1 mm in length prior to incubation (Rymer et al., 2005, Tagliapietra et al., 2010, Theodorou et al., 1994, Menke and Steingass, 1988). All operations were conducted under anaerobic conditions by flushing with nitrogen. Each bottle was pre-heated overnight at 39°C with the Rhodes grass hay substrate. On the day of incubation, each vessel was filled with 25 mL of homogenised rumen fluid and 105 mL of incubation media, keeping the headspace of the bottles continuously flushed with nitrogen. The vessels were then closed and placed in an oscillating incubator (Orbital Mixer Incubator, Ratek) at 39 ± 1°C. During incubation, gases in the headspace of the bottles were automatically released by opening a valve when a threshold pressure variation of ± 3.4 kPa was reached. The cumulative gas pressure was automatically recorded every 15 minute over the 72 hour incubation period. The gas pressure measured during the study was converted to moles of gas produced using the 'Ideal' gas law, and then converted to millilitres (mL) of gas produced using Avogadro's law.

Sub-samples of rumen fluid were collected from each bottle prior to and after 72 hours of incubation and stored at -20°C prior to analysis. 1 mL of metaphosphoric acid was added to each 4 mL sub-sample of rumen fluid prior to VFA analysis (Cottyn and Boucque, 1968). Acetic,

propionic, iso-butyric, butyrate, iso-valeric, and valeric acids and Total VFA were determined by Gas Chromatography (Cottyn and Boucque, 1968).

3.2.3.Statistical Analysis

Fixed effect in variance analysis was performed. The statistical model was treatment : time and the interaction between treatment * time. The student's t-test also were undertaken to compare means between treatments on specific times and VFA. S-Plus software version 8.0.4 (Insightful Corp., Seattle, USA) was used.

3.3.   Results

In vitro gas production of Goering-Van Soest and Kansas State incubation media over 72 hours are presented in Figure 3.1. The Goering-Van Soest media allowed greater gas production than the Kansas State media at 24, 48 and 72 hours of incubation (P = 0.001).   Total gas production for the Goering-Van Soest media was greater (P = 0.001; Table 3.3) than the total gas production for the Kansas State media. Cumulative gas production demonstrated day-related time effects by increasing in concentration with time (P = 0.001). There was no difference in internal bottle temperature between bottles.

Gas Production (mL/250mL)

Figure 3.1.Cumulative *In vitro* gas production (mean ± SEM) from rumen fluid incubated with

Kansas State media or the Goering-Van Soest media including the blanks for 72 hours using Rhode

grass (*Chloris gayana*) hay as substrate.

After 72 hours of incubation the pH of the Goering-Van Soest treatment was greater than

the Kansas State media (P = 0.001; 6.80 and 6.39 respectively).

Table. 3.3. *In vitro* total gas production, rumen fluid pH and incubation media pH after incubation
with the Kansas State media or Goering-Van Soest media for 72 hours using Rhode grass (*Chloris
gayana*) hay as substrate.

Variables	Treatments		P-value
	Kansas State Buffer	Goering-Van Soest Buffer	
Total gas production[*]	69.04 ± 1.18 [a]	98.05 ± 1.49 [b]	0.001
Medium pH after incubation	6.39 ± 0.01 [a]	6.80 ± 0.01 [b]	0.001

[*]Gas production data (mL/250mL) = GP of treatment – GP of control blanks
[a,b] means (± SEM) for individual GP and pH in same row with different superscripts differ (P<0.05)

The total concentration of VFA was higher (P = 0.004) in rumen fluid that was incubated

in the Goering-Van Soest media compared with the Kansas state media.   Acetic and butyric acids

concentrations were greater (P = 0.001; P = 0.03, respectively) in rumen fluid that was incubated

in the Goering-Van Soest treatment compared with the Kansas State treatment (Table 3.4). Propionic, iso-butyric, iso-valeric and valeric acids were similar between treatments.

Table 3.4. *In vitro* volatile fatty acid concentrations[1] (mean ± SEM) from rumen fluid incubated with the Kansas State media or the Goering-Van Soest media for 72 hours using Rhode grass (*Chloris gayana*) hay as substrate.

Variables	Treatments				[a]P - value
	Kansas Stated Media	Kansas Stated Blank	Goering-Van Soest Media	Goering-Van Soest Blank	
Total VFA (mmol/L)	51.8 ± 0.5[a]	16.36	54.2 ± 0.6[b]	15.21	0.004
Acetic (mmol/L)	35.1 ± 0.4[a]	11.36	37.6 ± 0.4[b]	11.22	0.001
Propionic (mmol/L)	11.0 ± 0.1	2.60	11.0 ± 0.1	2.37	0.75
Iso-butyric (mmol/L)	0.6 ± 0.0	0.32	0.6 ± 0.0	0.22	0.18
Butyric (mmol/L)	3.9 ± 0.0[a]	1.48	3.8 ± 0.0[b]	1.10	0.03
Iso-valeric (mmol/L)	0.7 ± 0.0	0.45	0.7 ± 0.0	0.31	0.15
Valeric (mmol/L)	0.4 ± 0.0	0.15	0.4 ± 0.0	0.00	0.48

[a]Student t-test comparison between Kansas State media and Goering-Van Soest media

3.4. Discussion

The Goering-Van Soest media demonstrated a greater buffering capacity for *in vitro* fermentation studies of Rhodes grass hay as demonstrated by constant pH, greater gas production and greater total VFA concentration compared to the Kansas state media. The pH was significantly different between treatments after incubation. The difference in pH may affect the rumen microorganism population during the incubation. The decline in pH may have affected the rumen microorganism population in the Kansas State media. Therefore, a lower gas production had been produced in Kansas State media. The GP curve for both treatments demonstrated a similar trend. However, the Goering-Van Soest media demonstrated a greater total GP after a 72 hours incubation period compared to the Kansas state media. This is important because cumulative *In vitro* GP is associated with feedstuff degradation by rumen microorganism activity (Mould et al. 2005).

In vitro rumen fluid VFA concentrations especially total VFA, acetic and butyric acid in the present study followed the same trend as gas production. Energy-yielding substrates universally limit the rate of growth of rumen microorganisms in open ecosystems (Hungate, 1984). The

VFA's acetic, propionic and butyric acids are the major waste products of fermentation from energy-yielding substrates (Hungate, 1984). In the present study, the Goering-Van Soest media caused greater concentrations of volatile fatty acids, with an exception of propionic acid, after 72 hours of incubation than the Kansas state media.

Differences in chemical composition of the incubation media (Table 3.4) appeared to influence the buffering capacity between media treatments. The Kansas State media predominantly contained phosphate and potassium. In comparison, the Goering-Van Soest media contained carbonate and sodium. The differences in composition of phosphate, carbonate and nitrogen affected buffering capacity consequently affecting microbial activities, volatile fatty acid and gas production (Carroll and Hungate, 1955, Mould et al., 2005). Hungate (1947) presumed that bicarbonate might be a more natural component than phosphate in an incubation medium because the salt is predominantly found in saliva.

The Goering-Van Soest buffer also contained some micro minerals which did not exist in the Kansas State buffer such as manganese, cobalt and iron. However, it has been argued that small differences in media composition are unlikely to affect end-point degradation values (Mould et al., 2005). The current study demonstrated that incubation media can effect *in vitro* GP and total VFA concentrations. Therefore, the recommendation from Mould *et al.* (2005) to simplify *in vitro* incubation media by excluding the micro minerals should be reviewed. Several researchers suggested also that the micro minerals could increase cellulose digestion especially for fiber digestion of forage substrates (Grant and Mertens, 1992, Martinez and Church, 1970).

The present study demonstrates that some incubation media, containing differences in phosphate:carbonate ratio, nitrogen and microminerals, resulted in differences in *in vitro* fermentative end-products. Therefore, when undertaking or comparing *in vitro* studies using cellulolytic substrates consideration needs to be given to the incubation media used. The Goering-van Soest media is preferred to be used for the next experiment (chapter 4).

CHAPTER FOUR

FEED AND WATER DEPRIVATION HAS A NEGATIVE BUT TRANSIENT EFFECT ON THE RUMEN KINETICS OF *BOS INDICUS* STEERS

ABSTRACT: The effects on rumen kinetics after feed and water had been deprived for 72 hours were studied using four fistulated *Bos indicus* steers. The steers were assigned in a 2 x 4 cross-over design with two treatments: feed and water *ad libitum* (control) and no feed and water for 72 hours (deprived) with four steers per treatment over two time periods. Rumen fluid was collected at day -6, 0, 4 and 9. Feed and water deprivation occurred from day -3 to day 0. Feed and water deprivation decreased the numbers of cellulolytic bacteria (1.4 v 0.4 colony-forming unit;cfu x 10^6/mL; P = 0.001), live (23.7 v 0.8 x 10^9/mL; P = 0.001), dead (12.7 v 0.5 x 10^9/mL; P = 0.001) and total bacterial counts (36.4 v 1.4 x 10^9/mL; P = 0.001) at day 0, compared with the control treatment. However, the deprived group had greater numbers of cellulolytic bacteria (2.7 v 50.1 cfu x 10^6/mL; P = 0.001), live (18.3 v 42.2 x 10^9/mL; P = 0.001), dead (6. 5 v 19.1 x 10^9/mL; P = 0.001) and total bacterial counts (24.8 v 61.3 x 10^9/mL; P = 0.001) from rumen fluid on day 4, compared with the control treatment. The numbers of protozoa in rumen fluid from the deprived group were less than (551.2 v 2.4 x 10^3/mL; P = 0.001) the control group on day 0. However, the deprived treatment had fewer protozoa in rumen fluid than the control treatment on day 4 (P = 0.001) and day 9 (P = 0.001). The concentrations of acetic, butyric, propionic and total VFA in rumen fluid from steers deprived of feed and water were less than the concentrations found in the control steers on day 0 (P = 0.001). The concentrations of VFA from deprived steers were not different to the concentrations of the same VFAs from the control steers at day 4 and 9. There were no differences between control and treatment groups for *in vitro* gas production (GP) on day -6. The cumulative *in vitro* GP from the control steers was greater than the deprived steers (P = 0.001) on day 0. However, the *in vitro* GP was not different for the following incubations on day 4 and 9. These results indicate that feed and water deprivation would have a negative but transient effect on the rumen kinetics of *Bos indicus* steers.

4.1. Introduction

Bos indicus cattle in northern Australia may be transported over long distances to finishing operations and as a consequence are deprived of feed and water for extended periods of time (Parker *et al.* 2003). Managers of pasture and feedlot finishing operations that utilise *Bos indicus* cattle anecdotally report that when the animals are exposed to a periods of feed and water deprivation of up to 72 hours, the rumen becomes dysfunctional and is unable to ferment and digest feedstuffs. Furthermore, the alleged rumen dysfunction is believed to contribute to a reduction in feed intake and subsequent liveweight gain in the newly received cattle.

Depriving *Bos taurus* cattle of feed and water for more than 32 hours has been reported to compromise the digestion and fermentation of substrates in the rumen (Galyean *et al.*(1981). Extended intervals of low nutrient availability can influence the survival of microorganisms and the ability of these organisms to respond when adequate nutrients become available again (Yokoyama and Johnson 1988). In addition, adaptation to diets has been demonstrated to take several days or weeks (Yokoyama and Johnson 1988). The change in the rumen environment such as micro-organism population, VFA, GP for *Bos taurus* cattle receiving a total mixed ration after feed and water deprivation differs among authors (Galyean et al., 1981, Fluharty et al., 1996). Furthermore, there is a lack of data for the change in the rumen environment of *Bos indicus* cattle fed tropical grass diets and deprived of feed and water for an extended period of time. Our working hypothesis was that feed and water deprivation would have a negative but transient effect on rumen kinetics of *Bos indicus* steers. In particular, we hypothesised the rumen microorganism populations and other measures of functionality such as gas and VFA production would recover within 96 hours after feed and water deprivation for 72 hours.

4.2. Materials and Methods

4.2.1. Animals and Management

All experimental procedures were reviewed and approved by the animal ethics committee at James Cook University (Approval # A1728). Four fistulated *Bos indicus* steers (4 years-of-age, mean ± SEM LW; 609 $\pm$ 31 kg) were used in this experiment. Throughout the experiment, the animals were allocated to individual pens (4.28 m x 1.26 m) within a covered cattle housing facility. The animals were given chaffed Rhodes grass (*Chloris gayana)* hay at three times (1000, 1300, 1800 h) each day in approximately equal amounts to ensure *ad libitum* intakes. The chemical composition of Rhodes grass (*Chloris gayana*) hay: DM 86.0%, Ash 6.0%, OM 80.1%, CP 12%, NDF 69.6%, ADF 38.6%. The amount of hay given to individual animals was calculated from the previous days actual intake + 20% extra hay (w:w). Water was available to the animals *ad libitum* in 25 L containers*.* Hay refusals were recorded once daily at 0800 h. The animals were familiarized with handling and sampling procedures before the start of the experiment.

Dry matter of Rhodes grass hay was determined by drying samples in an oven at 100 °C for 24 hours. Samples were then placed in a dessicator and allowed to cool before weighing. Organic matter was determined by ashing dried samples at 600 °C in a muffle furnace for three hours. Crude protein was determined by the Kjeldahl method for nitrogen (AOAC 1994). Neutral detergent and acid detergent fibre were determined according to the method of Goering and VanSoest (1970) modified by Mertens (2002) using the Foss fibre-cap system (Foss, 2012).

4.2.2. Experimental design

The animals were assigned in a 2 x 4 cross-over design with two treatments: feed and water *ad libitum* (control) and no feed and water for 72 hours (deprived) with four replicates (steers) per treatment. The experiment consisted of two periods, with each period comprising 9 days adaptation and 12 days sampling in individual pens. Both treatments were included at each

period. At the end of the first period the animals were placed on Rhodes grass hay for 42 days

before starting the second experimental period. Incubations of rumen fluid *In vitro*, consisted of

three replicates per animal at each sampling time (day -6, 0, 4 and 9).
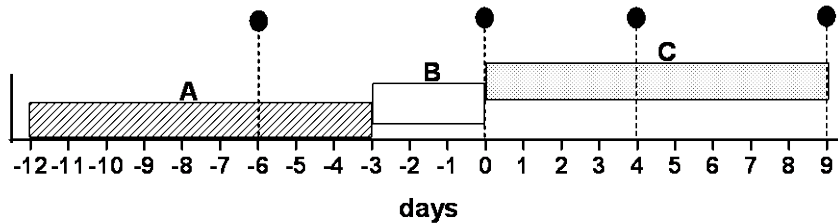
Figure 4.1. Schematic of the experimental design detailing: A. Adaptation period with feed and water available for 24h/day, B. Deprivation period that started from day -3 at 10.00am to day 0 at 10:00am, and C. Re-feeding period with feed and water available for 24h/day; ● Rumen fluid samples were collected at 10.00am after 2h of feed and water restriction in the morning.

4.2.3. Rumen fluid collection

On Days -6, 0, 4 and 9, approximately 1 L of rumen content was removed through the

fistula from each steer and treated as separate specimens. Rumen digesta collection was taken

from the dorsal and ventral sac of the rumen of each steer according to the method of Bueno et

al. (2005). The rumen contents were filtered through a 2.5 mm sieve, and the contents

transferred into cleaned thermal flasks preheated to $39 \pm 1\ ^{o}C$ with a sample of approximately 10

g rumen digesta. Thereafter, the solid rumen remnants were returned to the rumen of the animal

from which it was obtained through the fistula. The rumen contents were taken immediately to

the laboratory to conduct *in vitro* studies. At the same time each morning, the liveweights of all

animals were recorded on Days -6, 0, 4 and 9.

4.2.4. Measurement of microorganism numbers

Rumen fluid samples were homogenised using a Sunbeam 600 watt stick mixer under

anaerobic conditions by flushing the container with nitrogen gas. Fifty millilitres of homogenised

rumen fluid was separated to determine the numbers of viable cellulolytic, the live dead and total bacteria and numbers of protozoa per millilitre of whole rumen contents. The temperatures of the samples were maintained at 37 to 40 ^{o}C by placing the samples into a heated water bath.

