

EFFICACY AND SAFETY OF ACIDIFIED ENTERAL FORMULAE IN TUBE FED PATIENTS IN AN INTENSIVE CARE UNIT

Short Title: Acidified Formulae in ICU Patients

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fulfilment of the requirements for the degree of Master in Nutrition by

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DECLARATION OF ORIGINAL WORK

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously, in part or in its entirety, submitted it at any university for a degree.

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ABSTRACT

INTRODUCTION: The primary objective was to determine whether acidified formulae (pH 3.5 and 4.5) decreased gastric and tracheal colonisation, as well as microbial contamination of the enteral feeding delivery system, compared with a non-acidified control formula (pH 6.8) in critically ill patients. Secondary objectives included tolerance of the trial formulae and mortality in relation to the administration of acidified formulas.

DESIGN: The trial was a controlled, double-blinded, randomised clinical trial of three parallel groups at a single centre.

METHOD: Sixty-seven mechanically ventilated, medical and surgical critically ill patients were randomised according to their APACHE II scores and included in the trial. Patients received either an acidified (pH 3.5 or 4.5) or control polymeric enteral formula via an 8-Fr nasogastric tube at a continuous rate. Daily samples were taken for microbiologic analyses of the enteral formulae at various stages of reconstitution and at 6-hour and 24-hour intervals during administration thereof (feeding bottle and delivery set). Daily patient samples included nasogastric and tracheal aspirates, haematological evaluation and gastro-intestinal tolerance. The trial period terminated when patients were extubated, transferred from the ICU, enteral nutrition became contra-indicated, a patient died, or for a maximum of 21 days.

RESULTS: Gastric pH showed no significant difference ($p = 0.86$) between the 3 feeding groups [pH 3.5 ($n = 23$), pH 4.5 ($n = 23$) and pH 6.8 ($n = 21$)] at baseline prior to the administration of enteral formulae. After initiation of feeds, the gastric pH decreased significantly ($p < 0.0001$) in the acidified formulae as compared to the control formula during the trial period. Patients who received acidified enteral formulae (pH 3.5 and 4.5) had significantly less ($p < 0.0001$) contamination from the feeding bottles and delivery systems in respect of *Enterobacteriaceae*, and *Enterococcus*. The more acidified group (pH 3.5) showed significantly less gastric contamination ($p = 0.029$) with *Enterobacteriaceae*, but not for fungi. The 3.5 acidified group also had the lowest gastric growth in terms of colony counts ($\leq 10^4$) of these organisms, but not for fungi, when compared to the control group ($\leq 10^5$). Vomiting episodes were 22% and abdominal distension 12%, with a higher incidence in the control group. Adverse events occurred equally between the groups with a higher, but not significantly different incidence of 37% in the control group and 32% for the acidified groups. There was no evidence of gastro-intestinal bleeding in any patient. Overall, the mortality rate in this trial was 6%, with 6.5% for the acidified groups ($n=46$) and 4.8% for the control group ($n=21$), a statistically insignificant difference.

CONCLUSION: Acidified enteral formulae significantly decrease gastric colonisation by preserving gastric acidity that decreases the growth of *Enterobacteriaceae* organisms. Acidified formulae significantly decrease bacterial contamination of the enteral feeding system (bottle and delivery set) of *Enterobacteriaceae* and *Enterococcus* organisms. Acidified formulae are tolerated well in critically ill patients.

OPSOMMING

INLEIDING: Die hoofdoelwit van hierdie kliniese studie was om te bepaal of, in vergelyking met 'n nie-aangesuurde kontroleformule (pH 6.8), aangesuurde formules (pH 3.5 en 4.5) gastriese en trageale kolonisasie asook mikrobiële kontaminasie van die toedieningsstelsel vir enterale voeding by kritiek siek pasiënte verminder. Sekondêre doelwitte het toleransie van studieformules, asook mortaliteit in verhouding tot die toediening van aangesuurde voedings, ingesluit.

ONTWERP: Die studie was 'n gekontroleerde, dubbel-blinde, ewekansige ("randomised") kliniese studie van drie parallelle groepe by 'n enkele sentrum.

METODE: Sewe en sestig meganies geventileerde, mediese en sjirurgiese kritiek siek pasiënte is volgens die APACHE II-tellings ewekansig gemaak ("randomised") en ingesluit. Pasiënte het óf 'n aangesuurde (pH 3.5 of 4.5) óf 'n kontrole polimeriese formule via 'n 8-Fr nasogastriese buis teen 'n konstante tempo ontvang. Daaglikse monsters is vir mikrobiologiese ontleding van die formules op verskillende stadiums van rekonstitusie en op 6-uur- en 24-uur-intervalle tydens die toediening (voedingsbottel en toedieningstel) daarvan geneem. Daaglikse monsters het ook nasogastriese en trageale aspirate, hematologiese evaluering en gastro-intestinale toleransie ingesluit. Die studietydperk is beëindig wanneer pasiënte geëkstubeer is, na ander sale oorgeplaas is, enterale voeding gekontra-indikeerd is, pasiënte gesterf het, of vir 'n maksimum van 21 dae.

RESULTATE: Gastriese pH het geen statisties beduidende verskil ($p = 0.86$) tussen die 3 voedingsgroepe [pH 3.5 ($n = 23$), pH 4.5 ($n = 23$) and pH 6.8 ($n = 21$)] by basislyn, voor die toediening van die formules, getoon nie. Na die inisiëring van voedings, het die gastriese pH by die aangesuurde groepe statisties beduidend verminder ($p < 0.0001$), vergeleke met die kontrolegroep. Pasiënte wat aangesuurde formules (pH 3.5 en 4.5) ontvang het, het statisties beduidend minder ($p < 0.0001$) kontaminasie van die voedingsbottels en toedieningstelsel ten opsigte van *Enterobacteriaceae* en *Enterococcus* gehad. Die meer aangesuurde groep (pH 3.5) het statisties beduidend minder gastriese kontaminasie ($p = 0.029$) van *Enterobacteriaceae* gehad, maar nie van fungi nie. Die 3.5 aangesuurde groep het, vergeleke met die kontrolegroep se kolonietellings ($\leq 10^5$), ook die laagste gastriese groei in terme van kolonietellings ($\leq 10^4$) van hierdie organismes, maar nie vir fungi nie, gehad. Vomerings episodes was 22% en abdominale distensie 12%, met 'n hoër insidensie in die kontrolegroep. Ongunstige voorvalle het eweredig tussen die verskillende voedingsgroepe voorgekom met 'n hoër, maar statisties nie-beduidende, insidensie van 37% by die kontrolegroep en 32% by die aangesuurde groepe. Geen gastro-intestinale bloeding is by enige van die pasiënte waargeneem nie. Die mortaliteitsyfer vir die kliniese studie was 6%, met 6.5% vir die aangesuurde groepe ($n = 46$) en 4.8% vir die kontrolegroep ($n = 21$), 'n statisties nie-beduidende verskil.

GEVOLGTREKING: Aangesuurde enterale formules verminder gastriese kolonisasie beduidend deur die preserving van gastriese asiditeit wat die groei van *Enterobacteriaceae* organismes verminder. Aangesuurde enterale formules verminder bakteriële kontaminasie van die toedieningsstelsel vir enterale voeding (bottel en toedieningstel) van *Enterobacteriaceae* en *Enterococcus* organismes. Aangesuurde enterale formules word by kritiek siek pasiënte goed verdra.



DEDICATION

To my husband, Christopher, who motivates and inspires me and who made it possible for me to do this worthwhile project for the last 4 years. To my daughter, Anje and son, Rian who made me laugh when I needed to and reminded me of the important things in life in the midst of this project.



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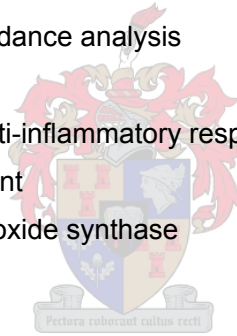
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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophic hormone
AE	Adverse event
AGCP	Applied good clinical practice
AGP	Alpha-acid glycoprotein
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ANCOVA	Analysis of covariance
APACHE	Acute physiology and chronic health evaluation
APR	Acute phase response
ARDS	Acute respiratory distress syndrome
AST	Aspartate Transaminase
ATP	Adenosine triphosphate
BEE	Basal energy expenditure
BGA	Blood gas analyzer
BIA	Bioelectrical impedance analysis
BMI	Body mass index
CARS	Compensatory anti-inflammatory response syndrome
CCP	Critical control point
cNOS	constitutive nitric oxide synthase
CRP	C-reactive protein
↓	Decreased
d	days
DXA	Dual-energy X-ray absorptiometry
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamide-tetra acetic acid
EEF	Early enteral feeding
EPA	Eicosapentaenoic acid
FFA	Free fatty acid
GALT	Gut associated lymphoid tissue
GGT	Gamma Glutamyl Transferase
GIT	Gastro-intestinal tract
HACCP	Hazard analysis critical control point process
ICU	Intensive care unit
IL-1	Interleukin 1
IL-2	Interleukin 2



IL-6	Interleukin 6
↑	Increased
IPPV	Intermittent positive pressure ventilation
ISE	Ion selective electrode
ITT	Intention to treat
Kg	kilogram
LOS	Length of stay
m	meters
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MODS	Multiple organ dysfunction syndrome
N ₂	Nitrogen
n-3	omega-3
n-6	omega-6
NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide hydrogenase
NHLS	National health laboratory services
NO	Nitric oxide
OKG	Ornithine α -ketoglutarate
PINI	Prognostic inflammatory and nutritional index
PMN	Polymorphonuclear cells
PROC MIXED	Mixed procedure
PVC	Polyurethane
RBC	Red blood count
RBP	Retinol binding protein
REE	Resting energy expenditure
RNA	Ribonucleic acid
SAE	Serious adverse event
SD	Standard deviation
SEC	Squamous epithelial cells
SIRS	Systemic inflammatory response syndrome
TNF α	Tumor necrosis factor α
UV	Ultraviolet
UTI	Urinary tract infection
VAP	Ventilator-associated pneumonia
WBC	White blood count
y	Years

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CHAPTER 1: LITERATURE REVIEW



1.1 THE ACUTE PHASE RESPONSE

Within 24 - 48 hours post injury, where an injury encompasses any injurious insult including infection and sepsis, a hypermetabolic response known as the acute phase response (APR) with hypercatabolism, hyperglycaemia, lipolysis, skeletal muscle proteolysis and increased vascular endothelial instability occurs ⁽¹⁾. Cuthbertson divided this response to injury into the ebb and flow phases with the following characteristics (Table 1.1) ⁽²⁾.

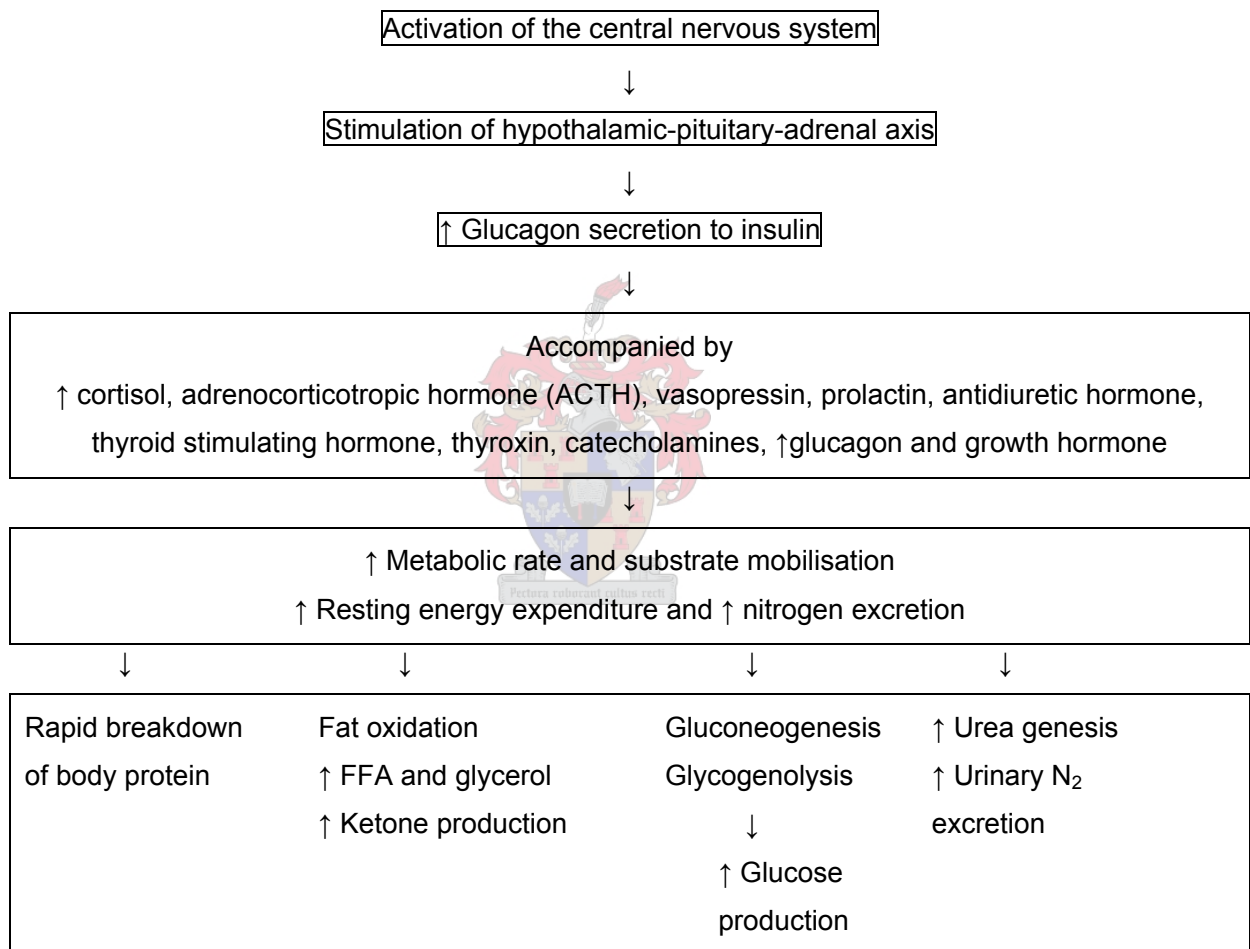
Table 1.1 Cuthbertson's definition of the acute phase response ⁽²⁾

Ebb Phase	Flow Phase
Hypometabolic	Hypermetabolic
Shock	↑ Catabolism, especially protein
↓ Energy Expenditure	↑ Energy Expenditure
↓ Cardiac output	↑ Cardiac output
↓ O ₂ Consumption	↑ O ₂ Consumption
↓ Tissue perfusion	↑ Glycogenolysis
↓ Blood pressure	↑ Gluconeogenesis
↓ Core temperature	↑ Core temperature
Normal glucose production	↑ Glucose production
↑ Blood glucose	Normal or ↑ Blood glucose
↑ Glucagon	↑ Glucagon
↓ Insulin concentration	↓ or ↑ Insulin concentration
↑ Catecholamines	↑, ↓ or normal catecholamines
Hypovolaemic shock	Insulin resistance
Cold and clammy extremities	Warm extremities
Fluid and electrolyte maintenance	Nutritional support
Mediated by central nervous system	Mediated by central nervous system and cytokines

1.2 PATHOGENESIS OF SEPSIS

Severe sepsis and septic shock are life-threatening complications of infections and the most common cause of death in intensive care units ⁽³⁾. The innate immune system is the first line of defence against infection and is activated when a pathogen crosses the host's natural defence barriers ⁽⁴⁾. The initial stress response that follows injury and infection, is hormonally or endocrine-driven (Table 1.2), followed by a cytokine mediated response that trigger the acute phase response.

Table 1.2 Neuroendocrine and hormonal events following injury/infection ^(5,6,7,8)



Innate immune responses must be tightly regulated as unbalanced inflammatory and immune reactions can result in either uncontrolled microbial growth or devastating inflammatory responses with tissue injury, vascular collapse and multi-organ failure ⁽⁹⁾. The pathophysiology of sepsis is characterised by a systemic inflammatory response reaction and concurrent activation of the host's compensatory anti-inflammatory response mechanisms. Strongly activated phagocytes and high levels of pro-inflammatory cytokines (Interleukin-1, Interleukin-6 and tumour necrosis factor α) occur in patients at risk of developing circulatory shock and multiple organ

dysfunctions. Extensive anti-inflammatory reaction caused by counter-inflammatory cytokines (Interleukin-4 and Interleukin-10) renders critically ill patients prone to secondary infections ⁽¹⁰⁾. It is thus of vital importance that the systemic inflammatory response syndrome (SIRS) and the compensatory anti-inflammatory response syndrome (CARS) and its components are in careful balance and well-controlled to lead to inflammation, repair and recovery. The evidence demonstrating the importance of nutritional measures in preventing and enhancing recovery from infection is encouraging ⁽¹¹⁾. Prevention remains the key and it has been shown that achieving the optimal fluid balance and oxygen delivery in all intensive care unit (ICU) patients during the initial “golden hours” will significantly improve outcome from severe sepsis ⁽¹²⁾.

1.3 BIOCHEMICAL MEDIATORS OF METABOLISM

Cytokines are involved with signalling between the cells of the immune system and in modifying the metabolism ⁽¹³⁾. The primary pro-inflammatory cytokines ⁽¹³⁾ are:

Interleukin-1 α and β (IL-1) causing fever, hypotension, increased glutamine transport, decreased gastro-intestinal glutamine utilization, increased ACTH release, increased insulin/glucagon release, inflammation, acute phase protein synthesis ⁽¹⁴⁾. **Interleukin-6 (IL-6)** causing fever, increased β cell proliferation, increased β cell immunoglobulin synthesis, increased acute phase protein synthesis, increased prostaglandin production, mediator of the acute phase response ⁽¹⁴⁾. **Tumour necrosis factor α (TNF α)** causing fever, cardiovascular collapse, increased glutamine transport, decreased lipopolisaccharide lipase activity, increased acute phase protein synthesis, increased collagen degradation, activates hypothalamic-pituitary-adrenal axis, induces interleukin-1 ⁽¹⁴⁾. These cytokines cause the main features accompanying injury/infection ^(14,15)

- Fever, loss of appetite, lethargy, weight loss
- Stimulation of synthesis of nitric oxide to damage the cellular integrity of the invading organism
- Production of reactive oxygen species to kill the invading organism
- Creating a hostile environment for pathogens
- Stimulation of the pituitary and adrenal glands

1.4 THE ACUTE PHASE RESPONSE AND MACRONUTRIENTS

Although the immune system plays a protective role within the host, some of the effects of the system have the potential to damage the host. In adults, prolonged infection will lead to large losses of muscle and adipose tissue and depletion of the micronutrient stores, as endogenous materials act as substrates for the immune system. Injury and infection result in fever and wasting of peripheral tissues. The wasting process facilitates the delivery of nutrients to the immune system, assists tissue repair, controls cytokine production, protects healthy tissue and removes from the bloodstream nutrients that may assist in the multiplication of pathogens. Thus, tissue-wasting is beneficial insofar as it facilitates the operations of the immune system in destroying pathogens and protecting the host ⁽¹³⁾. The cascade of events called the acute phase response eventually results in increased release of catabolic hormones and thus an increase in energy expenditure.

1.4.1 Energy Metabolism

An increase in resting energy expenditure (REE) occurs in trauma patients. Possible reasons for this increase are the following: a) Increased utilization of O₂ by injured tissue; b) increased energy expenditure by other organs; c) increased substrate recycling representing a net energy drain.

This hypermetabolism is mainly caused by cytokine activation, as well as energy dependent activation of metabolic pathways. REE is also influenced by pain, agitation, increased muscular tone, medication, supportive therapy and fever ⁽¹⁴⁾.

1.4.2 Protein Metabolism

In the acute phase, an increased loss of body protein, increased protein degradation, increased amino acid catabolism and N₂ loss occurs. Prolonged immobility of patients may exacerbate atrophy of skeletal muscle and so further contribute to nitrogen loss and a negative nitrogen (N₂) balance, even if total protein synthesis is increased ⁽¹⁶⁾. Amino acids, especially alanine and glutamine are mobilized from skeletal muscle and serve as substrates for the synthesis of acute phase proteins and for gluconeogenesis ⁽¹⁷⁾.

These acute phase proteins have the following main functions: ⁽¹⁸⁾

- Promoting tissue repair
- Assisting the host in adaptive defence
- Transporting antioxidant proteins
- Controlling tissue damage
- Inhibiting serine proteinases

Severe depletion of lean body mass is associated with an increase in morbidity and mortality in intensive care patients. The two most important factors determining the extent of protein loss are:

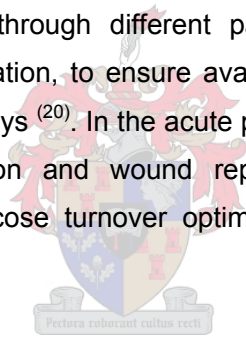
1) nutritional status prior to injury and 2) control of the inflammatory response as effectively and timeously as possible.

1.4.3 Carbohydrate Metabolism

In the acute phase, various degrees of hyperglycaemia, decreased glucose tolerance and insulin resistance occur as a result of increased glycogenolysis and non-suppressible gluconeogenesis from substrates that are mobilized peripherally ⁽¹⁷⁾. New glucose enters the plasma via the following routes: ⁽¹⁹⁾

- 1) External sources e.g. gastro-intestinal tract or intravenously,
- 2) Endogenous production from glycogen e.g. glycogenolysis,
- 3) Conversion of lactate in the Cori cycle,
- 4) Production from amino acids e.g. gluconeogenesis and
- 5) Synthesis from pyruvate.

The increase in hepatic glycogen breakdown and associated reduction in peripheral use of glucose, results in an increase in plasma glucose concentration and an increase in insulin release ⁽¹⁹⁾. Hepatic glucose production through different pathways remains increased, despite an increased blood glucose concentration, to ensure available glucose for the glucose dependent tissues such as the brain and kidneys ⁽²⁰⁾. In the acute phase, glucose becomes a primary fuel for the cells involved in inflammation and wound repair, and is predominantly metabolised anaerobically. This increased glucose turnover optimises host defences and ensures wound repair ⁽²¹⁾.



1.4.4 Lipid Metabolism

During the acute phase response, lipolysis of triglycerides is increased ⁽²⁰⁾. This results in production of free fatty acids (FFA) and glycerol. The glycerol can be used by for gluconeogenesis by the liver and the FFAs can be used as a fuel source ⁽¹⁹⁾. A high rate of fat oxidation is sustained or accelerated in seriously ill patients, which suggests that some fat is oxidized directly in tissue in which lipolysis occurs, because of the hypoperfusion of adipose tissue ⁽²²⁾. This increase in fatty acid oxidation is not substrate led, which suggest changes in intracellular fat metabolism ⁽²³⁾. This preference for fat as an energy substrate is more pronounced in septic than in trauma patients ⁽²⁴⁾. Cellular uptake of medium- and long-chain fatty acids is increased in infected and traumatized patients, suggesting an increased turnover rate relative to plasma concentration ⁽¹⁷⁾. Ketone bodies can serve as alternative energy substrates for many tissues, as they reduce whole-body glucose demand and gluconeogenesis from protein. In severe sepsis this response is blunted and nitrogen conservation is not optimal. Hepatic ketone production is increased, but plasma levels remain low, most likely because of increased insulin levels ⁽²⁵⁾.

1.4.5 The acute phase response and micronutrients

Micronutrients are intermediaries in metabolism and play potential roles in wound healing, cellular immunity and antioxidant activity. The micronutrients can be classified as follows:

Water-soluble vitamins:

They mainly act as co-enzymes in protein and energy metabolism.

Fat-soluble vitamins:

They are intermediaries in various cellular functions, differentiation and proliferation of cells, skeletal formation, immune function, antioxidant activity and coagulation.

Macrominerals and trace elements:

They acts as co-enzymes in various enzymatic reactions in the body ⁽²⁶⁾.

Characteristics of micronutrients are:

- They exist in pools in the body.
- They are bound to carrier proteins.
- An altered distribution occurs in the acute phase response.

The acute phase response/inflammation increases requirements of vitamins A, E, C, D, folate and B₆ and causes a decrease in plasma zinc, iron, copper and selenium because of a rapid redistribution that is mediated by cytokines. This redistribution is characterised in the body by decreased levels of serum-binding proteins (albumin, transferrin, retinol binding protein, and macroglobulin) ⁽²⁶⁾. The decrease in free circulating zinc and iron may have a beneficial effect for the host. Zinc is redistributed for tissue repair at the site of injury, protects the liver, can act as a co-factor for acute phase protein synthesis and can increase bactericidal capability ⁽¹⁷⁾. Iron is also moved into storage to decrease its availability in plasma for bacteria use, reduces oxidative damage to membranes or DNA by decreased free radical formation and thus plays a protective role in the host ^(27,28). Copper concentrations, on the other hand, rise in the acute phase as a result of the increased synthesis of its carrier protein, ceruloplasmin. A proposed benefit of increased ceruloplasmin concentration is that it may play a role in iron transport as a result of its ferro-oxidase activity ⁽²⁹⁾. Requirements of micronutrients are further elevated in critical illness and the acute phase response because of increased urinary, cutaneous and plasma losses, decreased bio-availability, disruptions in homeostasis, decreased gastro-intestinal absorption and an increased free radical formation and thus an increased requirements of antioxidants. It is therefore very important to closely monitor patients who are already depleted/malnourished and those with ongoing losses, as they have a high risk of developing micronutrient deficiencies.

As the precise requirements for micronutrients in the critically ill are not known yet, ⁽³⁰⁾ relatively conservative supplementation protocols together with astute interpretation of clinical and biochemical indices of micronutrient nutriture are recommended ⁽¹⁴⁾.

1.5 THE ROLE OF NUTRITION IN THE ICU SETTING

Factors contributing to the continuing high prevalence of malnutrition in the critical care setting include the aging of the population, the higher acuity level of patients seeking care and the treatment of chronic diseases. These factors are coupled with the continuing lack of attention to the nutritional status of patients at the time of admission ⁽³¹⁾. Resuscitation is the first priority in the management of injury, followed by specific treatment. A major factor that improves outcome in trauma, or injury in general, is the preservation of nutritional status ⁽⁶⁾. Critical illness has a significant impact on the nutritional status ⁽³²⁾. Post-injury hypermetabolism leads to malnutrition much more rapidly than simple starvation, and consequently nutritional support is an important part of the overall management of such patients ⁽²⁰⁾. It is known that appropriate nutrition support is positively associated with successful recovery ⁽³³⁾. It is imperative that nutrition support practitioners are able to identify a patient that is nutritionally at risk and have an understanding of the metabolic response to injury to intervene with specialized nutritional support during a prolonged course of hypermetabolism, immobilization and healing ⁽¹⁴⁾.

1.6 NUTRITIONAL STATUS ASSESSMENT IN THE ICU SETTING

Intensive care patients are a unique group, represented almost always by hypermetabolic individuals who usually suffer from acutization of previous illness, which could have compromised their nutritional status, or acute trauma. In any of the aforementioned situations, the nutritional status should be assessed, especially if nutritional therapy is foreseen to be necessary. It is difficult to assess the nutritional status of ICU patients, since their management usually includes ventilation, various drugs and abrupt and significant shifts in water between compartments ⁽³⁴⁾.

Unlike starvation or undernutrition, where the loss of protein is minimized by its reduced utilization as a source of energy, in hypercatabolic patients (post-operative, sepsis or polytrauma), protein catabolism occurs to provide energy and to support protein synthesis. Both visceral and muscle protein are broken down to provide fuel and metabolic substrate; the more severe and prolonged the hypermetabolic state, the greater the chances of malnutrition ⁽³⁵⁾. Thus, most ICU patients are at imminent risk of developing malnutrition and should have their nutritional status routinely assessed. However, at the moment there is no available test that is both sufficiently sensitive and specific for the assessment of malnutrition in critically ill patients (Table 1.3) ⁽³⁴⁾

Table 1.3: Tools for nutritional status assessment in ICU patients ⁽³⁴⁾

Method	Characteristic	Limitations
Anthropometry (Triceps skinfold; mid-upper arm circumference, body mass index (BMI))	Objective data Inexpensive Loss of body weight has been related to morbidity and mortality	Accuracy of weight loss not precise Oedema alters measurements Error factors inter and intra observers Comparative tables derived from healthy populations
Body Composition tests (DXA, BIA and others)	Define body composition dividing it in compartments BIA is good for clinical studies in ICU patients, but not accurate for one given individual	Difficult to perform in ICU patients Mostly expensive
Functional tests (Grip strength and respiratory muscle strength)	Represent cell ion uptake Linked to cell energetics	Muscle relaxants and other drug interferences
Immune tests (Lymphocyte count)	Express delayed cutaneous hypersensitivity Inexpensive	Situations that cause anergy influence results
Laboratory tests (Albumin, transferrin, prealbumin, prognostic inflammatory and nutritional index (PINI))	Dependent on liver metabolism half-lives of 21, 7 and 2 days Correlation between low concentrations and morbidity and mortality In acute stress albumin is usually ↓ due to ↑ degradation, transcapillary losses, fluid replacement and ↓ synthesis	Influenced by renal liver function In chronic malnutrition states, blood levels are usually normal Poor tools to assess nutritional deficiencies and measure effectiveness of nutritional support

Table 1.3: Tools for nutritional status assessment in ICU patients (cont.) ⁽³⁴⁾

Method	Characteristic	Limitations
Nitrogen excretion	Assess protein metabolism Estimates daily protein losses reasonably accurately Inexpensive	Inaccurate if counterregulatory hormones are negative Demands good nursing protocols for 24 hour urine collections
Subjective global assessment	Clinical Good sensitivity and specificity Inexpensive Identifies risk factors for malnutrition	Depends on patient's and family's cooperation Subjective Demands good training of interviewer Not initially described for ICU patients
Physical examination	Identifies muscle weakness Identifies signs of nutritional depletion	
Indirect Calorimetry	Accurate estimate of nutritional requirements through energy expenditure Convenient Considered to be the gold standard	Trained personnel to operate Expensive Not readily available

1.7 EARLY ENTERAL FEEDING IN THE ICU SETTING

It is through the realization of the importance of the supportive role of nutrition in the critically ill patients that has led to significant improvements in nutrition support practices including early enteral feeding (EEF). Research indicates that delaying administration of nutrition to patients who have multiple trauma can have potentially life threatening complications, including sepsis and the multiple organ dysfunction syndrome (MODS). Although stress induced hypermetabolism cannot be averted, the detrimental hypermetabolic effects and septic complications can be attenuated by delivering early enteral nutrition soon after the acute injury has occurred and before acute protein malnutrition ensues, lean body mass is lost beyond the levels which are compatible with survival and bacterial translocation occurs ⁽³⁶⁾ (Table 1.4). Most nutrition researchers advocate nutritional delivery within 72 hours after injury to facilitate improved clinical outcomes ⁽³⁷⁾. Later studies show EEF can be defined as starting nutrition as soon as 6 - 36 hours after injury.

Table 1.4 Benefits of early enteral feeding ^(38,39,40,41,42)

In the critically ill, it attenuates the stress response
Secretion of acute phase proteins is blunted and ↑ levels of synthetic proteins occur
↓ Counterregulatory hormone and C-reactive protein after exposure to endotoxin
↓ Rate of catabolism and energy expenditure
Improved gallbladder contraction, development of gallstones and acalculous cholecystitis less likely
↑ Pancreatic stimulation and ↓ functional inefficiency
Improved gut healing after surgical anastomosis
Beneficial in major burns if fed within 48 hours
Improves host immune function and organ function
↑ Cellular antioxidant systems
Preserves intestinal mucosal integrity
Improved patient outcome in trauma: ↑ Nitrogen balance, ↑ protein synthesis, ↑ wound healing and ↓ infection rates

Various studies have shown benefit with EEF and the malnourished patients need to be fed as soon as possible to prevent further complications. Patients can be fed gastrically without such major complications as vomiting, diarrhoea, aspiration or delayed gastric emptying. Parenteral supplementation can be given if the full requirements are not met via the enteral route ⁽⁴³⁾. EEF should be the first choice after gastrectomy or pancreaticoduodenectomy. Patients fed early (6 hours post-operatively) into the jejunum showed no anastomotic breakdown, despite proximal infusion of nutrients and no adverse effects in the absence of a decompression tube and no aspiration. Enteral nutrition did accomplish the nutritional goals for these patients ⁽⁴⁴⁾. Similar results have been reported for postoperative gastro-intestinal surgery patients who were fed duodenally or jejunally and septic complications and length of stay were reduced in this group of patients ⁽⁴⁵⁾. On the other hand, a study done by Ibrahim *et al.* in 150 medical ICU patients did not show positive results in terms of EEF. Patients were fed orogastrically and divided into 2 groups, namely early feeders (Day 1) and late feeders (Day 5). The early group showed an increase in incidence of ventilator-associated pneumonia, an increase in days of ventilation, more antibiotic days, increased length of stay, and an increase in *Clostridium difficile* diarrhoea. There was no difference in the incidence of hospital mortality between the 2 groups and both groups failed to

reach their nutritional goals ⁽⁴⁶⁾. The statement that EEF is safe for every patient in the ICU must be evaluated with care, as it seems that the literature is not yet convincing in this regard and that clear indications and contra-indications are to be considered (Table 1.5).

Table 1.5 Indications and contra-indications for early enteral feeding ⁽⁴⁷⁾

Indications	Contra-indications
Haemodynamically stable	Haemodynamically unstable
Stable spinal cord injuries on a vasopressor to maintain vascular peripheral tone	Patients requiring substantial amounts of inotropic agents, vasopressors and norepinephrine
Resuscitated septic patients	Patients requiring massive fluid resuscitation
Caution in abdominal distension – monitor patient closely for intolerance	Abdominal distension due to peritonitis
Proximal fistulas where a feeding tube can be placed beyond the fistula	High output fistula
Pancreatitis can be fed distal to the ligament of Treitz	Bowel ischemia
Head injuries without gastric ileus. If gastric ileus; endoscopic placement of feeding tube into the duodenum	Gut perforation
Caution in patients with pseudo-obstruction of the colon – monitor patient closely for intolerance	Mechanical obstruction of the GIT
Patients with postoperative ileus can be fed into the small bowel	Don't continue feeding if patients have not passed stools in 3 weeks
Patients with bowel anastomosis	
Burn Patients	

Current data is convincing regarding the benefits of early enteral feeding in surgical ICU and trauma patients, but more clinical trials are needed for conclusive evidence regarding medical ICU patients. EEF does decrease episodes of infection (direct impact) and septic and non-septic complications, resulting in an improved outcome. There is a trend towards a decrease in length of stay (secondary impact), but other factors make it difficult to attribute it to nutrition *per se*. It is of

the utmost importance to choose the patient who is most likely to benefit from EEF carefully and above all, to do no harm.

1.8 IMMUNONUTRITION

The role of certain nutrients that seem to have pharmacologic effects on immune and inflammatory parameters has been studied over the last two decades. This area of research is called immunonutrition ^(48,49). Nutrition support may have a modulating effect on the underlying illness by its salutary effect on the immune system and organ function. In this context, immunonutrition is appealing as a novel approach to favourably modulate the immune (dys)function associated with critical illness. The concept of “immunonutrition” has been developed to supply specifically defined substrates that promote certain biochemical pathways as they become depleted due to their extensive consumption ⁽⁵⁰⁾. Several specific substrates with immunological effects have been added, alone or in combination, to standard enteral products in an attempt to modify the immune response of patients. The number of these key nutrients, also called nutraceuticals or pharmaconutrients, is now increasing but glutamine, arginine, ornithine α -ketoglutarate (OKG), omega-3 (n-3) polyunsaturated fatty acids [eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA)] and nucleotides seem to play a primordial role in the regulation of immunological and inflammatory responses in critically ill patients (Tables 1.6 – 1.10). Recommendations regarding the use, dosages and possible contra-indications for the three immunonutrients mostly used, namely glutamine, arginine and n-3 fatty acids are outlined in Table 1.11. Immunonutrition enteral formulae have been used and researched in several studies (Tables 1.12 – 1.13) trying to demonstrate their beneficial effect on laboratory, immunological and clinical parameters in comparison with standard formulae in the critically ill ⁽⁵¹⁾.