Total viable, cultureable, cellulolytic bacteria was determined by the spread plate technique using rumen fluid-glucose-cellobiose agar (Holdeman *et al.*, (1977) in Ezaki *et al.*, (2006)). The media consisted of 0.25 g glucose, 0.25 g cellobiose, 0.5g soluble starch, 1.0 g (NH4)2SO4, 300 mL Rumen fluid, 0.5 g Cysteine-HCl·H2O, 4mL resazurin solution (25% resazurin), 500mL salts solution (0.2 g CaCl2, 0.2 g MgSO4, 1 g K2HPO4, 1 g KH2PO4, 10 g NaHCO3, 2 g NaCl and 1 L distilled water), 10 mL hemin solution (50 mg hemin, 1 mL 1 N NaOH and 100 mL distilled water), 20 g Agar, and 186 mL distilled water. The media were prepared 2 to 6 days before the incubations, while thioglycollate broths were prepared 1 day before the incubations. The temperature of the media and broths was maintained at 37 to 40 ^{o}C by placing them into the incubator before diluting and pouring samples. A 1 mL subsample was diluted in 9 mL thioglycollate broth and then the 10x dilutions were continued through a series of 900 µL tubes. Using the 10^{-4} through 10^{-6} of broth tubes, a volume of 100 µL was spread plated onto the agar to determine total viable cellulolytic bacterial populations. The bacterial plates were incubated for 48h in an anaerobic canister containing an anaerobic GasPak (GasPaK, Oxoid Limited, Hampshire, England) at 37 ± 1 ^{o}C. The process of diluting and pouring samples was completed in triplicate, taking less than 15 minutes for each sample.

The bacterial viability kit (L-7007 LIVE/DEAD® *Bac*Light^{TM}, Invitrogen Corp., Oregon, USA) was used to count total rumen bacteria, including live and dead bacteria. For enumeration of total rumen bacteria, 1 mL of the sample was diluted in 9 mL of 0.85% NaCl and then the 10x dilutions were continued through a series of 900 µL tubes. 3 µL of dye mixture were added to each of the 10^{-3} through 10^{-5} of the bacterial suspension tubes and then incubated at room temperature in the dark for at least 15 minutes. The bacteria were counted under a fluorescence microscope using a 100X objective lens.

For enumeration of rumen protozoa, 10 mL of homogenised rumen fluid was preserved by adding 10 mL of 50% formalin. A 1 mL aliquot of formalinized sample was taken into a 3 mL tube and then 200 µL of brilliant green dye was added. The contents were mixed and allowed to stand overnight at room temperature. The protozoa population was determined by following the procedure presented in Dehority (1984).

4.2.5. Gas Production measurement

Gas production (GP) was determined by measuring the *in vitro* GP of 25 mL of strained rumen fluid with 1 g DM of Rhodes grass hay ground through a 1 mm sieve as the substrate in a 250mL bottle (Rymer et al., 2005, Tagliapietra et al., 2010, Theodorou et al., 1994, Menke and Steingass, 1988). On the day of incubation, each vessel was filled with 25 mL of rumen fluid, 100 mL of mineral buffer solution and 5 mL of reducing solution (Goering and Van Soest, 1970). All operations were conducted under anaerobic conditions by flushing with nitrogen. A commercial wireless GP apparatus (Ankom Gas Production System, Macedon, NY, USA) consisting of 16 vessels equipped with pressure module sensors (pressure range: from −69 to +3447 kPa; resolution: 0.27 kPa; accuracy: ± 0.1% of measured value) and a wireless receiver and computer was used. The cumulative gas production was automatically recorded at 15 minute intervals over the 72 hour incubation period. The cumulative gas production measured during the study was converted to moles of gas produced using the 'Ideal' gas law, and then converted to mL of gas produced using Avogadro's law as described in Menke and Steingass (1988).

50mL and 4mL sub-samples of each rumen fluid sample were strained before and after incubation and placed in a suitably labelled polypropylene vial, then capped, stored and frozen at -20°C. 1 mL of metaphosphoric acid was added to each 4 mL sub-sample of rumen fluid prior to VFA analysis (Cottyn and Boucque, 1968). Acetic, propionic, iso-butyric, butyrate, iso-valeric, valeric and total VFA concentrations were determined by gas chromatography (Cottyn and Boucque, 1968).

4.2.6.Statistical Analysis

The data were analysed according to the mixed effects procedure of S-Plus (Insightful Corp., Seattle, USA).   Individual animals were assigned to the model as random effects and the treatments, periods, day and all combinations of interactions as the fixed effects. Bacteria numbers were transformed to the $\log_{10}$ for analysis. The data in tables represents the untransformed means. *In vitro* GP data were measured cumulatively, and as such separate analyses were undertaken to compare cumulative *in vitro* GP at three different incubation times: 24, 48 and 72. Significance was set at $P < 0.05$ with a 95% confidence interval. The student's t test was undertaken to compare means between treatments on specific days.

4.3.   Results

The effect of feed and water deprivation for 72 hours on the numbers of rumen cellulolytic bacteria in cattle is presented in Table 2. On day -6, the total plate counts for rumen cellulolytic bacteria (cfu x 10^6/mL) did not differ between the deprived and control groups (Table 4.1). The feed and water deprived group demonstrated a decrease ($P = 0.001$) in the number of cfu counted on plates at day 0 compared with the control treatment. The deprived group demonstrated greater cfu of cellulolytic bacteria than the controls on day 4 ($P = 0.001$). There were no differences between the deprived and control groups for celluloyltic total plate counts from rumen fluid at 9 days post-feed and water deprivation. There were no day-related time effects for cellulolytic rumen bacterial numbers.

The bacterial counts for the live, dead and total populations demonstrated a day effect ($P = 0.001$) and a treatment x day interaction ($P = 0.001$). The bacterial counts for the live, dead and total populations from rumen fluid were similar (Table 4.1) for the deprived and control groups on day -6. The live, dead and total bacterial counts for rumen fluid from the deprived group were less than the control group ($P = 0.001$) after 72 hours of feed and water deprivation on day 0.

However, the deprived group demonstrated greater numbers of live, dead and total bacterial counts from rumen fluid on day 4 compared with the control treatment. There were no differences in live, dead or total bacterial numbers between treatments on day 9.

Table 4.1. Total plate count, live, dead and total bacterial counts in rumen fluid from two groups of *Bos indicus* steers either deprived of feed and water for 72 hours (Deprived) or not (Control) at day -6, day 0, day 4 and at day 9 of the experimental period

Day	Treatment		P value
	Control	Deprived	(t-test)
Total plate count*			
-6	3.5 ± 1.0	4.1 ± 1.0	0.62
0	1.4 ± 0.1^a	0.4 ± 0.0^b	0.001
4	2.7 ± 0.5^a	50.1 ± 11.7^b	0.001
9	4.2 ± 1.2	2.6 ± 0.3	0.18
*Live bacteria***			
-6	23.1 ± 1.6	22.7 ± 3.2	0.91
0	23.7 ± 1.8^a	0.8 ± 0.0^b	0.001
4	18.3 ± 1.3^a	42.2 ± 2.4^b	0.001
9	22.9 ± 1.2	23.3 ± 1.3	0.82
*Dead bacteria***			
-6	9.5 ± 1.7	10.2 ± 1.2	0.76
0	12.7 ± 0.7^a	0.5 ± 0.02^b	0.001
4	6.5 ± 0.2^a	19.1 ± 0.2^b	0.001
9	9.2 ± 1.5	9.6 ± 1.9	0.86
*Total bacteria (live + dead)***			
-6	32.6 ± 1.5	32.9 ± 4.4	0.96
0	36.4 ± 1.1^a	1.4 ± 0.0^b	0.001
4	24.8 ± 1.3^a	61.3 ± 2.6^b	0.001
9	32.0 ± 2.5	32.9 ± 2.9	0.83

* mean ± SEM cfu x 10^6/mL; ** mean ± SEM x 10^9/mL
[a,b] Within a row means without a common superscript differ (P = 0.001)
Treatment effects for live, dead and total bacteria (P = 0.86, 0.57 and 0.69)
Day effect (P = 0.001)
Treatment : Day (P = 0.001)

On day -6, the numbers of protozoa did not differ between groups. The numbers of protozoa in rumen fluid from the deprived group were less than the control group (P = 0.001) after feed and water deprivation for 72 hours on day 0. There was an effect of day on the protozoal counts (P = 0.001) demonstrating a decrease in protozoa numbers for both treatments at day 4. However, a treatment x day interaction demonstrated that the deprived treatment had less protozoa numbers in rumen fluid than the control treatment on day 4 and day 9 (P = 0.001).

Table 4.2. Rumen protozoa counts in *Bos indicus* steers prior to (Day -6), immediately after (Day 0) and at four (Day 4) and nine days (Day 9) post feed and water deprivation for 72 hours (fasted group) or not (control group).

Day	Treatment		P value
	Control	Deprived	(t-test)
-6	666.1 ± 48.9	684.8 ± 53.5	0.80
0	551.2 ± 53.3[a]	2.4 ± 0.9[b]	0.001
4	355.1 ± 16.3[a]	121.0 ± 15.9[b]	0.001
9	770.3 ± 95.5[a]	273.2 ± 21.9[b]	0.001

[a,b] Within a row means (± SEM no. X 10^3/mL) without a common superscript differ (P = 0.001)
Treatment effect (P = 0.001)
Day effect (P = 0.001)
Treatment : Day (P = 0.001)

Seventy two hours of feed and water deprivation caused a treatment x day effect (P < 0.001; Table 4.5) for VFA concentrations in rumen fluid. The concentrations of acetic, butyric, propionic and total VFA in rumen fluid from steers deprived of feed and water were less than the concentrations found in the control steers on day 0 (P = 0.001). Iso-butyric and iso-valeric acids did not differ between treatments on day 0. On day -6, concentrations of acetic, propionic, iso-butyric, iso-valeric, valeric and total VFA in rumen fluid were similar for the deprived and control groups. Butyric acid concentrations in rumen fluid from the deprived group were greater than the butyric acid concentrations in rumen fluid from the control group on day -6 (P = 0.04).   The concentrations of acetic, butyric, propionic, iso-butyric, iso-valeric and total VFA in rumen fluid from steers deprived of feed and water were not different to the concentrations of the same

VFAs from the control steers at day 4 and 9 post feed and water deprivation. Day effects were found for all rumen fluid VFAs (P < 0.01).

A treatment x day interaction was demonstrated for *in vitro* VFA concentration (P = 0.001; Table 4.6), with the exception of iso-butyric.   On day -6, all *in vitro* VFA concentration was similar between the deprived and control treatments, except iso-butyric acid (P = 0.012).

Table 4.3. Volatile Fatty Acid (Mean ± SEM mmol/L) concentration and pH in rumen fluid from *Bos indicus* steers prior to (Day -6), immediately after (Day 0) and at Day 4 and Day 9 post-feed and water deprivation for 72 hours (deprived group) or not (control group)

Day	Treatment	Volatile Fatty Acid							pH
		Acetic (mmol/L)	Propionic (mmol/L)	Iso-butyric (mmol/L)	Butyric (mmol/L)	Iso-valeric (mmol/L)	Valeric (mmol/L)	Total (mmol/L)	
-6	Control	56.1 ± 2.0[a]	12.9 ± 0.4[a]	0.6 ± 0.1[a]	4.6 ± 0.1[a]	0.6 ± 0.0[a]	0.4 ± 0.0[a]	75.3 ± 2.6[a]	6.8[a]
	Deprived	56.4 ± 6.5[a]	11.5 ± 1.7[a]	0.6 ± 0.1[a]	5.2 ± 0.7[b]	0.7 ± 0.1[a]	0.5 ± 0.1[a]	74.9 ± 8.5[a]	6.7[a]
0	Control	56.0 ± 1.4[a]	13.1 ± 0.4[a]	0.5 ± 0.1[a]	4.4 ± 0.2[a]	0.7 ± 0.1[a]	0.4 ± 0.0[a]	75.1 ± 1.9[a]	6.8[a]
	Deprived	12.8 ± 2.2[b]	02.5 ± 0.6[b]	0.3 ± 0.1[a]	0.6 ± 0.1[b]	0.7 ± 0.1[a]	0.0 ± 0.0[b]	16.9 ± 3.1[b]	8.1[b]
4	Control	63.5 ± 1.6[a]	14.4 ± 0.3[a]	0.5 ± 0.1[a]	5.2 ± 0.2[a]	0.9 ± 0.0[a]	0.5 ± 0.0[a]	84.9 ± 2.2[a]	6.7[a]
	Deprived	61.1 ± 1.4[a]	15.7 ± 3.2[a]	0.4 ± 0.1[a]	4.3 ± 0.2[b]	0.9 ± 0.1[a]	0.4 ± 0.0[a]	82.7 ± 1.8[a]	6.6[a]
9	Control	62.8 ± 2.1[a]	14.3 ± 0.4[a]	0.7 ± 0.0[a]	5.3 ± 0.1[a]	0.9 ± 0.1[a]	0.6 ± 0.0[a]	84.6 ± 2.6[a]	6.6[a]
	Deprived	59.4 ± 1.2[a]	13.1 ± 0.2[b]	0.7 ± 0.0[b]	4.9 ± 0.2[a]	0.9 ± 0.1[a]	0.5 ± 0.0[a]	79.4 ± 1.5[a]	6.8[a]
Treatment effect		0.001	0.001	0.112	0.001	0.762	0.001	0.001	0.001
Day effect		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Treatment : Day		0.001	0.001	0.509	0.001	0.866	0.001	0.001	0.001

[a,b] means for individual VFA within a day without a common superscript differ ($P < 0.05$)

Table 4.4. *In vitro* volatile fatty acid (mean ± SEM mmol/L) concentration from rumen fluid taken from *Bos indicus* steers and incubated for 72 hours prior to (Day -6), immediately after (Day 0) and at Day 4 and Day 9 post feed and water deprivation for 72 hours (deprived group) or not (control group)

Day	Treatment	Acetic (mmol/L)	Propionic (mmol/L)	Iso-butyric (mmol/L)	Butyric (mmol/L)	Iso-valeric (mmol/L)	Valeric (mmol/L)	Total (mmol/L)
-6	Control	20.0 ± 0.9^a	7.8 ± 0.3^a	0.3 ± 0.02^a	1.8 ± 0.2^a	0.2 ± 0.0^a	0.3 ± 0.0^a	30.4 ± 1.3^a
	Deprived	22.0 ± 0.7^a	8.5 ± 0.1^a	0.2 ± 0.01^b	2.1 ± 0.1^a	0.1 ± 0.0^a	0.3 ± 0.0^a	33.2 ± 0.9^a
0	Control	25.0 ± 1.7^a	8.9 ± 0.7^a	0.2 ± 0.05^a	2.2 ± 0.3^a	0.3 ± 0.0^a	0.3 ± 0.0^a	36.9 ± 2.5^a
	Deprived	9.8 ± 1.1^b	3.5 ± 0.4^b	0.0 ± 0.0	0.7 ± 0.1^b	0.1 ± 0.0^b	0.0 ± 0.0^b	14.1 ± 1.5^b
4	Control	22.5 ± 0.5^a	8.3 ± 0.1^a	0.2 ± 0.1^a	2.1 ± 0.0^a	0.2 ± 0.0^a	0.4 ± 0.0^a	33.5 ± 0.7^a
	Deprived	21.1 ± 0.7^a	7.9 ± 0.3^a	0.2 ± 0.1^a	1.8 ± 0.1^b	0.2 ± 0.0^a	0.3 ± 0.0^a	31.8 ± 0.7^a
9	Control	24.2 ± 0.6^a	8.3 ± 0.1^a	0.3 ± 0.0^a	2.4 ± 0.1^a	0.3 ± 0.0^a	0.4 ± 0.0^a	35.8 ± 0.8^a
	Deprived	20.6 ± 1.8^a	7.1 ± 0.6^a	0.3 ± 0.0^a	1.8 ± 0.1^b	0.2 ± 0.0^b	0.3 ± 0.0^a	30.3 ± 2.6^a
Treatment effect		0.001	0.001	0.011	0.001	0.001	0.001	0.001
Day effect		0.001	0.001	0.001	0.001	0.002	0.001	0.001
Treatment : Day		0.001	0.001	0.053	0.001	0.001	0.001	0.001

[a,b] means for individual VFA within a day without a common superscript differ ($P < 0.05$)

The *in vitro* VFA concentration for rumen fluid taken from steers deprived of feed and water was less than the control rumen fluid on day 0 (P < 0.001).   The deprived and control group *in vitro* rumen fluid VFA concentration was similar on days four and nine. *In vitro* butyric acid concentration from the deprived steers was less than control steers on days four and nine. A day effect was demonstrated for rumen fluid VFAs.