Table 1.6 The functions and potential beneficial effects of Glutamine
(52,53,54,55,56,57,58,59,60,61,62)

FUNCTIONS AND CHARACTERISTICS
On the gut lumen interface: Glutamate transported in large amounts and used in oxidative processes or synthesis of glutathione, arginine, proline and citrulline. Gut utilises glutamine but cannot synthesise it
On the arterial interface: Glutamine is the major transported substrate across cell membranes. Arterial uptake is important for synthesis purposes
Dietary glutamine is utilised by the enterocytes and gut associated lymphoid tissue (GALT). An increased glutamine requirement by immune cells outside the gastro-intestinal tract (GIT) that must be met by the systemic circulation
Glutamate is used for: 1) the production of the antioxidant glutathione, 2) arginine synthesis and 3) transamination to α -ketoglutarate in the production of alanine
A conditional deficiency develops as endogenous supply cannot match the increased demand of glutamine in trauma and/or sepsis
↑ Requirement in ICU patients, as free glutamine pool is depleted in trauma and/or sepsis
Regulates purine, pyrimidine and nucleotide synthesis and ↑ DNA and protein content
Significant contributor to gluconeogenesis
Plays a role in acid-base balance in kidney (counteracts acidosis)
Central position in nitrogen-homeostasis in the liver
↑ Intestinal mucosal thickness (↓ atrophy) and ↓ bacterial translocation
Energy substrate for enterocytes and immune cells
Regulation of intracellular water content in skeletal muscle
Parenteral route seems to give a greater treatment effect, but both parenteral and enteral supplementation is advised
No evidence of harm demonstrated in glutamine supplementation in critically ill
Studies show ↓ mortality, ↓ complication rate and ↓ length of hospital stay

Table 1.7 The functions and potential beneficial effects of Arginine (63,64,50,56,65)

FUNCTIONS AND CHARACTERISTICS
Endogenous synthesis through Urea Cycle
Precursor of polyamine, histidine and nucleic acid synthesis
Promoter of thymic growth
Stimulates release of growth hormone, prolactin, insulin and glucagon
Metabolised to ornithine and urea and to citrulline
Effect of arginine is on parameters of cellular defence function, presumably by means of constitutive nitric oxide synthase (cNOS) mediated nitric oxide (NO) formation
Acts as substrate for NO synthesis which enhances the inflammatory response through unbalanced NO release
Basal NO production important in mucosal barrier dysfunction, but more advanced SIRS / sepsis may cause injury or dysfunction of the intestinal mucosal barrier
Enhances wound collagen synthesis
Preservation / enhancement of T lymphocytes (restores immune cell function)
Reduces protein catabolism during stress
Enteral arginine ↑ protein synthesis, woundhealing and net N ₂ retention
Precursor of growth factors e.g. spermine and spermidine
Through formation of glutamine yield ↑ amounts of proline and hydroxyproline for connective tissue synthesis
Studies show no direct positive effect on mucosal integrity
Studies have shown enhancement of depressed immune response of individuals suffering from injury, surgical trauma, malnutrition and sepsis

Table 1.8 The functions and potential beneficial effects of ornithine α -ketoglutarate (OKG) ^(66,67,68,69)

FUNCTIONS AND CHARACTERISTICS
Suggested to be a gut nutrient
Potentially a muscle catabolism reducing agent when given parenterally
Precursor to glutamate and glutamine synthesis in muscle
Central part of the tricarboxylic cycle
Enteral OKG metabolised directly in the intestinal mucosa to glutamine and provision of energy without \uparrow N ₂ load
Favourable effects on muscle protein synthesis (trauma and burns)
Sparses glutamine in plasma, liver, muscle and stimulates anabolic pathways through insulin and growth hormone secretion
Mostly animal studies that have shown positive glutamine sparing results
Easy administration
Wiren <i>et.al.</i> (2002) showed no benefit of giving OKG enterally via a jejunostomy postoperatively for 5 days after elective major abdominal surgery
No effect on length of stay (LOS) and rate of infectious complications
Clinical impact requires confirmation through controlled human trials
In the very catabolic patient: 30g/day. Higher doses cause diarrhoea

Table 1.9 The functions and potential beneficial effects omega-3 polyunsaturated fatty acids (EPA / DHA) ^(70,71,72,49,56)

FUNCTIONS AND CHARACTERISTICS
↓ Magnitude of inflammatory response through influence on function of specific and non-specific immune cells
Arachidonic acid (n-6 fatty acid) is a substrate for bioactive eicosanoids that are pro-inflammatory and that generate O ₂ radicals which causes oxidative damage
n-3 fatty acids act as arachadonic acid antagonists
n-3 compete with n-6 fatty acid for production of eicosanoids to form EPA eicosanoids that are less biologically active
EPA eicosanoids: ↓ production of pro-inflammatory cytokines (IL-1; IL-6: TNF)
EPA eicosanoids involved in modulating the intensity and duration of the inflammatory and immune response
A sensitive balance must exist between n-6 and n-3 fatty acids. Normally the ratio of n-6:n-3 is between 2:1 to 8:1.
Used as therapy for acute / chronic inflammation and inappropriately activated immune response
Act as intra- and intercellular fuel practitioners – lipid metabolism shifted from storage to supply
↓ Platelet aggregation and thrombogenesis, thus may predispose to vasodilatation and prolonged bleeding time
Studies showed ↑ immunity, but no change in clinical outcome, post-op infection, N ₂ balance or delayed cutaneous hypersensitivity
Could be beneficial in patients with, or at risk of, developing acute respiratory distress syndrome (ARDS)
Studies are small and done in combination with other immunonutrients, so single effect is not as clear

Table 1.10 The functions and potential beneficial effects of Nucleotides ^(73,74)

FUNCTIONS AND CHARACTERISTICS
Nucleotides are elements for synthesis of DNA, RNA and ATP
Absence of nucleotides results in loss of T helper lymphocytes and suppression of IL-2 production
Deficiency results in ↓ cellular immunity and ↓ resistance to infection
Immunostimulant properties on natural killer cells and T lymphocytes
Crucial for restoration of intestinal function and immune status
If protein intake is adequate, de novo synthesis occurs
↑ Demands in injury occurs
Little evidence in humans, more studies in animals
Studies are small and done in combination with other immunonutrients, so single effect is not as clear



Table 1.11 Indications, dosages and contra-indications for specific immunonutrients
(75,76,50,77,78)

ARGININE	GLUTAMINE	n-3 FATTY ACIDS
Indications	Indications	Indications
Elective surgery patients	Multiple trauma, sepsis, critically ill	Ventilated patients, critically ill
Potentially beneficial in other groups of ICU patients than mentioned in the contra-indications	Burns	Possibly ARDS Above have been found in product with borage oil, fish oil and anti-oxidants
Potentially indicated in renal failure	Pre- and post surgery	
Dosage	Dosage	Dosage
> 12g/L required per day. Intakes of 30g/day showed no adverse effect.	30 – 50 g/day or 0,3 - 0,65 g/kg/day (enteral) 0,3 – 0,57 g/kg/day (parenteral) Intakes of up to 0,57 g/kg/day considered being safe.	Optimal ratio: n-6:n-3 = 2:1 – 5:1 1g EPA/DHA per day
Contra-indications	Contra-indications	Contra-indications
Patients with SIRS, severe sepsis and multiple organ failure	Hepatic failure (total bilirubin > 10mg/dl)	Very few studies on single nutrient
Possibly hepatic failure (to be confirmed)	Renal impairment (creatinine clearance < 30ml/min)	Possibly longer bleeding times

Table 1.12 Recent reviews, meta-analyses and studies of immunonutrients ^(79,80,81)

<p>Beale <i>et al.</i> (1999) ⁽⁷⁹⁾</p> <p>Review of 15 randomised trials of critically ill patients.</p> <p>Impact and Immun-aid used (arginine / nucleotides / fish oils)</p> <p>Overall benefit for reducing infection rate, ventilator days and hospital length of stay (LOS) in critically ill. Benefit most marked in surgical group of patients.</p> <p>No evidence of detrimental effect</p>	<p>Galban <i>et al.</i> (2000) ⁽⁸⁰⁾</p> <p>Randomised, multicentre, unblinded trial.</p> <p>181 septic ICU patients (Impact used) with APACHE II score of ≥ 10 at baseline.</p> <p>Significant \downarrow ICU mortality.</p> <p>Treatment effect evident in patients with APACHE 10 – 15.</p> <p>No mortality advantage with higher APACHE scores at baseline.</p> <p>\downarrow frequency of bacteraemia and \downarrow rate of repeated nosocomial infections.</p> <p>No change in overall ICU acquired infectious morbidity and length of ICU stay.</p>	<p>Heyland <i>et al.</i> (2001) ⁽⁸¹⁾</p> <p>Systematic review of 22 descriptive human randomised trials.</p> <p>Elective surgery, critically ill with severe trauma, critically ill in ICU and critically ill with severe burns patients was studied.</p> <p>2 or more immunonutrients (glutamine, arginine, n-3, nucleotides)</p> <p>Aggregated results:</p> <p>No mortality advantage, fewer infectious complications and shorter hospital LOS, but significant heterogeneity in groups.</p> <p>Subgroups analyses results:</p> <p>Formulae \uparrow in arginine (Impact and Immun-aid): not associated with \uparrow mortality. Associated with significant \downarrow in infectious complications and length of hospital stay. These studies had combination of nutrients \rightarrow could be different dose of arginine or other nutrients.</p> <p>Elective surgery: no overall effect on mortality. Significant lower infectious complications and length of hospital stay.</p> <p>Critically ill: no overall effect on mortality, infectious complications, and length of ICU stay or duration of ventilation.</p> <p>Some evidence for harm. Products other than those \uparrow in arginine seem to be associated with \uparrow mortality and trend toward \uparrow complications.</p>
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Table 1.13 Recent reviews, meta-analyses and studies of immunonutrients (cont.) ^(76,51,78)

<p>Canadian guidelines (2003) ⁽⁷⁶⁾</p> <p>RCT's or meta-analyses of RCT's Vented ICU patients (elective surgery excluded) No arginine for the critically ill. Arginine supplemented trial from Keift <i>et al</i> (2003) showed no effect on mortality, complication rates or LOS. 3 randomised trials suggest excess mortality in arginine-supplemented diets in patients with underlying infection ⇒ strong signal not to be ignored. Because of current neutral effect, cost and possible harm, arginine is not recommended. Enteral glutamine indicated in burns and trauma patients. Parenteral glutamine if parenteral nutrition has been prescribed.</p>	<p>Montejo <i>et al.</i> (2003) ⁽⁵¹⁾</p> <p>Review of 26 clinical trials of the critically ill Infectious complication rate: no significant effect. No presentation on overall ratio of infected patients. Cost: 2 studies showed ↓ cost Mortality: no difference in surgical / burn / trauma subgroups, mixed patients ↑ in mortality Trauma: ↓ bacteraemia and intra-abdominal infections, ↓ ventilation, ↓ ICU stay, nosocomial pneumonia, urinary tract infection (UTI), wound infection and hospital stay. Sepsis: no difference. Surgical: ↓ wound infection and UTI, ↓ ICU stay, ↓ hospital stay Burns: ↓ nosocomial pneumonia Mixed group: ↓ bacteraemia Outstanding: best combination of nutrients and heterogeneity of populations remain a problem.</p>	<p>Mc Cowen & Bistran (2003) ⁽⁷⁸⁾</p> <p>An accumulating body of evidence suggests: Immunonutrition must be delivered sufficiently in advance of the surgical insult and that ≥ 800ml / day is required to maximise outcome. Data from Braga <i>et al.</i> (2002) and Senkal <i>et al.</i> (1997, 1999) suggests either that substantial amounts of the supplement must be absorbed before an effect is evident or that the timing of use is the key. Summary: (1) patients undergoing abdominal surgery for CA, especially malnutrition (pre + post-operatively) (2) ICU with APACHE 10 - 20 (3) multiple trauma. (4) arginine > 12g/l (5) duration > 3 days, preferably 5-10 days (6) 25 kcal/kg goal (7) ≥ 800 ml / day.</p>
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Suchner *et al.* (2002) showed that there was improvement in outcome only when critical amounts of the immune-modulating formulae were tolerated in patients classified as being malnourished⁽⁵⁰⁾. Gianotti *et al.* (2002) confirmed this and found that immunonutrition also decrease post-operative infections and LOS when compared to no nutritional support in the well nourished. Pre-operative administration of immunonutrients was found to be as effective as post-operative support⁽⁴⁴⁾. Immunonutrition needs time to influence immune and inflammatory parameters. Alvarez & Mobarhan (2003) showed post-operative immunonutrition seemed to decrease infections and/or LOS and that this effect was most noticeable several days after surgery. Immunonutrition did not prevent the initial adverse effect of surgical trauma on the immune system⁽⁴⁸⁾. In patients with severe sepsis, shock and organ failure, no benefit or even disadvantages were reported. The recommendation is made to exercise great caution when immune-enhancing substrates are used in patients suffering from SIRS, severe sepsis and organ failure⁽⁵⁰⁾. Griffiths (2003) concluded “confusing results do not warrant the universal use of immunonutrients at present”. It should be used in surgical and trauma patients where its clinical benefit has been shown⁽¹¹⁾. Results of trials on immunonutrition are controversial due to a number of reasons:^(51,50,58,82)

- Methodological limitations, e.g. inadequately powered, randomisation techniques, blindness
- Heterogeneity of the studied patient populations
- The same trial methodology are used as for new drugs (treatment not support) which is not practical for nutrition trials
- Hypothesis-generating rather than hypothesis-confirming
- Appropriate mortality and length of stay (LOS) endpoints
- Evidence-based medicine is contradictory, as authors are subjective
- Negative and positive studies should be evaluated
- Focus should be on patient centred outcomes e.g. quality of life
- Intention to treat (ITT) analyses should be included

In order to advance in the knowledge in this field, the evidence-based medicine methodology has to be applied⁽⁵¹⁾.

The way forward in terms of immunonutrition:

The timing of immunonutrition is crucial.

Sufficient amounts of immunonutrients must be given to benefit the patient⁽⁸³⁾.

Must be given for a period of 3 days, preferably for 5-10 days⁽⁸³⁾.

Scepticism needs to be overcome and a balanced approach should be developed.

Economic constraints must be kept in mind, as these formulae tend to be quite expensive.

Appropriate indications and patient populations must be defined and researched.

An individualised approach for patients in the ICU setting remains the golden rule and the blanket use of immunonutrients are not recommended at this stage.

1.9 BACTERIAL TRANSLOCATION

The intestinal epithelium forms the intrinsic barrier that separates the intestinal luminal contents and the surrounding tissue. Loss of this barrier may enhance the movement of intestinal bacteria or toxins across this barrier to local or regional tissue. This phenomenon is termed gut translocation of bacteria ⁽⁸⁴⁾ and is defined as the passage of viable enteric bacteria across the intact mucosa of the gastro-intestinal tract into normally sterile extra-intestinal tissues, e.g. lymph nodes. Animal studies regarding bacterial translocation are numerous, but human studies are few and this limits the recommendations for changes in clinical practice ⁽⁸⁵⁾. There is general consensus that if gastric acid secretion is unimpaired, the resting stomach is frequently sterile or colonized at a density of fewer than 10^3 organisms with specific species ⁽⁸⁶⁾. Similar flora is seen in the upper small bowel, which is sterile in up to 80% of individuals ⁽⁸⁷⁾. Therefore the indigenous flora of the gastro-intestinal tract in normal individuals exerts an important influence on immunological homeostasis: ⁽⁸⁸⁾

- Local immunity regulates growth of indigenous flora.
- Local immunity prevents adherence to enterocytes and colonization by enteric pathogens.
- Systemic immunity modifies the host's response to enterically administered antigens through the development of natural antibodies e.g. secretory IgA.
- Gastric acidity, pancreatobiliary secretion, intestinal immunological and intestinal peristalsis maintain microbiological gut ecology ⁽⁸⁹⁾.

Translocation of bacteria can probably occur throughout the small and large intestine, but evidence suggests that the distal ileum and cecum are sites associated with perhaps the greatest amounts of translocation. Translocation can occur because of direct injury or indirect injury to the mucosa and there are several predisposing factors (Table 1.11): ⁽⁹⁰⁾

A study by Sedman confirms that bacterial translocation does occur in humans, but sheds little light on its clinical significance. They also found that intestinal barrier function is not the most important determinant and that translocation is associated with a higher incidence of postoperative sepsis ⁽⁸⁵⁾. More recent studies have shown the gut barrier to be more of a functional than anatomic concept and that the major promoting mechanisms for bacterial translocation is the following: ⁽⁸⁹⁾

- Intestinal bacterial overgrowth
- Altered permeability of the intestinal mucosa
- Changes in villous architecture
- Deficiencies in host immune defences

Table 1.11 Causes of bacterial translocation ⁽⁹⁰⁾

Direct injury to the mucosal cells
• Irradiation
• Inhibitors of cell replication e.g. cyclophosphamide, methotrexate
• Chemicals
Indirect injury
• Reduced blood flow e.g. hypovolaemic shock, vasoconstrictors, endotoxin, intestinal ischemia and thermal injury
• Heat stress
Diseases associated with ulceration
• Crohn's Disease
• Ulcerative colitis
• Intestinal obstruction
• Malignant disease of the mucosa
Predisposing factors
• Immunosuppressive drugs
• Antibiotics causing alterations of the intestinal mucosa
• Malnutrition, not causative, but contributing
• Immune compromise e.g. trauma

There is much evidence from animal studies to support all these proposed mechanisms, but in human studies it is only the alterations in gastro-intestinal microflora, that has been shown to directly correlate with microbiologically confirmed bacterial translocation ⁽⁹¹⁾. Immune-enhancing substrates also appear to play a role in experimental studies in reducing bacterial translocation by activation of the immune response. As integrity of the mucosal barrier is the major determinant of translocation, measures taken to protect this integrity include administration of nutrients to reduce bacterial translocation and related complications ⁽⁹²⁾. Many studies have established an association between gastro-intestinal microflora and nosocomial infection, supporting the concept of the gut as a reservoir of bacteria and endotoxins. However, the evidence that bacterial translocation is the mechanism that accounts for this association between enteric organisms and subsequent sepsis remains, at least in humans, largely circumstantial ⁽⁹³⁾. The two most commonly cited factors representing alterations in intestinal barrier function are: 1) Changes in

villous structure and 2) intestinal permeability. There is no evidence to support the view that short-term absence of luminal nutrients (parenteral feeding versus enteral feeding) in humans will result in “feeding-induced” intestinal atrophy. Furthermore, there is also no evidence to support the view that changes in villous architecture *per se* will inevitably result in bacterial translocation. There is consensus that alterations in intestinal permeability do occur in ill patients. Although this must reflect one aspect of the intestinal barrier, there is no evidence that it is causally associated with translocation⁽⁹³⁾. There are a number of reasons why the gastro-intestinal tract has become one of the major foci in our search for explanations of why ICU patients get sick and die:⁽⁹⁴⁾

- Most infections in critically ill seem to be due to gut-derived microorganisms.
- Enteral feeding seems to reduce the incidence of infectious complications in some subsets of patients.
- Optimising splanchnic blood flow is associated with a decreased complication and death rate.
- Selective gut decontamination seems to reduce infections.
- Alterations in gut permeability to larger molecules in critically ill patients.
- The gut contains as much immune tissue as the rest of the body and modifications in gut immune function may be the single most important factor in the development of sepsis syndrome and organ failure.

However, it is very important to distinguish between the importance of the gastro-intestinal tract in the development of sepsis syndrome and the assumption that bacterial translocation is the primary cause⁽⁹⁴⁾. It now seems clearer that the gut plays a role in the development of sepsis and multiple organ failure (MOF), but that bacterial translocation is not a likely cause. More likely, it seems to be the alterations in the gut's immune function and the interaction between gut-associated immune tissue and the rest of the body.

1.10 NOSOCOMIAL INFECTIONS IN THE ICU

Ventilator-associated pneumonia (VAP) is the most common nosocomial infection in the intensive care unit. It is a pulmonary infection, caused mostly by *Staphylococcus aureus* and Gram-negative opportunists, which occurs after at least 48 hours of intermittent positive-pressure ventilation (IPPV) and is a leading cause of mortality and morbidity⁽⁹⁵⁾ (Table 1.12).

Risk factors for the development of nosocomial pneumonia are:⁽⁹⁶⁾

- Patients requiring mechanical ventilation
- The loss of the protective coughing and sneezing reflexes due to sedation or decreased level of consciousness
- Antibiotic therapy
- Invasive procedures where upper respiratory tract bacteria can be transferred to the lower airways

Factors related to the clinical course of the patient, rather than variables on the first day in ICU, have the greater influence on the development of nosocomial pneumonia ⁽⁹⁷⁾. Other variables associated with an increased risk of development of nosocomial pneumonia in trauma patients include: 1) H₂ receptor blocker use 2) Decreased consciousness 3) Prophylactic anti-microbial use 4) Massive gastric aspiration 5) Prolonged mechanical ventilation (≥ 24 hours) 6) Corticotherapy 7) Re-intubations 8) Tracheostomy and 9) Continuous enteral feeding ⁽⁹⁷⁾. However, further studies have shown that intermittent enteral feeding results in a small increase in intragastric pH without influencing rates of colonization and infection in the respiratory tract and is less well tolerated than continuous enteral feeding ⁽⁹⁸⁾.

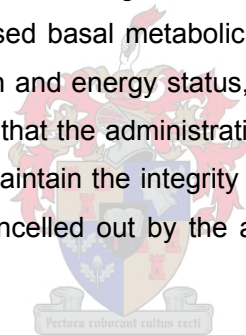
Table 1.12 Sources of pathogens in nosocomial pneumonia in the ICU patient ^(96,99)

<p>Aspiration of pathogens from the oropharynx</p> <p>The most important source of bacterial pneumonia, especially in ventilated patients who have a increased risk of aspiration of these pathogens.</p>
<p>Colonization of the oropharynx</p> <p>It increases the risk of developing pneumonia and Gram-negative bacilli replace the normal flora if the patient receives antibiotics.</p>
<p>Colonization of the stomach</p> <p>It occurs if patients receive drugs to suppress gastric acid secretion to prevent stress ulceration.</p>
<p>Endotracheal and tracheostomy tubes</p> <p>Irritation of the respiratory mucosa occurs and promotes Gram-negative colonization of the oropharynx. Contaminated secretions enter the trachea from the mouth and pharynx through secretions seeping down the trachea.</p>
<p>Contaminated ventilator circuits</p> <p>Cross-infection by delivering bacteria-laden air directly to the lower airways.</p>
<p>Nebulisers</p> <p>Aerosols of minute droplets penetrate deeply into the narrowest airways.</p>
<p>Humidification</p> <p>The condensate in the tubing can become heavily contaminated and can drain into the trachea, increasing the risk for infection.</p>
<p>Tracheo-bronchial suction</p> <p>Poor techniques transfer bacteria and damage the mucus membranes that \uparrow the risk of infection.</p>
<p>Bronchial occlusion with mucus plug</p> <p>The pooling of secretions in the airway distal to the obstruction, causes lung collapse, which is a favourable condition for bacterial growth.</p>

The most cost effective and important proven intervention to prevent aspiration and thus nosocomial pneumonia in the mechanically ventilated ICU patient is to ensure that the patient remains at a body position of not less than 45 degrees ^(100,101,102).

1.11 BACTERIAL CONTAMINATION OF ENTERAL FEEDS AND FEEDING SYSTEMS

The importance of optimal nutritional support is well documented and enteral nutrition has become the preferred route of feeding for the critically ill, but it comes with a price of complications other than the ones mentioned and includes the possible contamination of enteral feeds and feeding systems. The clinical consequences of the bacterial contamination of enteral feeds have been the subject of debate for many years. Infectious complications reported in patients following the administration of enteral feeds contaminated by bacteria include the following: 1) bacteraemia, 2) septicaemia, 3) pneumonia, 4) diarrhoea and 5) infectious enterocolitis ^(103,104). It is important to take into account that all infections, even if they are sub-clinical, decrease nutrient intake and increase nutrient losses ⁽¹⁰⁵⁾. Decreased intestinal absorption, direct loss of nutrients in the gut, internal diversion for metabolic responses to infection and fever induced increased basal metabolic rate, all lead to nutrient losses such that infection not only influences protein and energy status, but also that of most other nutrients ⁽¹⁰⁵⁾. Since there is increasing evidence that the administration of nutrients via the enteral, rather than the parenteral route may help to maintain the integrity of the gut mucosal tissue, it is even more important that this effect is not cancelled out by the administration of feeds contaminated with bacteria ⁽¹⁰⁶⁾ (Table 1.13).



Looking at the sources and routes of contamination, it is clear that it is imperative to have properly developed and applied feeding protocols which include the preparation and administration of feeds, the correct handling procedures and techniques regarding feeding systems, the proper management of enteral feeding tubes and enteral microbiological quality control procedures ⁽³²⁾. A logical approach to achieve this, is the application of the hazard analysis critical control point process (HACCP) to the preparation and administration of enteral feeds. HACCP is a rational, objective and systematic procedure widely used in the food industry to ensure food safety and quality. It involves a series of stages ⁽¹¹⁵⁾.

- Detailed analysis of the process from selection of raw materials to consumption.
- Identification and assessment of the hazards associated with the handling of the product at each stage in the process.
- Identification of the points where control over an identified hazard can be achieved (critical control points – CCP).
- Specification and implementation of monitoring and control procedures at each stage in the process.

The HACCP approach has thus shifted the emphasis from the classical approach to food safety, which relies heavily on end-product testing, to a system that places emphasis on the effective control of raw material and key processing operations. One of its major strengths is that it entails a team effort from the key personnel involved in the full range of activities associated with the product. A team of different disciplines with good communication enables good quality and cost effective nutritional support given to each patient ⁽¹⁰⁶⁾. The implementation of the HACCP system ⁽¹¹⁶⁾ will accelerate the development and evaluation of enteral feeding protocols in a wide variety of institutions, as each unit will be able to adapt the system to meet their own needs.

Table 1.13 Exogenous and endogenous sources and routes of contamination of enteral feeds

Feed preparation ^(107,104,108,109)
Incorrect handling of formulae during reconstitution.
Inadequate cleaning and disinfecting of mixing equipment and surfaces.
Powder formulae have the highest risk of contamination, then liquid feeds, then closed system feeds.
Contamination also higher for cans and tetrapacks than for crown-cap or screw-cap bottles. Recessed spikes and seals are better than exposed spikes, larger seals and caps.
Feed administration ^(106,110,111)
Hand contamination (From patient to nurses hands to feed).
Incorrect handling techniques of feeds during administration.
Feeding systems (Design, protective covers, single use only, assembly instructions).
Correlation of risk contamination of feeds with the type and number of manipulations of the feed and feeding systems between preparation and end of administration.
Colonization of enteral feeding tubes on the inside and outside of the tube.
Retrograde spread of bacteria ^(112,113,114,109)
Fine-bore nasogastric tubes versus wide-bore tubes.
Drip chamber can protect the nutrient container, but not the giving set.
Aspiration of stomach or intestinal contents.

1.12 ACIDIFIED FEEDS IN THE CRITICALLY ILL

Gastric acidity is the major factor inhibiting the growth of Gram-negative organisms in the stomach. Gram-positive organisms are more resistant to acid, and the *Candida* species can survive at a pH of 1⁽⁸⁶⁾. Colonization by yeasts occurs independently of pH, while Gram-negatives and Gram-positives proliferate in direct proportion to increases in pH⁽¹¹⁷⁾. Ablation of normal gastric acidity in the ICU setting has been shown to result in proximal gastro-intestinal overgrowth with Gram-negatives and elevated rates of Gram-negative pneumonia⁽¹¹⁸⁾. It has been described before that enteral feeds can support rapid bacterial growth, but a study done by Ovesen *et al.* demonstrated that not all enteral feeds support microbial growth. The study reported that bacterial growth was inhibited in a partially hydrolyzed soy protein diet with an acid pH of 4.2 as compared with a standard whole protein diet with a near neutral pH of 6.6. The suggestion arose that a product that inhibits microbial growth may also be useful in limiting levels of contamination of enteral feeds⁽¹¹⁹⁾. Another study done by Jacobs *et al.* in 1990 led to the hypothesis that continuous enteral feeding raised the gastric pH, allowing bacterial colonization to take place and suggested that this problem might be overcome by intermittent feeding⁽¹²⁰⁾. This hypothesis was tested in a study by Lee *et al.*, where patients were changed from continuous feeding to an intermittent feeding regime, resulting in a significant reduction in the number of patients with persistently high gastric pH and a reduction in the incidence of pneumonia. The regimen of intermittent feeding as a strategy to reduce nosocomial pneumonia caused by organisms from the stomach among ventilated ICU patients was thus proposed⁽¹²¹⁾. In an attempt to confirm previous data a study was performed by Gowardman *et al.* in 2003 where patients were divided into 3 groups: 1) intermittent gastric 2) continual gastric and 3) continual jejunal feeds. This study concluded that enteral nutrition delivered using an 8-hour feeding break failed to significantly acidify the gastric contents or alter overall bacterial colonization rates⁽¹²²⁾.

The other method to lower gastric pH would be to acidify the actual feed that is given to the patient. As most commercial standard enteral feeds have a pH of 6.0-7.0, it follows that alkalization of the stomach contents by these feeds may promote bacterial overgrowth. Heyland *et al.* tested this hypothesis in 1999. Two groups of patients were studied where the one group received pH 3.5 (acidified) and the other pH 6.5 (neutral). A total number of 120 patients were studied and the results of the acidified feeds showed the following⁽¹²³⁾.

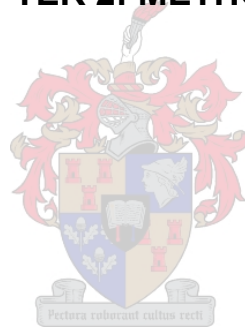
- Acidified enteral feeds preserve gastric acidity.
- Acidified enteral feeds alter the bacterial flora of critically ill patients.
- Acidified feeds dramatically reduce bacterial growth from aspirates of stomach contents.
- Less dramatic effect on tracheal bacterial growth, but lower rate of Gram-negative bacterial growth in tracheal secretions.
- **No significant** reduction in nosocomial pneumonia.

- More feeding protocol interruptions and episodes of high gastric residuals in the acidified group.
- The study shows that gastric colonization plays a significant role in the pathogenesis of subsequent infection.
- Preservation of gastric pH through acidified feeds is associated with a significant reduction in gastric colonization.

1.13 MOTIVATION FOR THE TRIAL

Bacterial contamination of the enteral feeding system has been described as a potential source of infection in critically ill patients. It can result in gastric colonization, especially as bacterial growth is pH dependent and most commercial formulae used in the ICU have a neutral pH. Retrograde transmission of contaminated gastric contents to the oropharynx and subsequent aspiration are thought to predispose the critically ill to pneumonia. By promoting a natural defence mechanism – gastric acidity – acidified feeds do alter gastric pH and intestinal flora⁽¹²³⁾. As no other trials, except for Dr Heyland, have been done on acidified feeds, it was plausible and warranted further investigation to establish efficacy and safety of acidified feed in the critically ill. The aim of this trial was to investigate if acidified feeds decrease gastric and tracheal colonisation, preserve gastric acidity and decrease contamination of the enteral feeding delivery system. The trial also aimed to investigate two different acidity levels (3.5 and 4.5) as compared with the acidity of a control formula (pH 6.8) to establish if the degree of acidification alters the defined outcomes. An additional area of investigation was to establish the microbiological safety of the reconstitution process of enteral feeds practiced in a third world setting where ready-to-hang products are often too expensive to use routinely. This clinical trial also aimed to establish the safety and tolerance of acidified feeds in the critical care setting.

CHAPTER 2: METHODOLOGY



2.1 AIM

The aim of this trial was to investigate that acidified enteral formulae would lower the incidence of bacterial contamination of the enteral feeding delivery system and potentially decrease infectious morbidity and mortality of critically ill patients.

2.2 OBJECTIVES OF THE TRIAL

2.2.1 Primary objective

- To determine if acidified formulae (pH 3.5 and pH 4.5) decrease microbial contamination of the enteral delivery system compared with a non-acidified control formula.

2.2.2 Secondary objectives

- To determine bacterial colonisation of the stomach and trachea,
- To determine acid-base status in relation to the administration of acidified formulae,
- To establish tolerance of the trial formulae,
- To determine morbidity and mortality in relation to the administration of acidified formulae.

2.3 METHODOLOGY

2.3.1 Trial design

The trial was a controlled, double-blinded, randomised clinical trial of three parallel groups at a single centre.



2.3.2 Sampling and randomisation

The primary outcome of this trial was to define the enteral nutrition delivery system contamination with $\alpha = 0.05$ and power = 80%. Using $\alpha=0.05$ (1-sided) and a power of 80%, 25 patients per pH-group needed (calculated by Pass6.0) to be included in the trial. The "lost to follow-up" patients were included in this number of 25 patients per pH-group. A maximum of 2 feeding bottles and 2 delivery sets were sampled from each patient per day. It was assumed that 25% of the patients (i.e. 6 patients) would only stay in the trial for a small number of days, on the basis of the records of the ICUs from which the the patients were recruited. These 6 patients would contribute 12 feeding bottles per pH group to the study. The remainder 75% of the patients (i.e. 19 patients) would each contribute 16 bottles. In total 376 bottles per pH-group would be sampled, which provided enough opportunity to sample feeding bottles and delivery sets for the purpose of contamination assessment. Subjects were randomised according to their APACHE II ⁽¹²⁴⁾ scores. This is a classification tool for severity of disease that describes patient acuity based on the worst data in the 24 hours after admission to the intensive care unit and includes acute physiology, Glasgow coma score, age and chronic health status (**Appendix A**). The

randomisation was done at baseline (Day 0) using “TrialBalance” (Nestle randomisation software), and then assigned to the three treatment groups:

- Group 1: Nutren pH 3.5
- Group 2: Nutren pH 4.5
- Group 3: Nutren pH 6.8

All trial personnel were blind as to the feed administered. All formulae were colour coded (red, white and blue) for the purposes of preparing the formulae, but all formulae were similar in final appearance, taste and smell. The code for the colour of the formulae was provided to the chief investigator in a sealed envelope, which was returned **sealed** to Nestle at the completion of the trial. The code was broken in Geneva, Switzerland; in the presence of witnesses **after** the data analysis had been completed.

2.3.3 Patients

Subjects received enteral nutrition for a minimum of 3 days and a maximum of 21 days. Patients received one of the trial enteral formulae: as long as it was clinically indicated, until they were transferred from the ICU, until enteral nutrition became contra-indicated, or until a patient died or alternatively for a maximum of 21 days. Patients were discontinued from the trial if any signs of intolerance to the formulae were elicited or if any other adverse gastro-intestinal symptoms or gastro-intestinal failure (e.g. an ileus) occurred.

2.3.3.1 Inclusion criteria

All mechanically ventilated surgical and medical ICU patients, aged 18 to 65 years were included in the trial. Expected time of mechanical ventilation had to be for > 48 hours. Patients had to be eligible to be enterally fed if more than 25% of energy requirements could be provided by enteral feeding within the expected minimum time of feeding of at least 3 days.

2.3.3.2 Exclusion criteria

Patients with overt gastro-intestinal bleeding or a clinically significant gastro-intestinal bleeding episode in the preceding 2 weeks of randomisation, with active peptic ulcer disease diagnosed endoscopically in the preceding 6 weeks of randomisation, any clinical setting associated with raised gastric luminal pH or any clinical setting requiring gastric acid secretion suppression, were excluded from the trial. Other exclusion criteria were the following:

- An arterial pH < 7.20 lasting > 24 hours.
- Renal and hepatic failure.
- Uncontrolled diabetic patients (type I and II) with complications.
- Severe electrolyte disturbances.

- Patients expected to receive enteral feeding for less than 3 days.
- Patients who participated or had participated in another clinical trial in the preceding 4 weeks of randomisation.

2.3.4 Product design

The trial formulae consisted of proteins, carbohydrates, fats, with vitamins and minerals in amounts intended for full nutritional support of patients on tube or oral feeding during pre- and post-operative nutrition. The formulae provided 100 kcal per 100 ml of reconstituted feed. All formulae were provided in colour tins in powder form and were reconstituted with tap water by the trial dietitian as per current protocol of the Tygerberg Academic Hospital (**Appendix B**). After reconstitution, the feeds were decanted into standard identical tube feed bottles that were labelled with the patient details, colour code of the formula, date of reconstitution and administration rate. The trial dietitian transported the bottles to the different wards where it was refrigerated until it was used within a 24-hour period.

2.3.5 Treatment administration

Enteral nutrition was initiated within 48 hours of admission to the ICU. Randomisation and baseline information were recorded on Day 0 of the trial and enteral nutrition with the assigned study formula initiated on Day 1 of the trial, and then continued daily. A dietician, registered with the Health Professions Council of South Africa, determined and documented the nutritional requirements of each patient on Day 0 of the trial and reassessed according to the condition of the patient. Energy requirements were calculated using the Harris Benedict equation ⁽¹²⁵⁾, together with the appropriate stress and activity factors (**Appendix C**). Enteral nutrition was administered to all patients via a nasogastric tube. The ICU physician or physicians placed the feeding tube in the stomach using standard procedures and as the patient's condition necessitated. Feeding tubes (thin bore, PVC, Tyco Healthcare Ltd) were replaced if and when necessary and any such changes in feeding tubes were recorded. Radiological confirmation of the position of the feeding tubes was obtained prior to initiating enteral nutrition and once weekly as necessary, depending on the type of patient or if feeding tubes had to be replaced. Additionally, the nursing sister marked the site of nasal entry of the tube and checked daily that there has been no tube displacement. A registered nurse was responsible for the administration of the enteral feeds over a 24-hour period at a constant infusion rate using an enteral feeding pump. The enteral feeding delivery system was cared for according to standard established procedures of the Department of Human Nutrition, Tygerberg Academic Hospital (**Appendix D**).

2.4 DATA COLLECTION

All data was captured daily in specially designed case report forms that were non-carbon, required paper and provided 3 copies of each page. Each patient had a numbered file with these case report forms (Nestec Ltd, Geneva, Switzerland), as well as a source document file where original copies were kept. The data was verified by the study monitor onsite at each visit.

Samples were collected in sterile, individually marked test tubes with screw caps (B&M Scientific, South Africa).

2.4.1 Enteral feeding reconstitution samples were taken daily as follows:

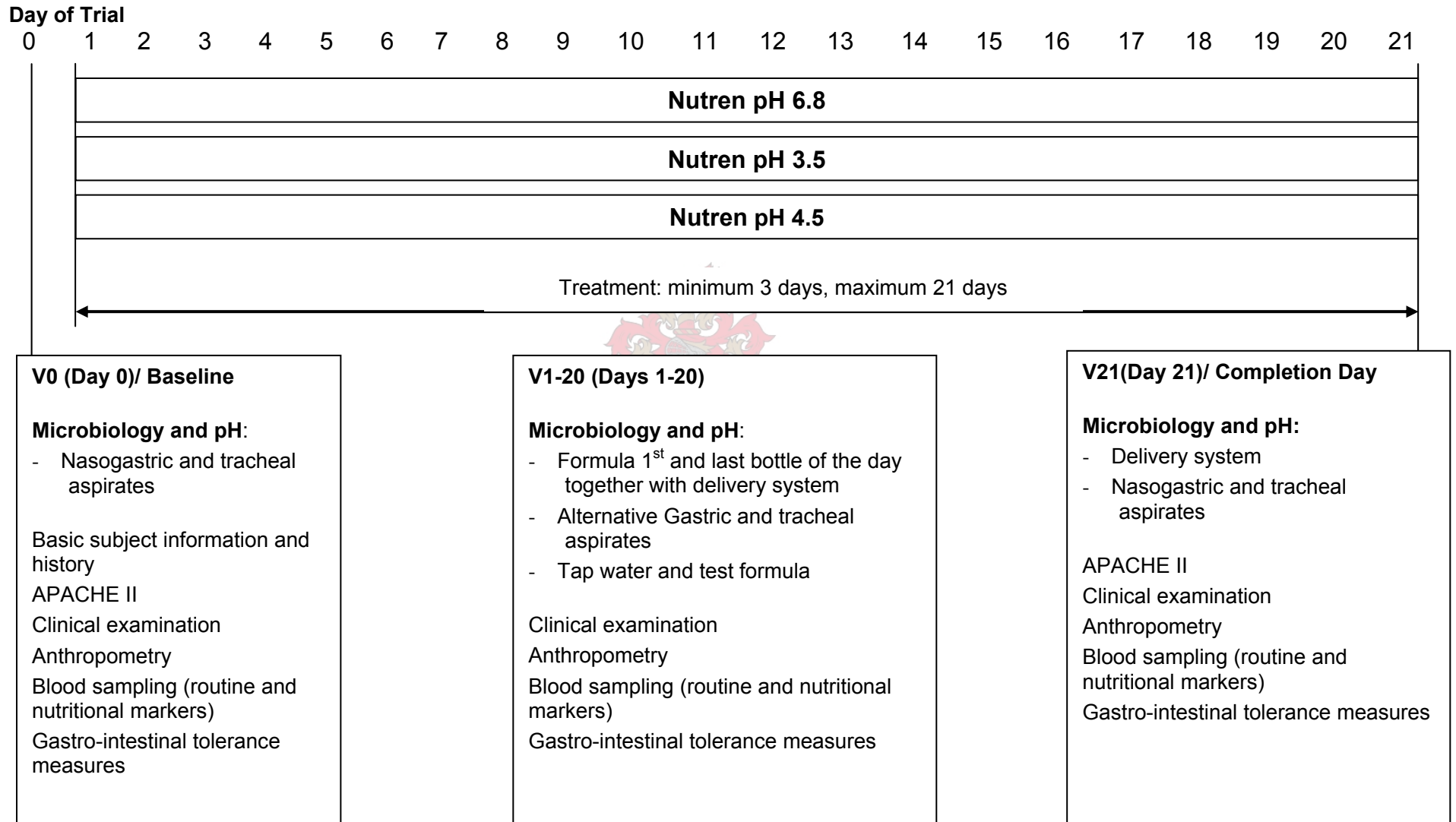
- Tap water prior to and at the end of the reconstitution process of the formulae.
- Dry powder of every opened tin.
- Each test formula after reconstitution for each subject.
- First bottle of the test formula after a hanging period of 6 hours on ward level for each of the subjects.
- The tap at the distal end of the enteral nutrition delivery system (where it connects to the feeding tube) after a hanging period of 6 hours for each of the subjects.
- Last bottle of the test formula when the feed is almost completed after 24 hours for each of the subjects.
- The tap at the distal end of the enteral nutrition delivery system (where it connects to the feeding tube) after 24 hours for each of the subjects.