There were no differences between control and treatment groups for *in vitro* gas production (GP) on day -6, (Figure 4.2A). After 72 hours of feed and water deprivation, (day 0; Figure 4.2 B), the cumulative *in vitro* GP from the control group was greater than the deprived group (P = 0.001). However, the *in vitro* GP was not different for the following incubations on day 4 and 9 (Figure 4.2 C and D respectively).
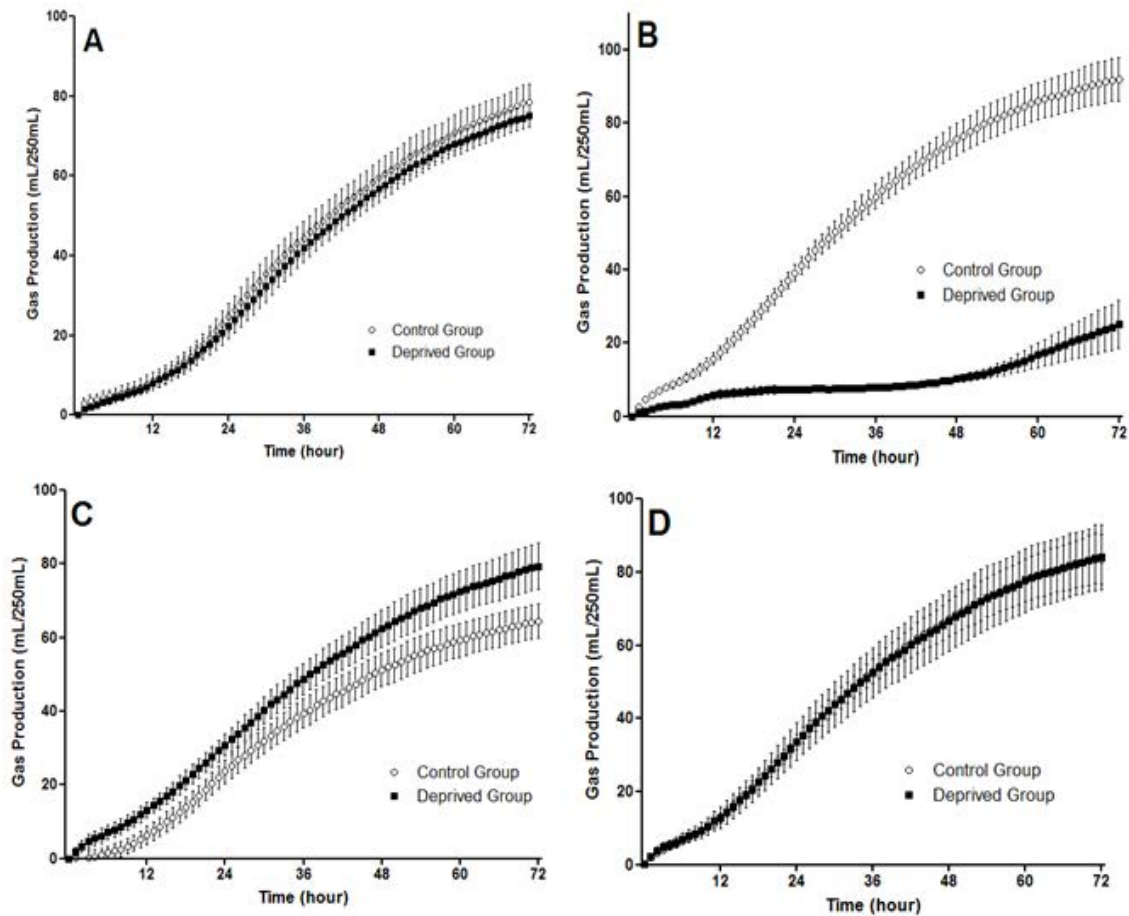
Figure 4.2. Cumulative *in vitro* gas production (mean ± SEM mL/250mL) from rumen fluid of two groups of *Bos indicus* steers (deprived of feed and water for 72 hours or control) at four times: A. prior to feed and water deprivation day -6 ; B immediately after feed and water deprivation: day 0 ; C: at four days after feed and water deprivation day 4 ; and D: at nine days after feed and water deprivation day 9.

During the pre-deprivation period, there were no differences between the deprived group and the control group for DMI. After 72 hours of feed and water deprivation, the intake of the deprived cattle was the same as the control group on the morning of day 1, 7.9 ± 0.4 kg and 7.5± 0.4 kg, respectively. However, the deprived group had less DMI (P=0.003) on day 2 compared to the control group and this contributed to the cumulative DMI of the deprived cattle being less than the control group for the remainder of the re-feeding period.

DM Intake (kg/head.day)

Cumulative DM Intake (kg/head)

Figure 4.3. Dry matter intake (DMI) and cumulative DMI (mean ± SEM kg DM/head/day) for two groups of *Bos indicus* steers after feed and water deprivation for 72 hours (■) or not (O).

4.4. Discussion

The greatest impact on rumen kinetics from feed and water deprivation in this study was the

decrease in bacteria and protozoa numbers at the end of the deprivation period.   Feed and water

deprivation for 72 hours decreased cellulolytic and total, live and dead bacteria in rumen fluid. A

number of factors may have contributed to the decrease seen in the microbial populations. Saliva flow

into the rumen is known to decrease with feed and water deprivation (Silanikove and Tadmor 1989).

However, the rumen fluid pH on Day 0 was 8.1 for the deprived cattle (P=0.001). In addition, it was observed that the rumen fluid from the deprived cattle was relatively clear and viscous compared to the control cattle. Furthermore, fluid outflow from the rumen is known to increase and digesta particle size decreases with extended feed and water deprivation (Silanikove and Tadmor 1989). This outflow effect and feed deprivation create a lack of substrate for the microorganisms and a reduced rumen motility ensues. It is likely that it is the sum of saliva inflow and digesta outflow that contributed to the decrease in bacterial and protozoa numbers in our experiment. Similar results have been demonstrated in younger feedlot cattle (Galyean et al., 1981). In contrast, Fluharty *et al.* (1996) failed to demonstrate a decrease in rumen bacterial numbers in cattle with increasing time off feed and water. A number of factors may have contributed to the contrasting reports regarding the effects of feed and water deprivation on rumen bacterial populations including the age of the cattle, their genotype, the time off feed and water and dietary regimen. However, the different sampling and enumeration procedures between the reported studies are notable. Studies utilising the most probable number method, following the procedure of Dehority et al. (1989), to measure the bacterial populations have been associated with large variances from the mean bacterial counts and hence a lack of significance in results (Fluharty *et al.* 1996). Whereas studies involving actual counts of bacteria (Galyean et al., 1981) demonstrate a difference between treatments for feed and water deprived or control animals.

The cellulolytic, total, live and dead bacterial counts showed an increase on day 4 after feed and water deprivation for 72 hours. Previous studies have demonstrated that the number of rumen bacteria are greater in the absence of rumen protozoa (Mathieu et al., 1996, Hegarty, 1999, Hegarty et al., 1994, Jouany et al., 1988, Coleman, 1979). The decrease in protozoa number at day 4 for the deprived group may partially explain the increase in bacterial numbers. However, we cannot explain why the increase in bacterial number at day 4 does not correspond with an expected increase in volatile fatty acid concentration in rumen fluid or gas production *In vitro* from the deprived group. Nonetheless, in agreement with other authors (Fluharty *et al.* 1996; Galyean *et al.* 1981), bacterial

numbers, VFA concentration and GP *in vitro* at day four would suggest recovery of rumen fluid functionality to pre-deprivation values.

A time effect for protozoa counts was demonstrated indicating a decrease in the protozoa counted for both groups with sampling time up to day 4. It is possible that our sampling regime introduced a bias into our study and therefore caution should be taken in interpreting the results. However, the treatment x time effects are consistent with other authors in that extended feed and water deprivation decreases total protozoa numbers in rumen fluid.   The recovery of protozoa numbers after feed and water deprivation appears to be dependent on the length of time that the feed and water deprivation was imposed.   Galyean *et al.* (1981) observed a faster recovery in protozoa populations from steers deprived for 32 hours than those that had been deprived of feed and water for 48 and 72 hours. In the present study, the rate of recovery for the protozoa population in the rumen of *Bos indicus* cattle deprived of feed and water for 72 hours showed that protozoa were increasing in numbers counted from day 4, but had not fully recovered by day 9.

In vivo and *in vitro* rumen fluid VFA concentrations in the present study followed the same trend as the bacterial populations. Energy-yielding substrates universally limit the rate of growth of bacteria in open ecosystems (Hungate 1984). The VFA's, acetic, propionic and butyric acids are the major waste products of fermentation from energy-yielding substrates in the rumen (Hungate 1984). Hence at day 0, where limited substrate was left in the rumen of the feed and water deprived steers, there was a decrease in the concentration of acetic, propionic, butyric and total acids. Likewise, as the bacterial populations increased at day 4, the VFA concentrations *in vivo* and *in vitro* were similar to pre-deprivation VFA concentrations. Feed deprivation alone has been demonstrated to negatively affect the rate of absorption of VFA from the rumen. The net absorption of acetate, propionate and butyrate was demonstrated to decrease by 56, 43 and 44% of control sheep given *ad libitum* access to feed (Gabel *et al*. 1993). A decreased absorption rate in combination with the low concentrations of VFA

after an acute reduction in feed and water intake in this study would have an impact on the nutrient status of an animal.

In the present study, acetic acid molar proportions were influenced by feed and water deprivation for 72 hours. However, others have demonstrated that acetic acid was not influenced by feed and water deprivation (Galyean et al., 1981, Cole and Hutcheson, 1985a). This could be due to the time off feed and water in the respective studies and or the diets fed before the deprivation.

In vitro GP in the present study followed the same trend as the bacterial populations.   Pre-deprivation GP was the same between treatment groups on day -6. After feed and water deprivation for 72 hours, incubation on day 0 showed that the deprived steers produced less *in vitro* GP compared with the control steers. Cole and Hutcheson, (1981, 1985b) incubated rumen fluid for 2 hours and reported that *in vitro* GP was less than pre-fast concentrations after five days of refeeding.   However, it is noteworthy that our data demonstrated no difference between treatments for *in vitro* GP at 2 hours into the incubation period on day 0. At 24 h onwards treatment differences were detected in cumulative GP using the Ankom[TM] apparatus in this experiment. As substrate availability was not limiting the *in vitro* system it appears that the quantity of bacteria and protozoa in the inoculum was having the greatest effect on rumen fluid functionality as measured by GP and VFA concentrations.   If the quantity of bacteria and other microorganism are a limiting factor in rumen function of feed and water deprived cattle then transfaunation of rumen contents from healthy cattle should correct rumen function immediately.

Feed and water deprivation has been reported to decrease the DMI of cattle, especially the day after the deprivation period (Cole and Hutcheson, 1985a). The depression in DMI often extends to the first 10 days after feed and water deprivation (Cole and Hutcheson, 1985a). Fluharty *et al.* (1996) stated that the DMI of calves deprived of feed and water for 48 or 72 hours was less than their control calves at day 1. In addition, Fluharty *et al.* (1996) demonstrated that, for the calves deprived for 72 hours, a depression in DMI persisted for 7 days. The cattle used in the present study demonstrated a

similar DMI to the control animals on day 1 after the 72 hour deprivation period. However, the deprived cattle subsequently decreased their DMI on the following day but continued to consume a similar DMI to the controls for the rest of the study. It is likely that the cattle in our study, when introduced to feed, consumed hay to pre-deprivation amounts for the first 24 hours but were limited in the subsequent two days dry matter intake due to a delayed rate of passage of digesta as microbial numbers increased and thereby assisted in the reduction in particle size of digesta leaving the rumen.

Seventy two hours of feed and water deprivation is an extreme event that may occur rarely in a managed production system in the dry tropics. However, *Bos indicus* genotypes are known for their tolerance of such conditions (Parker et al., 2004). In addition, *Bos indicus* cattle are the dominate cattle genotype in northern Australia and periods of feed and water deprivation do occur in Australian cattle production systems. The present study demonstrates that feed and water deprivation of 72 hours has a negative but transient effect on the rumen kinetics of *Bos indicus* steers. The greatest impact was the decrease in bacteria and protozoa numbers at the end of the deprivation period. However, the rumen microbiota populations and other measures of functionality such as VFA production and GP recover within 96 hours after feed and water deprivation of 72 hours in *Bos indicus* steers.

CHAPTER FIVE

RUMEN TRANSFAUNATION AFTER LONG DURATION TRANSPORTATION INCREASES DRY MATTER INTAKE BUT NOT MUSCULAR GLYCOGEN REPLETION IN *BOS INDICUS* BULLS

ABSTRACT: Twelve Bos indicus bulls were allocated to one of two treatment groups: rumen transfaunation (n = 6) given 10 kg of rumen fluid after transport or Control (n = 6), given 10 kg of deionised water after transport. Glycogen concentrations of the *M semimembranosus*, *M. semitendinosis* and *M. Longisimus dorsi* were measured before transport on day -7 and after transport on day 0, day 1, day 4 and day 9. Feed intake, liveweight gain, plasma metabolites and electrolytes were recorded on the sampling days. Rumen transfaunation increased the dry matter intake of treated bulls compared to the bulls treated with deionised water. Rumen transfaunation had no effect on glycogen concentrations of the *M. semimembranosus*, *M. semitendinosis* and *M. Longisimus dorsi* compared to the bulls treated with deionised water. The *M. semimembranosus* and the *M. semitendinosis* decreased in glycogen concentration immediately after transport on day 0 but repleted to pre–transportation concentrations within 1 day. However, the *M. Longisimus dorsi* demonstrated no change in glycogen concentration between the pre-transportation sample on day -7 and day 0 or day 1 after transportation. The *M. Longisimus dorsi* of the bulls increased in glycogen concentration between day 1 and day 4 after transportation. The bulls in this experiment demonstrated that muscle glycogen, as measured at the *M. longissimus dorsi,* cannot replete to sufficient concentrations (40 to 45 µmol of glycogen concentration per gram muscle) to ensure normal meat quality until between one and four days of rest when feeding Rhodes grass hay *ad libitum*.

5.1. Introduction

During long duration transportation, in general, cattle encounter short or long periods of feed and water deprivation and rapid changes in feeding. These changes lead to at least two negative effects on

appetite and body liveweight (Hutcheson and Cole, 1986). Various research regarding fasting and transported cattle using different kind of breeds and periods showed that the cattle could lose 3% to 17.3% of their initial liveweight   (Parker, 2004, Kirton et al., 1972, Self and Gay, 1972, Wythes et al., 1980, Smith et al., 1982, Earley et al., 2010, Leo-Penu et al., 2010). The combination of transportation and deprivation stressors leads the cattle to lose more weight compared to fasting alone. Phillips et al. (1985) reported that 68% of the liveweight loss was a result from the combined effects of fasting and transport within 46h. After physical activities, fasting or long transportation, a high degree of glycogen concentration in the muscle and liver also depletes (Aguera et al., 2001, McVeigh and Tarrant, 1982). In the liver, 5 to 8% of liver weight is glycogen and in the muscle it is up to 1 to 3% (Guyton and Hall, 2001). Glycogen is a key role for the homeostatic control of blood glucose and energy balance of animals. Glycogen insufficiency adversely affects flavour, colour and microbial acceptability in meat (Warriss, 1990, Purchas et al., 1999). However, few substrates have been tested to see if fermentative activity and glycogen concentration can be improved after long duration transportation and fasting period.

Some supplementation efforts with electrolyte solutions had been devoted to minimize the negative effects of transportation (Parker, 2004). The electrolyte supplementation during 18 days after long transportation of live export cattle resulted in a 2.9% liveweight advantages compared to control steers (Beatty et al., 2007). However, this weight advantage can be contributed by an increased water proportion due to an increase of isotonic fluid intake. Several attempts also have been made to improve the glucose supply to cattle during the marketing period (Hutcheson and Cole, 1986, Schaefer et al., 1990). Although, it was reported that glucose and electrolyte treatments appear to improve meat colour and reduce the amount of carcass loss by up to 3% (Schaefer et al., 1990), the effectiveness of oral glucose administration to ruminant animals is still questionable due to rumen degradation.

In fact, during exercise or deprivation and handling-transportation period, in the muscle metabolism, animals rely less on carbohydrate and more on fat utilization via operation of the glucose-fatty acid

cycle (Randle et al., 1964, Bergman, 1990). In ruminant, metabolism of fats and lipids mainly occur in adipose tissue rather than in liver (Cook and Miller, 1965, Knowles et al., 1974, Bergman, 1990). An increasing beta-oxidation due to exercise results in an increase in intramitochondrial citrate (Coggan et al., 1993), some of which escapes into the cytosol and be a potent inhibitor of phosphofructokinase (PFK) (Parmeggiani and Bowman, 1963). The inhibition of PFK leads to the accumulation of fructose 6-phosphate (F-6-P) as well as glucose 6-phosephate (G-6-P). The mechanism has been shown to result in muscle glycogen sparing during treadmill running in rats by elevating plasma free fatty acid (Hickson et al., 1977, Rennie et al., 1976). Meanwhile, oral acetic acid feeding is immediately absorbed and occur in adipose tissue (Bergman, 1990). Due to an increased citrate concentration and the inhibition of glycolysis in the muscle, this acetate might also increase the influx of G-6-P, through high activities of acetyl-CoA synthesis, into the glycogen pathway (Fushimi et al., 2001, Knowles et al., 1974). Thus, it is hypothesized that rumen transfaunation containing readily absorbed Volatile Fatty Acid (VFA) will preserve muscle glycogen stores in cattle exposed to feed and water deprivation during long duration transportation. In addition, rumen transfaunation might improve rumen kinetics by providing rumen microorganism for optimal recovery to normal rumen function.