2.4.2 Patient samples were taken as follows:

- Gastric aspirates at baseline (Day 0), at 24 hrs (Day 1) and every 48 hrs thereafter
- Tracheal aspirates at baseline (Day 0) and then every 48 hrs thereafter
- Feeding tube when removed or replaced

A flow diagram detailing the time-points, samples taken and the measurements of the trial was constructed (Table 2.1).

Table 2.1 Flow diagram for sample collection during the trial



2.4.3 Nasogastric Aspirates

A nursing sister drew all samples between 07:00 and 08:00. A 50 ml syringe (catheter tip, Tyco Healthcare Ltd, South Africa) was used to withdraw the initial 5 to 10 ml of the aspirate. This represented the fluid in the tubing and was discarded. A new, sterile 50 ml syringe (catheter tip, Tyco Healthcare Ltd) was used to aspirate the remainder of the stomach contents. Approximately 5-10 ml of this aspirate was put in two sterile, individually marked test tubes with screw caps (B&M Scientific, South Africa). One aliquot (5ml) of the specimen was processed according to standard laboratory techniques for microbiological counts of both bacteria and yeasts as well as the presence of potential pathogens. The other aliquot was used to determine the pH value.

2.4.4 Tracheal Aspirates

Samples were collected at baseline (Day 0) and at least in 48-hour intervals or more often as clinically indicated and were processed according to standard laboratory techniques for microbiological analysis, and pH measurements. (**Appendix E**). All Gram-negative bacteria, *Enterococcus* species, and *Staphylococcus aureus* were considered to be potentially pathogenic microorganisms.

2.4.5 Microbiological analysis

Gastric and tracheal aspirate, tap water, dry powder and reconstituted formula samples were analysed by the Department of Microbiology at Tygerberg Academic Hospital for total bacterial counts and potential pathogens (**Appendix E, point C**). If or when the feeding tube was removed from/replaced in the patient, as well as at discharge, discontinuation of enteral nutrition or death, it was sent to the microbiology laboratory for analysis of total bacterial counts and potential pathogens.

2.4.6 pH Determinations

Daily samples of tap water (one sample only), reconstituted formula, first and last bottle of test formula for each subject were taken to the Department of Human Nutrition for pH determination using a Radiometer pH meter (Model PHM64, Copenhagen, Lyon, France) with a combined pH electrode (Model pHC2005-7) measuring pH to the second decimal. One aliquot (5ml) of a nasogastric and a tracheal aspirate sample was taken and put into sterile containers for pH determinations, the reference of method being "pH theory and practice," (Radiometer Analytical, France). Two determinations were carried out in two separate sub-aliquots and the mean determined and recorded in the laboratory data sheets. The results were recorded in the case report forms only after the completion of the trial in order to ensure its blindness requirements.

2.4.7 Blood samples

Blood samples (15 ml) were drawn between 08:00 and 10:00 by a registered nurse and placed in a EDTA test tube (5ml) (Hemogard Lavender, The Scientific Group Ltd, South Africa), a gel and clot activator test tube (5ml) (Hemogard Gold, The Scientific Group Ltd, South Africa) and a glucose test tube (5ml) (Hemogard FL/OX grey, The Scientific Group Ltd, South Africa) at baseline and daily/weekly thereafter for the duration of the trial for the following analyses. All analyses were performed on site:

Blood biochemistry (daily/weekly)

Sodium, Potassium, Urea, Creatinine, Aspartate Transaminase, Alanine Aminotransferase, Alkaline Phosphatase, Total bilirubin, Phosphate, Magnesium, Total protein, Blood glucose (Advia®1650, Bayer Chemistry System).

Arterial blood gasses (IL 1312 Blood gas manager, ILEX, SA and Stat Profile pHox plus, Nova Biomedical, USA)

Full blood count (Advia®120, Bayer)

Blood glucose (Daily – Haemoglucotest, Accu-chek®, Roche, Ireland)

Principles of the above methods of analysis are detailed in **Appendix F** with measurements and coefficients of variation (Appendix F, Tables 1-4). These determinations were documented when they were routinely done as part of the patient's treatment and were usually performed daily or every 48 hours. The principle of analyses used for the above measurements was nephelometry (Nephelometer, Dade-Behring, BN100, coupled K, Apple). Details of the experimental procedure, including the principle of the method, reagents required, specimen treatment and the necessary steps that made up the analysis methodology, were located in the Dade-Behring antiserum kits (Table 2.2). If the patient received tube feeding for less than 1 week, the blood was drawn on the day the tube feeding was discontinued to ensure a follow-up value for comparison of improvement.

Table 2.2 Biochemical markers of nutritional status measurement and coefficient of variation

Measurement	Reference of method	Coefficient of variation
Pre-Albumin	OUIF 09	6.8%
Albumin	OSAL 15	4.4%
α_1 -acid-glycoprotein (AGP)	OSAW 15	3.8%
Retinol binding protein (RBP)	Ouvo 09	6.9%
C-reactive protein (CRP)	OQIY 21	4.7%

2.4.8 Anthropometry

Anthropometric measurements were taken in all oedema-free patients when possible. Oedema (or the expansion of the interstitial fluid volume) was elicited by applying moderate pressure over the lower tibial shaft for at least 10 seconds and was classified as pitting and non-pitting⁽¹²⁶⁾. Two research dietitians, who were duly trained and standardised, took three triceps skinfolds and three upper arm circumference measurements by using standard anthropometrical techniques (**Appendix G**). The measurements were taken at baseline, weekly thereafter, and at the cessation of enteral feeding. If a patient received tube feeding for less than a week, the measurements were taken on the last day of tube feed administration. For the calculation of the energy and protein requirements, the patient's height was determined as the distance from the midpoint of the sternal notch to the fingertip of the middle (third) finger, multiplied by two⁽¹²⁷⁾. Where the aforementioned height measurement could not be taken, the patient's height was determined in relation to the bed-length being 195cm, with the understanding that the patient was lying flat and straight on the bed and a non-stretch measuring tape used to measure the distance from head to toe of the patient⁽¹²⁸⁾. Weight was calculated from an estimated body mass index⁽¹²⁹⁾ ($BMI = \text{weight}/\text{height}^2$), when the height was measured. If a usual weight was obtainable from the patient's folder or history, the usual weight was used.

2.4.9 Gastro-intestinal function

The following data was monitored and recorded daily in the case report forms from the patient's clinical records:

- Number of stools, colour, consistency
- Abdominal pain
- Nausea
- Vomiting
- Bowel sounds
- Abdominal distension, as well as the measurement of abdominal circumference

For the purposes of this trial, diarrhoea was defined⁽¹³⁰⁾ as a score of >12 over a 24-hour period (Table 2.3).

Table 2.3 Scoring of incidence of diarrhoea according to consistency and volume

Consistency	Estimated volume		
	<200mL (+)	200-250mL (++)	>250mL (+++)
Formed	1	2	3
Semisolid	3	6	6
Liquid	5	10	15

2.4.10 Fluid balance

Volume and composition of the feed administered

Input and output

Nasogastric or any other fistulous drainage

Oedema

2.4.11 Vital signs

Clinical status and any change thereof

Temperature

2.4.12 Additional data

Medication and changes therein as well as concomitant medication.

Reasons for discontinuation of tube feeding.

2.4.13 Adverse events (AE)

An adverse event was defined as any untoward occurrence in a patient or clinical investigation subject administered an investigational product and which did not necessarily had to have a causal relationship with this treatment. Adverse events were recorded in the case report form for each patient and described according to duration, frequency, intensity and seriousness. Action taken, outcome and relation to the test product were also recorded.

2.4.14 Serious adverse events (SAE)

A serious adverse event was defined as death in this trial and was recorded in the case report forms. The treating physician of the patient documented complete information about the event, the clinical course of the event, causal relationship to the trial product, concomitant medication, treatment and further medical comments. The principal investigator signed the case report forms and faxed the appropriate documentation to Nestec Ltd within 48 hours of the event as per protocol. The principal investigator also informed the Human Research Committee of the Faculty of Health Sciences of the University of Stellenbosch of these serious adverse events as per University protocol.

2.5 ETHICS

The subject's confidentiality was protected at all times. The Human Research Committees of both the Faculty of Health Sciences of the University of Stellenbosch and Tygerberg Academic Hospital approved the trial. Applied Good Clinical Practice (AGCP) guidelines were adhered to in the duration of the trial. Each patient, his or her immediate family or the treating physician, in consultation with the Medical Superintendent of the hospital gave written informed consent where

family were not available. Complete written information regarding the trial was given to each patient or the immediate family after explanation of what the trial entailed.

2.6 STATISTICAL ANALYSIS

Data was captured electronically with Microsoft Excel[®] from the case report forms and regularly cross-referenced to ensure precision of data transfer. The University of Stellenbosch appointed a consultant statistician to assist with the analysis of the data using SAS 9.1.3 Service Pack 2, XP_PRO platform for Windows[®] (SAS Institute, 2005). Statistical analyses were based on an intention to treat (ITT) data set and were performed in all patients (n = 67) for the following parameters: demographics, non-nutritional related baseline characteristics, anthropometry, nutritional requirements, gastro-intestinal tolerance, blood biochemistry and adverse events. Statistical analyses were also completed for all enteral feed reconstitution and patient compartments (i.e. water, enteral feed powder, reconstituted formulae, feeding bottles, delivery sets and aspirates), as well as for microbiological safety profile and pH determinations. Means and standard deviations (SD) were calculated for all baseline parameters. Baseline data were compared using the Kruskal-Wallis test for non-parametric data for more than 2 independent groups with different sample sizes. Longitudinal data was compared by analysis of the PROC MIXED model (with repeated measures) for a wider class of mixed linear models to estimate the following: differences between the three study groups, the effect of time during the trial period, interaction between time and the feeding groups and the relationship with time in terms of the number of days on the trial. It is important to note that the ANCOVA illustrations/figures presented in this thesis are general linear ANCOVA's, showing interaction between groups and not relationship with time in terms of number of days on the trial. It is only of illustrative value and no significance testing was done with this method. Chi-squares were calculated to estimate if proportions or relationships between groups were the same. The level of significance was set at $p < 0.05$ and applied to all tests.

CHAPTER 3: RESULTS



3.1 BACKGROUND

An international monitor through 5 visits over the 20-month trial period monitored the trial. Verification of correct data entry in the case report forms (CRFs) was done at each visit for each subject through the source documents kept by the investigator. Full progress reports were written after each visit and submitted to the principal investigator and Nestec Ltd, Switzerland. Patients were recruited according to the inclusion and exclusion criteria from 4 different intensive care units within the Tygerberg Academic Hospital [from 1 May 2001 to 31 December 2002]. After written consent had been obtained, patients were randomised into 3 feeding groups, each group being fed with a formula which had a different pH, namely Group 1 (pH 3.5), Group 2 (pH 4.5) and Group 3 (pH 6.8).

3.2 DEMOGRAPHICS

A total of 567 patients were screened for inclusion in the trial, of whom only 67 met the entry criteria, were randomised and distributed in the 3 different feeding groups (Table 3.1). Patients were equally distributed between the 3 feeding groups. Twenty-four percent of the patients dropped out of the trial. The highest dropout rate of 12% was found in the acidified group with a pH of 3.5 (Table 3.1) with no significant difference (Chi square, $p = 0.26$) found between the 3 feeding groups. Sixteen patients were prematurely withdrawn from the trial and did not complete the study as outlined in the protocol. The main reason for premature withdrawal from all groups was early extubation ($n = 9$) (patients ventilated for 3 days or less). Other reasons for premature withdrawal from the trial were: inability to establish enteral feeding within 3 days ($n = 2$), prescription of gastric acid suppressive medication within the first 3 days ($n = 1$), acidosis ($\text{pH} < 7.2$) for $>$ than 24 hours within the first 3 days ($n = 1$), enteral feeding not administered ($n = 1$), or death ($n = 2$). Patient withdrawal due to feeding intolerance did not play a role. All of the patients ($n = 67$) were included in the analysis of the data, thus the statistical analyses were performed in an ITT data set.

Table 3.1 Distribution of trial patients in the different feeding groups

	Group 1 (pH 3.5)	Group 2 (pH 4.5)	Group 3 (pH 6.8)	Total sample	Chi- square (p-value)
Distribution [n (%)]	23 (34.3)	23 (34.3)	21 (31.4)	67	
Dropouts [n (%)]	8 (12)	5 (7.5)	3 (4.5)	16 (24)	0.26

The ethnic group distribution for the total sample ($n = 67$) included 1 White patient (1.5%), 16 Black patients (24%) and 50 Coloured patients (74.5%) (Table 3.2). Gender distribution was fairly

equal between the 3 groups, with a significant majority of 71% of total patients being male, reflecting the statistics of the ICU population in the Tygerberg Academic Hospital for the trial period.

Table 3.2 Gender distribution of trial patients at baseline (n = 67)

Variable	Group 1 (pH 3.5) [n (%)]	Group 2 (pH 4.5) [n (%)]	Group 3 (pH 6.8) [n (%)]	Total sample [n (%)]
n (%)	23 (34.3)	23 (34.3)	21 (31.4)	67
Male	14 (20.9)	18 (26.9)	16 (23.9)	48 (71.6)
Female	9 (13.4)	5 (7.5)	5 (7.5)	19 (28.4)

The past medical history of 43 (64.2%) of the patients was not known. Of the remaining 24 patients (35.8%), the following history was elicited: urogenital (n=2); Diabetes Mellitus type 1 (n=1); gastro-oesophageal reflux disease (n=1); rheumatoid arthritis (n=1); neurological (n=3); cardiology (n=6); bronchopulmonary (n=12) and ophthalmic (n=2). In cases where more than one history of disease was present in a patient, all diagnoses were taken into account and documented. In all cases, the medical examination was completed at baseline on admission to the ICU. In cases where more than one abnormality had been documented in respect of a patient, all abnormalities were documented as recorded by the treating physician in the patient's folder. The abnormalities were classified as follows: 32 (48%) neurological; 12 (18%) cardiology; 52 (79%) brochopulmonary; 9 (14%) dermatological; 7 (11%) ophthalmic; 9 (14%) ear, nose and throat; 16 (25%) abdomen; 9 (14%) throat; 2 (3%) lymph nodes. The admission diagnosis (Table 3.3) of the majority of patients was various types of trauma (n=41; 61%;), followed by respiratory patients (n = 19; 28.5%).

Table 3.3 The admission diagnosis of trial patients at baseline (n=67)

Admission diagnosis	n (%)
TRAUMA	41 (61)
Motor vehicle accidents	32 (47.7)
Polytrauma	2 (3)
Stab wounds	3 (4.5)
Trauma-related sepsis	2 (3)
Spinal fractures	2 (3)
RESPIRATORY DISEASE	19 (28.5)
Pneumonia	3 (4.5)
Tuberculosis	5 (7.5)
Status asmaticus	3 (4.5)
Respiratory failure	5 (7.5)
ARDS	1 (1.5)
Interstitial lung disease	1 (1.5)
Tension pneumothorax	1 (1.5)
VARIOUS	7 (10.5)
Overdose	2 (3)
Brain bleed	1 (1.5)
Tetanus	1 (1.5)
Rheumatoid Arthritis	1 (1.5)
Perforated sigmoid colon	1 (1.5)
Colon malignancy (Hemi-colectomy)	1 (1.5)

Twenty-seven patients (40%) had had no surgical procedures on admission. The remaining 40 patients (60%) had surgical procedures on admission at baseline, as well as intensive care unit procedures during the trial period (Table 3.4). If more than one procedure was performed on a patient, all procedures were documented.

Table 3.4 Surgical/ICU procedures on trial patients during the trial period

Surgical procedure	n (%)
Tracheotomy	3 (7.5)
Debridement	4 (10)
Laparotomy	7 (17.5)
Neck exploration	1 (2.5)
Orthopaedic procedures	8 (20)
Hysterectomy	1 (2.5)
Pulmonary embolisation	1 (2.5)
Pneumonectomy	1 (2.5)
Craniotomy	1 (2.5)
Sternotomy exploration	1 (2.5)
Thoracotomy	1 (2.5)
Neck exploration and tracheotomy	1 (2.5)
Brachial artery repair	1 (2.5)
Underwater drains inserted	2 (5)
Embolisation	2 (5)
Abscess drainage	1 (2.5)
Arteriography	2 (5)
Debridement and laparotomy	1 (2.5)
Underwater drains and tracheotomy	1 (2.5)

3.3 NON-NUTRITION-RELATED BASELINE CHARACTERISTICS

Patients in the different feeding groups were well matched for age, severity of illness classification, PINI scores and temperature, with no significant differences between the groups (Table 3.5). Average length of ICU stay ranged from 7.5 days for Group 1 (pH 3.5) to 9.3 days for Group 3 (pH 6.8) and did not differ significantly between the 3 groups (Kruskal Wallis, $p = 0.19$). The mean and range of APACHE II scores were similar in the 3 groups, with a slight, but not significant improvement in the scores between the baseline (14.3) and completion of the trial (12). Group 1 (pH 3.5) showed a higher, but non-significant apparent difference in APACHE scores at completion, which was probably due to the two deaths in this group in comparison with only one death each in the other two feeding groups.

Table 3.5 Non-nutrition-related baseline characteristics of trial patients (n = 67)

Variable	Units	Group 1 (pH 3.5) mean(SD)	Group 2 (pH 4.5) mean(SD)	Group 3 (pH 6.8) mean(SD)	Total sample mean(SD)	Kruskal Wallis (p-value)
Age	y	37.6 (13.1)	39.8 (11.3)	37.8 (14.3)	38.4 (12.8)	0.71
Length of stay	d	7.5 (5.4)	8.2 (4.5)	9.3 (3.9)	8.3	0.19
APACHE II Baseline	1 – 72	12.9 (5.6)	15.4 (4.8)	14.4 (8.1)	14.3 (6.2)	0.29
APACHE II Completion	1 – 72	15.4 (9.5)	10.3 (5.3)	10.6 (5.1)	12.0 (7.1)	0.27
PINI Score 1	*	35.9 (56.6)	37.6 (74.0)	35.6 (47.6)	36.4 (6.0)	0.81
PINI Score 2	*	22.9 (32)	22.9 (28.9)	20 (14.9)	21.9 (25.8)	0.66
PINI Score 3	*	42.8 (67.0)	38 (77.4)	16.6 (14.7)	29.0 (53.6)	0.85
Temperature	36.9 (°C)	37.1 (0.7)	37.3 (0.7)	37.3 (0.6)	37.3 (0.9)	0.85

Abbreviations: y = years; d = days; APACHE = Acute Physiology and Chronic Health Evaluation; PINI = prognostic inflammatory and nutritional index.

* PINI interpretation ⁽¹³²⁾: > 30 = life threatening; 21–30 = high risk; 11-20 – moderate risk; 1-10 = low risk; < 1 = non-infected subjects.

3.4 NUTRITION-RELATED BASELINE CHARACTERISTICS

3.4.1 Anthropometric data

Anthropometric measurements (Table 3.6) were taken in all patients at baseline and used for the calculation of nutritional requirements. Patients were well matched for height and weight, with no significant differences between the feeding groups. Other anthropometric measurements, e.g. mid-upper arm circumferences and triceps skinfold thickness, could only be determined in 3 oedema-free patients and could not be statistically analysed.

Table 3.6 Baseline anthropometric measurements of trial patients (n = 67)

Variable	Units	Group 1 (pH 3.5) mean(SD)	Group 2 (pH 4.5) mean(SD)	Group 3 (pH 6.8) mean(SD)	Total sample mean(SD)	Kruskal Wallis (p-value)
Height	M	1.74 (0.1)	1.72 (0.1)	1.69 (0.1)	1.72 (0.1)	0.25
Weight	Kg	69.5 (11.0)	72.1 (19)	67.2 (15.6)	69.6 (15.4)	0.81
BMI	18–25 (kg/m ²)	23.0 (3.6)	24 (4.2)	23.6 (5.6)	23.5 (4.5)	0.72

Abbreviations: m = metres; kg = kilogram; BMI = body mass index

3.4.2 Nutritional requirements and intake data

Nutritional requirements were calculated for each patient at baseline and intake of the enteral formulae was monitored and recorded daily (Table 3.7). Groups were well matched for nutritional requirements, with no significant differences between the feeding groups. The actual volume of formulae that was administered per day over the trial period ranged from 1000 ml to 1200 ml for the groups and was administered at a rate of between 44 to 50 ml per hour over 24 hours. Maximum calculated volume to be administered over 24 hours was on average 2000 ml for the three feeding groups, with no significant difference between the groups. Administration rate was calculated at 83ml per hour over 24 hours, with no significant differences between the groups. Energy received via the administration of IV fluids was also recorded daily and contributed on average an additional 400 kcal over 24 hrs for the different feeding groups, with no significant difference between the groups.

Table 3.7 Actual nutrient intake received in relation to calculated nutritional requirements for trial patients for the different feeding groups during the trial period

Variable	Group 1 (pH 3.5) mean (SD)		Group 2 (pH 4.5) mean (SD)		Group 3 (pH 6.8) mean (SD)	
	Rec	Calc	Rec	Calc	Rec	Calc
Energy (kcal) / 24 hrs	930 (537)	1933 (342)	1011 (422)	1955 (292)	1191 (337)	2010 (287)
Protein (g) / 24 hrs	42 (17)	80 (15)	48 (16)	83 (13)	48 (12)	81 (11)
Test formula administration rate per 24 hrs (ml/hr)	44 (18)	82 (11)	50 (17)	83 (10)	50 (13)	84 (10)
Test formula volume per 24 hrs (ml)	1056 (432)	1967 (253)	1200 (408)	1993 (242)	1200 (312)	2023 (232)

Abbreviations: rec = received, calc = calculated, g = gram

Patients received on average 53% of their energy requirements through enteral formulae alone, but this intake increased to 61% if the energy received from the IV fluids were taken into consideration (Figure 3.1).

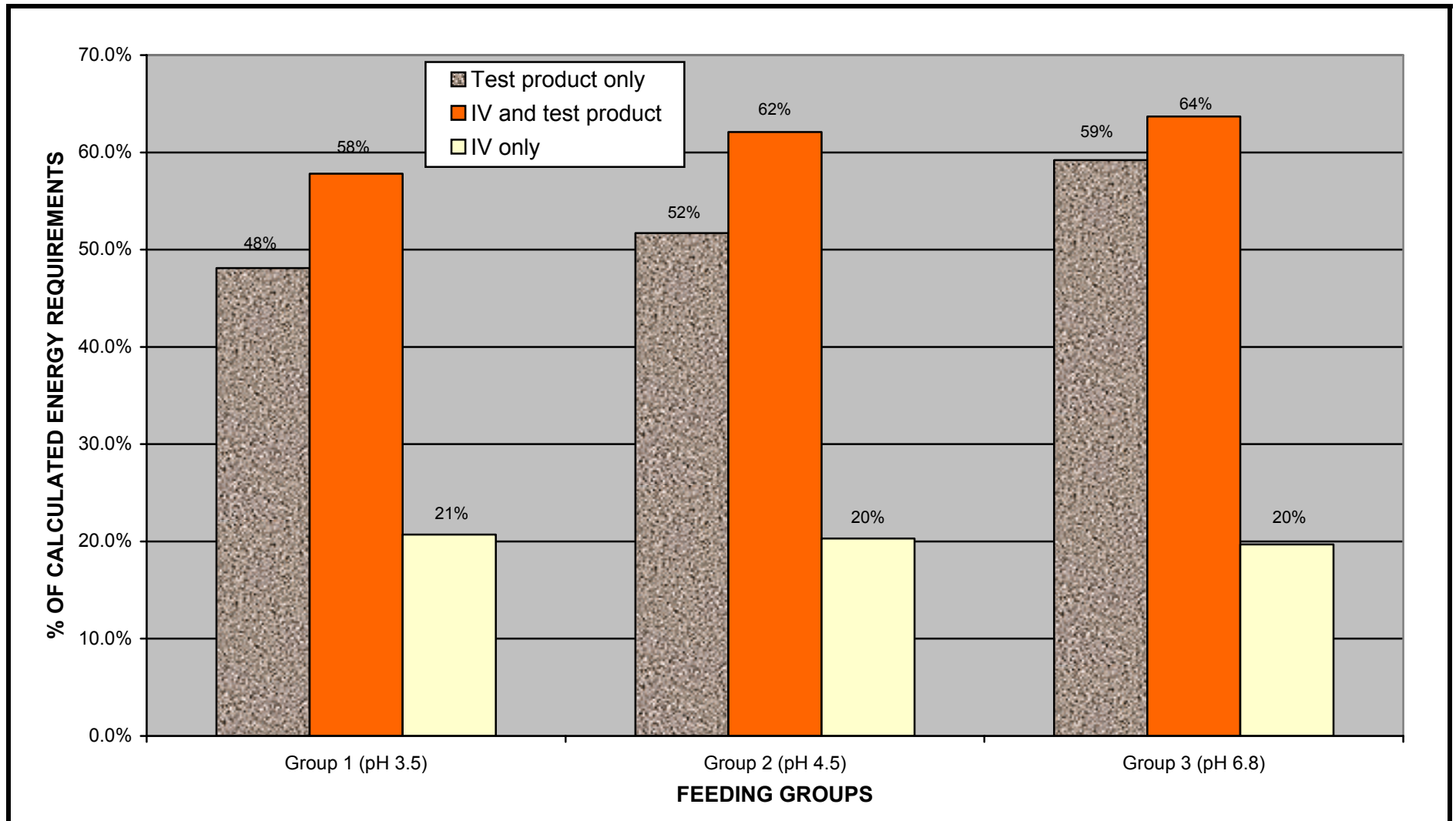


Figure 3.1 Average percentage of energy received from trial formulae and IV fluids in relation to the calculated *total* energy requirement for the different feeding groups during the trial period

3.5 TOLERANCE OF TRIAL FORMULAE

Daily monitoring of gastro-intestinal function of every trial patient established tolerance of the trial products. Overall, trial formulae were tolerated well in all 3 feeding groups (Table 3.8). Nausea episodes could only be documented in 9 patients (13.4%) who were awake on ventilation and were able to communicate. Vomiting episodes were documented in the case of 15 patients (22.3%) and abdominal distension in the case of 8 patients (12%), with 4 of these patients being in the control group (pH 6.8). The presence of abdominal pain was not known in the majority (77%) of trial patients and in 84% of trial patients oedema could be elicited. Three patients (4.5%) had to be withdrawn from the trial due to ileus. The control Group 3 (pH 6.8) had the higher incidence of ileus developing in 2 patients. Group 2 (pH 4.5) had 1 patient and no patients in Group 1 (pH 3.5). None of these differences were statistically significant.

Table 3.8 Tolerance of trial formulae in terms of gastro-intestinal parameters for the different feeding groups during the trial period

Variable	Group 1 (pH 3.5) (n = 23)	Group 2 (pH 4.5) (n = 23)	Group 3 (pH 6.8) (n = 21)	Total (n = 67)
Nausea episodes	3 (2)*	2 (2)*	5 (5)*	10 (9)*
Vomiting episodes	7 (5)*	10 (6)*	5 (4)*	22 (15)*
Abdominal distension	3 (2)*	3 (2)*	15 (4)*	21 (8)*
Ileus developed	0	1 (1)*	2 (2)*	3 (3)*

* Number of patients in whom the variable occurred.

The number of stools for the different feeding groups was documented as an indication of possible diarrhoea and intolerance to the trial formulae and showed no significant difference (chi-square $p = 0.62$) in the average stool number per group during the trial period (Table 3.9). The consistency and volume of stools were documented (Table 3.10) and no incidence of diarrhoea was found that warranted medication or further investigation.

Table 3.9 Average stool numbers per feeding group during the trial period

Stool number	Group 1 (pH 3.5) [n (%)]	Group 2 (pH 4.5) [n (%)]	Group 3 (pH 6.8) [n (%)]	Total for trial period (n)
1	16 (52)	7 (41)	22 (56)	45
2	11 (36)	5 (29)	9 (23)	25
3	2 (6)	4 (25)	5 (13)	11
4	1 (3)	0	1 (3)	2
5	0	1 (6)	2 (5)	3
6	1 (3)	0	0	1

Table 3.10 Stool consistency and volume for all the feeding groups during the trial period

Number	Consistency	Stool volume		
		< 200ml	200-250ml	> 250ml
1	Liquid	9	4	7
1	Soft	9	9	7
2	Liquid	7	13	9
2	Soft	5	14	0
2	Hard	2	0	0
3	Liquid	4	4	11
3	Soft	3	8	2
4	Liquid	0	0	3
5	Liquid	1	0	1
5	Soft	1	0	0
6	Liquid	2	0	0
6	Soft	4	0	0

3.6 BLOOD BIOCHEMISTRY

3.6.1. Baseline blood biochemistry results

The baseline (Day 0) nutrition-related biochemical variables (Table 3.11) were on average low according to the reference normal values. There were no significant differences between the different feeding groups for any of these parameters.

Table 3.11 Baseline nutrition-related biochemical variables of trial patients in the different feeding groups

Variable	Normal range (Units)	Group 1 (pH 3.5) [Mean (SD)]	Group 2 (pH 4.5) [Mean (SD)]	Group 3 (pH 6.8) [Mean (SD)]	Kruskal Wallis (p value)
Pre-albumin	25–45 (mg/dL)	10.3 (4.6)	10.6 (5.2)	11.7 (6.2)	0.9
RBP	3–6 (mg/dL)	2.1 (1.2)	1.9 (0.9)	2.2 (1.2)	0.62
AGP	0,4–1,3 (g/L)	1.6 (0.4)	1.6 (0.6)	1.7 (0.4)	0.65
Albumin	37–53 (g/L)	18.8 (5.6)	19.0 (6)	20 (6.1)	0.93

Abbreviations: RBP = Retinol binding protein; AGP = α_1 -acid-glycoprotein

The baseline full blood and differential counts (Table 3.12) indicated significant differences in red blood cells (Kruskal Wallis, $p = 0.05$), MCV ($p = 0.02$) and MCHC ($p = 0.02$) between the feeding groups. Clinically, this was not significant, as the MCV and MCHC values were still in the normal ranges. The following parameters were on average low at baseline for all 3 feeding groups: RBC, Haemoglobin, Haematocrit and Lymphocytes, with Neutrophils (%) and white blood cells being high according to the normal range.

Table 3. 12 Baseline full blood and differential counts of trial patients in the different feeding groups

Variable	Normal range (Units)	Group 1 (pH 3.5) [Mean (SD)]	Group 2 (pH 4.5) [Mean (SD)]	Group 3 (pH 6.8) [Mean (SD)]	Kruskal Wallis (p value)
WBC	4,0–10 (x 10 ⁹ /L)	13.9 (6.6)	14.6 (8.2)	12 (4.4)	0.92
RBC	3,8–4,8 (x 10 ¹² /L)	3.4 (0.7)	3.7 (0.7)	3.2 (0.7)	0.05
Haemoglobin	12,0–15,0 (g/dl)	9.8 (1.7)	10.9 (2.2)	9.7 (2)	0.13
Haematocrit	36-46 (L/L)	28.6 (8.5)	31.1 (9.3)	27.8 (9)	0.13
MCV	79,1–98,9 (fl)	87.5 (5.1)	87.6 (5.8)	91.1 (3.9)	0.02
MCH	27–32 (pg)	28.8 (2.1)	29.6 (2.1)	29.9 (1.7)	0.10
MCHC	32–36 (g/dl)	32.9 (1.4)	33.7 (1.2)	32.9 (0.9)	0.02
Lymphocytes	10–40 (%)	7.2 (4.4)	9.3 (5.9)	7.0 (5.2)	0.28
Neutrophils	20–75 (%)	87.1 (6.9)	85.8 (7.1)	87.5 (6.6)	0.69
Monocytes	1,8–8 (%)	7.1 (17.7)	2.5 (1.3)	3.1 (1.7)	0.26
Eosinophils	0–4,5 (%)	0.8 (0.8)	1.0 (1)	0.8 (0.7)	0.34
Platelets	178–400 (x 10 ⁹ /L)	235 (125.6)	201.4 (99.1)	194.7 (104.3)	0.52

Abbreviations: WBC = White blood cells; RBC = Red blood cells; MCV = Mean corpuscular volume; MCH = Mean corpuscular haemoglobin; MCHC Mean corpuscular haemoglobin concentrate

The baseline urea and electrolytes concentrations (Table 3.13) indicated a significant difference between the different feeding groups for potassium (Kruskal Wallis, p = 0.05), but the values were all still in the reference ranges for this parameter. The following parameters were on average low at baseline for all 3 feeding groups: Creatinine, Total Protein and Calcium. The liver enzymes (AST, ALT and GGT), as well as the inflammatory marker (CRP) concentrations were high for all 3 feeding groups at baseline.

Table 3.13 Baseline urea and electrolyte concentrations of trial patients in the different feeding groups

Variable	Normal range (Units)	Group 1 (pH 3.5) [Mean (SD)]	Group 2 (pH 4.5) [Mean (SD)]	Group 3 (pH 6.8) [Mean (SD)]	Kruskal Wallis (p value)
Sodium	135–147 (mmol/L)	139.7 (4.1)	140.1 (5.2)	140.4 (6.3)	0.73
Potassium	3,3–5,3 (mmol/L)	4.4 (0.6)	4.2 (0.6)	3.9 (0.6)	0.05
Urea	2,6–7,0 (mmol/L)	4.7 (1.9)	4.6 (2.4)	5.2 (3.4)	0.77
Creatinine	80–100 (µmol/L)	74.7 (16)	81.2 (23.6)	77.4 (25.1)	0.62
Total protein	60–85 (g/L)	46.1 (8.5)	47.7 (12)	45.6 (7.7)	0.59
Phosphate	0,8–1,4 (mmol/L)	0.9 (0.3)	1.0 (0.3)	1.0 (0.4)	0.74
Magnesium	0,65–1,1 (mmol/L)	0.8 (0.1)	0.7 (0.1)	0.7 (0.1)	0.42
Calcium	2,05–2,56 (mmol/L)	2 (0.1)	2.0 (0.1)	2 (0.1)	0.81
AST	8–20 (IU/L)	78.9 (60)	107.7 (101.1)	86.5 (104.5)	0.65
ALT	5–40 (IU/L)	52.3 (31)	95.9 (146)	48.8 (38.6)	0.62
Total bilirubin	1–17 (µmol/L)	13.1 (7.8)	16.9 (9.2)	23.3 (28.3)	0.42
ALP	40–120 (IU/L)	66.0 (30.2)	77.7 (59.5)	59.6 (18.4)	0.82
GGT	1–24 (IU/L)	29.5 (19)	55.4 (65.9)	35.3 (32.8)	0.80
CRP	≤ 3 (mg/L)	197.7 (110)	209.2 (118.6)	224.1 (114.2)	0.67
Glucose	4–11,1 (mmol/L)	7.5 (2)	6.8 (1.8)	7.3 (2.7)	0.50

Abbreviations: AST = aspartate transaminase, ALT = alanine aminotransferase, ALP = alkaline phosphatase, GGT = Gamma glutamyl transferase, CRP = C-reactive protein

Baseline blood gas pressures/concentrations (Table 3.14) indicated a significant difference (Kruskal Wallis, $p = 0.05$) only for base excess among the different feeding groups. Clinically, this was not significant, as the values were still within the normal range. The other blood gas

variables were all within the normal ranges, except for TCO₂ that was just above the normal range for all 3 feeding groups with no clinical significance.

Table 3.14 Baseline blood gas pressures/concentrations of trial patients in the different feeding groups

Variable	Normal range (Units)	Group 1 (pH 3.5) [Mean (SD)]	Group 2 (pH 4.5) [Mean (SD)]	Group 3 (pH 6.8) [Mean (SD)]	Kruskal Wallis (p value)
Base Excess	-4 - +2	-0.3 (2.6)	-0.3 (4.9)	1.6 (3.8)	0.05
pCO ₂	4,5–6,1 (kPa)	5.4 (1.5)	5.5 (1.6)	5.4 (1.2)	0.94
pH	7,37 – 7,43	7.4 (0.1)	7.4 (0.1)	7.4 (0.1)	0.54
pO ₂	11–15 (kPa)	15.7 (5.2)	14.2 (4.3)	14.8 (3.8)	0.56
Saturation	95 - 98 (%)	97.0 (2.9)	96.3 (2.4)	97.2 (2.8)	0.33
TCO ₂	21,0–25,0 (mmol/l)	25.1 (3.9)	25.2 (5.7)	26.8 (4.6)	0.23

3.6.2 Longitudinal treatment variables

Analyses of covariance (ANCOVA) were used to analyse the longitudinal treatment variables. Time was taken as the covariate to estimate whether the 3 regression lines (3 feeding groups) were parallel for time. The PROC MIXED model (with repeated measures) was used for a wider class of mixed linear models to estimate differences among the three trial groups. It is important to note that the ANCOVA illustrations/figures presented in this thesis are general linear ANCOVA's, showing interaction between groups and not number of days on the trial for repeated measures. It is only of illustrative value and no significance testing was done with this method. A similar analysis was performed for the nutrition-related variables. PROC MIXED analysis of the longitudinal nutrition-related variables (Table 3.15) showed no significant differences between the feeding groups. PROC MIXED analysis, however showed significant increases over time in the following parameters in all three feeding groups: Pre-albumin ($p = 0.004$) (Appendix H, Figure 1), retinol-binding protein ($p < 0.0001$) (Appendix H, Figure 2) and α_1 -acid-glycoprotein ($p < 0.0001$) (Appendix H, Figure 3) with no interaction between groups and a linear relationship over time.