5.2. Materials and Methods

5.2.1. Experimental Design

Twelve *Bos indicus* bulls of Brahman genotype (mean ± SEM liveweight; 482.00 ± 009.02 kg) were used in this study. A schematic of the experiment design is depicted in figure 5.1. The bulls grazed scenesed buffel grass (*Cenchrus ciliaris*) pasture at the Fletcherview research station, Charters Towers, Queensland Australia (20°04.603'S / 146° 15.802'E) before transportation. The bulls were allocated to one of two treatment groups: 1) rumen transfaunation (RT), offered water only for 12 hours then transported for 24 hours and given 10 kg rumen fluid immediately after transport (n=6) and 2) Control (C), offered water only for 12 hours then transported for 24 hours and given 10 kg of deionised water after transport (n=6). The bulls were also assigned at random to one of two periods of transportation.

At each transportation period six bulls were conveyed in a body truck with an eight tonne tare for approximately 1721 km. The second transportation period followed after the first 24 hour period. The truck stopped for 30 minutes every 5 hours due to re-fuelling and regulatory control of driver rest stops. The transportation period concluded at the School of veterinary and biomedical sciences, James Cook University, Townsville, Queensland, Australia. The bulls were unloaded from the truck where body weight and rectal temperature were recorded. In addition, muscle and blood samples were taken from the animals. Treatments were then delivered through an oral-gastric tube and pump to the respective bulls. Two fistulated *Bos indicus* steers that had been consuming Rhodes grass (*Chloris gayana*) hay were the donor animals for the RT group. The characteristics of the transferred rumen fluid are described in Table 5.1. All bulls were housed in individual pens and offered chaffed Rhodes grass (*Chloris gayana*) hay and water *ad libitum* (Table 5.2). Feed and water intake was recorded for ten days.

All experimental procedures have been reviewed and approved by the animal ethics committee at James Cook University (approval ID A1807).

Figure 5.1. Schematic of the experimental design detailing; A. Grazing on Buffel grass pasture, B. 12 h wet curfew in yards, C. 24 h transportation period, T. Treatment with rumen fluid or deionised water after sampling and D. repletion phase where bulls consumed Rhodes grass hay. Sampling of body weight, rectal temperature, muscle and blood are depicted with a solid circle (●).

Table 5.1. Characteristics of rumen fluid used in the rumen transfaunated *Bos indicus* bulls

pH	6.8
Total plate count (cfu x 10^5 /mL) of cellulolytic rumen bacteria[a]	5.10
Live bacteria (no. X 10^9/mL)[b]	27.6
Dead bacteria (no. X 10^9/mL) [b]	11.9
Total bacteria (live + dead) (no. X 10^9/mL) [b]	39.5
Protozoa (no. X 10^5/mL) [c]	6.63
Acetic acid (mmol/L)[d]	52.09
Propionic acid (mmol/L) [d]	12.31
Iso-butyric acid (mmol/L) [d]	0.67
Butyric acid (mmol/L) [d]	8.06
Iso-valeric acid (mmol/L) [d]	0.68
Valeric acid (mmol/L) [d]	1.06
Total VFA (mmol/L) [d]	74.87

[a] determined by the spread plate technique using rumen fluid-glucose-cellobiose agar (Holdeman *et al.*, (1977) in Ezaki *et al.*, (2006))
[b] determined by Bacterial Viability Kits L-7007 (LIVE/DEAD® *Bac*Light[TM], Invitrogen Corp., Oregon, USA).
[c] determined by following procedure in Dehority (1984).
[d] determined by Gas Chromatography (GC) (Cottyn and Boucque, 1968).

Table 5.2. The chemical composition of Rhodes grass (*Chloris gayana*) hay fed to *Bos indicus* bulls

DM (%)	86.0
Ash (%)	6.0
OM(%)	80.0
CP (%)	12.0
NDF (%)[a]	69.6
ADF (%)[a]	38.6

[a]NDF and ADF were determined by the procedures of Goering and Van Soest (1970) as modified by Mertens et al. (2002)

5.2.2. Sample Collections

Live weight, rectal temperature, muscle and blood samples were collected at day -7, 0, 1, 4 and 9. Feed and water intake were measured from day 1 to 9. Live weight was measured using digital scale. Blood samples were taken from the jugular vein at each sampling point using a 20 gauge needle and 10 mL Lithium heparin vacutainer (Beckton Dickson, Plymouth UK). The blood samples were immediately placed into an ice-water slurry after collection and then centrifuged at 3000 x g. The plasma was collected and frozen at -20° C for later analysis. Biopsy samples of *M. longissimus dorsi* (LD), *M. semimembranosis* (SM) and *M. semitendinosis* (ST) (± 300mg) were taken according to the method of Gardner *et al*. (2001). The biopsy site was alternated between the left and right hind limbs for the SM and ST muscles at each sampling. The biopsy site of the LD was also alternated between left and right sides at each sampling between the 12th and 13th rib. A local anaesthetic injection (2 mL of 2% Lignocaine) was used to anaesthetise the muscle before biopsy. Muscle biopsies were performed using a 12 V motorised biopsy drill with a simultaneous vacuum to hold the muscle sample within the biopsy needle (D. Pethick, Murdoch University, Western Australia). After removing all fat and connective tissue muscle samples were blotted with a gauze square to absorb any excess blood. Biopsy samples were then placed immediately into cryovials and submerged in liquid nitrogen. Muscle samples were later transferred to a -80°C freezer for storage until analysed for the determination of glucose and glycogen concentrations. After biopsy the wound was washed with a Betadine® solution and Chloromide® surface disinfectant was applied around the incision

5.2.3. Biochemical Analysis

Blood glucose, lactate, lactate dehygrogenase, creatinine kinase, creatine, phosphorous, potassium, sodium, albumin, carbon dioxide, total protein, calcium, potassium, magnesium, chlorine were measured using a Beckman Au 480 auto-analyser using Beckman spectrophotometric and enzymatic kits (Beckman Coulter, NSW, Australia).

Muscle glycogen, and free glucose was determined according to the method of Wulf *et al.* (2002). Frozen muscle samples were prepared on dry ice. Approximately 100 mg of muscle from each sample was transferred and homogenized in 0.5 mL of 0.6 N perchloric acid at a sample : perchloric acid ratio of 1:5 using a homogeniser (Polytron PT2100, Kinematica, Luzern, Switzerland) at maximum speed for 100 seconds. The sample was catalysed with amyloglucosidase (Sigma, catalog #10115) to glucose units. Free glucose (glucose-6-phosphate and glucose) was determined via NADP reduction using a glucose (HK) assay reagent (Sigma, catalog #G2020). The glucosyl units were spectrophotometrically measured by comparing the absorbance of homogenate sample to the absorption coefficient determined from a standard curve at 340 nm.

5.2.4. *Statistical Analysis*

General Linear Model with Univariate was performed including following factors: treatments, day, group and also the interactions between factors. Tukey test was undertaken in Post Hoc Tests to compare means between days. The student's t test was undertaken to compare body weight means between groups on specific days. SPSS version 22 (IBM Corp., US, 2013) was used for all statistical analysis.

5.3. Results

There were no treatment effects on glycogen (P=0.42, P=0.80 and P=0.40), free glucose (P=0.50, P=0.69 and P=0.89) and total glucosyl unit (P=0.45, P=0.80 and P=0.39) concentrations in the SM, ST, and LD respectively from the bulls subjected to the transportation regimen in this experiment (Figures 5.2, 5.3 and 5.4). There were day effects demonstrated for glycogen, free glucose and total glucosyl unit in any of the muscles sampled (P=0.001; P=0.009 and P=0.001).

Day effects were demonstrated for the glycogen concentration of the SM (Figure 5.2). The Day 0 glycogen concentration demonstrated a decrease from Day -7 (P=0.01). However, the glycogen concentration in the SM increased to pre-transportation concentrations at Day 1 after transport (P=0.006). The Day 4 glycogen concentrations were greater than Day -7 and Day 0 (P=0.01; P=0.004). The Day 9 glycogen concentrations did not differ to Day 4 (P=0.97). The total glucosyl unit has a similar trend to glycogen concentration. Glucose concentration in the SM was lower on Day 4 than Day -7, 0 and 1 (P=0.03; P=0.04; P=0.03). The Day 4 glucose concentrations did not differ to Day 9 (P=0.34).

(A)

Total glucosyl unit
(µmol/g wet tissue)

(B)

(C)

Glucose (µmol/g wet tissue)

Total glucosyl unit
(µmol/g wet tissue)
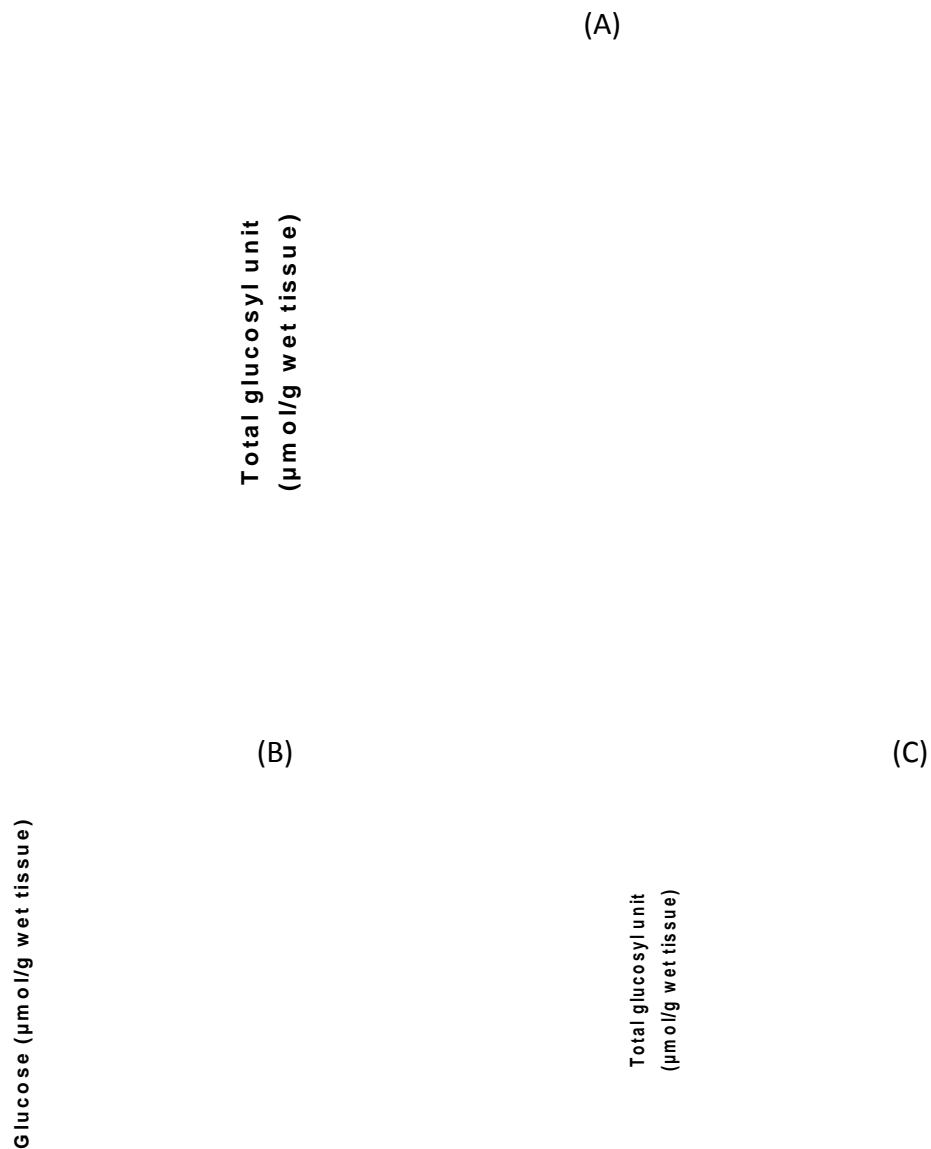
Figure 5.2. Glycogen (A); Free glucose (B); and Total glucosyl unit (C) concentrations (µmol/g wet tissue) of *M*. *semimembranosis* of *Bos indicus* bulls after a 12 hour wet curfew and 24 hours of transportation followed by treatments of rumen transfaunation (□) or deionised water (■).

Day effects were demonstrated for the glycogen concentration of the ST (Figure 5.3)*.* There were no day effects demonstrated for the muscle for Day -7, Day 0 and Day 1. The Day 0 glycogen concentrations in the ST did not differ to Day -7 and 1 (P=0.32; P=0.49). However, the Day 0 glycogen concentration in the ST was lower than Day 4 and 9 after transport (P=0.01; P=0.05).   The Day 9 glycogen concentrations were not different to Day 4 (P=0.91).   The total glucosyl unit has a similar

trend to glycogen concentration. Glucose concentration in the ST was lower on Day 4 and day 9 than

pre-transportation concentrations (P=0.01; P=0.02). The Day 4 glucose concentrations did not differ to

Day 9 (P=0.99).

(A)

Total glucosyl unit
(μmol/g wet tissue)

(B)   (C)

Glucose (μmol/g wet tissue)

Total glucosyl unit
(μmol/g wet tissue)
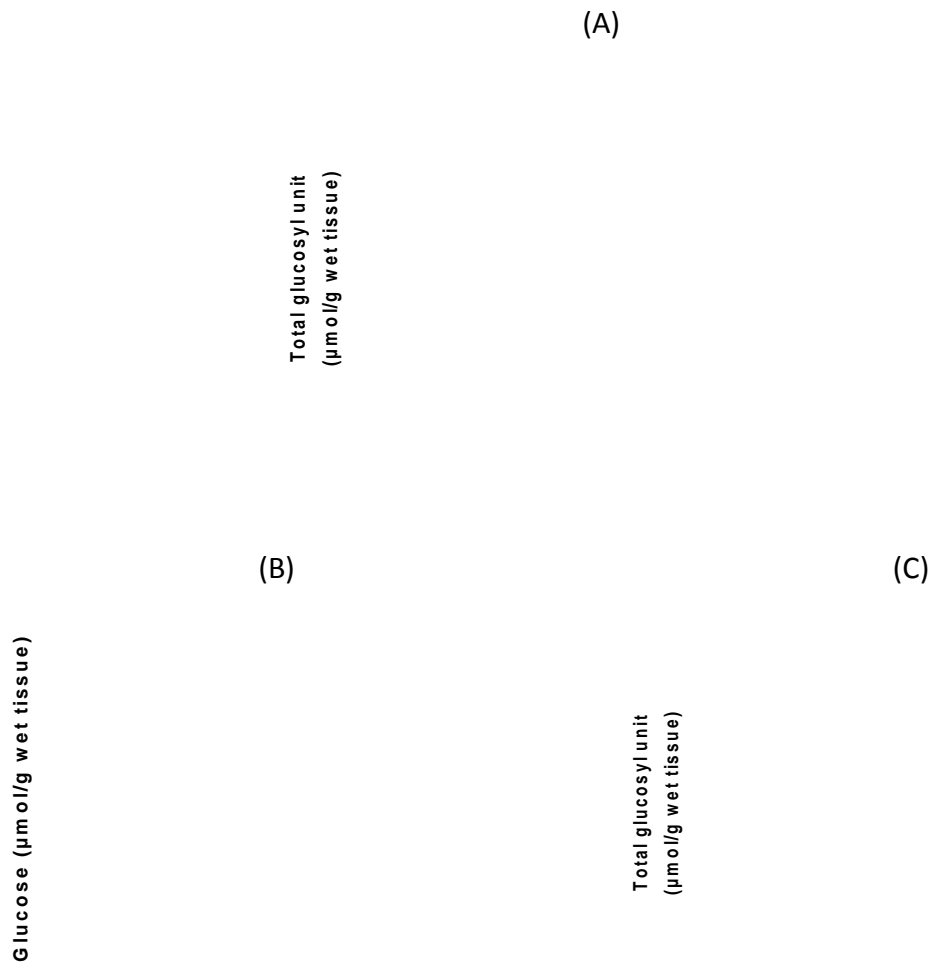
Figure 5.3. Glycogen (A); Free glucose (B); Total glucosyl unit (C) concentrations (μmol/g wet tissue) of
M. semitendinosis from *Bos indicus* bulls after a 12 hour wet curfew and 24 hours of transportation
followed by treatments of rumen transfaunation (□) or deionised water (■).

There were no day effects demonstrated for the LD (Figure 5.4) for Day -7, Day 0 and Day 1 (P=0.76 and

P=0.95). However, the glycogen concentration of the LD was greater for all bulls on Day 4 compared

with the glycogen concentrations at Day -7, Day 0 and Day 1 (P=0.03, P=0.005, and P=0.02).   In

addition, the glycogen concentration of the LD was greater for all bulls on Day 9 compared with the

glycogen concentrations at Day -7, Day 0 and Day 1 (P=0.004, P=0.001, and P=0.003). There were no day effects for the glycogen concentration of the LD between day 4 and Day 9.

(A)

Total glucocyl unit
(μmol/g wet tissue)

(B)

(C)

Glucose (μmol/g wet tissue)

Total glucocyl unit
(μmol/g wet tissue)
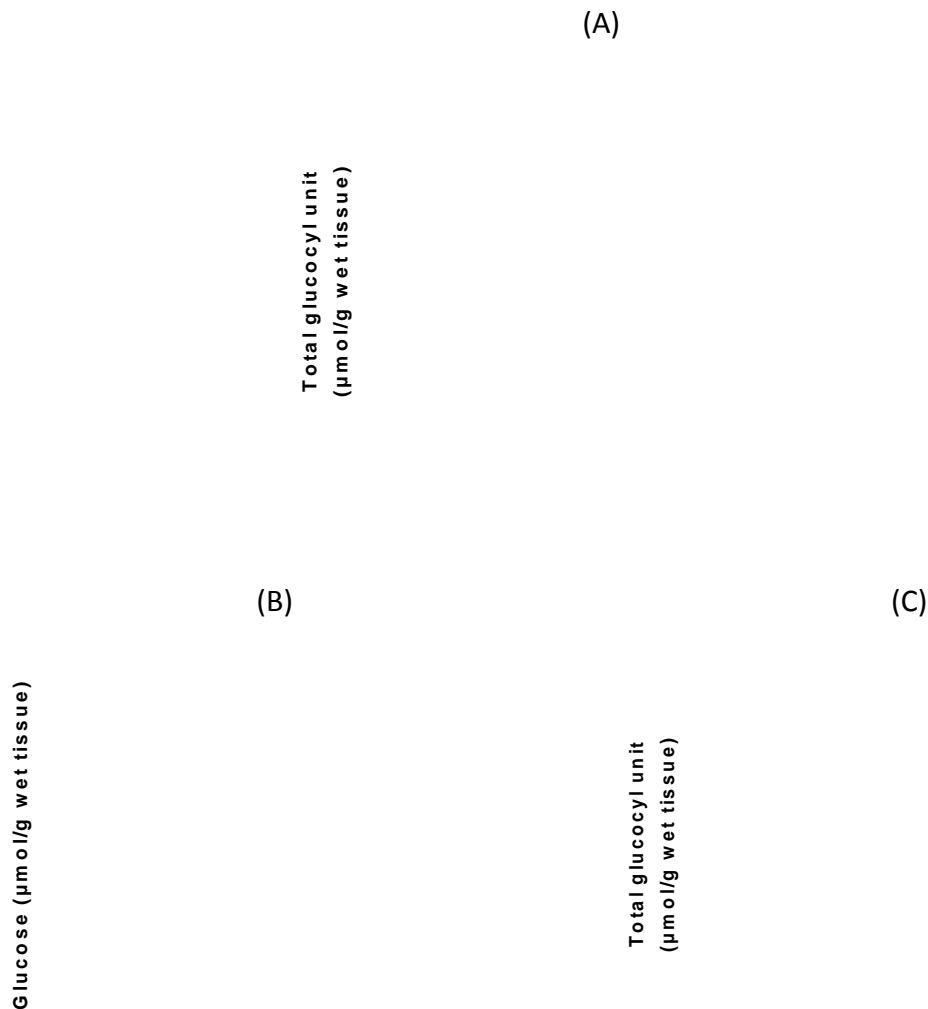
Figure 5.4. Glycogen (A); Free glucose (B); Total glucosyl unit (C) concentrations (μmol/g wet tissue) of *M. longissimus dorsi* of *Bos indicus* bulls after a 12 hour wet curfew and 24 hours of transportation followed by treatments of rumen transfaunation (□) or deionised water (■).

The RT treated bulls had a greater intake of Rhodes grass hay on day 2 compared to the control group and this contributed to the cumulative feed intake (P=0.002) of the control cattle being less than the transfaunated group for the remainder of the re-feeding period. There were no differences between the RT group and the C bulls for cumulative water intake (P=0.78).

Cumulative DM intake (kg/head)

Cumulative water intake (L/head)

Figure 5.5. Cumulative dry matter intake (mean ± SEM; kg as fed/head) and water (L/head) intake after a 12 hour wet curfew and 24 hours of transportation followed by treatments of rumen transfaunation (□) or deionised water (■).

A positive treatment effect was demonstrated for liveweight of the bulls (P=0.008). However student's test to compare both group on each day showed no difference between the C and RT bulls. There were day effects (P=0.001) demonstrated for body weight of the bulls indicating that the experimental regimen had an effect over all animals in the study. All animals lost weight during the wet curfew (Day -1) and during 24 hours of transport (Day 0). All animals subsequently gained weight on Day 1, Day 4 and Day 9. There were no treatment x day interactions for body weight (P=0.99). There were no treatment effects (P=0.37) between the RT group and the C group for rectal temperature.   However, there was a day effect demonstrated for rectal temperature (P=0.001). Rectal temperature decreased for both groups at Day -1.

Table 5.3. Liveweight and rectal temperature of *Bos indicus* bulls after a 12 hour wet curfew and 24 hours of transportation followed by treatments of rumen transfaunation (RT) or deionised water (C).

Day	Treatments	Liveweight*	Rectal Temperature**
-7	C	477.66 ± 9.84	39.25 ± 0.19
	RT	496.33 ± 8.20	39.03 ± 0.07
-1	C	455.83 ± 11.00	37.98 ± 0.28
	RT	472.50 ± 10.86	38.10 ± 0.19
0	C	441.66 ± 10.52	38.55 ± 0.08
	RT	455.00 ± 7.04	38.53 ± 0.13
1	C	455.33 ± 13.25	38.86 ± 0.29
	RT	478.33 ± 7.55	38.63 ± 0.10
4	C	485.66 ± 12.09	38.85 ± 0.15
	RT	495.50 ± 10.53	38.66 ± 0.08
9	C	493.33 ± 12.22	38.95 ± 0.26
	RT	513.00 ± 6.10	39.13 ± 0.13

* means ± SEM kg; ** means ± SEM °C

Treatment effect	0.008	0.37
Day effect	0.001	0.001
Treatment : Day effect	0.99	0.42

Plasma metabolites and electrolytes of *Bos indicus* bulls exposed to 24 hours of transportation are presented in Table 5.4 and Table 5.5 respectively. The RT groups had lower concentrations of plasma glucose (P=0.005) and lactate (P=0.001) compared to the C bulls. There were day effects demonstrated for all plasma metabolites. Plasma glucose, lactate, lactate dehydrogenase, creatine kinase , albumin, total protein and urea increased in concentration on Day 0 after transportation (P=0.001, P=0.01, P=0.005, P=0.001, P=0.01, P=0.01, P=0.002 and P=0.001 respectively). Plasma glucose and lactate concentrations returned to pre-transportation values on day 1. Day 4 and Day 9 plasma glucose concentrations were not different to Day 1 or Day -7.

Plasma lacatate dehydrogenase concentrations on Day 1 were not different from Day 0 concentrations. However, Day 4 and Day 9 were not different to Day -7. Creatine concentrations decreased on Day 0 compared to Day-7. Creatine concentrations on Day 1 were not different to Day 0 but remained lesser than Day -7. Creatine concentrations on Day 4 were less than Day -7 but not different to Day 0 or Day 1. There was no difference in creatine concentrations on Day 9 compared to Day -7. Creatinine kinase concentrations were greater at Day 1 compared to Day 0 and Day -7. Day 4 and day 9 demonstrated greater concentrations of CK than Day -7. Albumin, total protein and Urea demonstrated no differences at Day 1, Day 4 and Day 9 compared to day -7.

There were no differences between treatments for any of the electrolytes measured (Table 5.5). There were day effects demonstrated for P, Mg, K and Cl (P=0.001, P=0.001, P=0.001 and P=0.003). Plasma P, Mg and K concentrations decreased at Day 0 compared to Day -7. However, plasma concentrations of P, Mg and K were not different at Day 1, Day 4 and Day 9 compared to Day -7. Plasma Chloride concentrations increased at Day 0 compared to day -7.

Table 5.4. Plasma glucose, lactate, lactate dehydrogenase, creatine, creatinine kinase, albumin, CO_2, total protein and Urea (means ± SEM) in *Bos indicus* bulls subjected to transportation for 24 h followed by treatments of rumen transfaunation (RT) or deionised water (C).

Day	Treatments	Glucose (mmol/L)	Lactate (mmol/L)	Lactate Dehydrogenase (unit/L)	Creatine (μmol/L)	Creatine Kinase (unit/L)	Albumin (g/L)	CO2 (mmol/L)	Total Protein (g/L)	Urea (mmol/L)
day -7	C	4.4 ± 0.3	3.5 ± 0.8	789.8 ± 93.0	157.2 ± 9.9	68.7 ± 19.2	25.9 ± 0.7	25.0 ± 0.6	58.1 ± 2.4	4.9 ± 0.3
	RT	3.8 ± 0.2	2.3 ± 0.4	689.3 ± 93.6	160.2 ±12.0	77.9 ± 10.2	26.9 ± 0.8	24.0 ± 1.3	62.8 ± 1.9	5.1 ± 0.5
day 0	C	6.1 ± 0.3	4.9 ± 0.9	1102.0 ± 28.8	112.3 ± 6.5	335.7 ± 75.0	30.0 ± 1.0	20.8 ± 1.3	70.8 ± 2.7	5.1 ± 0.2
	RT	5.5 ± 0.3	2.6 ± 0.8	923.7 ± 64.2	114.3 ± 6.6	262.4 ± 72.9	29.5 ± 0.7	21.5 ± 0.7	71.1 ± 1.9	5.3 ± 0.3
day 1	C	4.4 ± 0.3	2.0 ± 0.4	982.2 ± 57.8	112.3 ± 9.3	553.6 ± 109.2	27.9 ± 1.0	19.6 ± 0.7	64.9 ± 2.6	4.2 ± 0.3
	RT	4.1 ± 0.3	1.4 ± 0.1	995.0 ± 71.7	116.5 ± 6.9	538.0 ± 107.5	27.4 ± 0.5	21.1 ± 1.1	66.9 ± 0.8	4.4 ± 0.2
day 4	C	4.5 ± 0.3	2.5 ± 0.6	957.3 ± 54.5	128.5 ± 6.0	130.9 ± 13.9	26.9 ± 0.5	22.2 ± 0.9	64.6 ± 2.1	4.2 ± 0.2
	RT	4.2 ± 0.4	1.9 ± 0.4	704.4 ±114.4	130.0 ±14.0	86.2 ± 12.2	26.6 ± 2.1	23.0 ± 1.1	64.6 ± 3.6	3.9 ± 0.2
day 9	C	4.4 ± 0.2	3.1 ± 0.9	740.6 ± 23.2	143.7 ± 3.4	169.0 ± 92.4	26.9 ± 0.8	21.4 ± 1.1	63.6 ± 2.2	4.7 ± 0.2
	RT	3.6 ± 0.2	1.4 ± 0.1	834.1 ±162.7	129.7 ± 7.6	169.0 ± 84.9	25.4 ± 1.5	19.2 ± 1.0	60.8 ± 3.7	4.7 ± 0.3
Treatment effect		0.005	0.001	0.11	0.91	0.56	0.61	0.75	0.60	0.79
Day effect		0.001	0.01	0.005	0.001	0.001	0.01	0.001	0.002	0.001
Period effect		0.14	0.001	0.01	0.29	0.16	0.62	0.02	0.95	0.04

Table 5.5. Plasma electrolytes (means ± SEM; mmol/L) in *Bos indicus* bulls subjected to 24 h transportation followed by treatments of rumen transfaunation (RT) or deionised water (C).

Day	Treatments	Phosphorous (mmol/L)	Calcium (mmol/L)	Magnesium (mmol/L)	Sodium (mmol/L)	Potasium (mmol/L)	Chloride (mmol/L)
day -7	C	1.8 ±0.2	1.9 ±0.0	0.9 ±0.0	121.2 ±2.6	4.0 ±0.2	83.0 ±1.6
	RT	1.9 ±0.1	2.0 ±0.1	0.9 ±0.0	123.3 ±2.6	4.0 ±0.1	86.3 ±2.3
day 0	C	1.5 ±0.1	2.1 ±0.0	0.7 ±0.01	131.3 ±1.5	3.6 ±0.1	96.5 ±1.4
	RT	1.4 ±0.1	2.0 ±0.0	0.7 ±0.02	130.5 ±1.9	3.5 ±0.1	97.3 ±1.7
day 1	C	1.7 ±0.1	1.9 ±0.0	0.7 ±0.02	123.7 ±3.5	3.9 ±0.1	93.0 ±2.7
	RT	1.8 ±0.1	1.9 ±0.1	0.7 ±0.02	125.5 ±3.7	4.0 ±0.2	93.3 ±2.7
day 4	C	2.0 ±0.1	2.0 ±0.0	0.7 ±0.01	128.4 ±2.0	4.02 ±0.1	93.7 ±1.1
	RT	1.8 ±0.2	2.0 ±0.1	0.6 ±0.05	123.6 ±8.2	3.9 ±0.3	91.2 ±5.7
day 9	C	2.0 ±0.1	2.0 ±0.0	0.7 ±0.02	129.0 ±1.6	4.2 ±0.2	94.8 ±0.6
	RT	1.9 ±0.1	1.9 ±0.1	0.6 ±0.03	117.9 ±4.9	4.0 ±0.3	88.2 ±3.9
Treatment effect		0.72	0.78	0.21	0.31	0.64	0.60
Day effect		0.001	0.57	0.001	0.22	0.01	0.003
Period effect		0.92	0.63	0.04	0.45	0.01	0.93

5.4. Discussion

The administration of rumen fluid to *Bos indicus* bulls after a 12 h wet curfew and 24 h of transportation had an advantage on hay intake. Consequently, the transfaunated animals were heavier during the refeeding period than non-transfaunated control bulls. Previous studies have demonstrated a higher feed intake after rumen transfaunation of dairy cows after surgical correction of left-sided displacement of the abomasum (Rager et al., 2004). However, rumen transfaunation failed to increase muscle glycogen concentrations in the *M. semitendinosis, M. semimembranosis and M. longissimus dorsi* of *Bos indicus* bulls subjected to 24 hours of transportation. There may be a number of reasons that explain the lack of effect on muscle glycogen repletion due to the RT treatment. It is possible that the five muscle biopsies and sampling protocol in this experiment created a stress on the bulls that hindered repletion rates of glycogen. However, sequential muscle biopsies have been demonstrated to have little impact on glycogen concentration when performed up to four times in steers (Gardner et al. 2001). Furthermore to eliminate psychological and physical stressors associated with re-grouping bulls care was taken to house and sample bulls individually. In addition the bulls remained docile throughout the experiment.

In general, glycogen repletion of muscles is influenced by the ME value of the total diet (Tudor *et al*. 1996; Pethick *et al*. 1999). The daily total intake of ME for the bulls in this study was insufficient to meet the maintenance requirements of all the bulls until six days after transportation and may have hindered the repletion rate of glycogen in the muscles sampled. The ME maintenance (ME_m) requirements for a 500 Kg bull is 47MJ/day (SCARM 1994). A 500 kg bull would need to have consumed 7 kg DM of Rhodes grass hay to meet the ME_m requirement. The intakes of hay for the first five days for the RT group were 2.3,

5.7, 6.1, 6.6, and 7.5 kg DM and 1.8, 3.6, 5.4, 6.6, and 6.3 kg DM for the C group. Both groups consumed the ME_m requirements or greater for a 500 kg bull after day 5. It is possible that due to the bulls dietary experience before transportation the efficiency of utilisation of net energy for maintenance from Rhodes grass hay was increased allowing for a small increase in glycogen at day 4.