Table 3.15 Treatment differences in the longitudinal nutrition-related variables of the different feeding groups during the trial period estimated by PROC MIXED

Variable	Differences in feeding groups	Effect of time during trial period	Interaction between time and groups and non-linear relationship over time
Pre-albumin	No	Increase (p = 0.004)	No interaction, linear over time
RBP	No	Increase (p<0.0001)	No interaction, linear over time
AGP	No	Increase (p<0.0001)	No interaction, linear over time
Albumin	No	No	No interaction, linear over time

Abbreviations: RBP = retinol binding protein; AGP = α_1 -acid-glycoprotein

The longitudinal full blood and differential count differences (Table 3.16) showed no significant differences among any of the feeding groups with significant increases over time for platelets (Figure 3.2) (p < 0.0001) and WBC (Figure 3.3) (p = 0.05), as well as interaction between groups for the same two variables. A non-linear relationship was found over time for the different feeding groups for platelets (p = 0.02)

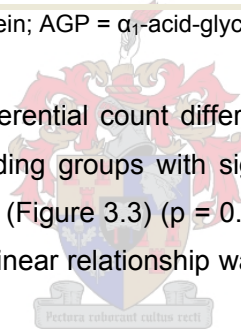


Table 3.16 Treatment differences in the longitudinal full blood and differential counts of the different feeding groups during the trial period estimated by PROC MIXED

Variable	Differences in feeding groups	Effect of time during trial period	Interaction between time and groups and non-linear relationship over time
Platelets	No	Increase ($p < 0.0001$)	Yes, interaction, lines not parallel ($p = 0.003$). Non-linear over time ($p = 0.02$)
WBC	No	Increase ($p < 0.05$)	Yes, interaction, lines not parallel ($p = 0.008$). Linear over time
MCV	No	No	No interaction, linear over time
MCH	No	No	No interaction, linear over time
MCHC	No	No	No interaction, linear over time
Haemoglobin	No	No	No interaction, linear over time
RBC	No	No	No interaction, linear over time
HCT	No	No	No interaction, linear over time
Lymphocytes	No	No	No interaction, linear over time
Neutrophils	No	Decrease ($p = 0.001$)	No interaction, linear over time
Monocytes	No	No	No interaction, linear over time
Eosinophils	No	No	No interaction, linear over time

Abbreviations: WBC = White blood cells; RBC = Red blood cells; MCV = Mean corpuscular volume; MCH = Mean corpuscular haemoglobin; MCHC Mean corpuscular haemoglobin concentrate

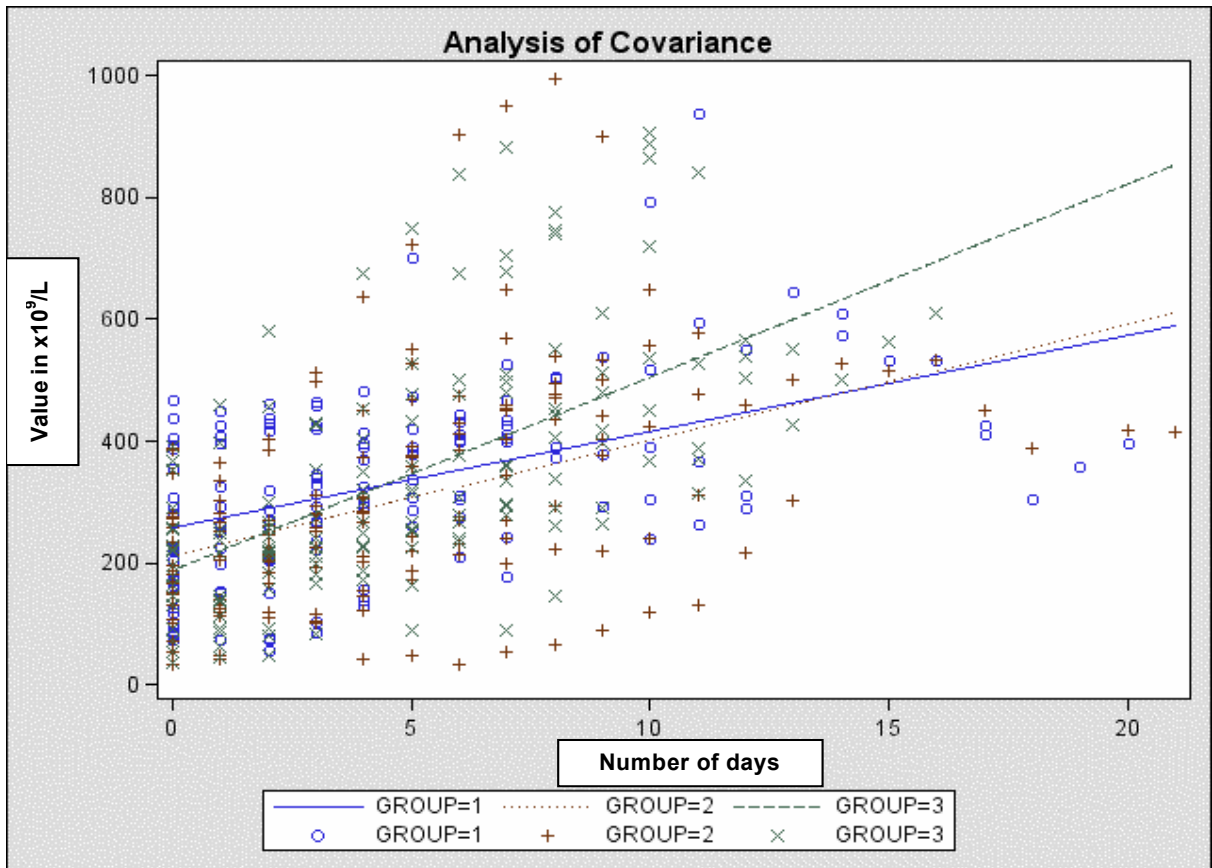


Figure 3.2 ANCOVA for platelets in the different feeding groups during the trial period

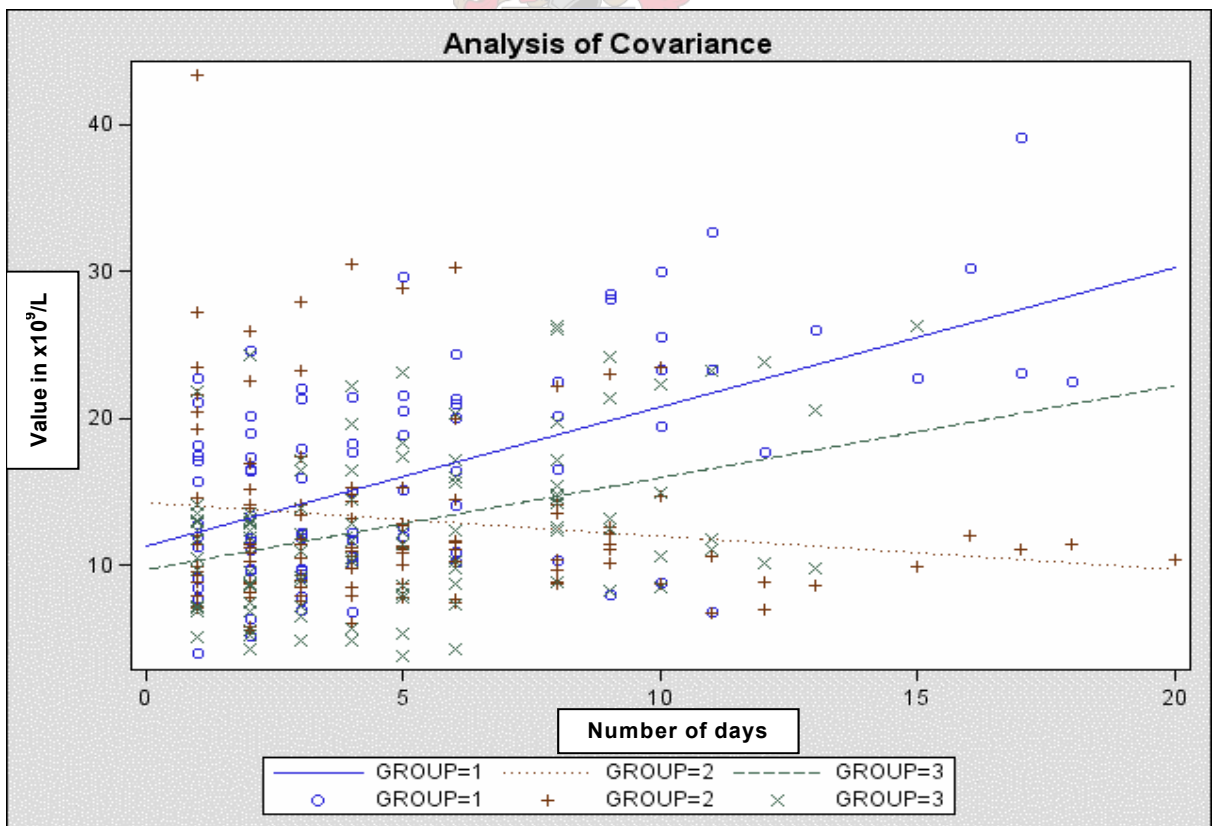


Figure 3.3 ANCOVA for white blood cells in the different feeding groups during the trial period

The longitudinal urea and electrolyte concentration differences (Table 3.17, PROC MIXED analysis) showed no significant differences among the feeding groups, except for the acidified group (pH 4.5) and the control group for GGT ($p = 0.043$) (Figure 3.4). Significant increases were found over time for the following parameters: GGT ($p < 0.0001$), potassium ($p = 0.002$) (Figure 3.5), total protein ($p < 0.0001$) (Appendix H, Figure 9), Phosphate ($p < 0.0001$) (Appendix H, Figure 10), Calcium ($p = 0.0004$) (Appendix H, Figure 11), ALP ($p < 0.0001$) (Appendix H, Figure 12). A decrease over time was found in AST ($p = 0.009$) (Appendix H, Figure 13) and CRP ($p = 0.0001$) (Appendix H, Figure 14). Non-linear relationships were found for urea ($p = 0.05$), AST ($p = 0.03$) and CRP ($p = 0.02$). An interaction between groups was found only for potassium ($p = 0.04$), as lines between groups were not parallel.

Table 3.17 Treatment differences in the longitudinal urea and electrolytes concentrations of the different feeding groups during the trial period estimated by PROC MIXED

Variable	Differences in feeding groups	Effect of time during trial period	Interaction between time and groups and non-linear relationship over time
Sodium	No	No	No interaction, linear over time
Urea	No	No	No interaction, non-linear over time ($p = 0.049$)
Creatinine	No	No	No interaction, linear over time
ALT	No	No	No interaction, linear over time
GGT	Group 2 (pH 4.5) differs from Group 3 (pH 6.8) ($p = 0.043$)	Increase ($p < 0.0001$)	No interaction, linear over time
Glucose	No	No	No interaction, linear over time
Potassium	No	Increase ($p = 0.002$)	Yes, interaction, lines not parallel ($p = 0.032$). Linear over time
Total protein	No	Increase ($p < 0.0001$)	No interaction, linear over time

Table 3.17 Treatment differences in the longitudinal urea and electrolytes concentrations of the different feeding groups during the trial period estimated by PROC MIXED (cont')

Variable	Differences in feeding groups	Effect of time during trial period	Interaction between time and groups and non-linear relationship over time
Phosphate	No	Increase (p<0.0001)	No interaction, linear over time
Calcium	No	Increase (p = 0.0004)	No interaction, linear over time
AST	No	Decrease (p = 0.009)	No interaction, non-linear over time (p = 0.029)
ALP	No	Increase (p<0.0001)	No interaction, linear over time
CRP	No	Decrease (p = 0.0001)	No interaction, non-linear over time (p = 0.0198)
Magnesium	No	No	No interaction, linear over time
Billirubin	No	No	No interaction, linear over time

Abbreviations: AST = aspartate transaminase, ALT = alanine aminotransferase, ALP = alkaline phosphatase, GGT = gamma glutamyl transferase, CRP = C-reactive protein

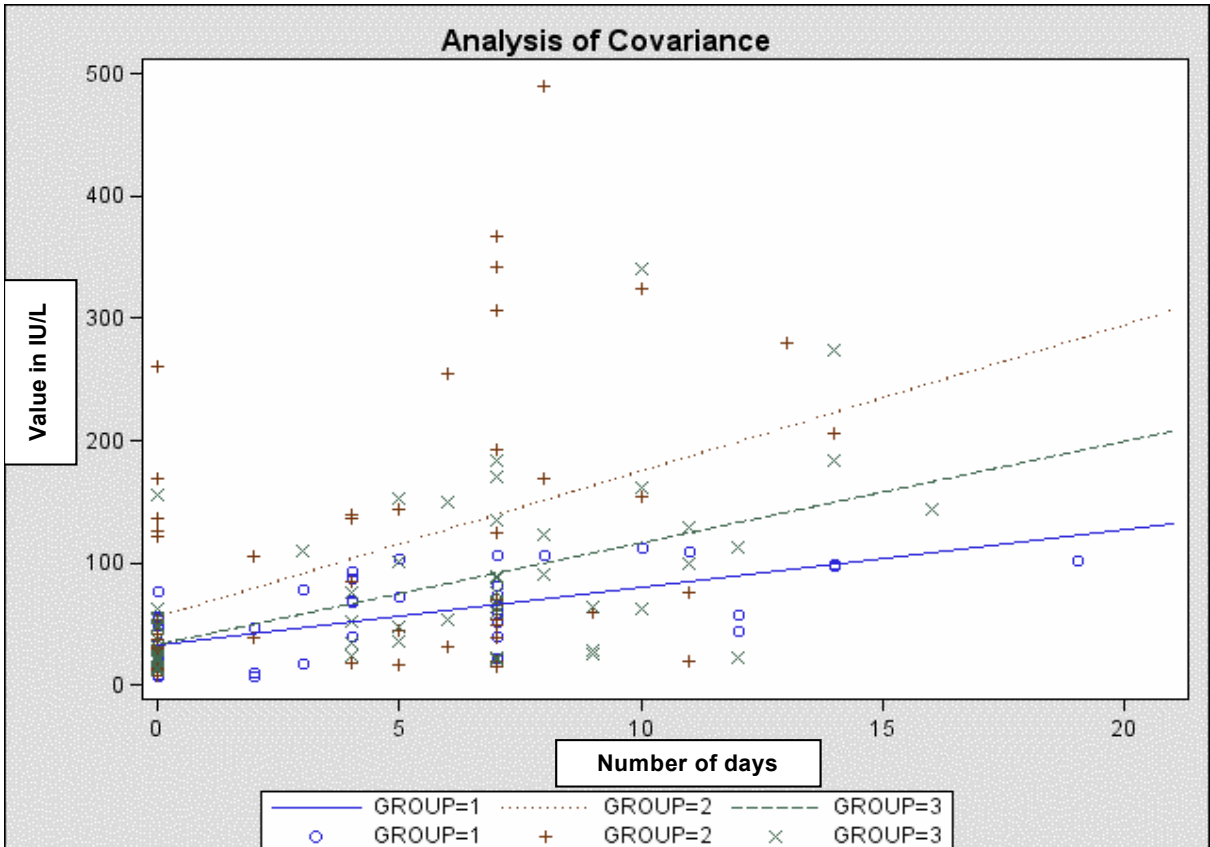


Figure 3.4 ANCOVA for GGT in the different feeding groups during the trial period

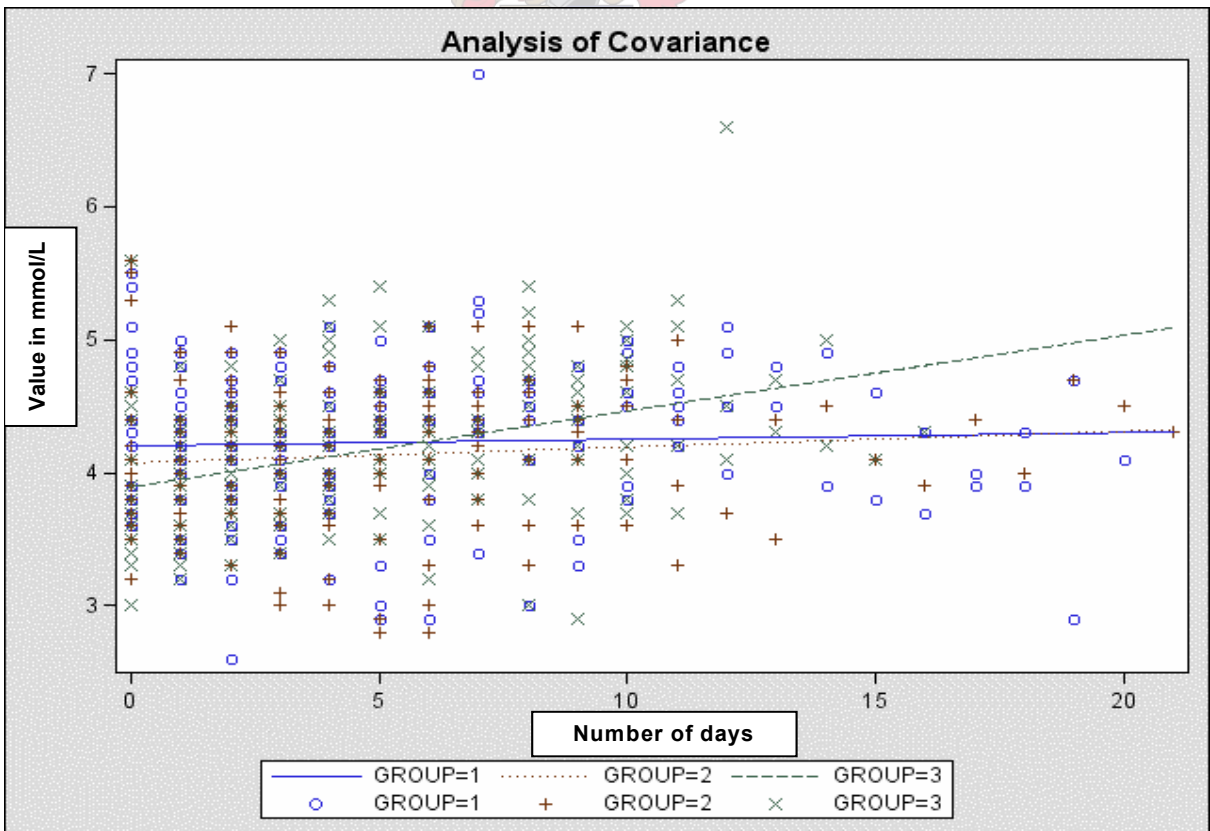


Figure 3.5 ANCOVA for potassium in the different feeding groups during the trial period

The longitudinal blood gas pressures/concentration differences (Table 3.18, PROC MIXED) showed no significant differences between any of the feeding groups. Significant increases over time were found for base excess ($p < 0.0001$) (Appendix H, Figure 15) and TCO_2 ($p < 0.0001$) (Appendix H, Figure 16), but the difference was of no clinical significance. No interactions were found between groups, but non-linear relationships over time for the following parameters were seen: base excess ($p = 0.004$), TCO_2 ($p = 0.04$) and pO_2 ($p = 0.04$).

Table 3.18 Treatment differences in the longitudinal blood gas pressures/concentrations of the different feeding groups during the trial period estimated by PROC MIXED

Variable	Differences in feeding groups	Effect of time during trial period	Interaction between time and groups and non-linear relationship over time
Base Excess	No	Significant increase over time ($p < 0.0001$)	No interaction, non-linear over time ($p = 0.004$)
pH	No	No	No interaction, linear over time
TCO_2	No	Significant increase over time ($p < 0.0001$)	No interaction, non-linear over time ($p = 0.04$)
pO_2	No	No	No interaction, non-linear over time ($p = 0.04$)
pCO_2	No	No	No interaction, linear over time
Saturation	No	No	No interaction, linear over time

3.7 pH VARIABLES

The pH values of different variables were done daily for the duration of the trial period. A summary was made of the main changes (Table 3. 19) with a more detailed discussion of each variable following.

Table 3.19 Treatment differences in the longitudinal pH values of the different feeding groups during the trial period estimated by PROC MIXED

pH	Differences in feeding groups	Effect of time during trial period	Interaction between time and groups and non-linear relationship over time
Tap water before	No	Increased ($p = 0.03$)	Yes, interaction, lines not parallel ($p < 0.0001$). Non-linear over time ($p = 0.04$)
Reconstituted formulae	Group 1 (pH 3.5) and Group 2 (pH 4.5) differs from Group 3 (pH 6.8) ($p < 0.0001$)	No	No interaction, linear over time
Delivery system	Group 1 (pH 3.5) and Group 2 (pH 4.5) differs from Group 3 (pH 6.8) ($p < 0.0001$)	No	No interaction, linear over time
Feeding bottle	Group 1 (pH 3.5) and Group 2 (pH 4.5) differs from Group 3 (pH 6.8) ($p < 0.0001$)	No	No interaction, linear over time
Nasogastric aspirate	Group 1 (pH 3.5) differs from Group 3 (pH 6.8) ($p < 0.005$)	No	Yes, interaction, lines not parallel ($p = 0.0008$). Linear over time
Tracheal aspirate	No	No	No interaction, linear over time

3.7.1 pH of tap water

Powdered trial formulae were reconstituted daily with tap water and showed no significant difference among the feeding groups (Table 3.19). An increase in pH was found over the trial period ($p = 0.03$). An interaction between the groups was found, as the lines are not parallel ($p < 0.0001$) with a non-linear relationship over the number of days during the trial period time ($p = 0.04$) (Figure 3.6). As the pH of the tap water ranged on average from 7.66 (Group 2), to 7.97 (Group 1) to 8.11 (Group 3), it had an effect on the pH of the reconstituted feeds. The following changes in the average pH values of the different trial formulae after reconstitution were found: Group 1 (pH 3.5) became pH 3.63, Group 2 (pH 4.5) became pH 4.09 and Group 3 (pH 6.8) became 6.97. The pH of the powdered trial formulae of the different groups was accepted as given by the manufacturer and was not determined before reconstitution.

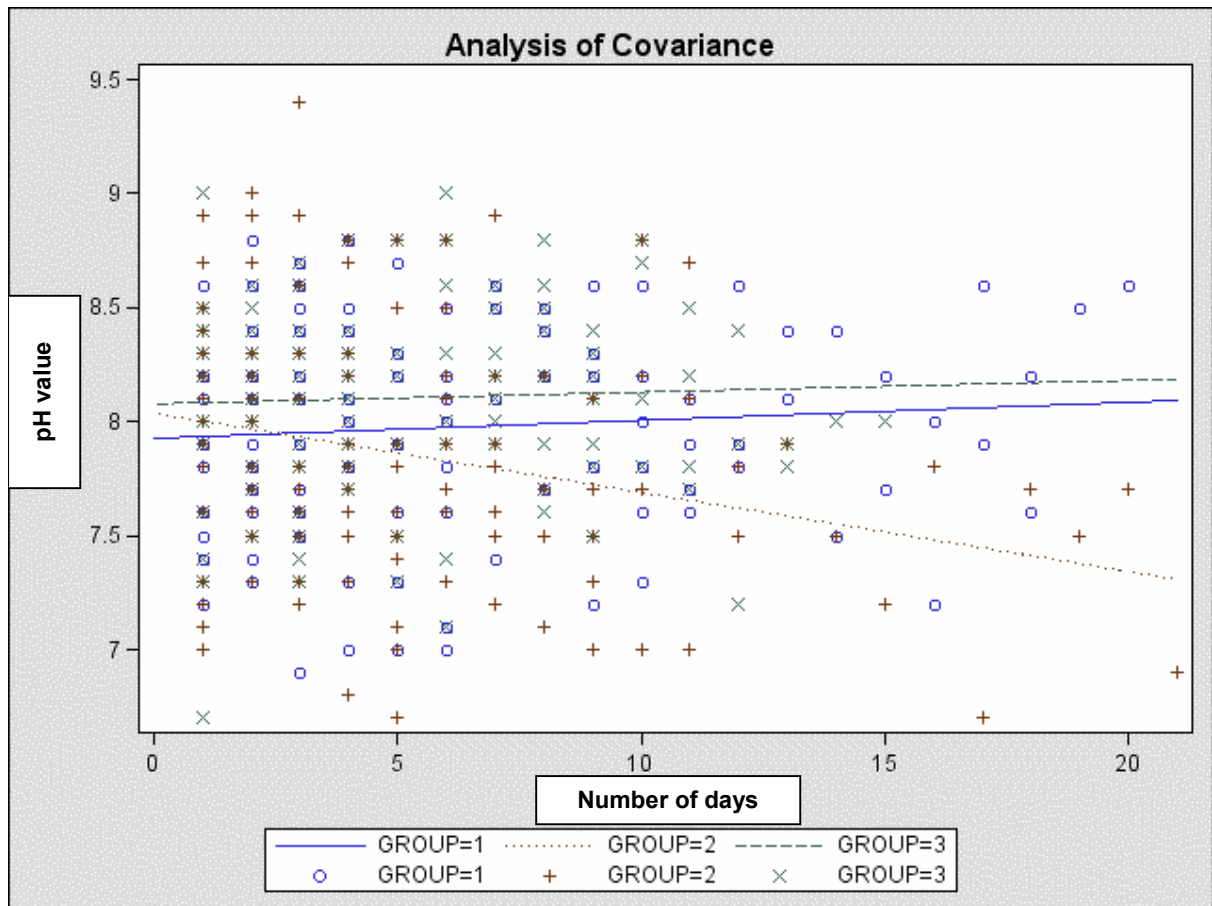


Figure 3.6 ANCOVA differences in the pH for tap water used for reconstitution of trial formulae during the trial period

3.7.2 pH of reconstituted formulae

Although the tap water did slightly alter the pH of the different trial formulae, the pH for the reconstituted trial formulae was still highly significant ($p < 0.0001$) (Table 3.19) for both the acidified formulae with pH 3.5 and pH 4.5 when compared with the control Group 3 (pH 6.8) (Figure 3.7). Time had no effect on the results. No interaction between groups was found and the relationship over time was linear.

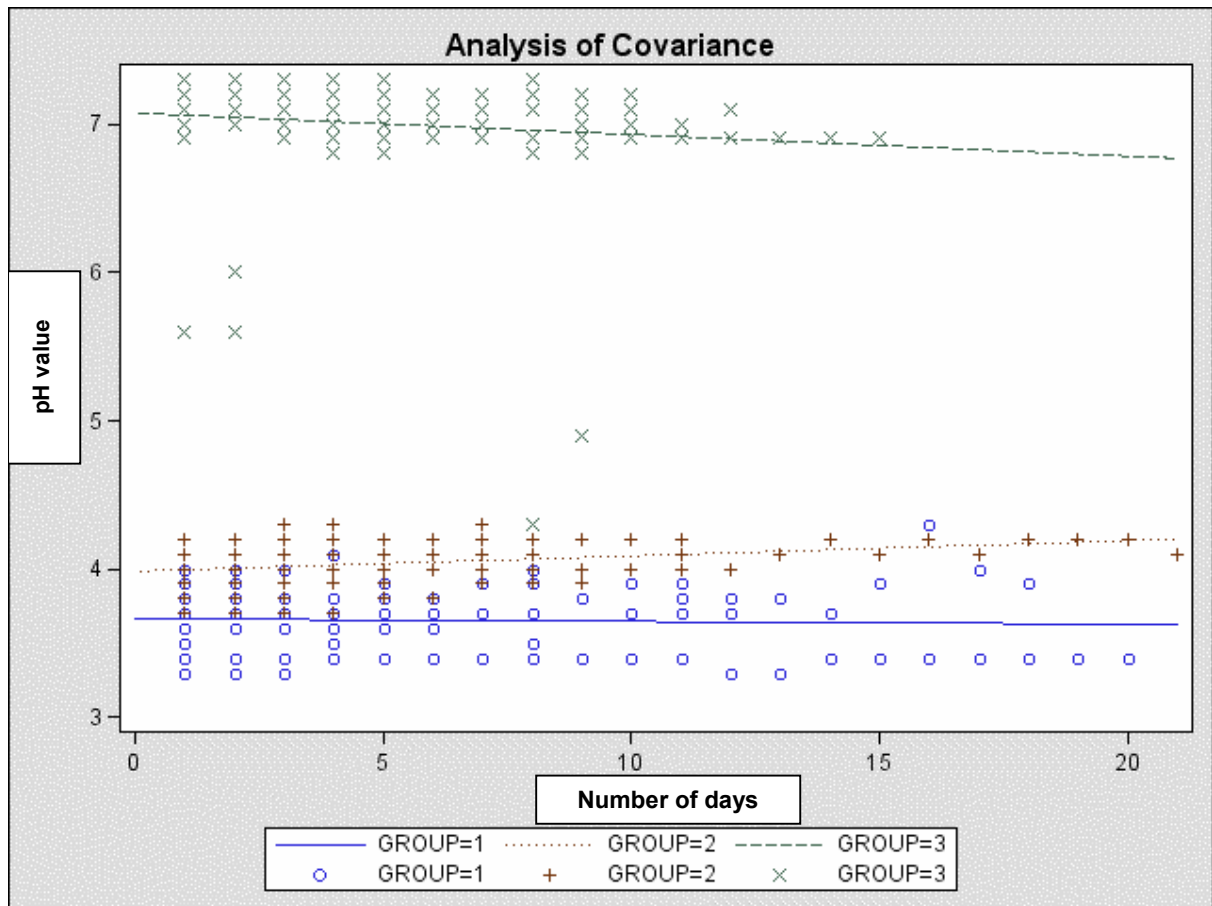


Figure 3.7 ANCOVA differences in the pH for the reconstituted trial formulae during the trial period

3.7.3 pH of formulae in the delivery systems and feeding bottles

During daily administration of enteral feeds, the pH of the trial formulae in the delivery system and feeding bottle of each patient was determined, to ensure that patients received the assigned formula according to randomisation. The pH of the trial formulae did not change during administration and remained significant ($p < 0.0001$) for both the acidified formulae with pH 3.5 and pH 4.5 when compared with the control Group 3 (pH 6.8) (Figure 3.8 – 3.9). Time had no effect on the results. No interaction between groups was found and the relationship over time was linear.

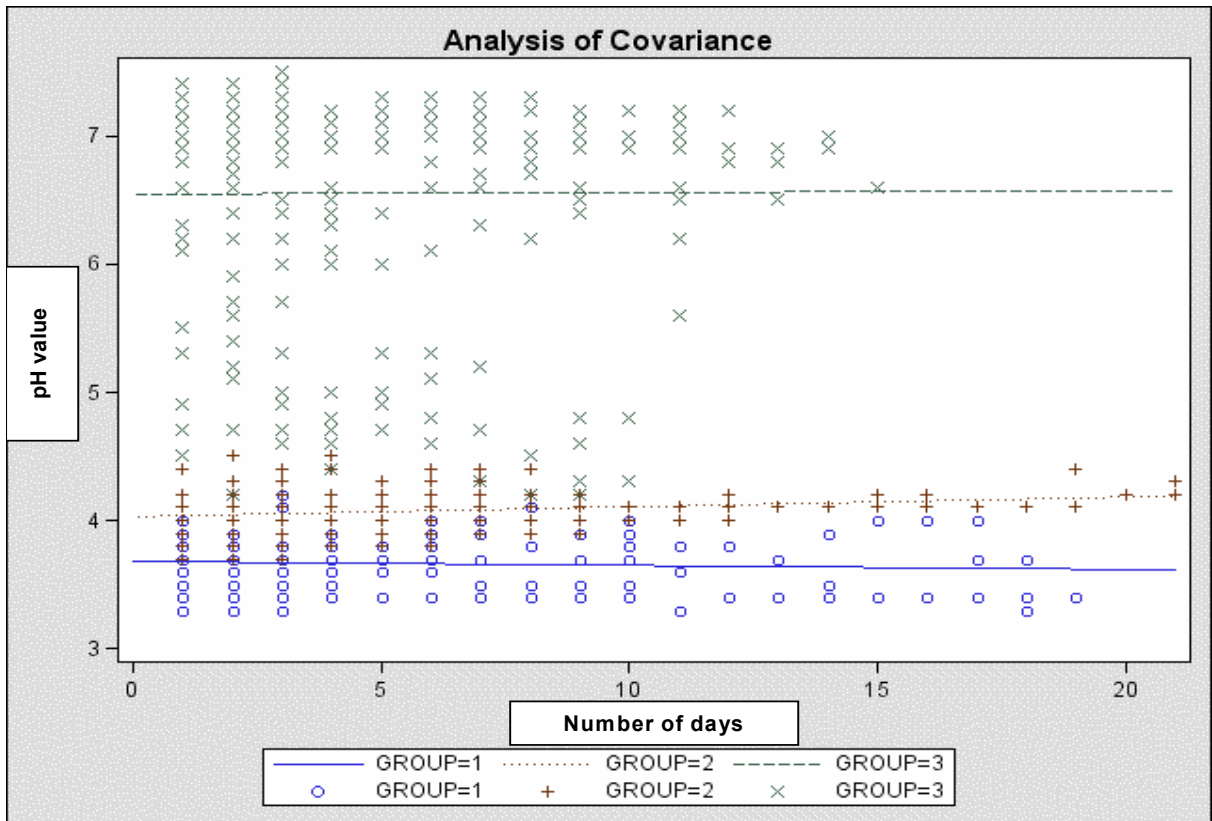


Figure 3.8 ANCOVA differences in the pH of trial formulae in the delivery systems during the trial period

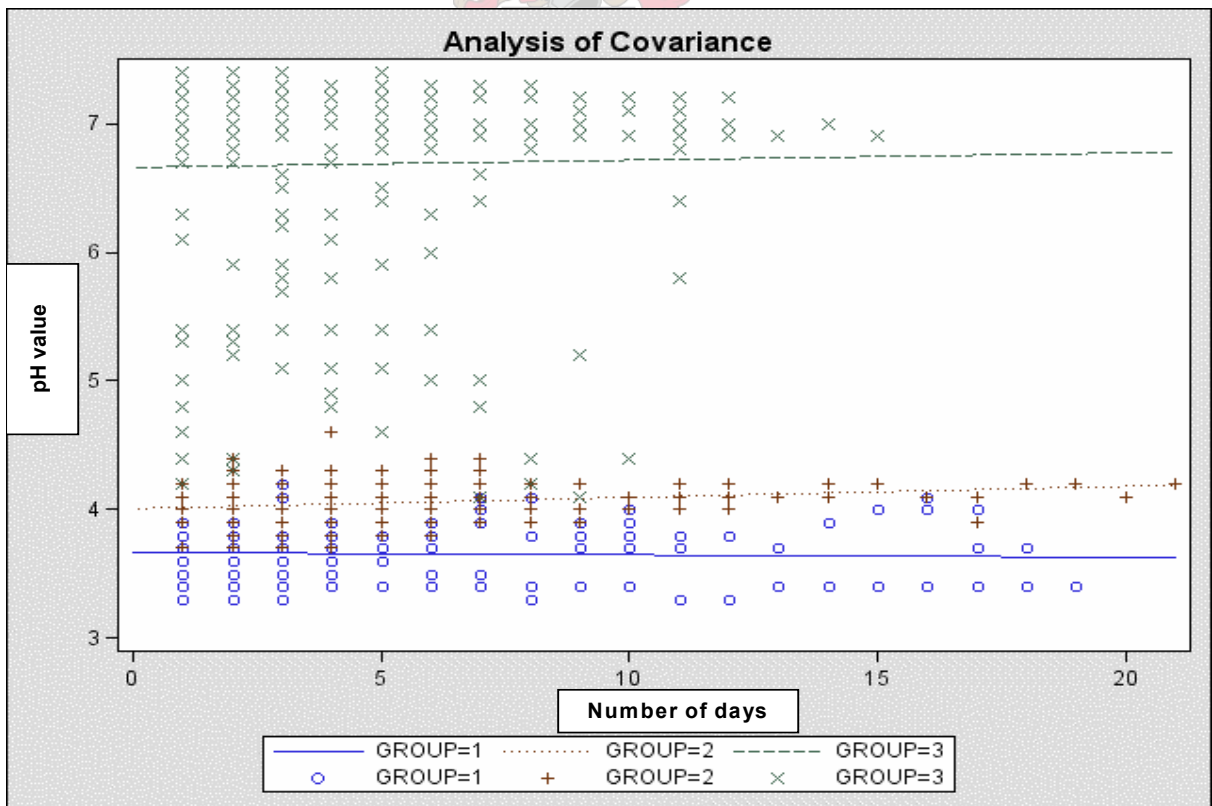


Figure 3.9 ANCOVA differences in the pH of the trial formulae in the feeding bottles during the trial period

3.7.4 pH of the nasogastric aspirates

At Baseline (Day 0), before the initiation of enteral feeds, the gastric pH of the three feeding groups was not significantly different (Kruskal Wallis, $p = 0.86$). After the trial formulae had been administered from Day 1 onwards, the average follow-up gastric pH decreased for Group 1 and Group 2 to 3.66 and 4.16 respectively and increased for Group 3 (control) at 4.62 and it was statistically significant (Kruskal Wallis, ANCOVA, $p < 0.0001$) (Table 3.20). Comparing mean gastric pH before and after the administration of trial formulae showed that acidified feeds (both groups) resulted in further acidification of gastric contents, while the control group resulted in gastric alkalinisation.

Table 3.20 Gastric pH changes for the different feeding groups before and after the administration of trial formulae

	Group 1 (pH 3.5) [Mean (SD)]	Group 2 (pH 4.5) [Mean (SD)]	Group 3 (pH 6.8) [Mean (SD)]	p-value
Gastric pH at baseline	4.15 (1.6)	4.57 (2.1)	4.18 (2.0)	0.86
Gastric pH on follow up	3.60 (0.2)	4.16 (1.3)	4.62 (1.6)	< 0.0001

The daily gastric pH for the trial period estimated by PROC MIXED was significantly different between Group 1 (pH 3.5) and Group 3 (pH 6.8) ($p = 0.005$) (Table 3.19). Time had no effect on the results. An interaction between the groups was found, as the lines are not parallel ($p = 0.0008$) with a linear relationship over time (Figure 3.10).