Diet type and nutrient restriction is known to effect liver and muscle glycogen concentrations in cattle. The bulls in this experiment were grazing senesced Buffel grass (*Cenchus ciliaris*) pasture at the end of the dry season in northern Australia when dietary protein and energy deficiencies predominate in the rangelands. The pre-transport *M. longissimus dorsi* glycogen concentration for the RT and C bulls was low (36.38 ± 2.61 μmol/g wet tissue) compared with values reported in other studies. The mean *M. longissimus dorsi* glycogen concentration from unstressed *Bos taurus* bulls fed a barley based diet *ad libitum* has been reported to be 87μmol/g wet tissue (McVeigh and Tarrant 1983) to 94μmol/g wet tissue (McVeigh *et al.* 1982). Similarly, Crouse *et al.* (1984), feeding a corn-silage based total mixed ration, reported mean pre-fasting glycogen concentrations from the *M. longissimus dorsi* of *Bos taurus* bulls of 86 and 82μmol/g wet tissue for the control and fasted treatment groups respectively. In addition, the pre-transportation glycogen concentration of the *M. semimembranosis and M. semitendinosis* were also less in this study than other reported values in *Bos taurus* steers (Gardner *et al*. 2001, Knee *et al*. 2007). It is noteworthy that the glycogen concentrations of the *M. longissimus dorsi* and *M. semitendinosis* were not decreased by the transportation regimen and remained at the same values for 24 hours after transportation. However, the glycogen concentration of the *M. semimembranosis* was decreased by transportation but repleted within 24 hours to pre-transportation (day -7) values. Gardner *et al.* (2001) found that the

M. semimembranosis was more responsive to repletion compared with the *M. semitendinosis* after steers were exercised. In the study of Gardner *et al.* (2001) the glycogen concentration of the *M. semitendinosis* remained at a depleted level for 72 hours despite a dietary ME concentration of 11.3 MJ/kg DM. In the present study, the low quality diet and low muscle glycogen concentrations before transportation may have affected the depletion and repletion rates of muscle glycogen especially in the *M. longissimus dorsi.* Fasting for three days has been demonstrated to decrease the rate of repletion of muscle glycogen in the *M. Longissimus dorsi* of beef heifers (McVeigh and Tarrant 1982). The bulls in this experiment demonstrated that muscle glycogen, as measured at the *M. longissimus dorsi,* cannot recover to sufficient concentrations of 40 to 45 μmol of glycogen concentration per gram muscle (Immonen *et al.*, 2000) to ensure normal meat quality until between one and four days of rest when fed Rhodes grass hay *ad libitum.*

Plasma glucose and lactate demonstrated differences between treatments. The C bulls were consistently greater than the RT bulls for plasma glucose and lactate. However, both treatments (RT and C) were within normal physiological ranges for these metabolites. In light of the other metabolic and electrolyte variables where there was no treatment effect demonstrated, it would appear that RT provides no further advantage for these variables than treatment with water alone during the transportation regimen implemented in this experiment. The transportation regimen had an effect on all the bulls at day 0 for all metabolites sampled. Plasma albumin, total protein and urea were greater on day 0 than day -7, 1, 4 and 9. Transportation stress has been demonstrated to cause dehydration that may manifest itself as a hyperproteinemia in cattle (Parker *et al.* 2003). Lactate and Lactate dehydrogenase also increased on day 0. The constant standing and bracing during transportation is known to elevate lactate concentrations in the blood of cattle (Tarrant

1990). The electrolytes magnesium, potassium and phosphorous decreased and sodium
and chloride increased on day 0 in line with the known effects of stress on cattle (Parker *et
al.* 2003).

The present study demonstrates that rumen transfaunation after a wet curfew of
12 hours and 24 hours of transportation increases dry matter intake and live weight during
a ten day recovery period. There was no treatment effect demonstrated for RT on muscle
glycogen although there were time related effects on muscle glycogen concentrations
caused by the transportation regimen. Glycogen concentration of the *M. Longissimus dorsi*
and *M. semitendinosis* did not exceed pre-transportation concentrations until between one
and four days after transportation. However, the glycogen concentrations of the *M.
semimembranosus*, repleted to pre-transportation concentrations within 24 hours after
transportation. The bulls in this experiment demonstrated a mild dehydration immediately
after transportation by the recorded plasma metabolites and electrolytes. The current
practice of resting cattle for 24 hours prior to slaughter when subjected to long duration
transportation may not replete muscle glycogen concentrations adequately to prevent high
pH measurements at the *M. Longisimus dorsi.*

CHAPTER SIX
GENERAL DISCUSSION

Bos indicus cattle in northern Australia may be transported over long distances in order to reach markets or abattoirs. As a consequence the animals are deprived of feed and water for extended periods of time. The stressors of handling, novel environments, and mixing with unfamiliar animals challenge homeostasis in cattle. Managers of pasture and feedlot finishing operations that utilise *Bos indicus* cattle anecdotally report that when the animals are exposed to extended periods of feed and water deprivation of up to 72 hours, the rumen becomes dysfunctional and is unable to ferment and digest feedstuffs. The rumen dysfunction is believed to contribute to a lack of feed intake and subsequent body weight gain in finishing operations. Low nutrient avaliability will influence the survival of rumen microorganisms and the ability of these organisms to respond when adequate nutrients become available again. In addition, adaptation to diets by cattle can take up to 21 days depending upon the type and amount of dietary change (Yokohama and Johnson 1988). There is a lack of data on the change in the rumen environment from long duration deprivation of feed and water in *Bos indicus* cattle fed tropical grass diets.

A negative but transient effect on the rumen kinetics of *Bos indicus* steers exposed to feed and water deprivation for 72 hours has been demonstrated in chapter four. The greatest impact to deprived animals was the decrease in bacteria and protozoa numbers at the end of the deprivation period. However, the rumen microbiota populations and other measures of functionality such as volatile fatty acid production and gas production recover within 96 hours after the deprivation period. It was probable that a decrease in salivary flow into the rumen, a decreased particle size of the digesta and an increase in fluid and

digesta outflow created an environment unsuitable for rumen micro-organisms at the end

of 72 hours of feed and water deprivation. However, rumen bacteria numbers returned to

baseline counts by 96 hours after re-feeding the steers with Rhodes grass hay. Volatile

fatty acid concentrations in the rumen fluid followed a similar trend to the bacteria

populations in both in vivo and in vitro assays. On day 0 with limited substrate there was a

decrease in propionic, acetic, butyric and total acids. Four days after re-feeding the VFA

concentrations were similar to pre-deprivation concentrations in the deprived steers. The

absorption of VFA from the rumen has been found to decrease with feed deprivation

(Gabel *et al.* 1993). When cattle are challenged with periods of low nutrient availability

combined with a decreased absorption of VFA from the rumen the animal is in a negative

energy status. Furthermore the quantity of bacteria and other microbiota appear to be a

limiting factor to rumen function of feed and water deprived cattle. Therefore strategies

that increase and/or sustain bacterial numbers and VFA concentrations in the rumen during

or after feed and water deprivation are likely to have the greatest effects on rumen

function and hence the energy status of cattle.

A common effect of a negative energy status on cattle is a decrease in glycogen

concentrations in the muscle and liver of the animal (Aguera et al., 2001, McVeigh and

Tarrant, 1982, Immonen et al., 2000). Glycogen stores play a key role in the homeostatic

control of blood glucose and energy balance in cattle. In addition, insufficient skeletal

muscle glycogen adversely affects flavour and colour acceptability and microbial stability in

meat (Warriss, 1990, Purchas et al., 1999). Some authors have tried to address the

negative energy balance in cattle with glucogenic substrates. Several attempts have been

made to improve the glucose supply to cattle during the marketing period (Hutcheson and

Cole, 1986, Schaefer et al., 1990). However, the effectiveness of oral glucose

supplementation to ruminant animals in increasing muscle glycogen after fasting and

transportation appears questionable. Although small live weight gains (2.9%) have been

reported from feeding electrolyte solutions on-board ships (Beatty et al., 2007) the weight

advantage from feeding electrolytes may be solely due to the fact that the electrolyte

treated animals drink more solution than the animals given water only. In most published

data sets, involving supplementation with electrolyte and glucose solutions during or after

transportation, muscle glycogen and rumen parameters are not measured (Hutcheson and

Cole, 1986; Schaefer et al., 1990; Beatty et al. 2007).

Ruminant animals derive a majority of their energy requirements from the VFA

produced by the fermentation of substrates by the rumen microflora.   If the rumen

microflora is absent after fasting a period of time between one and four days is required for

the rumen kinetics of *Bos indicus* cattle to stabilise back to pre-deprivation conditions. This

led to the hypothesis that rumen fluid from unstressed donor cattle may improve the

rumen environment adequately to correct the negative energy balance and allow for an

increase in skeletal muscle glycogen after transportation. Rumen transfaunation has been

recommended for cows with acidosis-related rumen stasis and also after surgical correction

of left sided displacement of the abomasum (Rager et al., 2004). In addition, acetic acid

feeding the major short chain fatty acid in rumen fluid has been found to enhance the rate

of muscle glycogen synthesis in exercised rats and horses (Waller et al., 2009, Fushimi and

Sato, 2005, Fushimi et al., 2001).

In chapter 5, rumen transfaunation was successful in increasing dry matter intake (DMI)

after transportation compared to control animals given an equivalent amount of water.

Consequently, the increase in DMI resulted in a greater live weight for the transfaunated

group. These results agree with previous studies evaluating rumen transfaunation on Holstein cows after abomasal surgery (Rager et al., 2004). This result is important because rumen transfaunation may expedite DMI in cattle immediately after transportation to feed yards and therefore may assist in the transition to full feed. However, rumen transfaunation had no effect on increasing muscle glycogen concentration in any of the muscles sampled. Rumen transfaunation failed to increase muscle glycogen concentrations in the *M. semitendinosis, M. semimembranosis and M. longissimus dorsi* of *Bos indicus* bulls subjected to 24 hours of transportation.

The first biopsy collection after 24h of lairage and refeeding might be too long to detect the influence of the treatment on the glycogen concentration. Although, a slow rate of glycogen repletion in mixing stressed Friesian bulls fed total mixed ratio had been reported to up to 7 days of refeeding period (McVeigh et al., 1982),

Considering the effect of feed and water deprivation, the rumen transfaunation might not enough to bring a significant difference among the groups after deprivation and transportation period in further variables such as muscle glycogen concentration. The frequency in rumen fluid administration might not enough to grant further effects on glycogen concentration as only once 10 kg of rumen fluid administered after deprivation and transportation period. Other studies found that certain deprivation and transportation stressors undoubtedly caused changes in rumen kinetics driven by ultrastructural changes in the proximal region of the basal cell cytoplasm of ruminal epithelium, the decrease in saliva flow in and out, the increase in rumen pH, the decrease in ruminal microflora, thereby decrease in ruminal volatile fatty acid (VFA) concentration and absorption (Tamate et al., 1974, Silanikove, 1989, Galyean et al., 1981, Fluharty et al., 1996, Gabel et al., 1993). Ruminal VFA concentration was found no difference between group of cattle exposing to

32h of fasting accompanied by 28h of transportation and control group at 10h of refeeding period (Galyean et al., 1981). However, a lower VFA absorption had been found in beef cattle exposed to short term feed restriction (Zhang et al., 2013). In the present study, the effort to increase total VFA concentration, transfaunated rumen and lower the pH rumen for stimulating absorption and transport of VFA (Stevens and Stettler, 1966) by administration of rumen fluid might not enough to supply more VFA into glucose-fatty acid cycle for further influence in the muscle glycogen. The future study is needed to investigate the effect of more frequent administrations of rumen fluid and also a shorter frequency of biopsy collections.

Diet type and nutrient restriction is known to effect liver and muscle glycogen concentrations in cattle.   The bulls in this experiment were grazing senesced Buffel grass (*Cenchus ciliaris*) pasture at the end of the dry season in northern Australia when dietary protein and energy deficiencies predominate in the rangelands. The pre-transport *M. longissimus dorsi* glycogen concentration for the RT and C bulls was low (36.38 ± 2.61 µmol/g wet tissue) compared with values reported in other studies. In addition, the pre-transportation glycogen concentration of the *M. semimembranosis and M. semitendinosis* were also less in this study than other reported values in *Bos taurus* steers (Gardner *et al*. 2001, Knee *et al*. 2007). It is noteworthy that the glycogen concentrations of the *M. longissimus dorsi* and *M. semitendinosis* were not decreased by the transportation regimen and remained at the same values for 24 hours after transportation. However, the glycogen concentration of the *M. semimembranosis* was decreased by transportation but repleted within 24 hours to pre-transportation (day -7) values. Gardner *et al.* (2001) found that the *M. semimembranosis* was more responsive to repletion compared with the *M. semitendinosis* after steers were exercised. In the study of Gardner *et al.* (2001) the

glycogen concentration of the *M. semitendinosis* remained at a depleted level for 72 hours despite a dietary ME concentration of 11.3 MJ/kg DM. In the present study, the low quality diet and low muscle glycogen concentrations before transportation may have affected the depletion and repletion rates of muscle glycogen especially in the *M. longissimus dorsi*. Fasting for three days has been demonstrated to decrease the rate of repletion of muscle glycogen in the *M. Longissimus dorsi* of beef heifers (McVeigh and Tarrant 1982). The bulls in this experiment demonstrated that muscle glycogen, as measured at the *M. longissimus dorsi*, cannot recover to sufficient concentrations of 40 to 45 µmol of glycogen concentration per gram muscle (Immonen *et al.*, 2000) to ensure normal meat quality until between one and four days of rest when fed Rhodes grass hay *ad libitum*.

A further consideration of the results from chapter 5 may question that the amount and frequency of rumen fluid administered to the bulls was not sufficient to demonstrate the hypothesised effects of muscle glycogen concentrations repletion. In chapter 5 a once off treatment of 10kg of rumen fluid administered after transportation was given. However, repeated doses or a greater volume may have been required from healthy donor steers to effect a change. Moreover, the volatile fatty acid composition and total quantity of fatty acids could be greater if the donor steers were consuming a total mixed ration with concentrates instead of a tropical grass hay.

In conclusion, the present studies have enhanced our knowledge in the physiological responses in particular the rumen kinetics and muscle glycogen concentration of *Bos indicus* cattle exposed to feed and water deprivation and long duration transportation. Rumen transfaunation will improve the optimal recovery of the cattle by increasing DMI early in the transition period of a feedlot. The results from this thesis

highlight the need for the Australian red meat industry to take into account the recovery period for glycogen if only Rhodes grass is fed to *Bos indicus* genotypes in lairage if the dark cutting beef condition is to be avoided.

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CHAPTER THREE ANOVA TABLES

Table A3.1. Fixed effect ANOVA of treatment, time and interaction between treatment and time for *in vitro* gas production from rumen fluid incubated with Kansas State media or the Goering-Van Soest media including the blanks for 72 hours using Rhode grass (*Chloris gayana*) hay as substrate.

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	7058.3 7058.30	695.386	0.0000	
Time	7	120829.2	17261.31	1700.591	0.0000
Treatment:Time	7	3423.6	489.08	48.185	0.0000
Residuals	112	1136.8	10.15		

CHAPTER FOUR ANOVA TABLES

Table A4.2. ANOVA of treatment, day, period and all interactions for total plate count of cellulolytic rumen bacteria from *Bos indicus* steers prior to (day -6), immediately after (day 0) and at 4 and 9 days post-feed and water deprivation for 72 hours (deprived group) or not (control group)

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Period	1	5.929853e+014	5.929853e+014	69.1694	3.594650e-010
Day	3	5.967398e+015	1.989133e+015	232.0244	0.000000e+000
Treatment	1	1.796995e+015	1.796995e+015	209.6123	0.000000e+000
Period:Day	2	1.670759e+015	8.353796e+014	97.4437	7.000000e-016
Period: Treatment	1	6.477221e+014	6.477221e+014	75.5542	1.158063e-010

```
Day:
Treatment    3    6.228452e+015   2.076151e+015   242.1747
             0.000000e+000

Period:Day:
Treatment    2    1.611854e+015   8.059269e+014   94.0082
             1.200000e-015

Residuals   39    3.343449e+014   8.572947e+012
```

Table A4.3.1. ANOVA of treatment, day, period and all interactions for total bacterial counts (Log10) in rumen fluid from two groups of *Bos indicus* steers either deprived of feed and water for 72 hours (Deprived) or not (Control).