3.7.5 pH of the tracheal aspirates

The average tracheal aspirate pH for the trial period showed no significant difference (PROC MIXED, Table 3.19) between any of the different feeding groups, time had no effect on the results and no significant interaction between groups was found with a linear relationship over time (Figure 3.11).

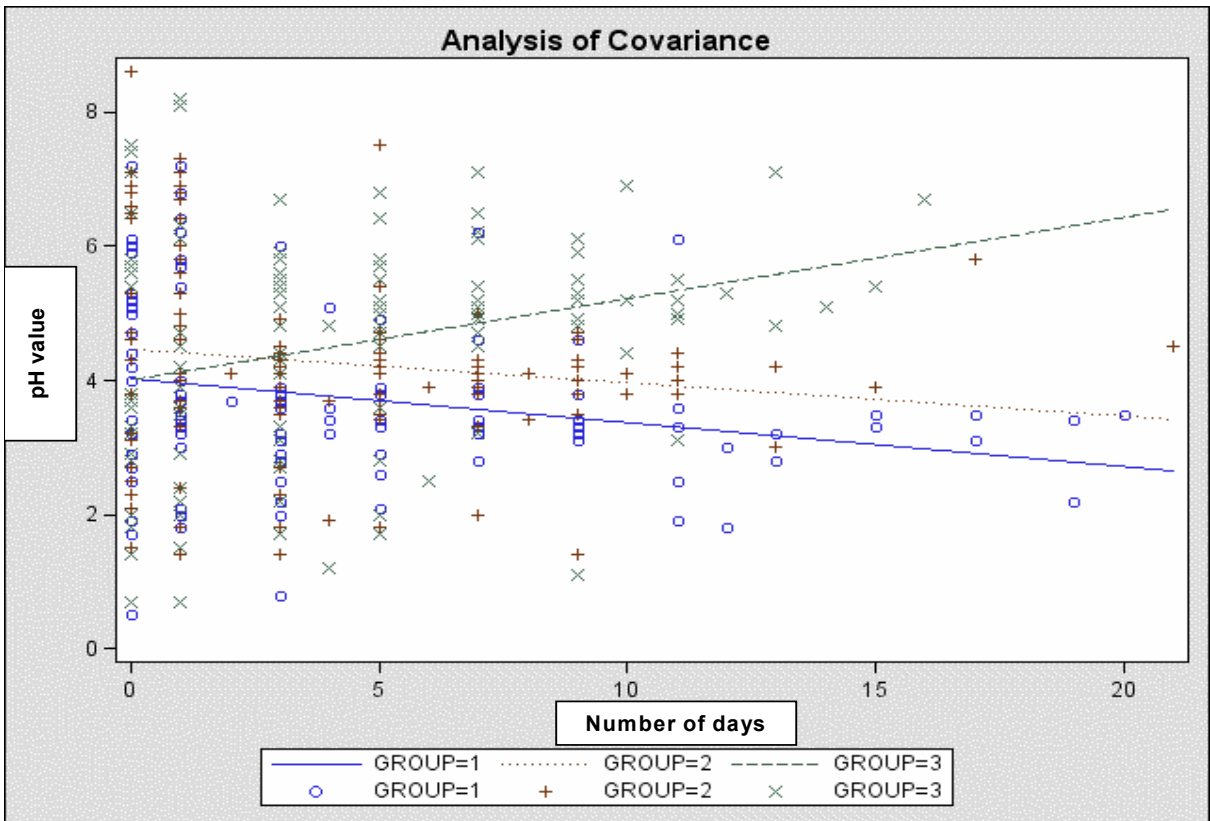


Figure 3.10 ANCOVA difference in pH for the nasogastric aspirates for the different feeding groups during the trial period

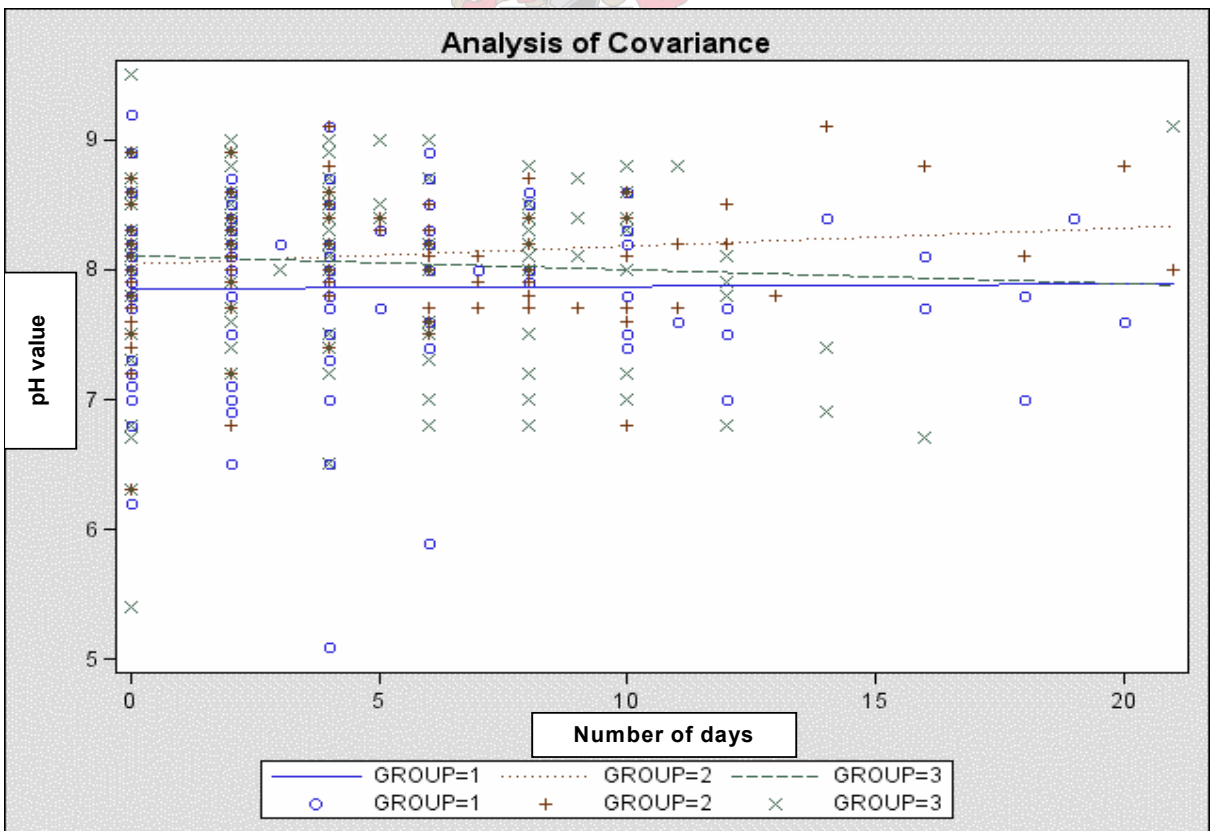


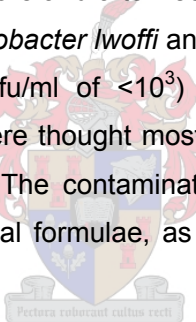
Figure 3.11 ANCOVA differences in the pH of the tracheal aspirates for the different feeding groups during the trial period

3.8 MICROBIOLOGIC VARIABLES

Microbiological analyses of the trial formulae, enteral feeding administration system, nasogastric and tracheal aspirates were done on a daily basis for each patient. Contamination was defined as the number of pathogenic organisms detected, as well as organism growth through colony counts and given in colony forming units per millilitre (cfu/ml). Unacceptable contamination was defined as $\geq 10^5$ cfu/ml using the Centre for Disease Control's definition of food-borne disease ⁽¹³³⁾. The PROC MIXED model (with repeated measures) was used for a wider class of mixed linear models to estimate differences between the three study groups. It is important to note that the ANCOVA illustrations/figures presented in this thesis are general linear ANCOVA's, showing interaction between groups and not number of days during the trial period for repeated measures. It is only of illustrative value and no significance testing was done with this method.

3.8.1 Tap water contamination

Microbiological samples of tap water before and after reconstitution of trial formulae were taken for each patient. Three of 1347 samples (0.2%) were contaminated. In two samples cfu/ml of $<10^4$ were grown in the tap water before and after reconstitution in the same patient on the same day with the same organisms (*Acinetobacter lwoffii* and *Citrobacter freundii*). In the third sample of tap water *Acinetobacter species* (cfu/ml of $<10^3$) was grown. All 3 contaminated samples occurred in Group 3 (pH 6.8) and were thought most likely due to cross-contamination from the researcher to the tap or tap water. The contamination of the tap water did not influence the contamination of the reconstituted trial formulae, as the organisms were not transferred to the reconstituted feeds.



3.8.2 Powdered trial formulae contamination

Microbiological samples of the dry-powder trial product were taken for each patient. Five of 487 samples (1%) were contaminated. Group 1 (pH 3.5) had 1 contaminated sample (cfu/ml of $<10^5$), Group 2 (pH 4.5) had no contaminated samples and Group 3 (pH 6.8) had 5 contaminated samples with the following colony counts respectively: 2×10^3 , 2×10^3 and 1×10^4 . Only 2 of these contaminated samples in Group 3 (pH 6.8) resulted in the same colony count in the reconstituted trial formulae on the same day.

3.8.3 Reconstituted trial formulae contamination

Microbiological samples were also taken from the reconstituted trial formulae before they were administered to the patients. Forty of 903 samples (4%) were contaminated, with the acidified Group 1 (pH 3.63) having the least contamination ($\leq 10^3$), followed by the acidified Group 2 (pH 4.09). The control Group 3 (pH 3.97) had the highest contamination and the heavier growth in terms of colony counts ($\leq 10^4$) (Table 3.21).

Table 3.21 Contamination in terms of severity of growth of the reconstituted trial formulae for the different feeding groups during the trial period

Cfu/ml	Group 1 (pH 3.5)	Group 2 (pH 4.5)	Group 3 (pH 6.8)
$\leq 10^3$	4	5	7
$\leq 10^4$	2	8	11
$\leq 10^5$	1	0	1
Total	7	13	20

3.8.4 Delivery systems and feeding bottles contamination

Microbiological samples were taken from 1711 delivery systems and from 1718 feeding bottles of patients after hanging times of 6 hrs and 24 hrs respectively. Patients who received acidified enteral formulae (pH 3.5 and 4.5) had significantly less contamination ($p < 0.0001$, PROC MIXED) of the feeding bottles and delivery systems (Figures 3.12 - 3.13), when compared with the control formula. Indicating that acidification of enteral feeds did inhibit bacterial growth in terms of contamination (Table 3.22). Time had no effect on the results with no interaction between groups and a linear relationship over time.

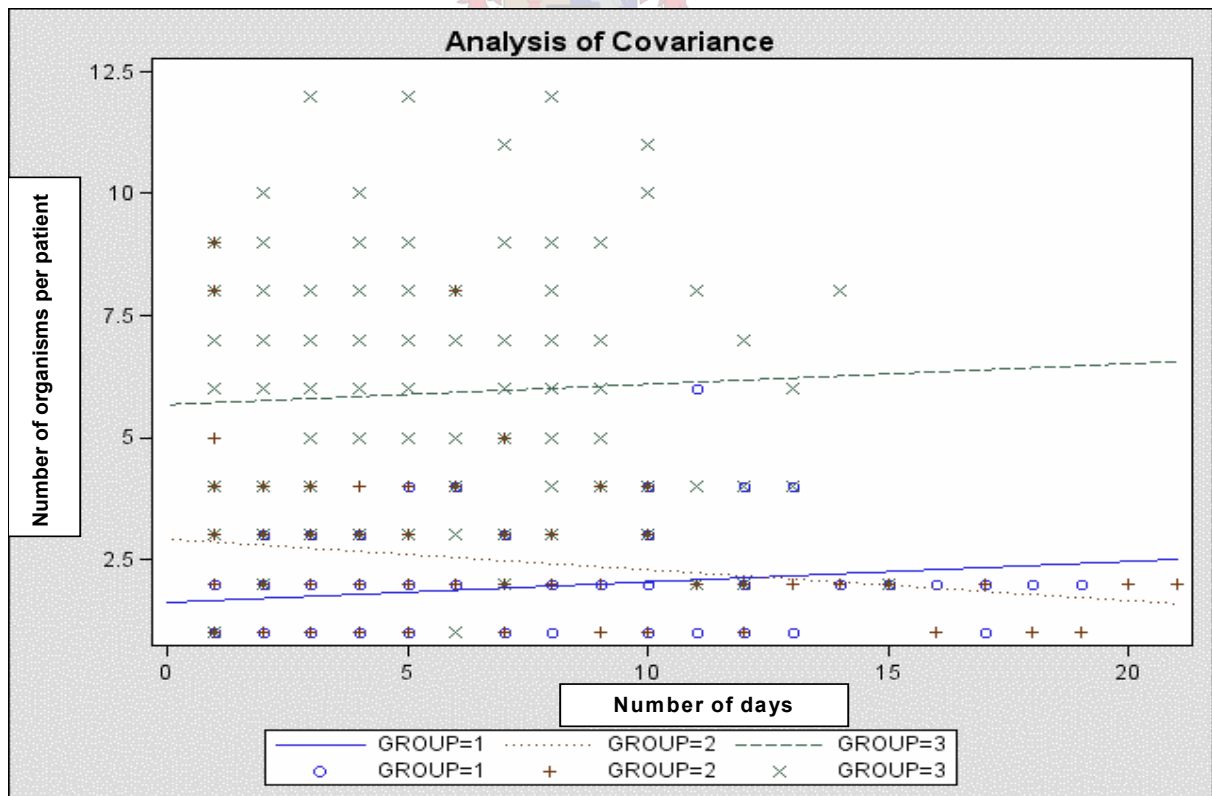


Figure 3.12 ANCOVA for number of pathogenic organisms per patient per day in the delivery systems of the different feeding groups during the trial period

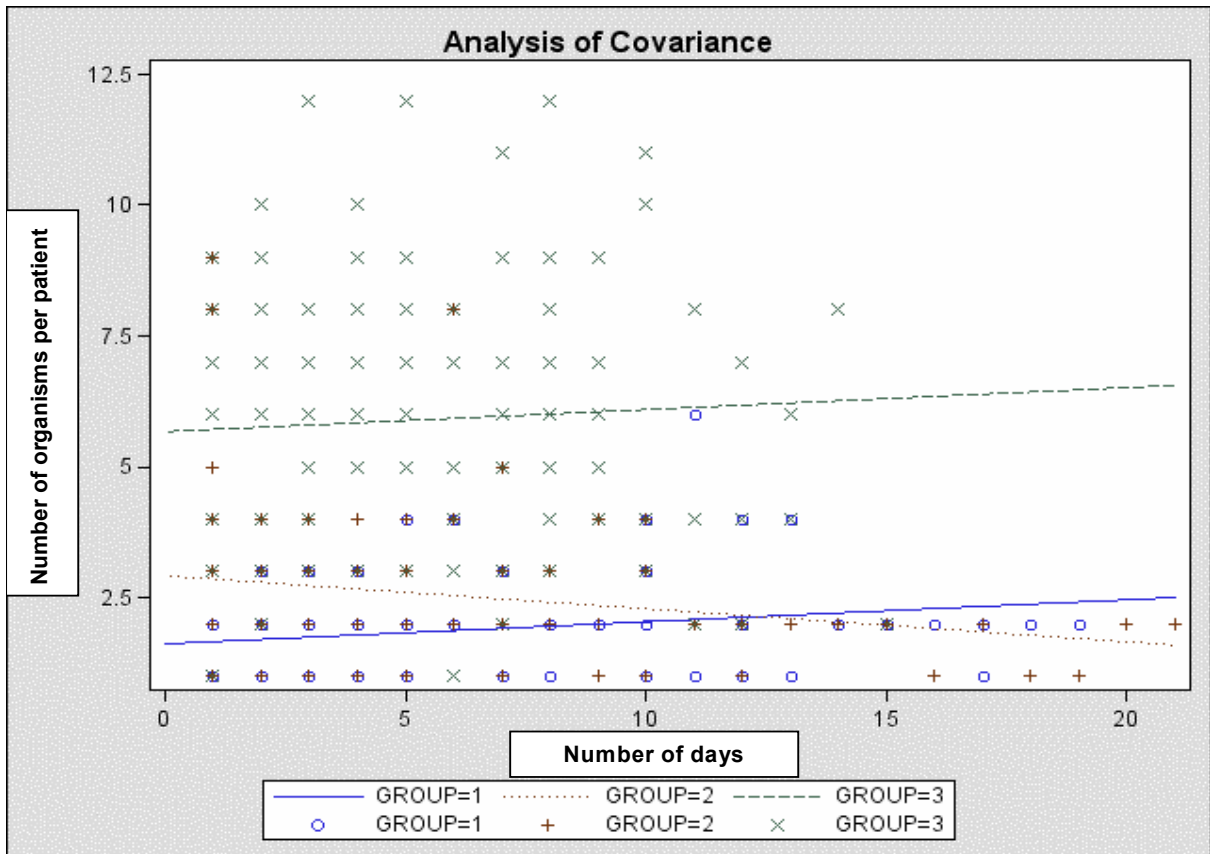


Figure 3.13 ANCOVA for number of pathogenic organisms patient per day in the feeding bottles of the different feeding groups during the trial period

3.8.5 Nasogastric aspirate contamination

Before the initiation of enteral feeds, the gastric pH of patients in Group 1 (pH 3.5) and Group 3 (pH 6.8) was almost the same at 4.15 and 4.18 respectively. The average pH over the study period was 3.60 for Group 1 and 4.62 for Group 3 and the difference was statistically significant (Kruskal Wallis, ANCOVA $p < 0.0001$). Significant differences (Table 3.22, PROC MIXED) were found between the acidified groups and the control group for contamination per patient per day for nasogastric aspirates ($p < 0.0001$) (Figure 3.14) and severity of growth in cfu/ml ($p = 0.0164$) (Figure 3.15). Time had no significant effect on the results with no interaction between groups and a linear relationship over time.

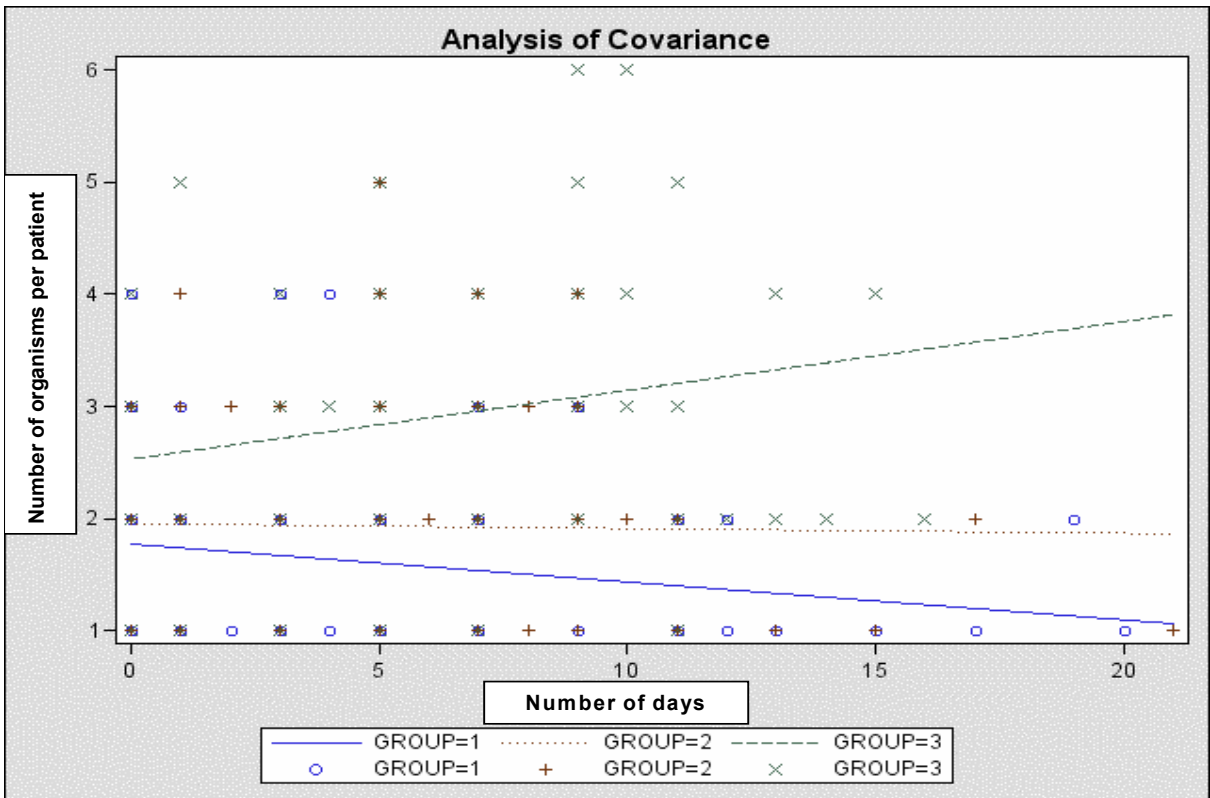


Figure 3.14 ANCOVA for contamination per patient per day in nasogastric aspirates of the different feeding groups during the trial period

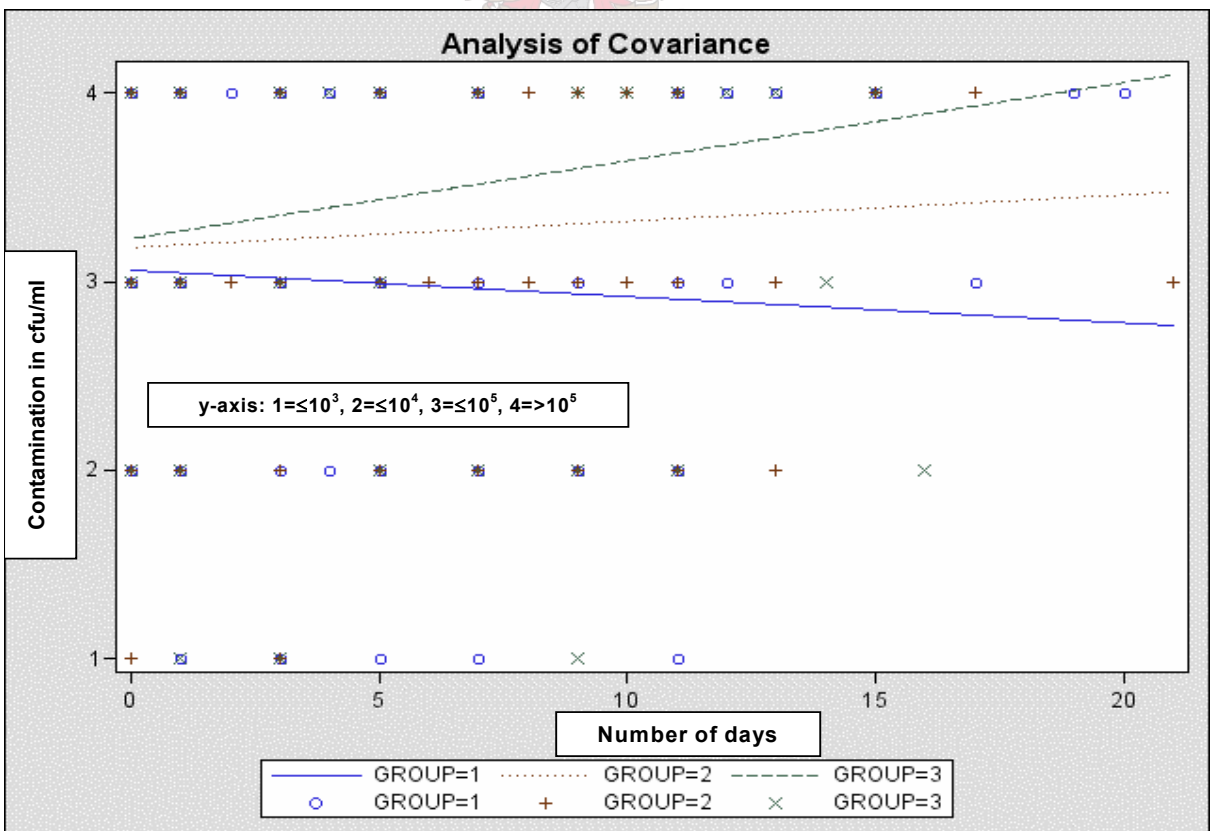


Figure 3.15 ANCOVA for contamination per day in terms of severity of growth (cfu/ml) in nasogastric aspirates of the different feeding groups during the trial period

Table 3.22 Treatment differences in the different feeding groups for microbiologic contamination during the trial period estimated by PROC MIXED

Variable	Differences in feeding groups	Effect of time during trial period	Interaction between time and groups and non-linear relationship over time
Delivery system	Group 1 (pH 3.5) and Group 2 (pH 4.5) differs from Group 3 (pH 6.8) ($p < 0.0001$)	No	No interaction, linear over time
Feeding bottle	Group 1 (pH 3.5) and Group 2 (pH 4.5) differs from Group 3 (pH 6.8) ($p < 0.0001$)	No	No interaction, linear over time
Nasogastric Aspirate	Group 1 (pH 3.5) and Group 2 (pH 4.5) differs from Group 3 (pH 6.8) ($p < 0.0001$)	No	No interaction, linear over time
Tracheal aspirate	No	Increased ($p = 0.005$)	No interaction, linear over time

3.8.6 Tracheal aspirate contamination

Microbiological analyses (PROC MIXED) of the tracheal aspirates showed no significant difference in contamination in terms of number of organisms or growth in terms of colony counts among any of the different feeding groups (Table 3.22). An increase in contamination over time was found ($p = 0.005$) with no interaction between groups and a linear relationship over time (Figure 3.16).

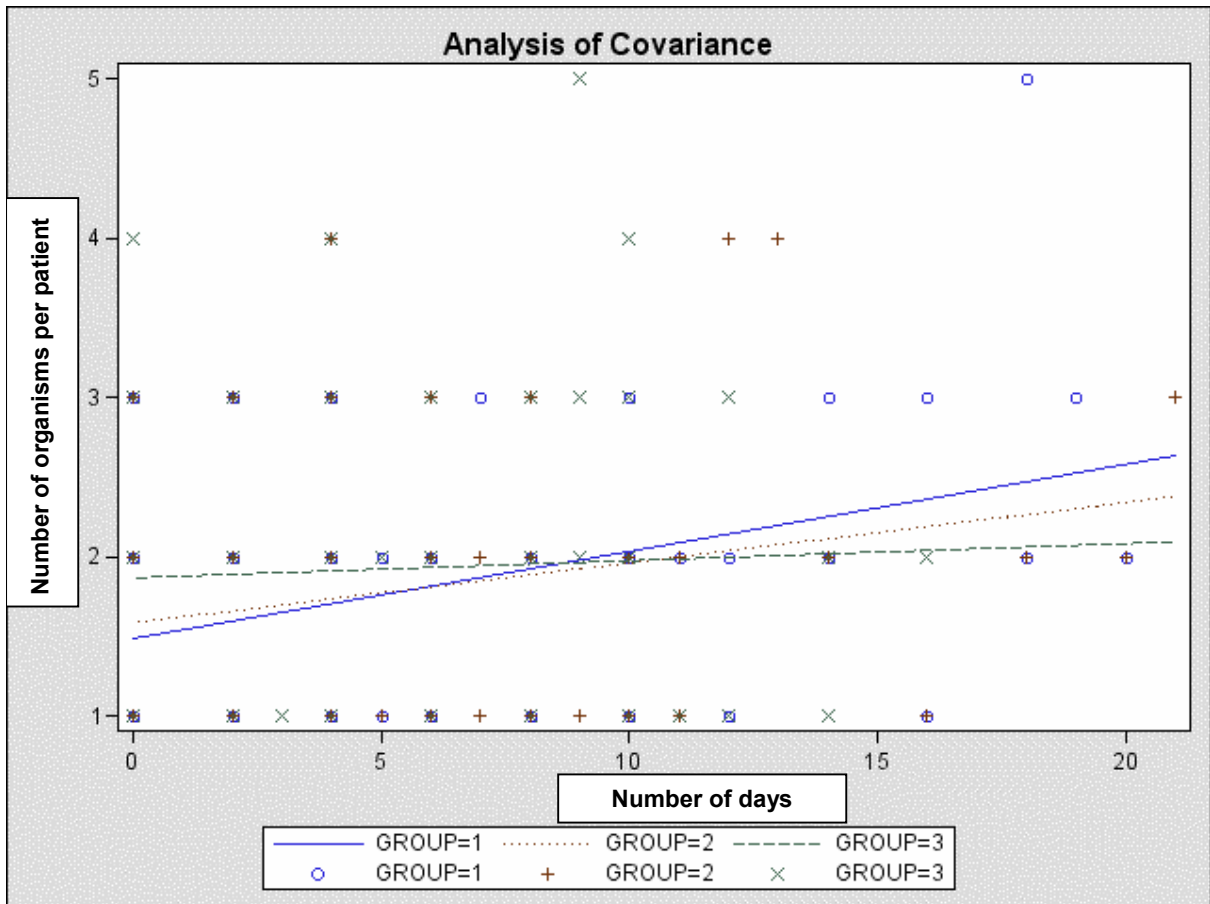


Figure 3.16 ANCOVA for contamination per patient per day in terms of number of pathogenic organisms in the tracheal aspirates of the different feeding groups during the trial period

The total number of pathogenic organisms (Figure 3.17) for the different sample types for the different feeding groups during the trial period indicated that (in relation to Figure 3.17) the contamination in the nasogastric aspirates and enteral feeding systems was the highest in the control feeding group when compared with the two acidified feeding groups.

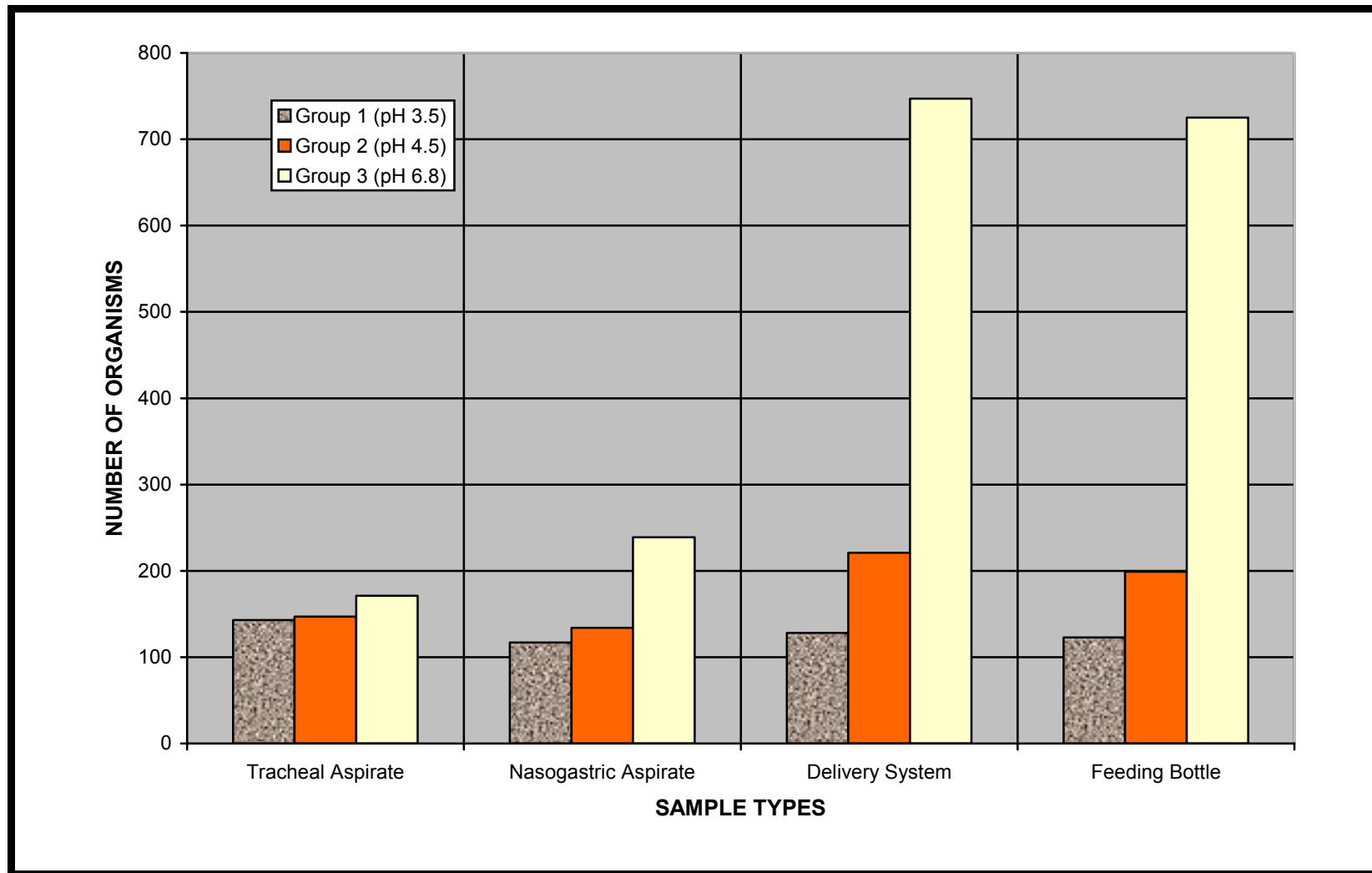


Figure 3.17 Total number of pathogenic organisms in the aspirates and enteral feeding delivery system in the different feeding groups during the trial period.

3.8.7 Classification of organism strains

Total pathogenic organisms were classified according to different groups of microbial strains namely: *Enterobacteriaceae*, *Enterococcus spp*, *Fungi*, *Non-fermenting Gram-negative bacilli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenza*, *Moraxella catarrhalis*, *Neisseria meningitidis*, *Aeromonas* and *Salmonella*. The first 4 groups were further analysed in detail because *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenza* and *Moraxella catarrhalis* were identified in tracheal aspirates of patients at baseline, before enteral feeding was initiated. *Staphylococcus aureus* was identified in 3 nasogastric aspirates of patients on Day 1 and 3. In one patient, *Neisseria meningitidis* was identified in the tracheal aspirate on Day 2. The treating physician was informed and the patient monitored closely for any clinical symptoms of meningitis. Patients who received acidified formulae had significantly less *Enterobacteriaceae* and *Enterococcus* in the feeding system (bottle and delivery set), but higher levels of fungi in the feeding bottle. No *Enterobacteriaceae* was found in the pH 3.5 Group for the delivery system. Significantly less *Enterobacteriaceae* was also found in the nasogastric aspirates for the acidified groups. *Enterobacteriaceae* was the organisms with the highest levels in the nasogastric aspirates and the feeding system, as they were commonly found in the stomach and enteral formulae. No significant difference was found between the groups for tracheal aspirates, with gram-negative bacilli being the strain with the highest levels.

Enterobacteriaceae was most frequently found in the aspirates and enteral feeding systems (both delivery sets and bottles). This group included the following organisms: *Escherichia coli*, *Citrobacter species*, *Enterobacter species*, *Klebsiella species*, *Proteus species*, *Providencia species*, *Serratia species* and *Morganella species*. No *Enterobacteriaceae* was found in the delivery system for Group 1 (pH 3.5) and there was a significant difference (PROC MIXED, $p = 0.03$) found between Group 2 (pH 4.5) and the control Group 3 in the delivery system (Table 3.23) (Figure 3.18). Significantly less *Enterobacteriaceae* was found in the feeding bottles of the acidified group 2 (pH 4.5) compared with the control Group 3 (PROC MIXED, $p = 0.002$) (Figure 3.19). A significant difference (PROC MIXED, $p = 0.034$) was found between the acidified groups and the control group for *Enterobacteriaceae* in the nasogastric aspirates (Figure 3.20). No statistical difference in *Enterobacteriaceae* growth was found between any of the feeding groups for tracheal aspirates. No difference was found between the groups over time with no interaction between the groups and a linear relationship over time (Table 3.23).

Table 3.23 Treatment differences in the different feeding groups for *Enterobacteriaceae* during the trial period estimated by PROC MIXED

Variable	Differences in feeding groups	Effect of time during trial period	Interaction between time and groups and non-linear relationship over time
Delivery system	Group 1 (pH 3.5) and Group 2 (pH 4.5) differs from Group 3 (pH 6.8) (p < 0.03)	No	No interaction, linear over time
Feeding bottle	Group 1 (pH 3.5) and Group 2 (pH 4.5) differs from Group 3 (pH 6.8) (p < 0.002)	No	No interaction, linear over time
Nasogastric Aspirate	Group 1 (pH 3.5) and Group 2 (pH 4.5) differs from Group 3 (pH 6.8) (p < 0.034)	No	No interaction, linear over time
Tracheal aspirate	No	No	No interaction, linear over time

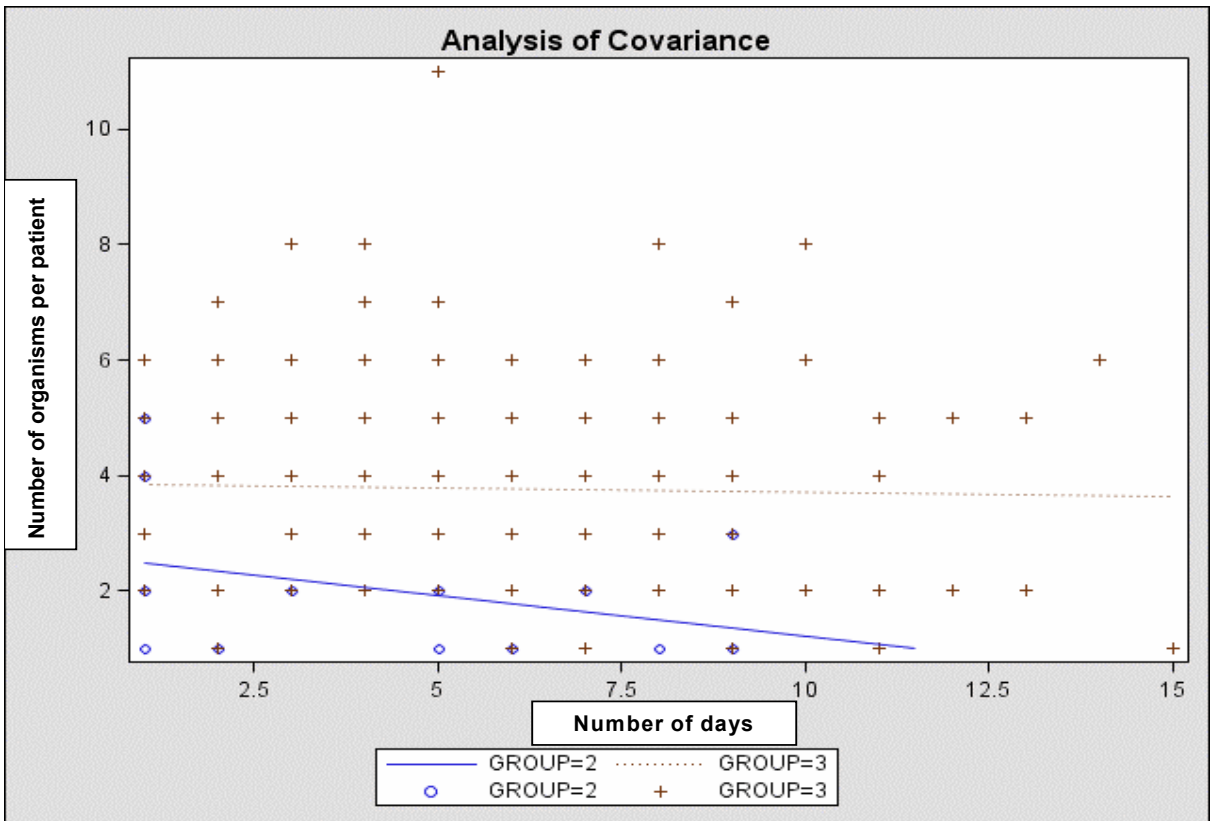


Figure 3.18 ANCOVA for *Enterobacteriaceae* growth per patient per day in the delivery systems of the different feeding groups during the trial period

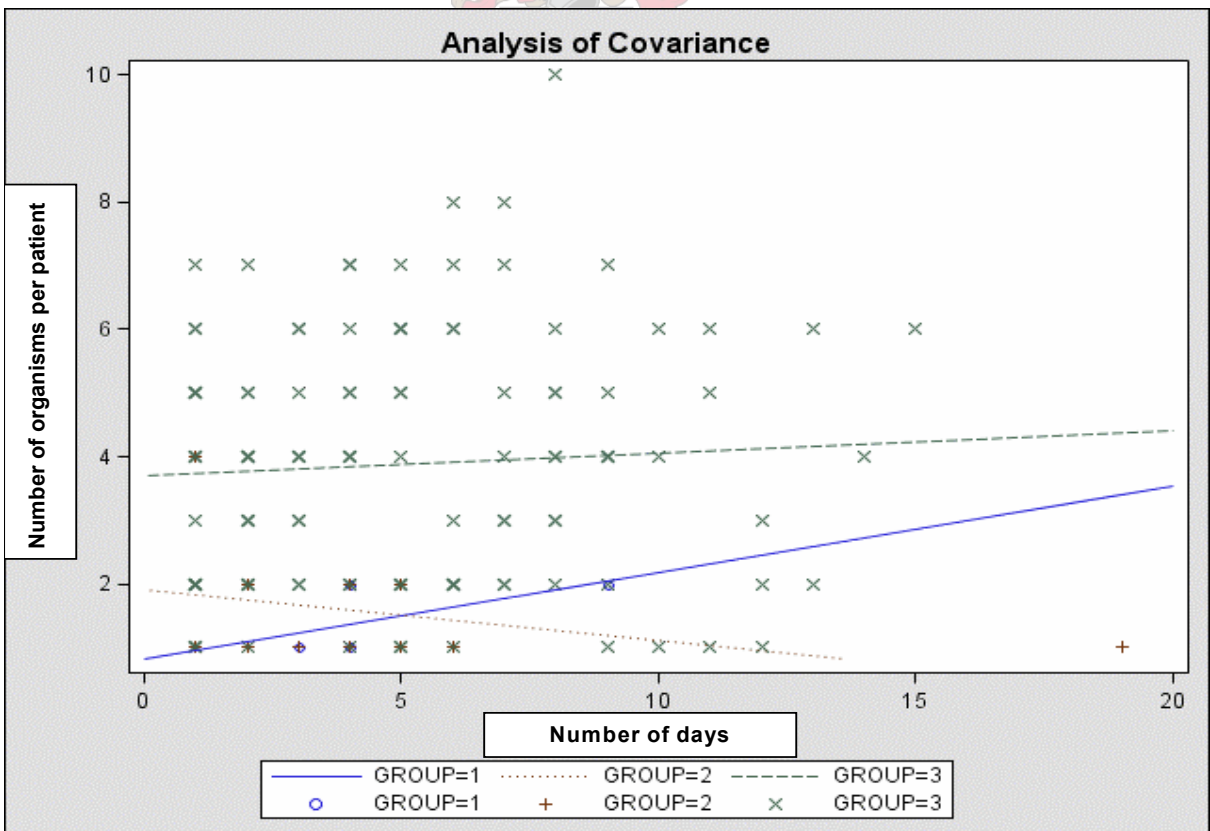


Figure 3.19 ANCOVA for *Enterobacteriaceae* growth per patient day in the feeding bottles of the different feeding groups during the trial period

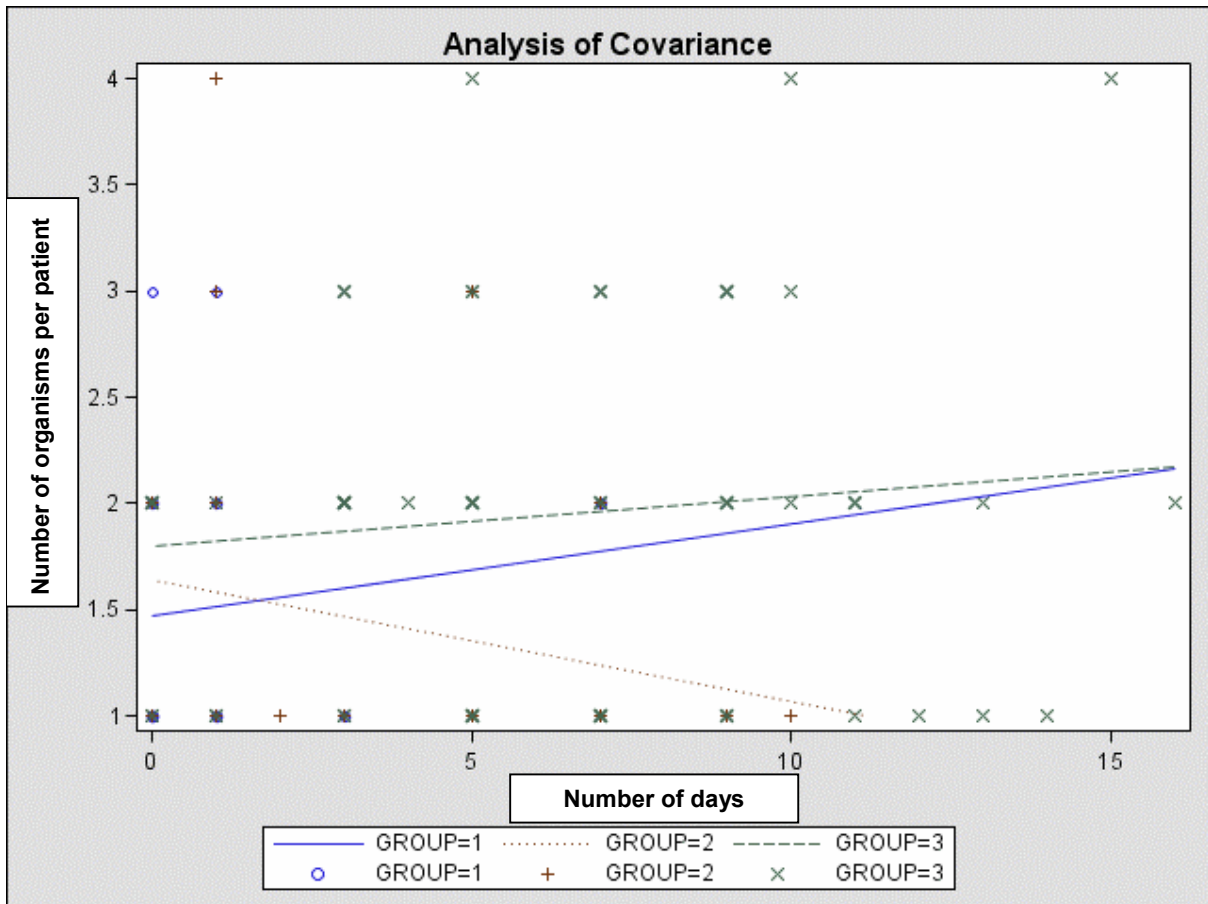


Figure 3.20 ANCOVA for *Enterobacteriaceae* growth per patient per day in the nasogastric aspirates of the different feeding groups during the trial period

There was a significant difference (PROC MIXED, $p = 0.02$) between the acidified groups and the control Group 3 in the delivery system for *Enterococcus* (Table 3.24) (Figure 3.21). Significantly less *Enterococcus* was found in the feeding bottles of the acidified groups when compared with the control Group 3 (PROC MIXED, $p = 0.002$) (Figure 3.22).

No significant differences in *Enterococcus* growth were found between any of the feeding groups for the nasogastric aspirates and tracheal aspirates. No significant difference was found between the groups over time with no interaction between the groups and a linear relationship over time (Table 3.24).

Table 3.24 Treatment differences in the different feeding groups for *Enterococcus* during the trial period estimated by PROC MIXED

Variable	Differences in feeding groups	Effect of time during trial period	Interaction between time and groups and non-linear relationship over time
Delivery system	Group 1 (pH 3.5) and Group 2 (pH 4.5) differs from Group 3 (pH 6.8) ($p < 0.02$)	No	No interaction, linear over time
Feeding bottle	Group 1 (pH 3.5) and Group 2 (pH 4.5) differs from Group 3 (pH 6.8) ($p < 0.002$)	No	No interaction, linear over time
Nasogastric Aspirate	No	No	No interaction, linear over time
Tracheal aspirate	No	No	No interaction, linear over time

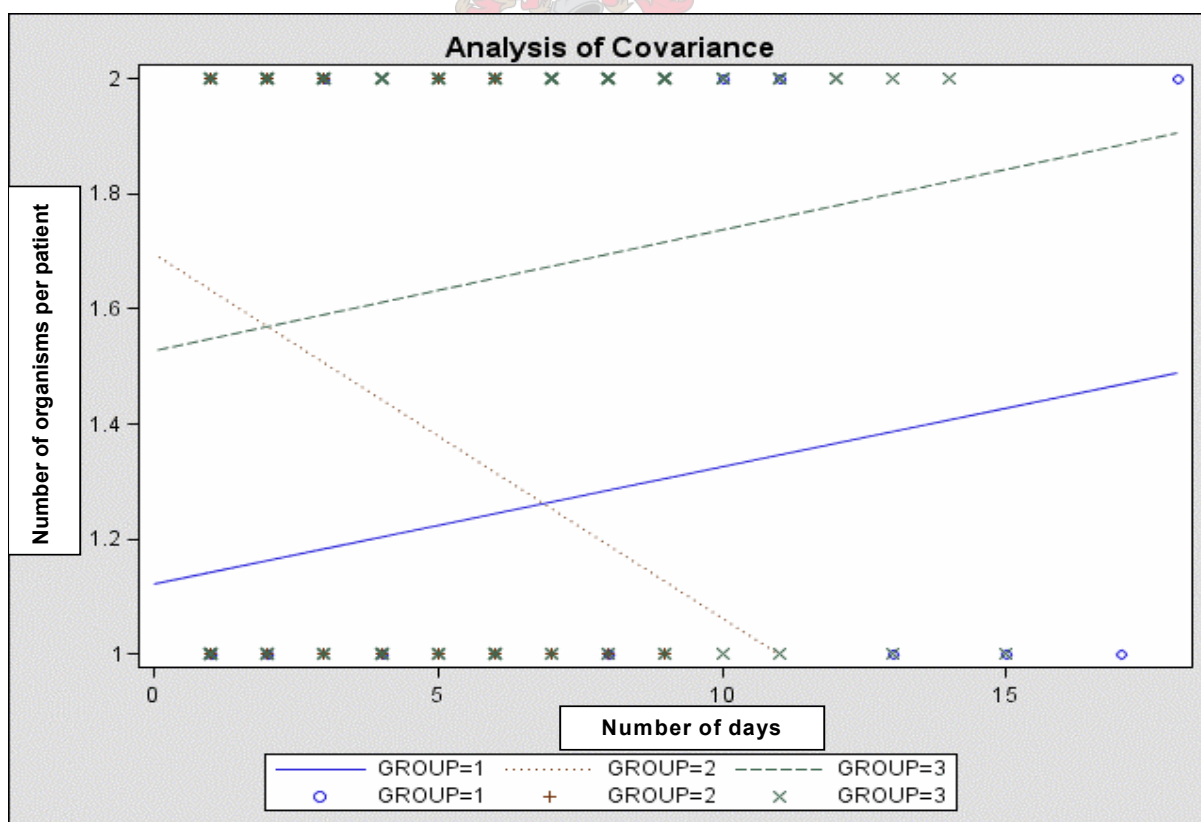


Figure 3.21 ANCOVA for *Enterococcus* growth per patient per day in the delivery systems of the different feeding groups during the trial period

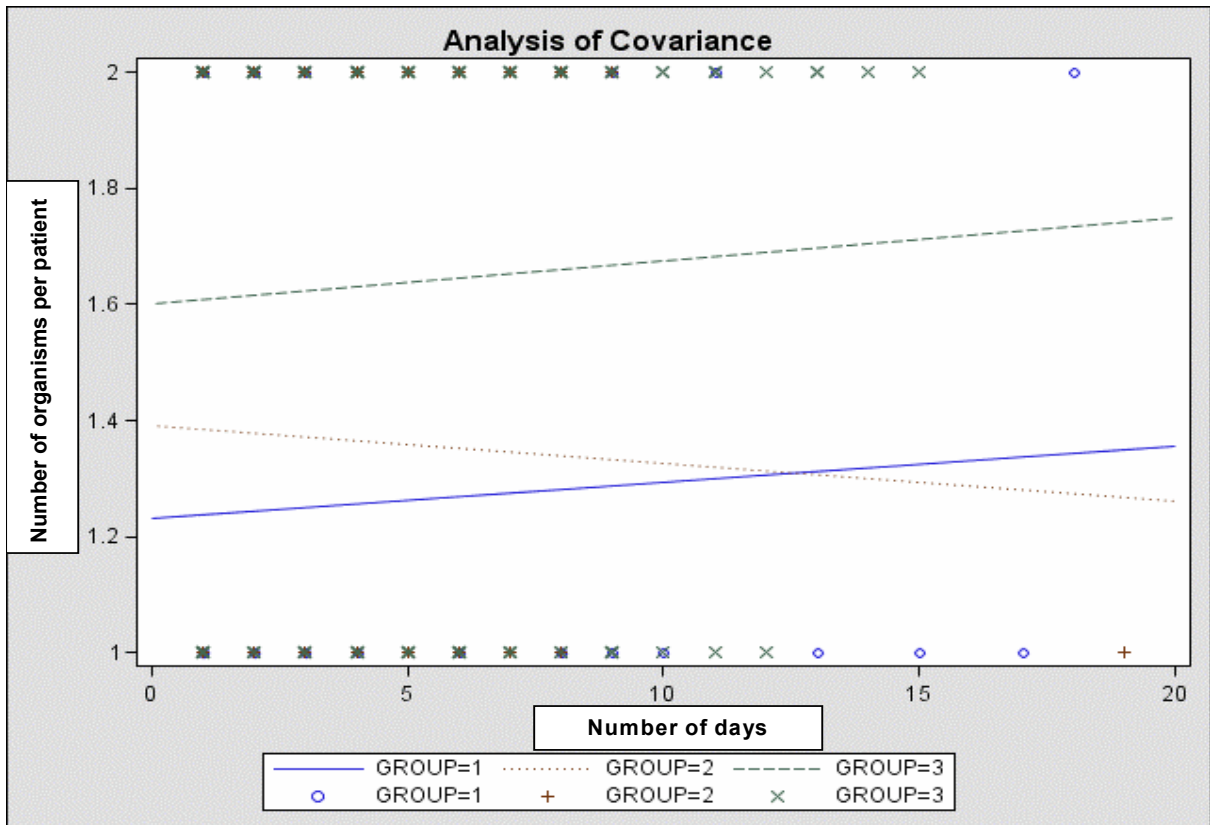


Figure 3.22 ANCOVA for *Enterococcus* growth per patient per day in the feeding bottles of the different feeding groups during the trial period

The fungi group included *Candida species* and *Candida albicans*. A significant difference (PROC MIXED, $p = 0.02$) for fungi was found only in the feeding bottles of the acidified groups compared with the control group, with higher levels in the acidified groups (Figure 3.23). No statistically significant differences in fungi growth were found among any of the feeding groups for the tracheal aspirates, but an interaction between groups (PROC MIXED, $p = 0.04$) was shown (Figure 3.24) for the tracheal aspirates. No difference was found between the groups over time, with no interaction between the groups and a linear relationship, except for the delivery system ($p = 0.03$) over time (Table 3.25).

Table 3.25 Treatment differences in the different feeding groups for *fungi* during the trial period estimated by PROC MIXED

Variable	Differences in feeding groups	Effect of time during trial period	Interaction between time and groups and non-linear relationship over time
Delivery system	No	No	No interaction, non-linear over time (p = 0.03)
Feeding bottle	Group 1 (pH 3.5) and Group 2 (pH 4.5) differs from Group 3 (pH 6.8) (p < 0.02)	No	No interaction, linear over time
Nasogastric Aspirate	No	No	No interaction, linear over time
Tracheal aspirate	No	No	Yes, interaction, lines not parallel (p = 0.04). Linear over time

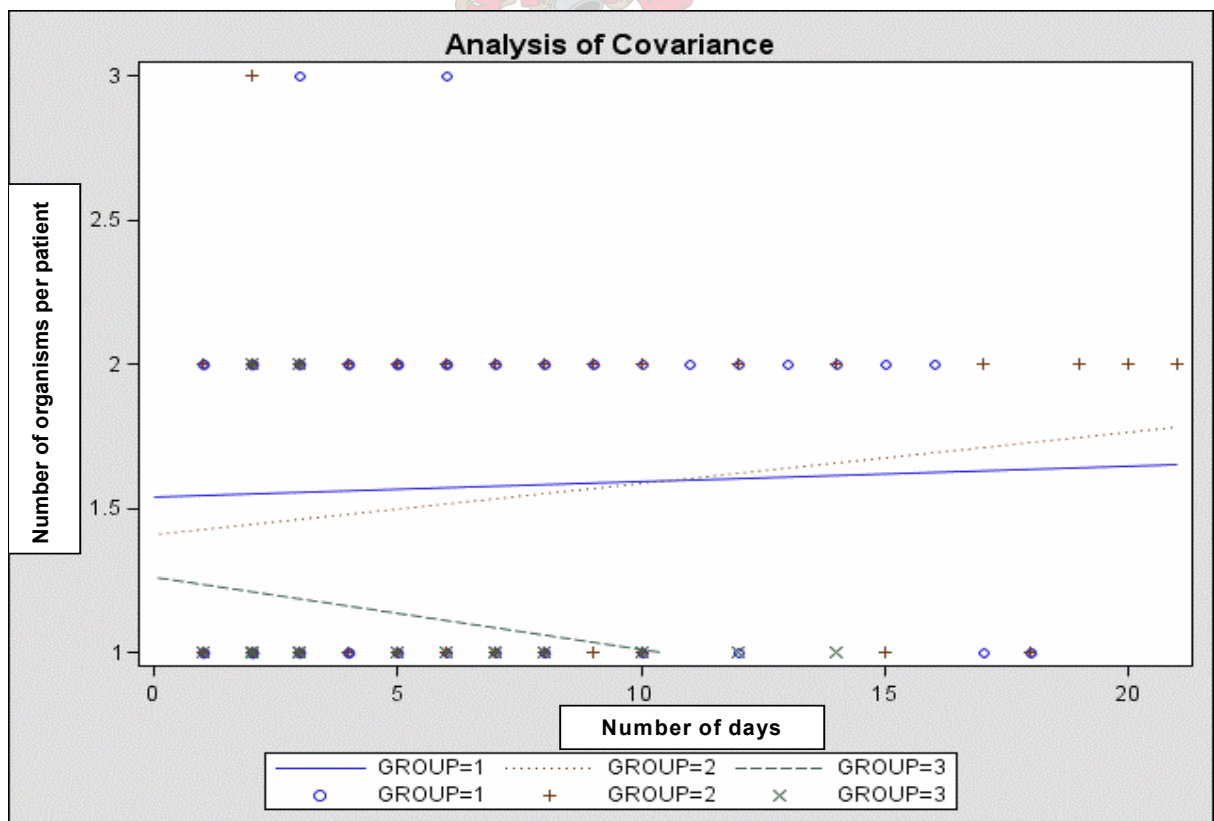


Figure 3.23 ANCOVA for fungi growth per patient per day in the feeding bottles of the different feeding groups during the trial period

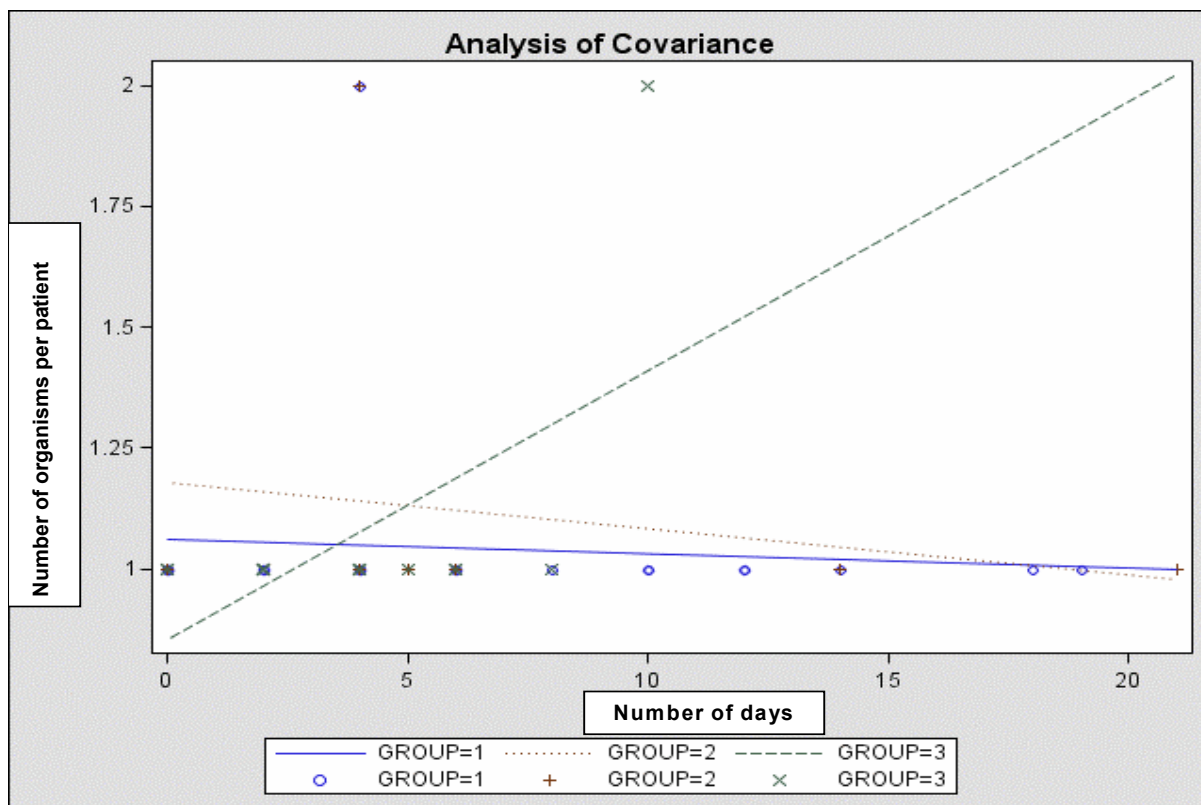


Figure 3.24 ANCOVA for fungi growth per patient per day in the tracheal aspirates of the different feeding groups during the trial period

The non-fermenting Gram-negative bacilli group analysis included the following organisms: *Acinetobacter species*, *Pseudomonas species*, *Burkholderia cepacia*, *Cryseobacterium species* that includes the *Flavobacterium species* and *Stenotrophomonas maltophilia*. No statistically significant differences (Table 3.26) were found between any of the groups for non-fermenting Gram-negative bacilli in the delivery system or feeding bottle, with no interaction between the groups and a linear relationship over time. An almost significant difference (PROC MIXED, $p = 0.052$) was found between the acidified feeding groups and the control group for the nasogastric aspirates, but an interaction between groups was shown (PROC MIXED, $p = 0.003$) with a non-linear relationship over time ($p = 0.04$) (Figure 3.25). No difference was found between the groups for tracheal aspirate, but a significant increase (PROC MIXED, $p = 0.03$) was found over time (Figure 3.26).

Table 3.26 Treatment differences in the different feeding groups for non-fermenting *Gram negative bacilli* during the trial period estimated by PROC MIXED

Variable	Differences in feeding groups	Effect of time during trial period	Interaction between time and groups and non-linear relationship over time
Delivery system	No	No	No interaction, linear over time
Feeding bottle	No	No	No interaction, linear over time
Nasogastric Aspirate	No	No	Yes, interaction, lines not parallel ($p = 0.003$). Non-linear over time ($p = 0.04$)
Tracheal aspirate	No	Increased ($p = 0.03$)	No interaction, linear over time

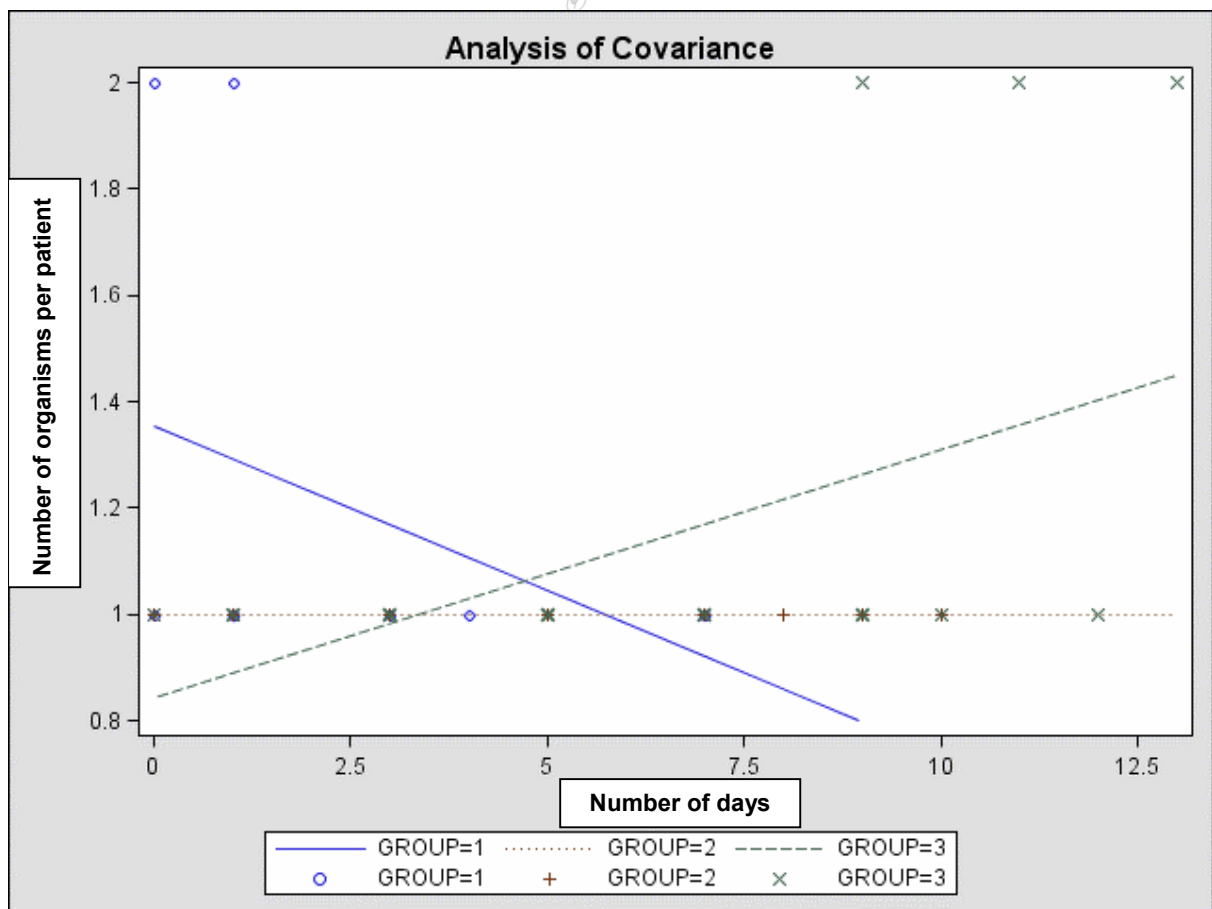


Figure 3.25 ANCOVA for non-fermenting *Gram-negative bacilli* growth per patient per day in the nasogastric aspirates of the different feeding groups during the trial period

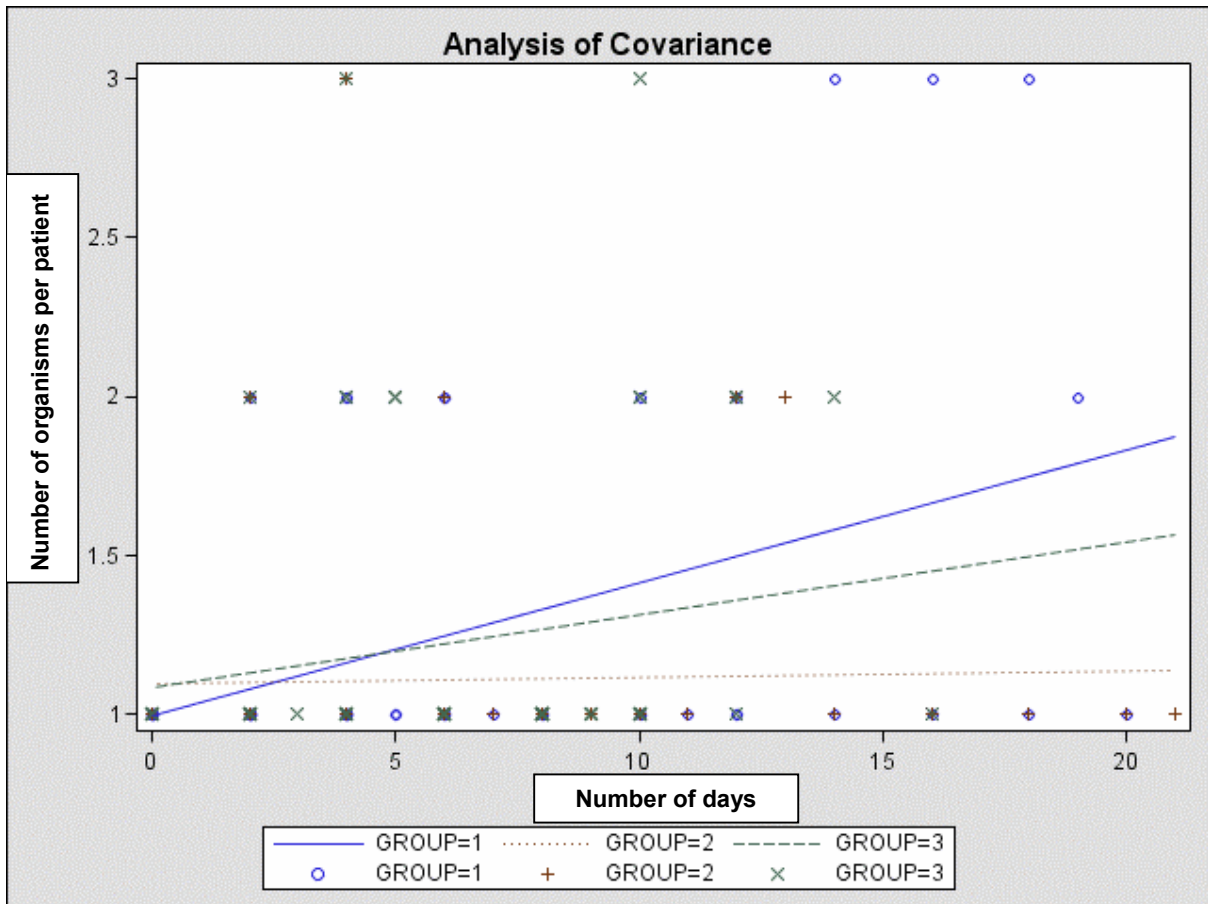


Figure 3.26 ANCOVA for non-fermenting Gram-negative bacilli growth per patient per day in the tracheal aspirates of the different feeding groups during the trial period



3.9 ADVERSE EVENTS AND MORTALITY

Morbidity was not investigated and was not documented during the trial period.

3.9.1 Adverse events (AEs)

All adverse events, single or multiple, were documented in every patient. Thirty-eight adverse events were documented in 25 patients during the course of the trial period (Table 3.27). Adverse events occurred equally between the different feeding groups with a higher, but not significant incidence of 37% in the control group, with pH 6.8 and 32% for the different acidified feeding groups. The intensity of the AEs was described as mild (symptoms hardly perceived, only slight impairment of general well-being) in 13 cases (34%), moderate (clearly noticeable symptom, but tolerable without immediate relief) in 20 cases (53%) and severe (overwhelming discomfort) in 5 cases (13%). Thirty-one (81.5%) of the total 38 AEs were unrelated to the trial product and 7 (18.5%) were unlikely to be related to the trial product. There was no causal relationship between any of the AE's and the trial products. The treatment of the adverse events was managed as per trial protocol (Table 3.28). In 25 (66%) of the 38 adverse events documented over the trial period,

the trial product had to be discontinued temporarily due to transient intolerance. Subsequently the AE disappeared in 16 (64%) of these 25 cases, but remained in 9 of these cases. The trial product was reintroduced within 24 hours in 13 (52%) of these 25 AEs, in the remaining 12 the trial product was reintroduced after 24 hours. In only 1 case did the AE reoccur and feeding was discontinued indefinitely. In 10 of the 38 adverse events, the trial was discontinued and the patients withdrawn because of the occurrence of an adverse event as per protocol (Table 3.29). For the different feeding groups, the withdrawals in relation to total trial patients (n = 67) were evenly distributed, with a slightly higher incidence in the control group (pH 6.8) (6%) as compared with in each of the acidified feeding groups (4.5% for each group).

Table 3.27 Distribution of adverse events for the different feeding groups during the trial period

Adverse events	Group 1 (pH 3.5)	Group 2 (pH 4.5)	Group 3 (pH 6.8)	Total [n (%)]
Vomiting	4	4	3	11 (29)
PH < 7.2	4	3	1	8 (21.5)
↑ Nasogastric drainage	1	1	3	5 (13)
Ileus developed	0	1	3	4 (11)
↓ Oxygenation	1	1	1	3 (8)
Hypokalemia	0	1	1	2 (5)
Acute renal failure	1	1	0	2 (5)
Aspiration	0	0	2	2 (5)
Hyponatremia	1	0	0	1 (2.5)
Total [n (%)]	12 (31.5)	12 (31.5)	14 (37)	38

Table 3.28 Management of adverse events in relation to trial formulae during the trial period

Variable	Group 1 (pH 3.5) n (%)	Group 2 (pH 4.5) n (%)	Group 3 (pH 6.8) n (%)	Total n (%)
Product discontinued temporarily	6	8	11	25 (66)
Disappearance of AE	3	5	8	16 (64)
Reintroduction of product within 24 hrs	2	3	8	13 (52)
Reappearance of AE	0	0	1	1

Table 3.29 Distribution of adverse events resulting in patients who were withdrawn from the trial during the trial period

Adverse Event	Group 1 (pH 3.5)	Group 2 (pH 4.5)	Group 3 (pH 6.8)	Total (n)
PH < 7.2 for > 24 hrs	2	1	1	4
Ileus developed	0	1	2	3
Renal failure	1	1	0	2
↓ Oxygenation	0	0	1	1
Trial discontinued	3	3	4	10

3.9.2 Mortality

Overall, out of 67 patients, there were 4 deaths (6%) during this clinical trial. For the different feeding groups, there were 2 deaths (8.7%) in the pH 3.5 group (n=23), 1 death (4.3%) in the pH 4.5 group (n=23) and 1 death (4.8%) in the pH 6.8 group (n=21). Should the groups be further combined into acidified versus control feeding groups, the mortality was 6.5% for the acidified group (n=46) and 4.8% for the control group (n=21), an insignificant statistical difference. The treating intensivist carefully reviewed the causes of the 4 fatalities in this trial and all deaths were deemed to be related to the underlying disease process and/or consequences of the clinical course of the patient during hospitalisation. No deaths were directly or indirectly related to a complication of the trial products. The 4 diagnoses at death were: cardio respiratory arrest (n = 1), chronic obstructive airway disease with sepsis (n = 1), ischemic heart disease (n = 1) and a brain haemorrhage (n = 1).

CHAPTER 4: DISCUSSION



This double-blinded, randomised controlled clinical trial (n=67) was designed to compare the effects of acidified enteral feeding formulae (pH 3.5 and pH 4.5) with a non-acidified control enteral feeding formula (pH 6.8) on:

- bacterial colonisation of the stomach and preserving of gastric acidity
- the microbial contamination of the enteral feeding delivery system
- bacterial colonisation of the trachea
- acid-base status in relation to the administration of the acidified formulae
- safety and gastro-intestinal tolerance of the trial formulae

All patients (n=67) were critically ill, ventilated and admitted to either the medical or surgical intensive care units of the Tygerberg Academic Hospital. The enteral feeds were of a polymeric type and the feeding protocol was designed so that patients would only receive their full energy requirements by day 4 following admission in the ICU. This less strict feeding protocol aimed to accommodate the greater number of trauma patients who had intra-abdominal surgery. Patients were fed for a minimum of 3 days. The average time on the enteral feeding protocol was 8.3 days. The trial period terminated as soon as patients were extubated, transferred from the ICU, enteral nutrition became contraindicated, a patient died or alternatively for a maximum of 21 days. Enteral feeds were prepared in sterile bottles by adding tap water to powdered formulae. According to the feeding regimens, a 24-hour supply of pre-prepared feeds, ranging from 500 – 2000 ml and divided into 4 bottles per patient, were delivered to the intensive care units and refrigerated until used. All feeds were discarded after 24 hours and new feeds and feeding delivery systems were introduced every 24 hours. Patients received 53% of their daily energy requirements through enteral feeds alone, and 61% through enteral feeds and glucose containing intravenous fluids. Sucralfate was administered prophylactically to prevent gastric stress ulceration. No cases of upper gastro-intestinal bleeding were reported in either of the trial groups.