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	0.528779	0.528779	147.0957	0.0000000
Day	3	2.868334	0.956111	265.9707	0.0000000
Period	1	0.000335	0.000335	0.0933	0.7639808
Treatment: Day	3	3.845287	1.281762	356.5601	0.0000000
Treatment: Period	1	0.008414	0.008414	2.3406	0.1455687
Day:Period	3	0.002620	0.000873	0.2429	0.8651160
Treatment: Day:Period	3	0.024262	0.008087	2.2497	0.1218903
Residuals	16	0.057517	0.003595		

Table A4.3.2. ANOVA of treatment, day, period and all interactions for live bacterial counts (Log10) in rumen fluid from two groups of *Bos indicus* steers either deprived of feed and water for 72 hours (Deprived) or not (Control).

```
            Df    Sum of Sq   Mean Sq   F Value     Pr(F)
Treatment    1    0.599511    0.599511  108.9894  0.00000
Day          3    3.297710    1.099237  199.8382  0.00000
Period       1    0.001314    0.001314  0.2388  0.6316713
```

```
Treat:Day      3      3.856705    1.285568    233.7127 0.00000
Treat:Period   1      0.002323    0.002323    0.4222 0.5250404
Day: Period    3      0.001567    0.000522    0.0950 0.9617172
Treatment:
Day: Period    3      0.009374    0.003125    0.5681 0.6439967
Residuals     16      0.088010    0.005501
```

Table A4.3.3. ANOVA of treatment, day, period and all interactions for dead bacterial counts (Log10) in rumen fluid from two groups of *Bos indicus* steers either deprived of feed and water for 72 hours (Deprived) or not (Control).

```
                Df    Sum of Sq   Mean Sq    F Value      Pr(F)
Treatment       1     0.367462    0.367462   46.3468 0.000004
Day             3     2.064869    0.688290   86.8118 0.000000
Period          1     0.000325    0.000325   0.0410 0.8420656
Treatment:Day   3     3.894497    1.298166   163.7335 0.00000
Treat:Period    1     0.037568    0.037568   4.7383 0.0448170
Day:Period      3     0.005192    0.001731   0.2183 0.8822604
Treatment:
Day:Period      3     0.109864    0.036621   4.6189 0.0164120
Residuals      16     0.126856    0.007929
```

Table A4.5.1. ANOVA of treatment, day, period and all interactions for acetic acid of rumen fluid from two groups of *Bos indicus* steers either deprived of feed and water for 72 hours (Deprived) or not (Control).

```
                     Df  Sum of Sq Mean Sq    F Value  Pr(F)
Treatment            1   2371.664  2371.664   124.6495 0.0000000
day                  3   8125.795  2708.598   142.3581 0.0000000
Period               1   1.550     1.550      0.0814   0.7765781
Treatment:day        3   5169.181  1723.060   90.5603  0.0000000
Treatment:Period     1   238.885   238.885    12.5553  0.0008918
day:Period           3   44.902    14.967     0.7867   0.5072747
Treatment:day:Period 3   232.173   77.391     4.0675   0.0118193
Residuals           48   913.280   19.027
```

Table A4.5.2. ANOVA of treatment, day, period and all interactions for propionic acid of rumen fluid from two groups of *Bos indicus* steers either deprived of feed and water for 72 hours (Deprived) or not (Control).

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	140.1997	140.1997	13.32426	0.0006457
day	3	479.8549	159.9516	15.20143	0.0000004
Period	1	48.5396	48.5396	4.61310	0.0368056
Treatment:day	3	327.3631	109.1210	10.37061	0.0000225
Treatment:Period	1	34.8115	34.8115	3.30841	0.0751675
day:Period	3	98.5472	32.8491	3.12190	0.0344368
Treatment:day:Period	3	69.0489	23.0163	2.18741	0.1016677
Residuals	48	505.0627	10.5221		

Table A4.5.3. ANOVA of treatment, day, period and all interactions for iso-butyric acid of rumen fluid from two groups of *Bos indicus* steers either deprived of feed and water for 72 hours (Deprived) or not (Control).

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	0.114860	0.114860	2.61811	0.1122037
day	3	0.932629	0.310876	7.08608	0.0004895
Period	1	1.129896	1.129896	25.75473	0.0000062
Treatment:day	3	0.103052	0.034351	0.78298	0.5092966
Treatment:Period	1	0.143155	0.143155	3.26307	0.0771278
day:Period	3	0.463509	0.154503	3.52172	0.0218274
Treatment:day:Period	3	0.027843	0.009281	0.21155	0.8879146
Residuals	48	2.105827	0.043871		

Table A4.5.4. ANOVA of treatment, day, period and all interactions for butyric acid of rumen fluid from two groups of *Bos indicus* steers either deprived of feed and water for 72 hours (Deprived) or not (Control).

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	19.52134	19.52134	113.4477	0.0000000
day	3	70.53584	23.51195	136.6390	0.0000000
Period	1	0.04625	0.04625	0.2688	0.6065457
Treatment:day	3	40.52839	13.50946	78.5099	0.0000000
Treatment:Period	1	1.75813	1.75813	10.2173	0.0024619
day:Period	3	1.61330	0.53777	3.1252	0.0343059
Treatment:day:Period	3	0.96863	0.32288	1.8764	0.1461672
Residuals	48	8.25953	0.17207		

Table A4.5.5. ANOVA of treatment, day, period and all interactions for iso-valeric acid of rumen fluid from two groups of *Bos indicus* steers either deprived of feed and water for 72 hours (Deprived) or not (Control).

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	0.0018770	0.0018770	0.09268	0.7621092
day	3	0.6212699	0.2070900	10.22559	0.0000255
Period	1	0.1102949	0.1102949	5.44609	0.0238473
Treatment:day	3	0.0146992	0.0048997	0.24194	0.8666452
Treatment:Period	1	0.4278932	0.4278932	21.12832	0.0000314
day:Period	3	0.3581772	0.1193924	5.89530	0.0016471
Treatment:day:Period	3	0.0931404	0.0310468	1.53302	0.2179917
Residuals	48	0.9721018	0.0202521		

Table A4.5.6. ANOVA of treatment, day, period and all interactions for valeric acid of rumen fluid from two groups of *Bos indicus* steers either deprived of feed and water for 72 hours (Deprived) or not (Control).

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	0.2824951	0.2824951	67.05446	0.0000000
day	3	0.9944277	0.3314759	78.68080	0.0000000
Period	1	0.0006829	0.0006829	0.16209	0.6890285
Treatment:day	3	0.3678031	0.1226010	29.10121	0.0000000
Treatment:Period	1	0.0671485	0.0671485	15.93870	0.0002234
day:Period	3	0.0262802	0.0087601	2.07934	0.1153355
Treatment:day:Period	3	0.0119564	0.0039855	0.94601	0.4258708
Residuals	48	0.2022202	0.0042129		

Table A4.5.7. ANOVA of treatment, day, period and all interactions for total VFA of rumen fluid from two groups of *Bos indicus* steers either deprived of feed and water for 72 hours (Deprived) or not (Control).

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	4327.76	4327.760	123.3153	0.0000000
day	3	14826.48	4942.159	140.8219	0.0000000
Period	1	88.70	88.699	2.5274	0.1184505
Treatment:day	3	9308.48	3102.828	88.4120	0.0000000
Treatment:Period	1	148.19	148.190	4.2225	0.0453551
day:Period	3	215.29	71.765	2.0449	0.1200704
Treatment:day:Period	3	288.65	96.218	2.7416	0.0533717
Residuals	48	1684.56	35.095		

Table A4.6.1. ANOVA of treatment, day, period and all interactions for *in vitro* acetic acid of rumen fluid from two groups of *Bos indicus* steers either deprived of feed and water for 72 hours (Deprived) or not (Control).

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	497.840	497.8396	45.75000	0.000000
Day	3	358.531	119.5104	10.98266	0.000004
Period	1	31.187	31.1871	2.86600	0.0944096
Treatment:Day	3	1007.384	335.7947	30.85854	0.000000
Treatment:Period	1	65.752	65.7518	6.04240	0.0161546
Day:Period	3	220.595	73.5315	6.75733	0.0004083
Treatment:Day:Period	3	79.028	26.3428	2.42083	0.0721778
Residuals	79	859.658	10.8817		

Table A4.6.2. ANOVA of treatment, day, period and all interactions for *in vitro* propionic acid of rumen fluid from two groups of *Bos indicus* steers either deprived of feed and water for 72 hours (Deprived) or not (Control).

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	58.4733	58.47335	47.98327	0.0000000
Day	3	59.7818	19.92728	16.35234	0.0000000
Period	1	3.7237	3.72369	3.05566	0.0843414
Treatment:Day	3	124.9858	41.66194	34.18781	0.0000000
Treatment:Period	1	19.7900	19.78999	16.23967	0.0001275
Day:Period	3	9.7004	3.23346	2.65338	0.0542736
Treatment:Day:Period	3	29.2419	9.74730	7.99864	0.0001012
Residuals	79	96.2710	1.21862		

Table A4.6.3. ANOVA of treatment, day, period and all interactions for *in vitro* iso-butyric acid of rumen fluid from two groups of *Bos indicus* steers either deprived of feed and water for 72 hours (Deprived) or not (Control).

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	0.0799018	0.0799018	6.67442	0.011624
Day	3	0.5074865	0.1691622	14.1305	0.000000
Period	1	0.1827313	0.1827313	15.2640	0.000196
Treatment:Day	3	0.0956437	0.0318812	2.6631	0.053629
Treatment:Period	1	0.0014895	0.0014895	0.12442	0.725230
Day:Period	3	0.4333148	0.1444383	12.0653	0.000001
Treatment:Day:Period	3	0.0475623	0.0158541	1.32434	0.272395
Residuals	79	0.9457361	0.0119713		

Table A4.6.4. ANOVA of treatment, day, period and all interactions for *in vitro* butyric acid of rumen fluid from two groups of *Bos indicus* steers either deprived of feed and water for 72 hours (Deprived) or not (Control).

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	6.09191	6.091908	19.35435	0.0000336
Day	3	6.87876	2.292921	7.28475	0.0002246
Period	1	0.04555	0.045552	0.14472	0.7046512
Treatment:Day	3	10.01203	3.337343	10.60294	0.0000062
Treatment:Period	1	0.21194	0.211937	0.67334	0.4143595
Day:Period	3	1.49623	0.498744	1.58454	0.1997147
Treatment:Day:Period	3	0.21191	0.070635	0.22441	0.8791612
Residuals	79	24.86576	0.314756		

Table A4.6.5. ANOVA of treatment, day, period and all interactions for *in vitro* iso-valeric acid of rumen fluid from two groups of *Bos indicus* steers either deprived of feed and water for 72 hours (Deprived) or not (Control).

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	0.1510295	0.1510295	55.1195	0.000000
Day	3	0.0430474	0.0143491	5.23684	0.002388
Period	1	0.0148140	0.0148140	5.40651	0.022629
Treatment:Day	3	0.1369014	0.0456338	16.6544	0.000000
Treatment:Period	1	0.0061674	0.0061674	2.25083	0.137529
Day:Period	3	0.0425072	0.0141691	5.17113	0.002581
Treatment:Day:Period	3	0.0065370	0.0021790	0.79524	0.500151
Residuals	79	0.2164629	0.0027400		

Table A4.6.6. ANOVA of treatment, day, period and all interactions for *in vitro* valeric acid of rumen fluid from two groups of *Bos indicus* steers either deprived of feed and water for 72 hours (Deprived) or not (Control).

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	0.2707666	0.2707666	74.6650	0.000000
Day	3	0.4794138	0.1598046	44.0668	0.000000
Period	1	0.0094584	0.0094584	2.60820	0.110299
Treatment:Day	3	0.3567899	0.1189300	32.7954	0.000000
Treatment:Period	1	0.0153235	0.0153235	4.22553	0.043124
Day:Period	3	0.0089068	0.0029689	0.81869	0.487362
Treatment:Day:Period	3	0.0332941	0.0110980	3.06033	0.032959
Residuals	79	0.2864869	0.0036264		

Table A4.6.7. ANOVA of treatment, day, period and all interactions for *in vitro* total VFA of rumen fluid from two groups of *Bos indicus* steers either deprived of feed and water for 72 hours (Deprived) or not (Control).

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	1130.233	1130.233	51.1803	0.000000
Day	3	897.983	299.328	13.5544	0.000000
Period	1	44.280	44.280	2.00515	0.160696
Treatment:Day	3	2227.285	742.428	33.6193	0.000000
Treatment:Period	1	175.791	175.791	7.96035	0.006044
Day:Period	3	354.593	118.198	5.35235	0.002084
Treatment:Day:Period	3	194.257	64.752	2.93218	0.038561
Residuals	79	1744.584	22.083		

Table A4.7. ANOVA of treatment, day, period and all interactions for cumulative *in vitro* gas production from rumen fluid of two groups of *Bos indicus* steers (deprived of feed and water for 72 hours or control).

	numDF	denDF	F-value	p-value
(Intercept)	1	68	272.2460	<.0001
Period	1	68	58.5103	<.0001
Treatment	1	68	84.0893	<.0001
Day	3	68	51.9243	<.0001
Period:Treatment	1	68	0.8151	0.3698
Period:Day	3	68	21.5921	<.0001
Treatment:Day	3	68	52.7446	<.0001
Period:Treatment:Day	3	68	0.9232	0.4344

Table A4.8. ANOVA of treatment, day, period and all interactions for cumulative dry matter intake for two groups of *Bos indicus* steers subjected to feed and water deprivation for 72 hours or not.

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	4941.1	4941.07	107.339	0.000000
Period	1	0.0	0.03	0.0006	0.979839
day	20	265140.9	13257.05	287.993	0.000000
Treatment:Period	1	3869.1	3869.06	84.0508	0.000000
Treatment:day	20	6241.1	312.06	6.7791	0.000000
Period:day	20	60.8	3.04	0.0661	1.000000
Treatment:Period:day	20	621.6	31.08	0.6752	0.839498
Residuals	84	3866.7	46.03		

CHAPTER FIVE ANOVA TABLES

Table A5.1.1. ANOVA of treatment, day, group and all interactions for glycogen concentration of *M. semimembranosis* of *Bos indicus* bulls subjected to a 12 hour wet curfew and 24 hours of transportation followed by treatments of rumen transfaunation or deionised water.

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	5583.552[a]	15	372.237	4.216	.000
Intercept	113098.241	1	113098.241	1280.914	.000
Treatment	58.166	1	58.166	.659	.423
Day	5144.326	4	1286.081	14.566	.000
Group	234.907	1	234.907	2.660	.113
Treatment * Day	117.504	4	29.376	.333	.854
Treatment * Group	91.585	1	91.585	1.037	.316
Day * Group	37.910	2	18.955	.215	.808
Treatment * Day * Group	30.788	2	15.394	.174	.841
Error	2825.438	32	88.295		
Total	131947.700	48			
Corrected Total	8408.989	47			

Table A5.1.2. ANOVA of treatment, day, group and all interactions for free glucose concentration of *M. semimembranosis* of *Bos indicus* bulls subjected to a 12 hour wet curfew and 24 hours of transportation followed by treatments of rumen transfaunation or deionised water.

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	30.335[a]	15	2.022	3.374	.002
Intercept	365.091	1	365.091	609.054	.000
Treatment	.280	1	.280	.467	.499
Day	9.816	4	2.454	4.094	.009
Group	.442	1	.442	.738	.397
Treatment * Day	1.176	4	.294	.491	.743
Treatment * Group	1.051	1	1.051	1.753	.195
Day * Group	15.291	2	7.645	12.754	.000
Treatment * Day * Group	.902	2	.451	.752	.480
Error	19.182	32	.599		
Total	413.893	48			
Corrected Total	49.517	47			

Table A5.1.3. ANOVA of treatment, day, group and all interactions for total glucosyl unit of *M. semimembranosis* of *Bos indicus* bulls subjected to a 12 hour wet curfew and 24 hours of transportation followed by treatments of rumen transfaunation or deionised water.

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	5186.257[a]	15	345.750	4.054	.000
Intercept	126312.290	1	126312.290	1480.897	.000
Treatment	50.268	1	50.268	.589	.448
Day	4783.072	4	1195.768	14.019	.000
Group	214.964	1	214.964	2.520	.122
Treatment * Day	112.691	4	28.173	.330	.856
Treatment * Group	73.017	1	73.017	.856	.362
Day * Group	58.622	2	29.311	.344	.712
Treatment * Day * Group	21.707	2	10.853	.127	.881
Error	2729.422	32	85.294		
Total	145235.207	48			
Corrected Total	7915.679	47			

Table A5.2.1. ANOVA of treatment, day, group and all interactions for glycogen concentration of *M. semitendinosis* of *Bos indicus* bulls subjected to a 12 hour wet curfew and 24 hours of transportation followed by treatments of rumen transfaunation or deionised water.

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	2279.258[a]	15	151.951	1.631	.124
Intercept	66827.091	1	66827.091	717.137	.000
Treatment	5.527	1	5.527	.059	.809
Day	1509.308	4	377.327	4.049	.010
Group	257.797	1	257.797	2.766	.107
Treatment * Day	348.024	4	87.006	.934	.458
Treatment * Group	4.194	1	4.194	.045	.833
Day * Group	269.797	2	134.898	1.448	.251
Treatment * Day * Group	73.618	2	36.809	.395	.677
Error	2795.580	30	93.186		
Total	77302.099	46			
Corrected Total	5074.838	45			

Table A5.2.2. ANOVA of treatment, day, group and all interactions for free glucose concentration of *M. semitendinosis* of *Bos indicus* bulls subjected to a 12 hour wet curfew and 24 hours of transportation followed by treatments of rumen transfaunation or deionised water.

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	28.726[a]	15	1.915	3.074	.004
Intercept	411.368	1	411.368	660.215	.000
Treatment	.100	1	.100	.160	.692
Day	9.484	4	2.371	3.805	.012
Group	4.209	1	4.209	6.756	.014
Treatment * Day	1.889	4	.472	.758	.560
Treatment * Group	.297	1	.297	.477	.495
Day * Group	11.677	2	5.838	9.370	.001
Treatment * Day * Group	.302	2	.151	.242	.786
Error	19.939	32	.623		
Total	475.282	48			
Corrected Total	48.665	47			

Table A5.2.3. ANOVA of treatment, day, group and all interactions for total glucosyl unit of *M. semitendinosis* of *Bos indicus* bulls subjected to a 12 hour wet curfew and 24 hours of transportation followed by treatments of rumen transfaunation or deionised water.