The findings of the present clinical trial confirmed the previously documented, but limited, experience in the literature ^(123,135), which indicates that acidified formulae:

- preserved gastric acidity,
- decreased gram-negative and gram-positive bacterial colonisation of the stomach significantly.
- were associated with lesser bacterial contamination of the enteral feeding delivery system, and that
- the decreased incidence in gastric and feeding delivery system colonisation did not translate into lesser tracheal contamination, with *non-fermenting gram-negative bacilli* being the most significant organism strain in the tracheal aspirates.

Bacterial colonisation

The literature confirms that a gastric pH < 4 is bactericidal ⁽¹³⁶⁾ and suggests that bacterial growth is suppressed at a gastric pH < 3.5 ⁽¹¹⁷⁾. The findings of this trial confirm and extend this relationship, since only the more acidified Group 1 patients receiving the more acidified formula (pH 3.5) showed a significant reduction in bacterial growth in their nasogastric aspirates. The pH 3.5 formula also showed to be superior to the pH 4.5 formula regarding bacterial growth, since a pH of 4.5 in the feed did not show a significant reduction in bacterial growth. When gastric acid secretion is unimpaired, the stomach is frequently sterile ⁽⁸⁶⁾. Alternatively, it may be colonized with very low density (less than 10³) organisms such as *α-haemolytic streptococci*, *lactobacilli*, *S epidermidis* and *Candida species* ⁽¹³⁶⁾. Gastric acidity is the major factor inhibiting the growth of gram-negative organisms in the stomach ⁽⁸⁶⁾. Probiotics, which are often found in fermented acidified milk, are also thought to protect the host against pathogens via 2 major mechanisms: the barrier effect or colonisation resistance and modulation of the host's own defence mechanism. These effects can be attained through direct bacterial antagonism and by enhancing the immune response ⁽¹³⁴⁾. The addition of lactic acid producing bacteria for acidification could possibly explain the lower incidence of gastro-intestinal intolerance in this trial.

Most bacteria cannot tolerate an acid pH, but Gram-positive and other organisms, especially yeasts / fungi, are more resistant to an acid environment. *Candida Albicans* is the most frequent offending organism among the fungi group. It is a normal inhabitant of the oral cavity, gastro-intestinal tract and vagina in many individuals. Under certain circumstances, such as immunosuppression, this organism may be responsible for clinical conditions ranging from superficial inflammation to fatal blood-borne systemic infection ⁽¹³⁷⁾. Fungal colonisation has also been reported to account for 9% of nosocomial infections ⁽¹³⁸⁾. Risk factors for opportunistic fungal infections include: treatment with antimicrobials, cancer chemotherapy, indwelling intravascular catheters, neutropaenia, prior haemodialysis, previous fungal colonisation, abdominal surgery and candiduria. Most of these factors are very common in hospitalised patients, especially in high-risk settings, such as ICUs ⁽¹³⁸⁾. *Candida species* can survive at a pH of 1 ⁽⁸⁸⁾. Hilman documented that, while gram-negative bacilli and gram-positive cocci proliferated in direct proportion to the increase in pH, colonisation of yeasts occurred independently of pH ⁽¹¹⁷⁾. Many factors contribute to *Candida* proliferation in the gastro-intestinal tract (GIT). Antibiotics, especially broad spectrum antibiotics, eliminate much of the normal microbiota in the GIT which attenuates the normal flora defence mechanisms, thus allowing yeasts to proliferate ⁽¹³⁸⁾. The use of antibiotics is common in the critically ill and could possibly be the main reason for fungi growth in this trial. Fungi (*Candida Albicans* and *Candida species*) growth was significantly increased in the acidified formulae groups compared to the control group, a finding that is in line with that of Heyland ⁽¹²³⁾, who also documented increased growth of *Candida* in patients receiving

an acidified formula group (52%) when compared with a control non-acidified enteral formula group (35%).

Delivery systems

The association between bacterial contamination of the enteral feeding delivery system and infection in the critically ill patient has been well described ⁽¹³⁹⁾. The specific risk of bacterial contamination of the enteral feed and the feeding delivery system depends on:

- the pH of the feed⁽¹¹⁹⁾
- the time span of feeding
- the duration of the use of the feeding delivery systems, and ⁽¹⁰⁶⁾
- the type and number of manipulations from preparation to the end of administration of the feed and feeding delivery system⁽¹⁰⁶⁾

Ovesen *et al.* ⁽¹¹⁹⁾ reported that not all enteral feeds support microbial growth. They found that bacterial growth was inhibited in a partially hydrolysed soy protein diet with an acid pH (4.2) as compared to a standard whole-protein diet with a near neutral pH (6.6). They suggested that products that inhibited microbial growth, for instance acidified formulae, might limit the level of contamination of enteral feeds. Anderton *et al.* recommended that enteral feeds should be discarded and enteral feeding delivery systems changed every 24 hours because of the progressive risk of contamination and colonisation over time due to the type and number of manipulations of the enteral feed and feeding delivery systems over a 24-hour period ⁽¹⁰⁶⁾. In this trial, patients who received acidified enteral formulae had significantly less contamination of feeding bottles and delivery systems with *Enterobacteriaceae*, *Enterococcus* and *non-fermenting Gram-negative bacilli* throughout the trial period. All feeding groups had a marked increase in organism growth (cfu/ml) over 24 hours (6 hours *versus* 24 hours) from the feeding systems (bottle and delivery system). Better control over the changing of enteral feeding delivery sets every 24 hours and hygienic handling of enteral formulae by nursing personnel is essential ⁽¹⁰⁶⁾. The enteral feeding delivery set must be changed every 24 hours in every patient, as staff shortages and lack of proper enteral feeding administration protocols could result in higher bacterial counts ⁽¹⁰⁶⁾.

Tracheal contamination

Despite the significant lesser colonisation of gastric content with both gram-negative and gram-positive organisms in critically ill patients receiving acidified enteral feeds, no significant difference in tracheal contamination was found between the three feeding groups, with the non-fermenting gram-negative bacilli being the organism most frequently grown. Neither did the higher incidence of gastric colonisation with *Candida species* translate into higher incidence of tracheal contamination with *Candida*. The lack of correlation between gastric colonisation and tracheal colonisation is surprising, as the concept that nosocomial pneumonias result from micro-

aspiration of microorganisms that originate from the enteral canal ^(96,99). appear to be well documented in the literature. Other variables associated with an increased risk of development of nosocomial pneumonia in trauma patients include: 1) H₂ receptor blocker use 2) decreased consciousness 3) prophylactic anti-microbial use 4) massive gastric aspiration 5) prolonged mechanical ventilation (≥ 24 hours) 6) corticotherapy 7) re-intubations 8) tracheostomy and 9) continuous enteral feeding ⁽⁹⁷⁾. It may therefore be that the most cost effective and important proven intervention to prevent aspiration and thus nosocomial pneumonia in the mechanically ventilated ICU patient is to ensure that the patient remains at a body position of not less than 45 degrees when receiving enteral feeding ^(100,101,102).

Nutritional requirements

Patients received on average 53% of their energy requirements through enteral feeds alone and 61.1% through enteral feeds and glucose containing intravenous fluid. Patients were fed on average for 8.3 days. These findings compare favourably with those of a prospective survey in Australia in which patients received only 51% of their energy requirement over a 7-day period ⁽¹⁴⁰⁾. The energy intake in this trial, however was less than that in the Heyland *et al.* study ⁽¹²³⁾, in which patients received 65.8% of their requirements through enteral feeds alone. This can possibly be explained by the difference in the feeding protocol used in the Heyland trial ⁽¹²³⁾ which was designed to provide full energy requirements within 24 hrs after enteral feeding had been initiated. The feeding protocol followed in this trial ensured that patients received their full energy requirements on day 4, with considerably less interruptions in feeding, due to high nasogastric drainage and vomiting, and better gastro-intestinal tolerance in the acidified groups than in the Heyland trial ⁽¹²³⁾.

Acidaemia

Six patients (9%) in this clinical trial developed acidaemia with a pH < 7.2, with no significant difference between the different feeding groups. This incidence of acidaemia in this trial was lower than that reported by Heyland (12.6%) ⁽¹²³⁾. Of these 6 patients who did develop acidaemia in the present trial, only 4 had a pH < 7.2 for more than 24 hrs and they were withdrawn from the trial as per protocol. The patients withdrawn were distributed almost evenly among the feeding groups as follows: 2 patients in Group 1 (pH 3.5), 1 patient in Group 2 (pH 4.5) and 1 patient in Group 3 (pH 6.8).

Tolerance and mortality

Acidified formulas for infants and small children have been on the market for 40 years and have proved to be safe ⁽¹⁴¹⁾. In this clinical trial the use of acidified enteral feeds for adults were well tolerated, with very few complications such as emesis and gastro-intestinal intolerance, and proved to be safe for use in critically ill patients. Mortality in this clinical trial was 6%, which was

much lower than the 18.3% reported in the Heyland trial ⁽¹²³⁾. This could possibly be because of a lower average APACHE II score of 12-14 in this trial compared to an APACHE II score of 21.5 in the Heyland trial, as well as patients being much younger at a mean age of 38 versus 55 in the Heyland trial. The mortality rate was evenly distributed between the acidified and control groups, with a much lower mortality rate than that found in the Heyland trial. This trial had an average mortality rate of 6.5% (3/46) for the acidified groups and 4.7% (1/21) for the control group versus the 24.1% for the acidified group and 11.9% in the control group of the Heyland trial ⁽¹²³⁾.

By promoting and preserving a natural defence mechanism – gastric acidity – acidified enteral formulae do alter gastric pH and flora ⁽¹²⁴⁾. The important observation of the lowered pH on organism growth may provide a safe alternative in preparation and administration of enteral formulae in a setting with financial and resource constraints. This trial confirmed that acidified formulae preserve gastric acidity, which plays a major role in determining the gastric microbial milieu and may therefore have an impact on subsequent colonisation and risk of infection.



CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS



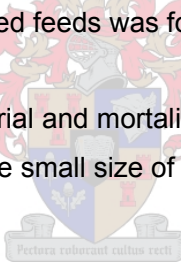
5.1 CONCLUSIONS

Acidified enteral formulae significantly decreased gastric colonisation in terms of number of organisms and growth in colony forming unit (cfu/ml) by preserving gastric acidity and decreasing the growth of *Enterobacteriaceae*, an organism implicated in the aetiology of nosocomial pneumonia, a leading cause of death in the ICU⁽⁹⁵⁾. By contrast, the protective effect of acidified enteral feeds did not have a significant effect on decreasing tracheal colonisation.

Although significantly higher levels of fungal growth was found only in the feeding bottles of acidified formulae, a trend towards increased growth never the less was seen compared with the control formula. Although this observation did not seem to have any clinical significance, it warrants further investigation.

Acidified enteral formulae also significantly decreased the bacterial contamination of the enteral feeding delivery system (bottle and delivery set) in terms of the number of *Enterobacteriaceae*, and *Enterococcus* organisms found, as well as the severity of growth in cfu/ml. Acidified enteral formulae were well tolerated by critically ill patients in this clinical trial. This trial also had a lower incidence of bacteraemia than was reported in other acidified clinical trials^(123,135). No clinically relevant acidosis in patients on acidified feeds was found

Morbidity was not determined in this trial and mortality is a difficult end-point to achieve, given the high-risk trial population, as well as the small size of the trial population.



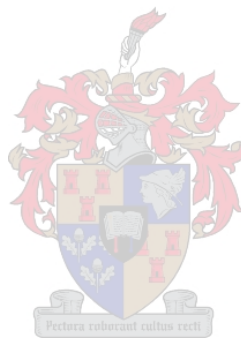
5.2 RECOMMENDATIONS

The goal of this clinical trial was to evaluate the effect of acidified enteral formulae on gastric and tracheal colonisation, as well as bacterial contamination of the enteral feeding delivery system in a critically ill population. This trial confirmed that patients in both the pH 3.5 and pH 4.5 formulae groups do have decreased gastric bacterial growth, but had no significant effect on tracheal growth. The trial also confirmed that acidified formulae decreased bacterial growth in the enteral feeding delivery system significantly (bottle and delivery set). The 2 acidified formulae groups compared well with each other in terms of all parameters (e.g. blood biochemistry, tolerance and mortality) but the pH 3.5 group had an advantage in terms of diminished bacterial growth. Both acidified formulae lowered gastric pH. However, the patients in the pH 3.5 group were able to maintain a lower gastric pH when compared with that of the pH 4.5 group.

More acid gastric pH was associated with lower bacterial counts in terms of colony forming units per millilitre in the reconstituted formula, delivery set, feeding bottle and nasogastric aspirate.

Further research into whether this translates into fewer episodes of nosocomial pneumonia and sepsis in critically ill patients needs to be conducted. Potentially this could be an easy and cost effective way to improve the outcome of critically ill patients. This trial did not have sufficient power to determine the effect on mortality which was not part of its objectives, but deserves further investigation. Morbidity was also not investigated and should be included in any such future clinical trials.

The findings of the present trial indicate that if acidified feeds are reconstituted with tap water, the pH of the powdered formulae should be determined to monitor the change in pH during the reconstitution process. It remains also unclear if and how the acidification process during the manufacturing of enteral products changes the bioavailability of certain nutrients, especially vitamins and minerals, and this needs to be investigated. Furthermore, antibiotic use and its impact on the proliferation of fungi in the critically ill also warrants further investigation.



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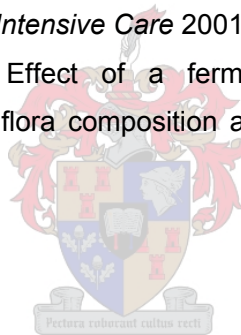
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APPENDICES



APPENDIX A

THE APACHE II SEVERITY OF DISEASE CLASSIFICATION SYSTEM ⁽¹²⁴⁾

PHYSIOLOGIC VARIABLE	HIGH ABNORMAL RANGE				NORMAL		LOW ABNORMAL RANGE		
	+4	+3	+2	+1	0	+1	+2	+3	+4
TEMPERATURE (° C)	≥41°	39°-40.9°		38.5°-38.9°	36°-38.4°	34°-35.9°	32°-33.9°	30°-31.9°	≤29.9°
MEAN ARTERIAL PRESSURE – Mm Hg	≥160	130-159	110-129		70-109		50-69		≤49
HEART RATE (ventricular response)	≥180	140-179	110-139		70-109		55-69	40-54	≤39
RESPIRATORY RATE – (non-ventilated or ventilated)	≥50	35-49		25-34	12-24	10-11	6-9		≤5
OXYGENATION (kPa) Intubated: PAO ₂ – PaO ₂	≥66.6	46.6-66.59	26.6-46.5		< 26.6				
PAO ₂ =FiO ₂ [PB-H ₂ O]-[PaCO ₂ ÷RQ]					PO ₂ >9.3	PO ₂ 8.1-9.3		PO ₂ 7.3-8	PO ₂ < 7.3
ARTERIAL pH	≥7.7	7.6-7.69		7.5-7.59	7.33-7.49		7.25-7.32	7.15-7.24	< 7.15
SERUM SODIUM (mMol/L)	≥180	160-179	155-159	150-154	130-149		120-129	111-119	≤110
SERUM POTASSIUM (mMol/L)	≥7	6-6.9		5.5-5.9	3.5-5.4	3-3.4	2.5-2.9		< 2.5
SERUM CREATININE (μmol/L) (Double point score for acute renal failure)	≥350	200-340	150-190		60-140		<60		
HEMATOCRIT (%)	≥60		50-50.9	46-49.9	30-45.9		20-29.9		< 20
WHITE BLOOD COUNT (total/mm ³) (in 1 000s)	≥40		20-39.9	15-19.9	3-14.9		1-2.9		< 1
Serum HCO ₃ (venous mMol/L)	≥52	41-51.9		32-40.9	22-31.9		18-21.9	15-17.9	< 15

TOTAL ACUTE PHYSIOLOGY SCORE (APS) _____

I. GLASCOW COMA SCORE*	
A. Eye Opening	Circle ONE response
___ Spontaneous (4)	Score = _____
___ To sound (3)	
___ To pain (2)	
___ None (1)	
B. Motor Response	Circle ONE response
___ Obeys commands (6)	Score = _____
___ Localizes pain (5)	
___ Normal flexion - withdrawal (4)	
___ Abnormal flexion - decortications (3)	
___ Extension - decerebration (2)	
___ None (1)	
C. Verbal Response [∇]	Circle ONE response
___ Oriented (5)	Score = _____
___ Confused conversation (4)	
___ Inappropriate words (3)	
___ Incomprehensive sounds (2)	
___ None (1)	
GLASCOW COMA SCORE CONTRIBUTION = A+B+C = _____ (II)	

III. AGE POINTS	
Circle the appropriate age range and enter the score	
Age Range	Score
___ <44	0
___ 45 - 54	2
___ 55 - 64	3
___ 65 - 74	5
___ >75	6
AGE POINTS CONTRIBUTION = _____ (III)	

* For those subjects who have a decreased level of consciousness secondary to medications (i.e. paralytics, anaesthesia, sedatives) the Glasgow Coma Score should NOT be calculated. For these subjects the contribution of the Glasgow Coma Score to the APACHE II will be zero.

∇ For intubated subjects the following scoring system should be used for verbal response: A score of (5) should be indicated for those subjects who are oriented or can write. A score of (3) should be indicated for those subjects who have responsive orientation and the ability to communicate is in question. A score of (1) should be indicated for those subjects who are disoriented.

IV. CHRONIC HEALTH POINTS*			
If the subject has a history of severe organ system insufficiency or if the subject is immunocompromised, he/she will have the following Chronic Health point assigned to their APACHE II score:		D. RENAL	Score = _____
5 points – for non-operative or emergency post-operative subjects		Receiving chronic dialysis	
2 points – for elective post-operative subjects		E. IMMUNOCOMPROMISED	Score = _____
DEFINITIONS: Organ insufficiency or immunocompromised state must have been evident prior to this hospital admission and conforming to one of the criteria listed below.		The subject has received therapy that suppresses resistance to infection, e.g. immunosuppression, chemotherapy, radiation, long term or recent high dose steroids, or has a disease that is sufficiently advanced to suppress resistance to infection, e.g. leukaemia, lymphoma, AIDS.	
A. LIVER	Score = _____	Total chronic health points contribution = A+B+C+D+E = _____ (iv)	
Biopsy proven cirrhosis and documented portal hypertension, or prior episodes of past upper GI bleeding attributed to portal hypertension, or prior episodes hepatic failure / encephalopathy / coma.		APACHE II SCORE = SUM OF:	Score = _____
B. CARDIOVASCULAR	Score = _____	I. Acute physiology score	
		II. Glasgow coma score [∇]	Score = _____
New York Heart Association Class IV		III. AGE POINTS	Score = _____
C. RESPIRATORY	Score = _____	Iv. Chronic health points	Score = _____
Chronic restrictive, obstructive, or vascular disease resulting in severe exercise restriction, i.e. unable to climb stairs or perform household duties; or documented chronic hypoxia, hypercapnia, secondary polycythemia, severe pulmonary hypertension (>40 mmHg), or respirator dependency.		TOTAL APACHE II SCORE = _____	

*Chronic Health Points should be calculated only for those subjects with a history of severe organ system insufficiency or who are immunocompromised.

∇ For those subjects who have a decreased level of consciousness secondary to mediations (i.e. paralytics, anaesthesia, sedatives) the Glasgow Coma Score should NOT be calculated. For these subjects the contribution of the Glasgow Coma Score to the APACHE II (item II) will be zero.

APPENDIX B

PROTOCOL FOR THE RECONSTITUTION OF TRIAL FORMULAE:

Put on a disposable apron and a cap.

1. Wash hands thoroughly with soap and water. Dry hands well, using paper towels.
2. Wash all work surfaces using the Biocide/Jik solution.
3. Place all apparatus (metal mixing bowls, whisk, measuring jug, funnel) in the Biocide/Jik solution and leave to soak for 15 minutes.
4. Rinse all feeding bottles and tops in the Biocide/Jik solution.
5. Label bottles.
6. Weigh the powder carefully (to the nearest gram) on a digital scale (Snowrex NHV Balance, Clover Scales Ltd, South Africa) for each patient and complete the logbook.
7. Transfer the powder into the mixing bowl and add a small amount of water. Mix with the whisk to form a paste.
8. Transfer the mixture into a measuring jug and add water until the required volume is reached.
9. Transfer mixture back into mixing bowl.
10. Divide the volume equally among 4 bottles and transfer into the bottles, using the funnel.
11. Put tops on the bottles.



APPENDIX C ⁽¹²⁵⁾**CALCULATION OF NUTRITIONAL REQUIREMENTS*****Harris Benedict Equation for Basal Energy Expenditure (BEE)***

BEE (Male) = $66,47 + 13,75 \text{ (kg)} + 5,0 \text{ (cm)} - 6,76 \text{ (years)}$

BEE (Female) = $655,1 + 9,56 \text{ (kg)} + 1,85 \text{ (cm)} - 4,68 \text{ (years)}$

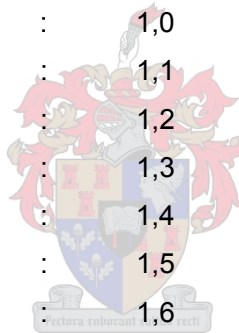
Where kg = weight; cm = height; years = age

Activity factors:

Bedridden : 1,2
 Ventilated : 1.05

Stress factors:

Absence of complications : 1,0
 Post-operative / cancer : 1,1
 Fractures : 1,2
 Sepsis : 1,3
 Peritonitis : 1,4
 Multiple traumas : 1,5
 Multiple traumas + sepsis : 1,6
 Weight gain : 2,0

***Protein Calculation***

1,5 – 2,0 g/kg body weight per day

APPENDIX D

NURSING PROCEDURES FOR PATIENTS ON ENTERAL FEEDS

Patients **must** be nursed at a 30 ° angle.

If the feeding tube has to be removed during working hours (07:30 – 16:00), please keep the tube for microbiological analysis and contact the dietician immediately. Pass a new feeding tube and resume feeding as soon as possible after the removal of the previous tube.

The dietician will give the instruction when feeding may begin (radiological confirmation of the position of the feeding tube must be obtained).

Initiation of the Feed:

1. Wash hands thoroughly with hand soap and water.
2. Put on gloves.
3. **Administer water at 20 ml/h for 6 hrs (using standard procedure).**
4. After 6 hours, check gastric aspirate and **record on the bed chart.**
5. If gastric aspirate is <200ml, start enteral feeding **immediately** with trial formula.
6. Gather the following equipment:
 - Fresenius Frentamat® Pump-Feeding Set, Fresenius Frentamat® Feeding Pump (Fresenius, Germany), Trolley, Feed (in ward kitchen fridge)
7. **Connect feeding set (according to ward protocol).**
8. Starting flow rate is 20 ml/hr.
9. Please record the time that the feed is started.
10. Please ensure that the correct formula is given to the correct patient.
11. The flow rate will be adjusted by the dietitian every 24 hours. **Please do not adjust the flow rate.**
12. Please ensure that a new bottle is connected **promptly** every 6 hours.
13. Flush feeding tube 6 hourly with 10 ml water (before new bottle is connected).
14. The delivery system (excluding the feeding tube) **MUST** be replaced every 24 hours.

APPENDIX E

All samples were collected in sterile, individually marked containers and immediately transported to the laboratory.

TRACHEAL ASPIRATE COLLECTION AND PROCESSING

A. COLLECTION:

The specimen was collected early morning, preferably between 08:00 and 10:00. The specimen was collected by the nursing sister who was familiar with the techniques of collecting the specimen through a tracheostomy or endotracheal tube.

The endotracheal opening had to be cleaned with sterile normal saline and sterile gauze swabs prior to inserting the “collecting” catheter.

1. Materials:

- a) Mucus extractor FG6 (2mm) (Roynhardt Ltd, South Africa)
- b) 20 ml Syringe (Promex, Sekunjalo Manufacturing Ltd, South Africa) or intermittent suction device

2. Method:

- a) Carefully pass the polyethylene catheter through the site and into the trachea.
- b) Aspirate material from the trachea by using the syringe or intermittent suction device.
- c) Remove the catheter, and disengage the syringe or device.
- d) Seal the sputum trap (seal provided with sputum trap).
- e) Label the specimen with the patient's name and folder number.

3. Transport:

- a) Do not refrigerate the specimen.
- b) Transport the specimen to the microbiology laboratory as quickly as possible.

B. COMMENTS:

1. Aspirates obtained through endotracheal tubes present the same problem as nasopharyngeal aspirates: the catheter must pass through densely colonised areas, making culture interpretation difficult.
2. Since tracheostomy sites rapidly become colonised with Gram-negative bacteria, the presence of these organisms in culture may or may not indicate the aetiology of pneumonia. Thus, no real significance can be ascribed to either the presence of large numbers of a pathogen or the pathogen's association with an inflammatory response.
3. Prevent excessive dilution of the specimen with saline.

C. PROCESSING IN THE LABORATORY:

Microbiological cultures

All samples were cultured aerobically at 37°C for 24 - 48 hours using blood agar and MacConkey agar media. Potential bacterial and fungal pathogens were identified by standard techniques.

Bacterial colony counts:

Viable bacterial counts were made on serial 10-fold dilutions of the enteral feed samples in nutrient broth. Aliquots of 0,1 ml were spread over the surface of blood agar plates and incubated at 37°C for 48 hours. Colony counts were expressed as colony forming units (cfu/ml).

Gram stain

Purulent portion of the specimen for Gram-stain was selected.

Low-power magnification (x10) was used and 10 representative fields examined to determine the ratio of polymorphonuclear cells/ "pus cells" (PMNs) to squamous epithelial cells (SECs).

>10 SECs and/or <25 PMN/10x field: indicates contamination with saliva.

Semi-quantitative types of bacteria were observed.

Culture of tracheal aspirates

Blood agar medium + satellitism + optochin

McConkey agar medium

All potentially pathogenic organisms were reported if a heavy predominant growth was obtained.

List of potential pathogens:

Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis, Staphylococcus aureus, Streptococcus pyogenes, Enterococcus faecalis, Gram-negative bacilli (Enterobacteriaceae),



APPENDIX F

BLOOD BIOCHEMISTRY ANALYSES AND COEFFICIENTS OF VARIATION

The determinations that follow were performed by the National Health Laboratory Services (NHLS) at the Department of Chemical Pathology and Haematology Departments of the Tygerberg Academic Hospital using standardised techniques and following the principle of defined laboratory practices.

Sodium

The sodium method is based on an indirect potentiometric procedure using an ion selective electrode (ISE). The sodium ISE responds selectively to sodium ions according to the Nernst equation.

Potassium

The potassium method is based on an indirect potentiometric procedure using an ion selective electrode (ISE). The potassium ISE responds selectively to potassium ions according to the Nernst equation.

Urea Nitrogen

The urea nitrogen method is based on the Roch-Ramel enzymatic reaction utilizing urease and glutamate dehydrogenase. Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide. The ammonia reacts with 2-oxo-glutarate in the presence of glutamate dehydrogenase and NADH. The oxidation of NADH to NAD is measured as an inverse reaction at 340nm.

Creatinine

The creatinine method is based on the reaction of picric acid with creatinine in an alkaline medium as described in the original procedure of Jaffe. The creatinine reacts with the alkaline picric acid and forms a coloured complex. The rate of complex formation is measured at 505nm and is proportional to the creatinine concentration.

Magnesium

The magnesium method is based on the modified xylidyl blue reaction that was first described C.K. Mann and J.H. Yoe. Magnesium ions react with xylidyl blue in an alkaline medium to form a water-soluble purple-red chelate. The increase in absorbancy of xylidyl blue at 505nm is proportional to the concentration of magnesium in the sample.

Calcium

The calcium method is based on the work of Critelman where calcium ions form a violet complex with α -cresolphthalein complexone in an alkaline medium and measured at 545nm.

Phosphate

The inorganic phosphorus method is based on the Daly and Ertinghausen procedure that relies on the formation of a UV absorbing complex between phosphorus and molybdate. It is measured as an endpoint reaction at 340nm.

Total Protein

The total protein method is based on the method of Weichselbaum utilising biuret reagent (cupric sulphate) in an alkaline solution. The protein peptide bonds interact with the cupric ions to form a purple complex that is measured as an endpoint reaction at 545nm.

Alanine Aminotransferase (ALT)

The ALT method is based on the work done by Wroblewski and LaDue. The reaction is initiated by the addition of α -Ketoglutarate as a second reagent. The concentration of NADH is measured by its absorbance at 340nm and the rate of absorbance decrease is proportional to the ALT activity.

Aspartate Transaminase (AST)

The AST method is based on the work done by Karmen. The procedure was later modified by H.U. Bergmeyer. The concentration of NADH is measured by its absorbance at 340nm and the rate of absorbance decrease is proportional to the AST activity. The reaction is initiated by the addition of α -Ketoglutarate as a second reagent.

Alkaline Phosphatase (ALP)

In the ALP (DEA) method, the sample is added to a *p*-nitrophenyl phosphatase (PNPP) substrate. DEA buffer is used to maintain the reaction pH at 9.7 to 9.8. Magnesium ions are added to DEA buffer to activate and stabilise the enzyme. During the reaction, ALP hydrolyzes the PNPP to form *p*-nitrophenyl that is yellow in alkaline solutions and can be measured photometrically at 410nm. The reaction rate follows zero order kinetics.

Gamma Glutamyl Transferase (GGT)

The GGT method is based on the procedure done by Szasz. In the reaction with synthetic substrate, glycylglycine acts as an acceptor for the gamma glutamyl residue and 5-amino-nitrobenzoate (ANB) is liberated. The liberated product has an absorbance maximum near 400nm; the rate of formation is measured photometrically at 410nm as a zero order kinetic assay.

Total Billirubin

The bilirubin method is based on the procedure of Jendrassik and Grof. The bilirubin is reacted with diazo sulfanilic acid at a low pH to produce azobilirubin. In the absence of caffeine only a rapid reaction with conjugated (direct) bilirubin occurs. The absorbance of the azo complex is measured as an endpoint reaction at 545nm.

Blood glucose

The glucose oxidation method is based on the modified method done by Keston. Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The formed hydrogen peroxide reacts under catalysis of peroxidase with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as indicator.

Table 1 Blood biochemistry measurements and coefficient of variation

Measurement	Coefficient of variation
Sodium	1.7 – 1.8%
Potassium	3.0 – 2.7%
Urea Nitrogen	2.4 – 2.2%
Creatinine	3.8 – 3.7%
Magnesium	2.3 - 2.0%
Calcium	2.7 – 3.5%
Phosphate	1.7 – 2.6%
Total Protein	1.8%
ALT	5.6 – 1.9%
AST	3.9 – 1.7%
ALP	3.9 – 3.1%
GGT	5.3 – 1.8%
Total bilirubin	7.6 – 2.2%
Glucose	1.5 – 1.4%

Arterial Blood gasses (pH, pCO₂, pO₂, Base excess, Actual bicarbonate, O₂ saturation)

A completely automatic blood gas analyzer (BGA) machine analyses the blood gases. All operative functions are controlled by a microcomputer. The procedure starts with a heating phase, then complete calibration of electrodes (2PTCAL) with buffers and calibration of gasses. At the end of the calibration cycle, a one-point calibration is done and the blood samples are brought to the electrodes for analyses.

Table 2 Arterial blood gasses measurements and coefficient of variation

Measurement	Coefficient of variation
pCO ₂	1.0 – 3.0%
pO ₂	1.5 – 3.0%

Full Blood Count

White blood count (WBC): The peroxidase method is the primary method used. It is a two-stage method utilising 3 reagents to stain intra-cellular myeloperoxidase and then passes the cells through a flowcell where light scatter and absorption is used to determine each cell's size and level of staining. This achieves values for the absolute WBC, Neutrophils, Monocytes, Lymphocytes and Eosinophils.

Table 3 White blood and differential cell count measurement and coefficient of variation

Measurement	Coefficient of variation
WBC	2.7%
Neutrophils	1.6%
Monocytes	6.9%
Lymphocytes	2.9%
Eosinophils	8.8%

Red blood cells (RBC), Haemoglobin, Haematocrit, MCV, MCH, MCHC, Platelets: The RBC/Platelets method makes use of Mie's Theory of light scatter of spheres. Utilising the laser optics low angle and high angle scatter to determine the size and haemoglobin content of each RBC. Other RBC parameters are generated from the histograms produced by the optical analysis of each cell e.g. MCV, MCH and MCHC.

Table 4 Full blood count measurement and coefficient of variation

Measurement	Coefficient of variation
RBC	1.20%
Haemoglobin	0.93%
MCV	0.78%
Platelets	2.93%



APPENDIX G

PROCEDURE FOR UPPER ARM CIRCUMFERENCE AND TRICEPS SKINFOLD MEASUREMENTS IN A BEDRIDDEN PATIENT:

UPPER ARM CIRCUMFERENCE: ⁽¹³¹⁾

1. Patient must lie on his/her left side.
2. Right arm must be bent at the elbow to form a 90° angle.
3. Find the midpoint between the corner of the acromium and the point of the olecranon of the humerus, in the posterior position and mark this point.
4. Turn the patient on his/her back with his/her arms straight along the sides of the body.
5. Right arm must be raised slightly at the elbow to lift it off the surface of the bed.
6. Take the measurement of the circumference of the right upper arm in a horizontal line at the marked midpoint, using a flexible, non-stretch tape measure.
7. Ensure that tissue is not being pinched by tape measure.
8. Take an average of 3 measurements to the nearest millimetre.

TRICEPS SKINFOLD: ⁽¹³¹⁾

1. Patient must lie on his/her left side with his/her legs bent.
2. Place a pillow under the patient's head and place his/her left hand under the pillow.
3. Right arm must rest on the patient's body with the palm facing downwards.
4. Measurement is taken at the marked midpoint of the right upper arm.
5. The skinfold is parallel to the length-axis of the arm.
6. Measurement is taken in the posterior position, not laterally, using a Harpenden skinfold calliper (British Indicators Ltd, England).
7. Skin and subcutaneous fat are lifted 1cm above the mark where the measurement is supposed to be taken.
8. Place the calliper blades in the middle of the base and top of the skinfold.
9. Hold the skinfold while the measurement is taken.
10. Release the calliper blades and take the measurement within 2-3 seconds thereafter, to the nearest 0,2 millimetre.
11. Take an average of 3 measurements.

Please note: Measurements were taken on the **right** side of patients and not the left as described in the literature, as the reference tables used are for the right side of the body.