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	2071.840[a]	15	138.123	1.457	.185
Intercept	77385.482	1	77385.482	816.230	.000
Treatment	6.340	1	6.340	.067	.798
Day	1418.214	4	354.553	3.740	.014
Group	195.082	1	195.082	2.058	.162
Treatment * Day	354.077	4	88.519	.934	.458
Treatment * Group	2.015	1	2.015	.021	.885
Day * Group	184.977	2	92.489	.976	.389
Treatment * Day * Group	64.651	2	32.326	.341	.714
Error	2844.252	30	94.808		
Total	88197.505	46			
Corrected Total	4916.092	45			

Table A5.3.1. ANOVA of treatment, day, group and all interactions for glycogen concentration of *M. longisimus dorsi* of *Bos indicus* bulls subjected to a 12 hour wet curfew and 24 hours of transportation followed by treatments of rumen transfaunation or deionised water.

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	3303.654[a]	15	220.244	2.914	.006
Intercept	63836.068	1	63836.068	844.590	.000
Treatment	54.115	1	54.115	.716	.404
Day	2826.673	4	706.668	9.350	.000
Group	26.251	1	26.251	.347	.560
Treatment * Day	94.256	4	23.564	.312	.868
Treatment * Group	129.141	1	129.141	1.709	.201
Day * Group	19.665	2	9.832	.130	.878
Treatment * Day * Group	78.285	2	39.143	.518	.601
Error	2343.052	31	75.582		
Total	77055.728	47			
Corrected Total	5646.707	46			

Table A5.3.2. ANOVA of treatment, day, group and all interactions for free glucose concentration of *M. longisimus dorsi* of *Bos indicus* bulls subjected to a 12 hour wet curfew and 24 hours of transportation followed by treatments of rumen transfaunation or deionised water.

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	26.511[a]	15	1.767	2.891	.006
Intercept	243.972	1	243.972	399.130	.000
Treatment	.011	1	.011	.018	.894
Day	10.441	4	2.610	4.270	.007
Group	.065	1	.065	.106	.747
Treatment * Day	2.142	4	.535	.876	.489
Treatment * Group	.065	1	.065	.106	.747
Day * Group	12.735	2	6.367	10.417	.000
Treatment * Day * Group	.633	2	.316	.518	.601
Error	19.560	32	.611		
Total	295.777	48			
Corrected Total	46.071	47			

Table A5.3.3. ANOVA of treatment, day, group and all interactions for total glucosyl unit of *M. longisimus dorsi* of *Bos indicus* bulls subjected to a 12 hour wet curfew and 24 hours of transportation followed by treatments of rumen transfaunation or deionised water.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3007.826[a]	15	200.522	2.619	.011
Intercept	71937.097	1	71937.097	939.406	.000
Treatment	57.420	1	57.420	.750	.393
Day	2572.737	4	643.184	8.399	.000
Group	25.238	1	25.238	.330	.570
Treatment * Day	91.313	4	22.828	.298	.877
Treatment * Group	138.368	1	138.368	1.807	.189
Day * Group	4.254	2	2.127	.028	.973
Treatment * Day * Group	63.041	2	31.520	.412	.666
Error	2373.893	31	76.577		
Total	85371.657	47			
Corrected Total	5381.719	46			

Table A5.4.1. ANOVA of treatment, day, group and all interactions for cumulative Rhodes grass (*Chloris gayana*) hay intake after a 12 hour wet curfew and 24 hours of transportation followed by treatments of rumen transfaunation or deionised water.

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
treatment	1	630.53	630.533	10.0505	0.002237
day	8	36278.63	4534.829	72.2843	0.000000
group	1	209.66	209.660	3.34195	0.071677
treatment:day	8	134.53	16.816	0.26804	0.974262
treatment:group	1	58.16	58.158	0.92702	0.338859
day:group	8	37.80	4.725	0.07532	0.999690
treatment:day:group	8	32.92	4.115	0.06560	0.999815
Residuals	72	4516.99	62.736		

Table A5.4.2. ANOVA of treatment, day, group and all interactions for cumulative water intake after a 12 hour wet curfew and 24 hours of transportation followed by treatments of rumen transfaunation or deionised water.

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
treatment	1	565	565.4	0.07791	0.780952
day	8	1462452	182806.5	25.1898	0.000000
group	1	65810	65809.5	9.06824	0.003586
treatment:day	8	568	71.0	0.00979	0.999999
treatment:group	1	34121	34121.1	4.70173	0.033437
day:group	8	12510	1563.7	0.21547	0.987185
treatment:day:group	8	6983	872.9	0.12029	0.998278
Residuals	72	522514	7257.1		

Table A5.5.1. ANOVA of treatment, day, group and all interactions for body weight of *Bos indicus* bulls subjected to a 12 hour wet curfew and 24 hours of transportation followed by treatments of rumen.

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatments	1	5117.35	5117.347	7.61132	0.008184
Day	5	24701.07	4940.214	7.34786	0.000035
Group	1	2508.68	2508.681	3.73130	0.059317
Treatments:Day	5	332.07	66.414	0.09878	0.991881
Treatments:Group	1	1449.01	1449.014	2.15520	0.148610
Day:Group	5	147.07	29.414	0.04374	0.998821
Treatments:Day:Group	5	838.40	167.681	0.24940	0.938087
Residuals	48	32272.00	672.333		

Table A5.5.2. ANOVA of treatment, day, group and all interactions for rectal temperature of *Bos indicus* bulls subjected to a 12 hour wet curfew and 24 hours of transportation followed by treatments of rumen.

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatments	1	0.088469	0.088469	0.79076	0.378400
Day	5	9.344308	1.868862	16.7042	0.000000
Group	1	3.817177	3.817177	34.1186	0.000000
Treatments:Day	5	0.559906	0.111981	1.00091	0.427671
Treatments:Group	1	1.139082	1.139082	10.1813	0.002525
Day:Group	5	1.059151	0.211830	1.89338	0.113426
Treatments:Day:Group	5	0.184937	0.036987	0.33060	0.891949
Residuals	47	5.258333	0.111879		

Table A5.6.1. ANOVA of treatment, day, group and all interactions for plasma glucose in *Bos indicus* bulls subjected to transportation for 24 h followed by treatments of rumen transfaunation or deionised water.

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	3.96294	3.962940	8.64447	0.005428
Day	4	26.16646	6.541615	14.2694	0.000000
Group	1	1.00363	1.003627	2.18924	0.146811
Treatment:Day	4	0.62229	0.155573	0.33936	0.849776
Treatment:Group	1	2.93046	2.930460	6.39229	0.015511
Day:Group	4	0.55814	0.139535	0.30437	0.873323
Treatment:Day:Group	4	0.96977	0.242443	0.52885	0.715161
Residuals	40	18.33747	0.458437		

Table A5.6.2. ANOVA of treatment, day, group and all interactions for plasma lactate in *Bos indicus* bulls subjected to transportation for 24 h followed by treatments of rumen transfaunation or deionised water.

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	27.35100	27.35100	14.8222	0.000416
Day	4	25.05053	6.26263	3.39388	0.017575
Group	1	24.43540	24.43540	13.2421	0.000775
Treatment:Day	4	8.96921	2.24230	1.21516	0.319549
Treatment:Group	1	8.88580	8.88580	4.81544	0.034073
Day:Group	4	2.80871	0.70218	0.38053	0.821200
Treatment:Day:Group	4	2.27051	0.56763	0.30761	0.871177
Residuals	40	73.81087	1.84527		

Table A5.6.3. ANOVA of treatment, day, group and all interactions for plasma lactate dehydrogenase in *Bos indicus* bulls subjected to transportation for 24 h followed by treatments of rumen transfaunation or deionised water.

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	108622	108621.6	2.60518	0.114377
Day	4	718196	179548.9	4.30630	0.005442
Group	1	270373	270372.8	6.48463	0.014834
Treatment:Day	4	235765	58941.2	1.41364	0.247018
Treatment:Group	1	153389	153388.9	3.67888	0.062264
Day:Group	4	75775	18943.8	0.45434	0.768630
Treatment:Day:Group	4	50126	12531.5	0.30055	0.875839
Residuals	40	1667776	41694.4		

Table A5.6.4. ANOVA of treatment, day, group and all interactions for plasma creatine in *Bos indicus* bulls subjected to transportation for 24 h followed by treatments of rumen transfaunation or deionised water.

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	6.67	6.667	0.01256	0.911323
Day	4	16635.77	4158.942	7.83621	0.000092
Group	1	589.07	589.067	1.10991	0.298423
Treatment:Day	4	679.17	169.792	0.31991	0.862958
Treatment:Group	1	45.07	45.067	0.08491	0.772251
Day:Group	4	284.43	71.108	0.13398	0.968923
Treatment:Day:Group	4	801.43	200.358	0.37751	0.823318
Residuals	40	21229.33	530.733		

Table A5.6.5. ANOVA of treatment, day, group and all interactions for plasma creatinine kinase in *Bos indicus* bulls subjected to transportation for 24 h followed by treatments of rumen transfaunation or deionised water.

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	9310	9310.1	0.33560	0.565629
Day	4	1765246	441311.6	15.9078	0.000000
Group	1	55462	55461.8	1.99921	0.165117
Treatment:Day	4	13778	3444.4	0.12416	0.972937
Treatment:Group	1	111733	111732.6	4.02759	0.051553
Day:Group	4	112332	28083.1	1.01230	0.412596
Treatment:Day:Group	4	140996	35249.0	1.27061	0.297554
Residuals	40	1109672	27741.8		

Table A5.6.6. ANOVA of treatment, day, group and all interactions for plasma albumin in *Bos indicus* bulls subjected to transportation for 24 h followed by treatments of rumen transfaunation or deionised water.

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	1.8375	1.83750	0.24981	0.619945
Day	4	102.7757	25.69392	3.49315	0.015436
Group	1	1.7682	1.76817	0.24038	0.626605
Treatment:Day	4	9.4883	2.37208	0.32249	0.861227
Treatment:Group	1	2.7735	2.77350	0.37706	0.542655
Day:Group	4	29.7143	7.42858	1.00993	0.413803
Treatment:Day:Group	4	21.9190	5.47975	0.74498	0.567119
Residuals	40	294.2200	7.35550		

Table A5.6.7. ANOVA of treatment, day, group and all interactions for plasma CO_2 in *Bos indicus* bulls subjected to transportation for 24 h followed by treatments of rumen transfaunation or deionised water.

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	0.7042	0.70417	0.10179	0.751343
Day	4	148.8740	37.21850	5.38046	0.001457
Group	1	39.5282	39.52817	5.71436	0.021624
Treatment:Day	4	25.5000	6.37500	0.92159	0.460946
Treatment:Group	1	0.0602	0.06017	0.00869	0.926160
Day:Group	4	8.4993	2.12483	0.30717	0.871467
Treatment:Day:Group	4	46.4907	11.62267	1.68022	0.173571
Residuals	40	276.6933	6.91733		

Table A5.6.8. ANOVA of treatment, day, group and all interactions for plasma total protein in *Bos indicus* bulls subjected to transportation for 24 h followed by treatments of rumen transfaunation or deionised water.

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	10.923	10.9227	0.27649	0.601911
Day	4	779.954	194.9886	4.93589	0.002493
Group	1	0.150	0.1500	0.00379	0.951172
Treatment:Day	4	91.026	22.7564	0.57605	0.681617
Treatment:Group	1	195.843	195.8427	4.95751	0.031673
Day:Group	4	165.342	41.3354	1.04635	0.395539
Treatment:Day:Group	4	32.836	8.2089	0.20779	0.932598
Residuals	40	1580.167	39.5042		

Table A5.6.9. ANOVA of treatment, day, group and all interactions for plasma urea in *Bos indicus* bulls subjected to transportation for 24 h followed by treatments of rumen transfaunation or deionised water.

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	0.02773	0.027735	0.06930	0.793708
Day	4	11.54017	2.885043	7.20885	0.000180
Group	1	1.69680	1.696802	4.23979	0.046038
Treatment:Day	4	0.63537	0.158843	0.39690	0.809648
Treatment:Group	1	1.71704	1.717042	4.29037	0.044822
Day:Group	4	0.58501	0.146252	0.36543	0.831761
Treatment:Day:Group	4	2.77283	0.693208	1.73211	0.161947
Residuals	40	16.00833	0.400208		

Table A5.7.1. ANOVA of treatment, day, group and all interactions for plasma Phosphorous in *Bos indicus* bulls subjected to 24 h transportation followed by treatments of rumen transfaunation or deionised water.

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	0.009102	0.0091020	0.12979	0.720544
Day	4	2.351675	0.5879189	8.38346	0.000052
Group	1	0.000687	0.0006868	0.00979	0.921661
Treatment:Day	4	0.191700	0.0479249	0.68338	0.607617
Treatment:Group	1	0.066867	0.0668668	0.95349	0.334700
Day:Group	4	0.234236	0.0585589	0.83502	0.511052
Treatment:Day:Group	4	0.203116	0.0507789	0.72408	0.580677
Residuals	40	2.805135	0.0701284		

Table A5.7.2. ANOVA of treatment, day, group and all interactions for plasma Calcium in *Bos indicus* bulls subjected to 24 h transportation followed by treatments of rumen transfaunation or deionised water.

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	0.002574	0.00257415	0.07458	0.786178
Day	4	0.102002	0.02550044	0.73887	0.571066
Group	1	0.007912	0.00791202	0.22924	0.634688
Treatment:Day	4	0.084534	0.02113361	0.61234	0.656181
Treatment:Group	1	0.047096	0.04709602	1.36460	0.249657
Day:Group	4	0.106642	0.02666039	0.77248	0.549588
Treatment:Day:Group	4	0.105126	0.02628156	0.76150	0.556545
Residuals	40	1.380508	0.03451270		

Table A5.7.3. ANOVA of treatment, day, group and all interactions for plasma Magnesium in *Bos indicus* bulls subjected to 24 h transportation followed by treatments of rumen transfaunation or deionised water.

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	0.0099588	0.00995882	1.5604	0.218861
Day	4	0.3412514	0.08531286	13.367	0.000000
Group	1	0.0269240	0.02692402	4.2187	0.046556
Treatment:Day	4	0.0141598	0.00353994	0.5546	0.696755
Treatment:Group	1	0.0004004	0.00040042	0.0627	0.803497
Day:Group	4	0.0048756	0.00121889	0.1909	0.941714
Treatment:Day:Group	4	0.0116008	0.00290021	0.4544	0.768570
Residuals	40	0.2552820	0.00638205		

Table A5.7.4. ANOVA of treatment, day, group and all interactions for plasma Sodium in *Bos indicus* bulls subjected to 24 h transportation followed by treatments of rumen transfaunation or deionised water.

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
Treatment	1	95.256	95.2560	1.04463	0.312889
Day	4	539.546	134.8865	1.47924	0.226613

```
Group                  1    51.894   51.8940    0.56909 0.455035
Treatment:Day          4   365.374   91.3435    1.00172 0.418016
Treatment:Group        1    27.744   27.7440    0.30425 0.584295
Day:Group              4   276.409   69.1023    0.75781 0.558896
Treatment:Day:Group    4   315.949   78.9873    0.86621 0.492557
Residuals             40  3647.453   91.1863
```

Table A5.7.5. ANOVA of treatment, day, group and all interactions for plasma Potasium in *Bos indicus* bulls subjected to 24 h transportation followed by treatments of rumen transfaunation or deionised water.

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                      Df  Sum of Sq  Mean Sq   F Value    Pr(F)
Treatment              1   0.033135  0.033135  0.21439 0.645855
Day                    4   2.232773  0.558193  3.61165 0.013230
Group                  1   1.096202  1.096202  7.09270 0.011098
Treatment:Day          4   0.217907  0.054477  0.35247 0.840754
Treatment:Group        1   0.137282  0.137282  0.88824 0.351610
Day:Group              4   1.133473  0.283368  1.83346 0.141388
Treatment:Day:Group    4   1.399193  0.349798  2.26328 0.079276
Residuals             40   6.182133  0.154553
```

Table A5.7.6. ANOVA of treatment, day, group and all interactions for plasma Chloride in *Bos indicus* bulls subjected to 24 h transportation followed by treatments of rumen transfaunation or deionised water.

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                      Df  Sum of Sq  Mean Sq    F Value    Pr(F)
Treatment              1    13.920   13.9202    0.27432 0.603335
Day                    4   953.977  238.4943    4.69999 0.003330
Group                  1     0.337    0.3375    0.00665 0.935407
Treatment:Day          4   169.377   42.3443    0.83447 0.511380
Treatment:Group        1     8.438    8.4375    0.16627 0.685616
Day:Group              4   163.933   40.9833    0.80765 0.527679
Treatment:Day:Group    4    97.487   24.3717    0.48029 0.749990
Residuals             40  2029.740   50.7435
```