APPENDIX H

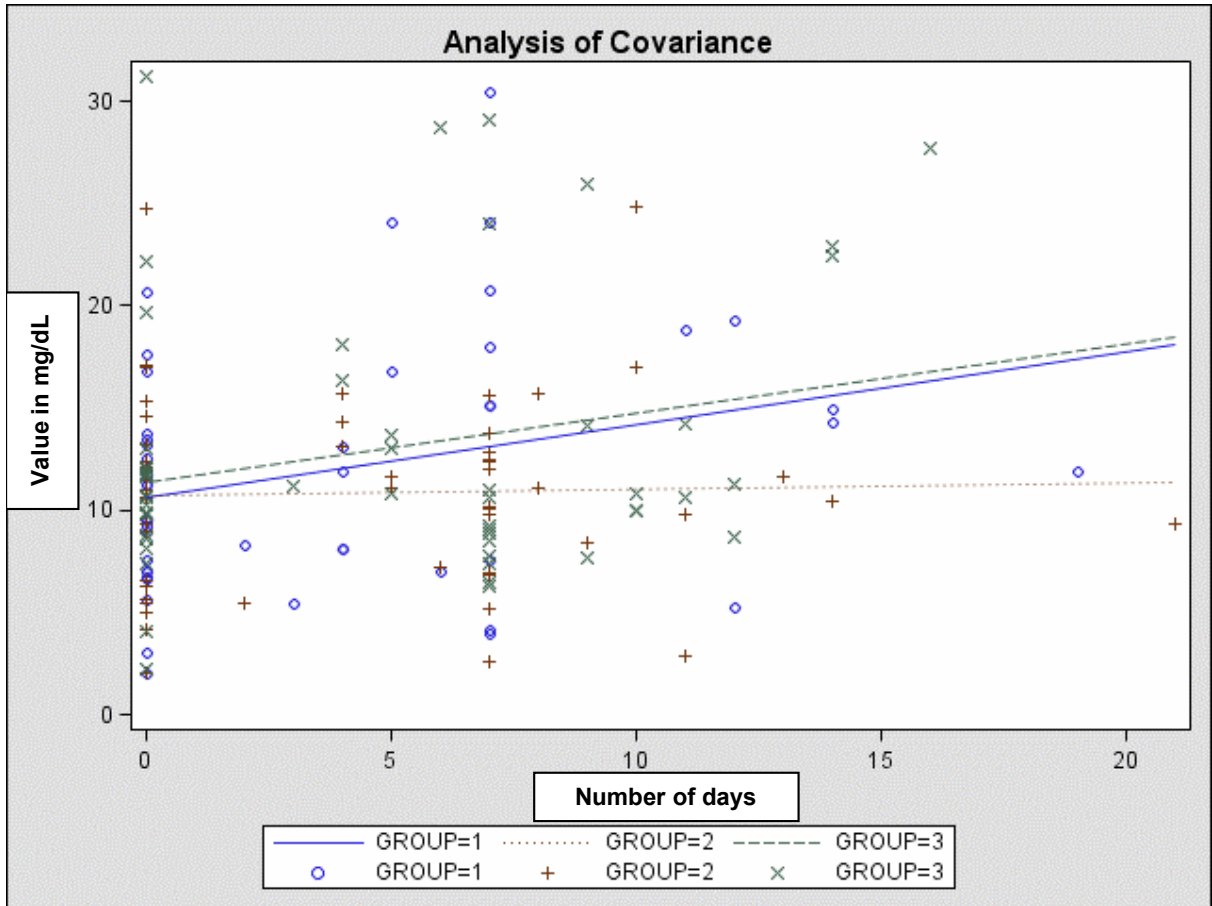


Figure 1 ANCOVA for Pre-albumin per day in the different feeding groups during the trial period

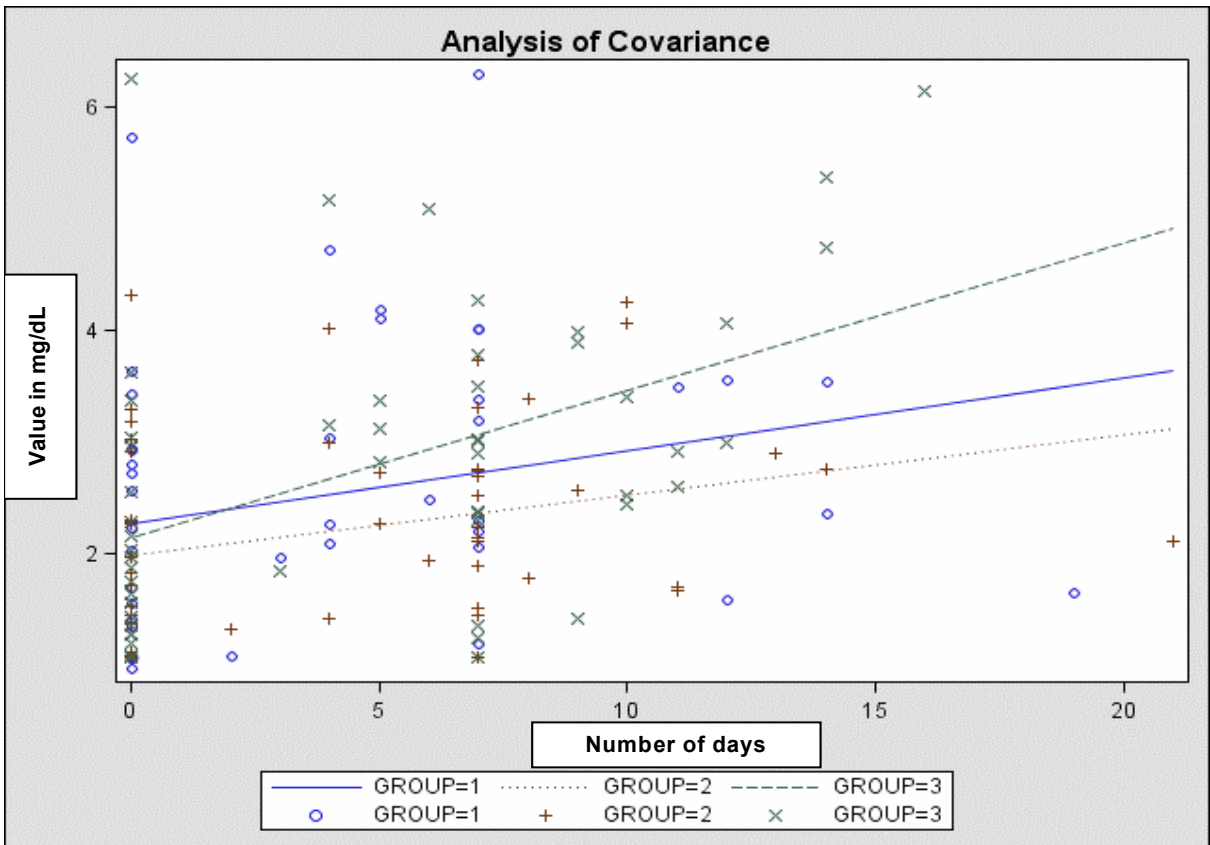


Figure 2 ANCOVA for retinol-binding protein per day in the different feeding groups during the trial period

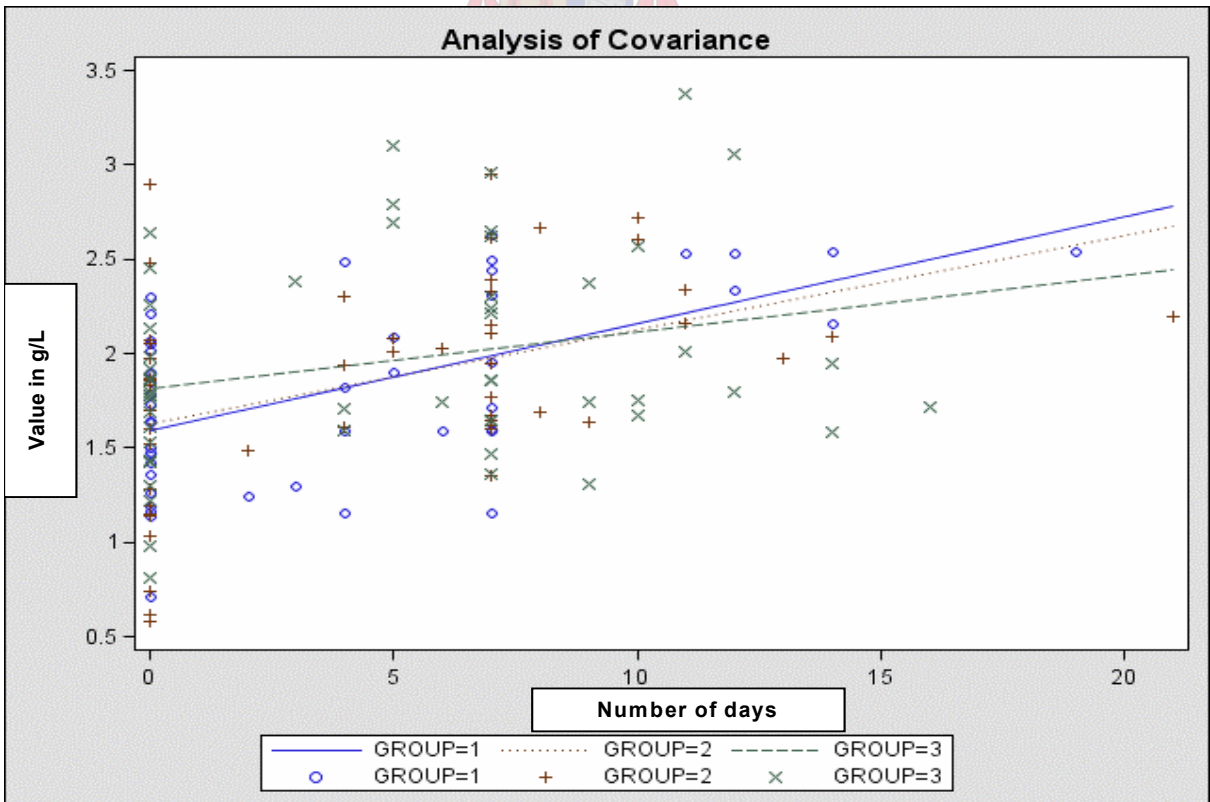


Figure 3 ANCOVA for α_1 -acid-glycoprotein per day in the different feeding groups during the trial period

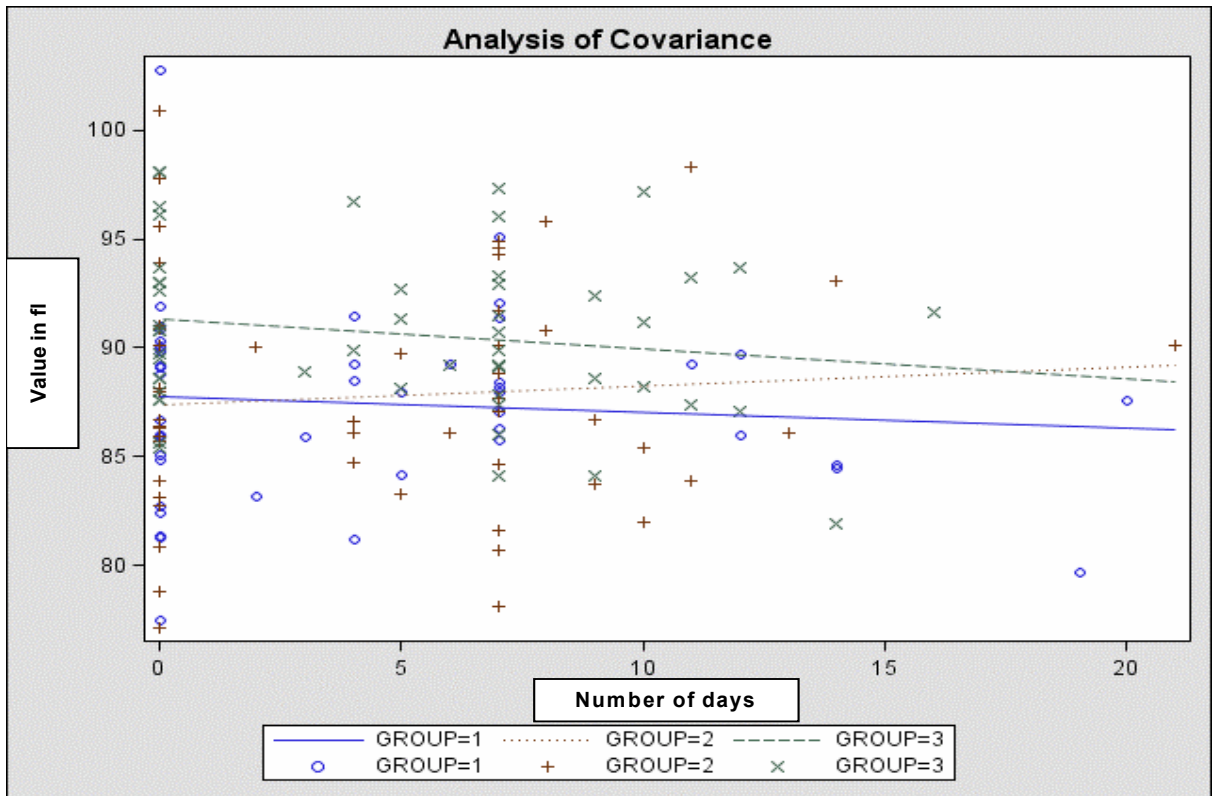


Figure 4 ANCOVA for MCV per day in the different feeding groups during the trial period

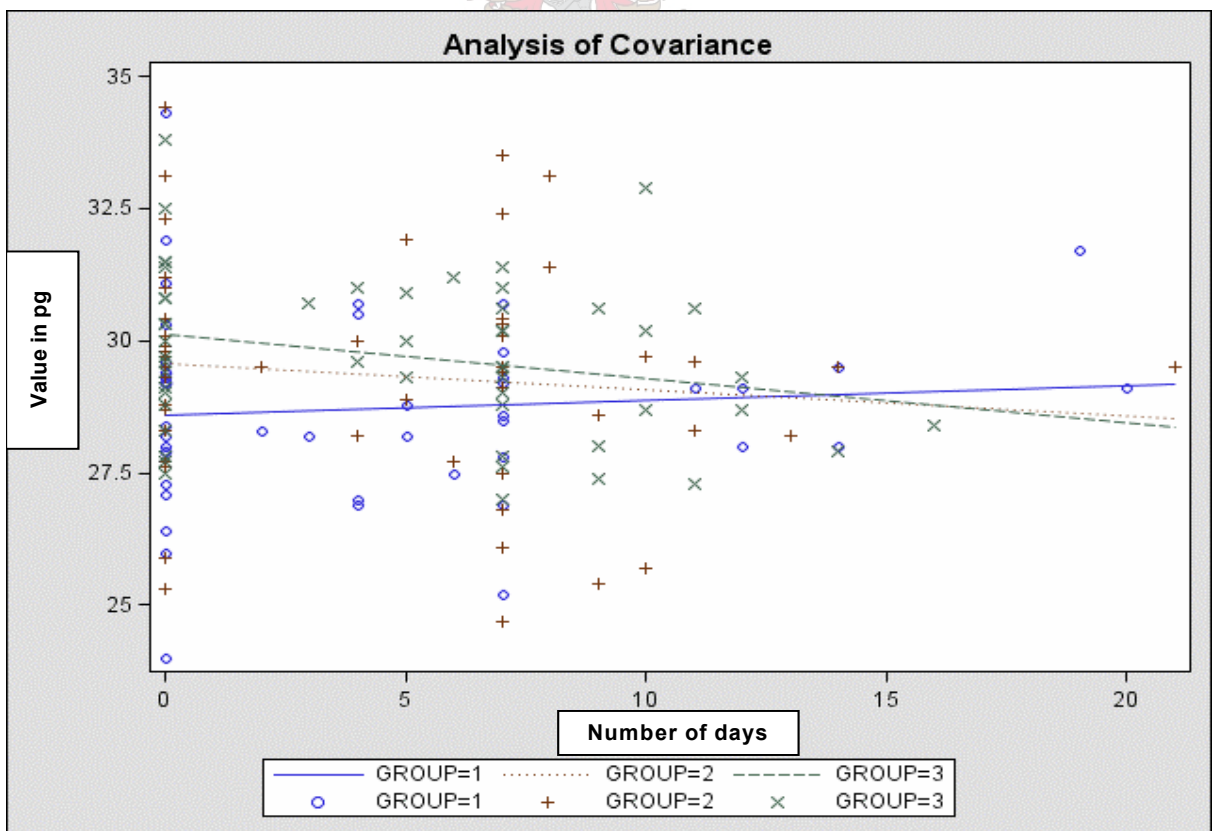


Figure 5 ANCOVA for MCH per day in the different feeding groups during the trial period

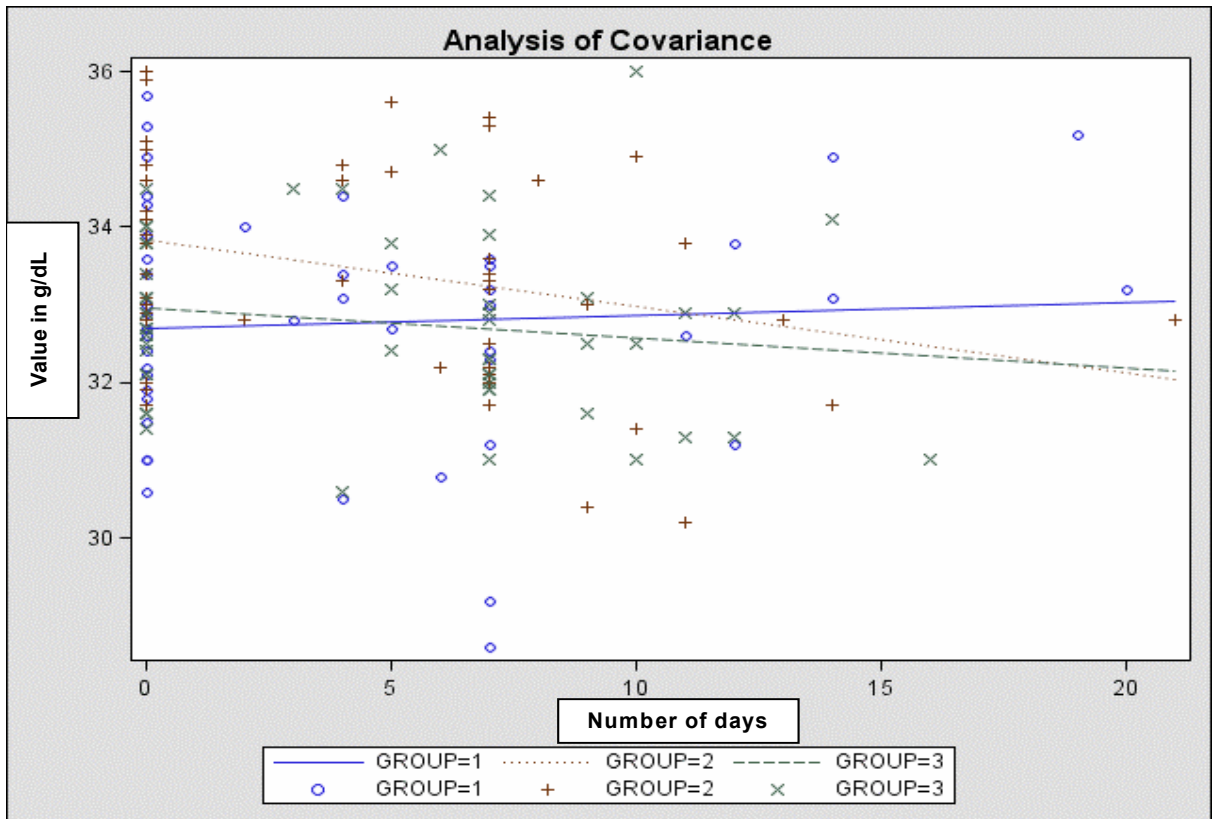


Figure 6 ANCOVA for MCHC per day in the different feeding groups during the trial period

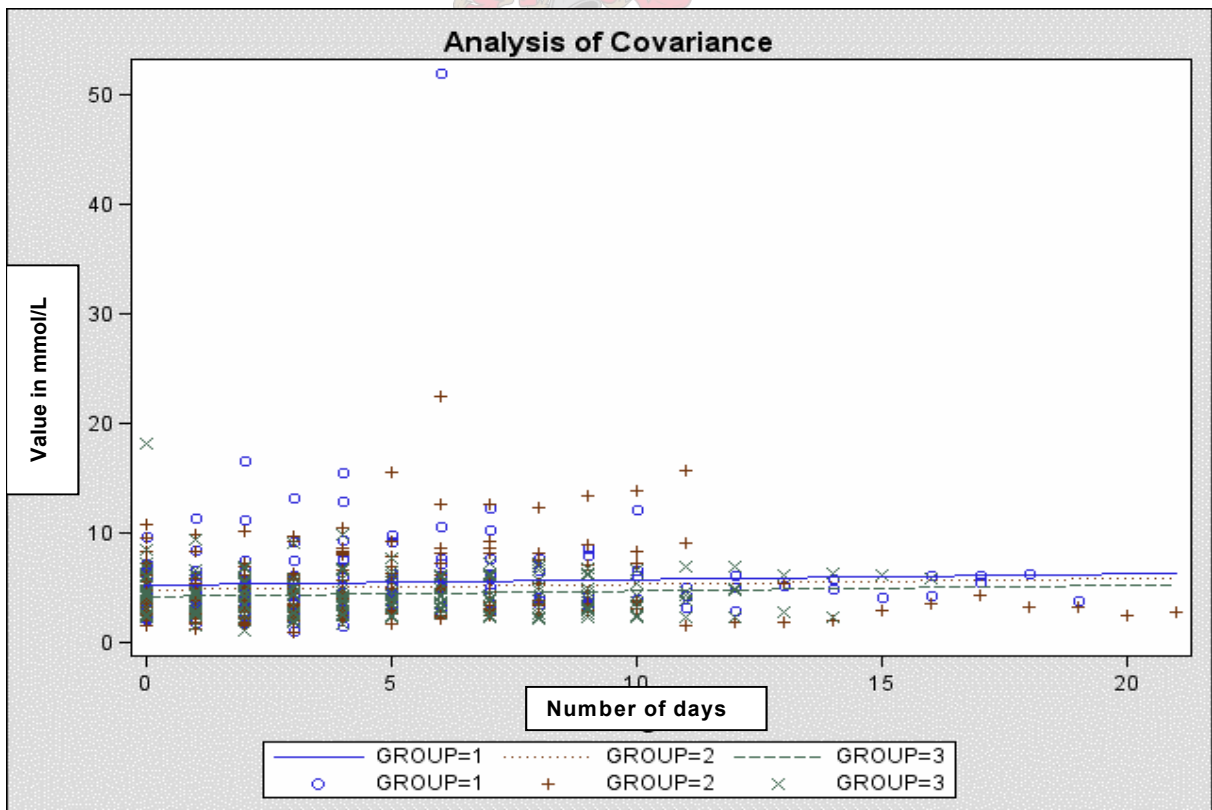


Figure 7 ANCOVA for urea per day in the different feeding groups during the trial period

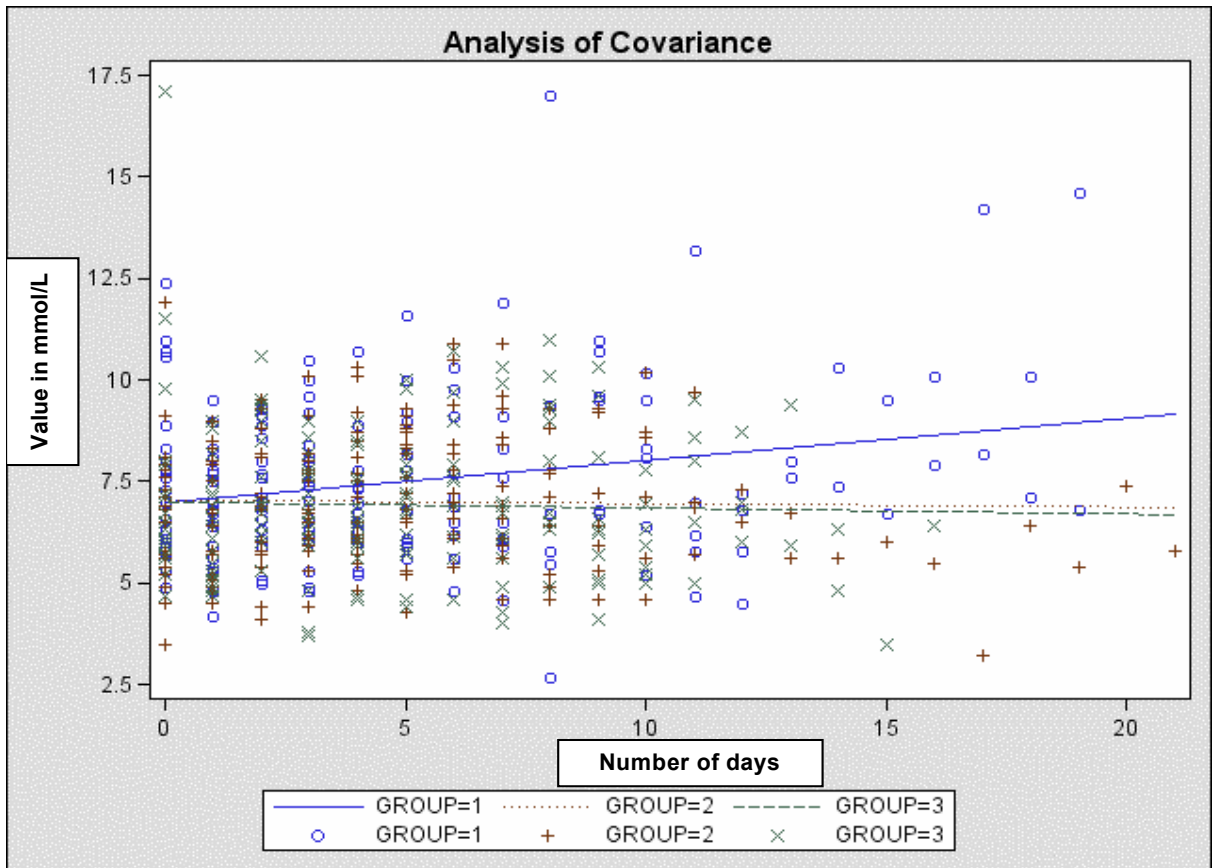


Figure 8 ANCOVA for glucose per day in the different feeding groups during the trial period

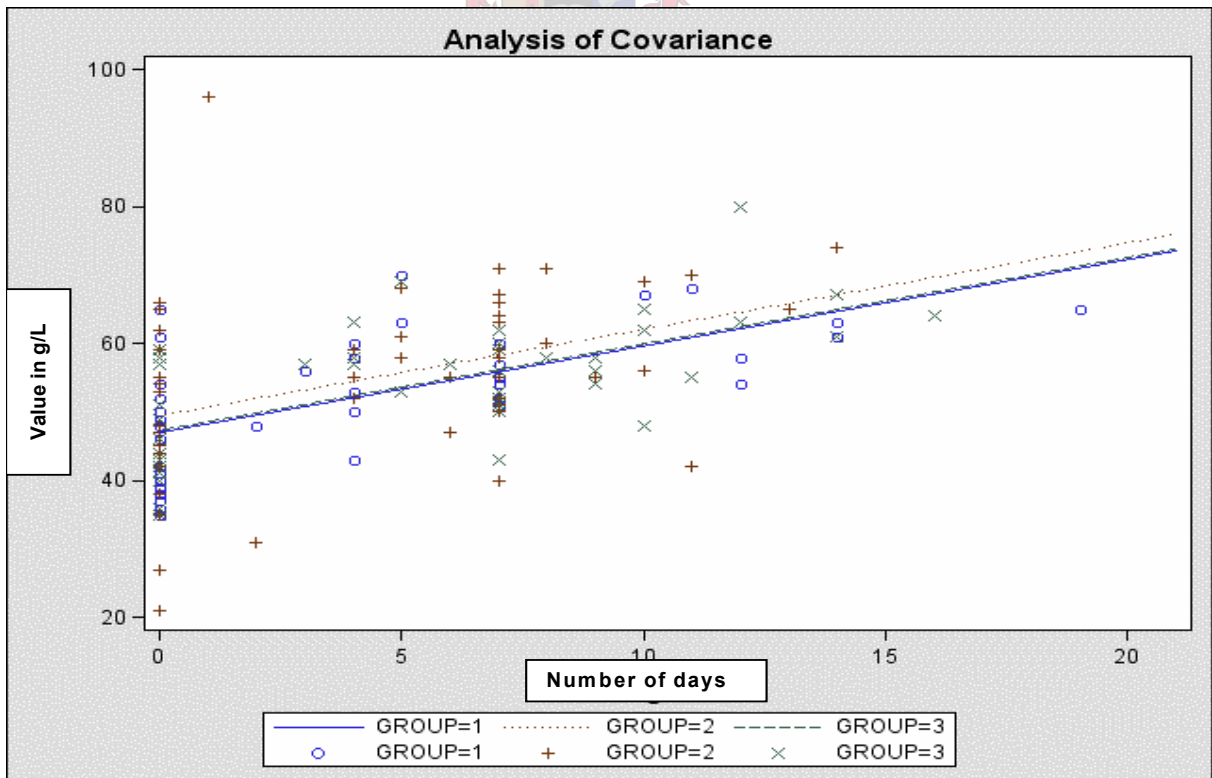


Figure 9 ANCOVA for total protein per day in the different feeding groups during the trial period

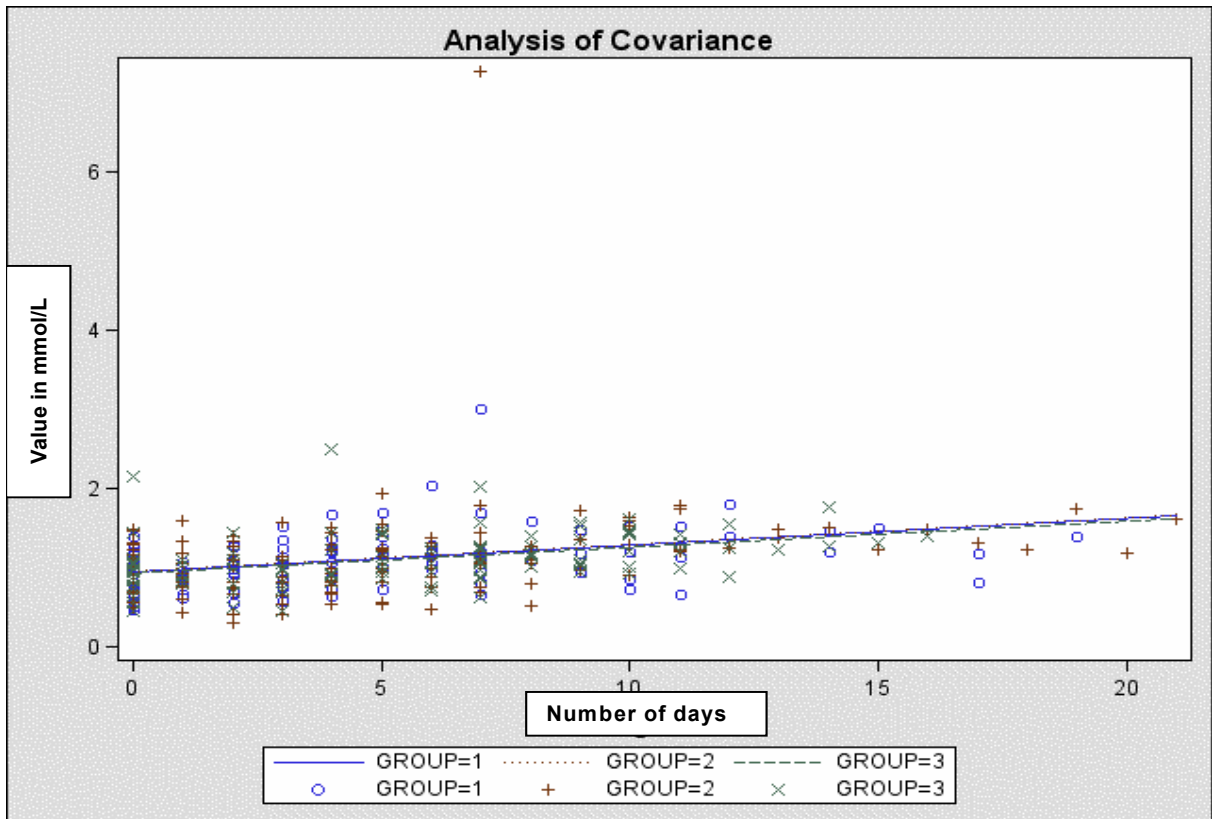


Figure 10 ANCOVA for phosphate per day in the different feeding groups during the trial period

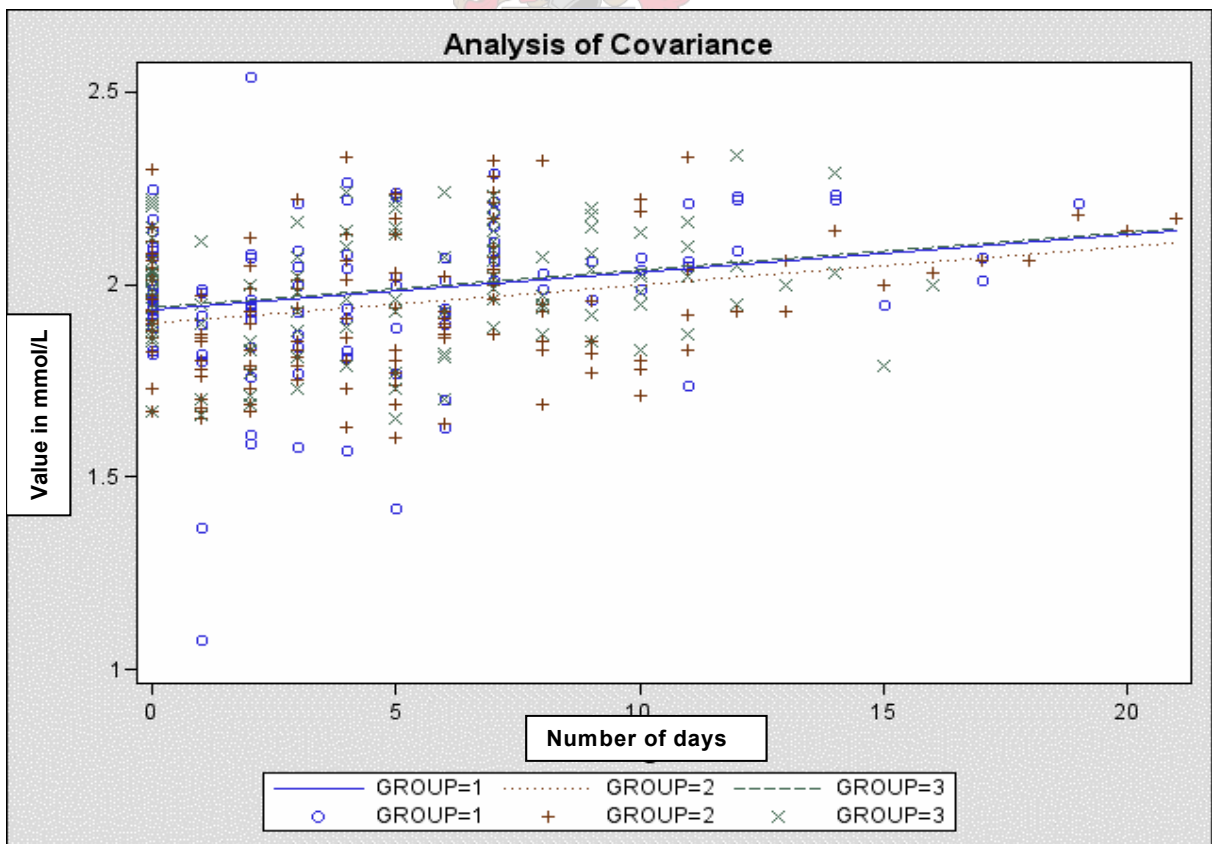


Figure 11 ANCOVA for calcium per day in the different feeding groups during the trial period

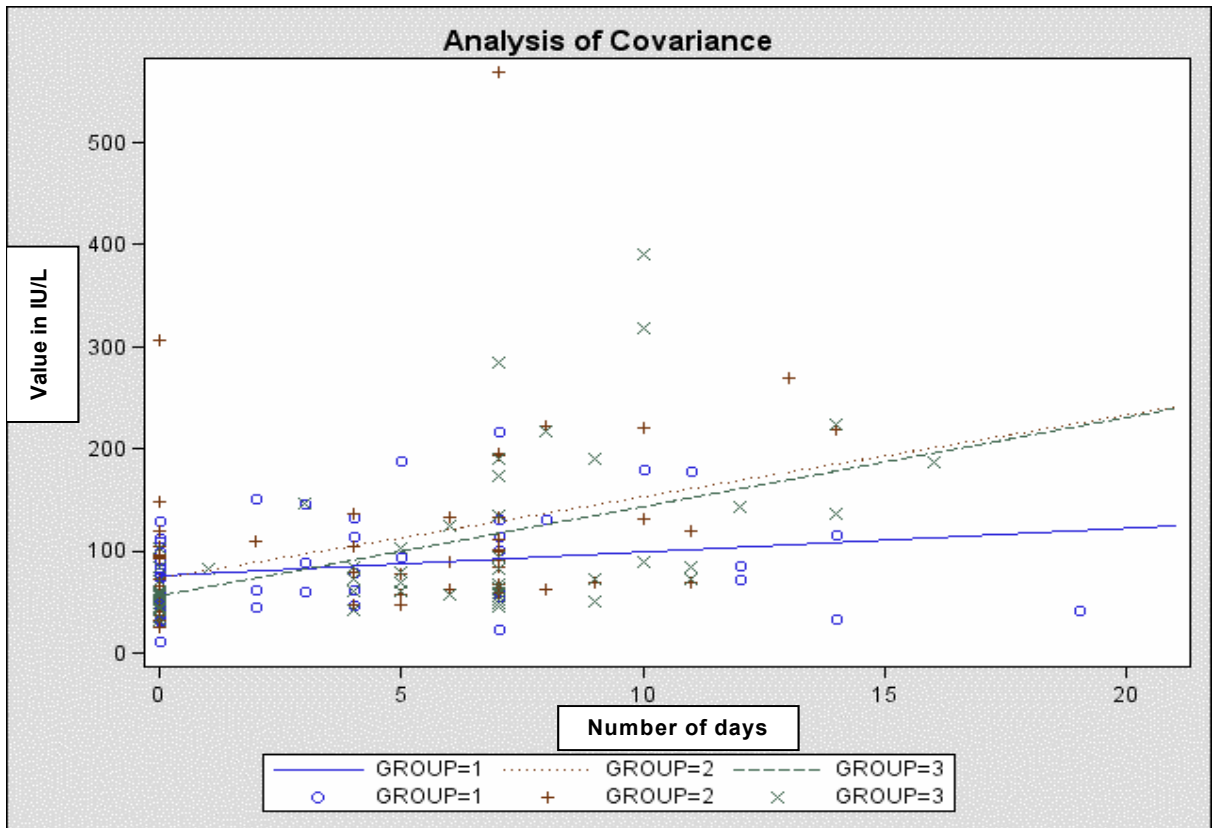


Figure 12 ANCOVA for ALP per day in the different feeding groups during the trial period

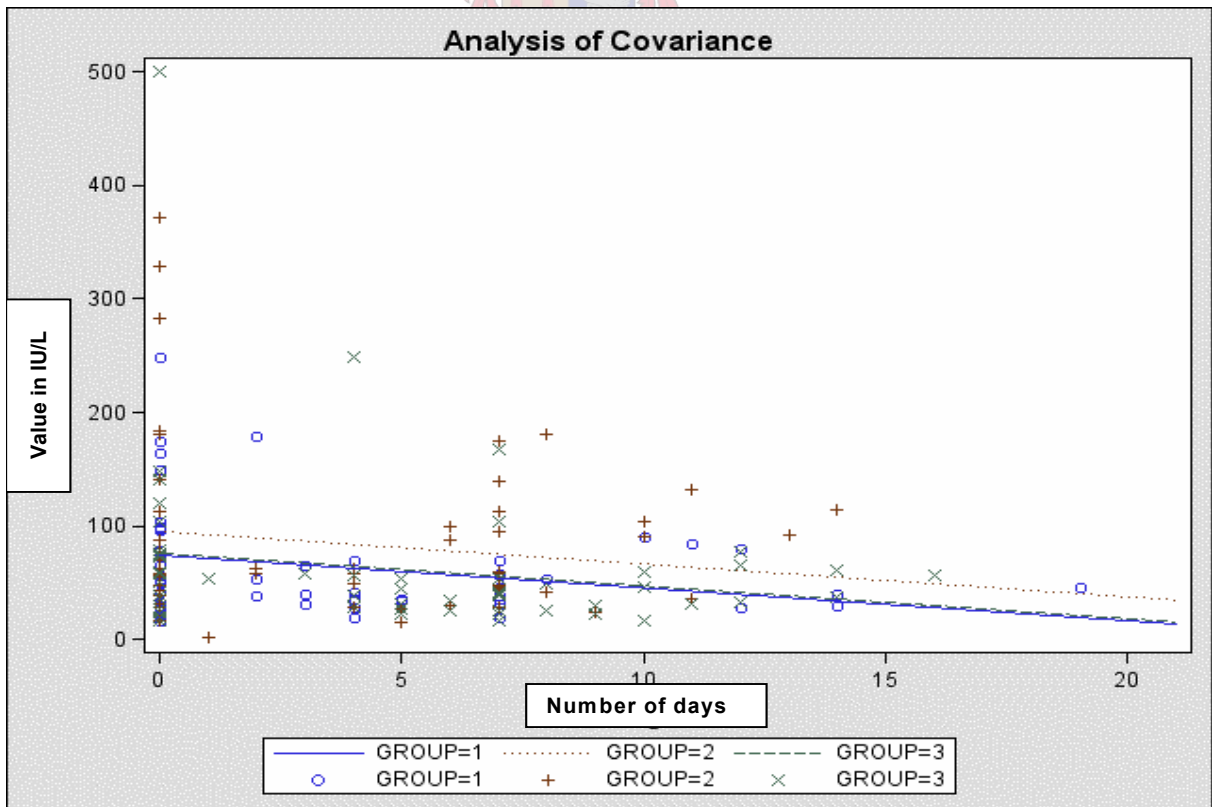


Figure 13 ANCOVA for AST per day in the different feeding groups during the trial period

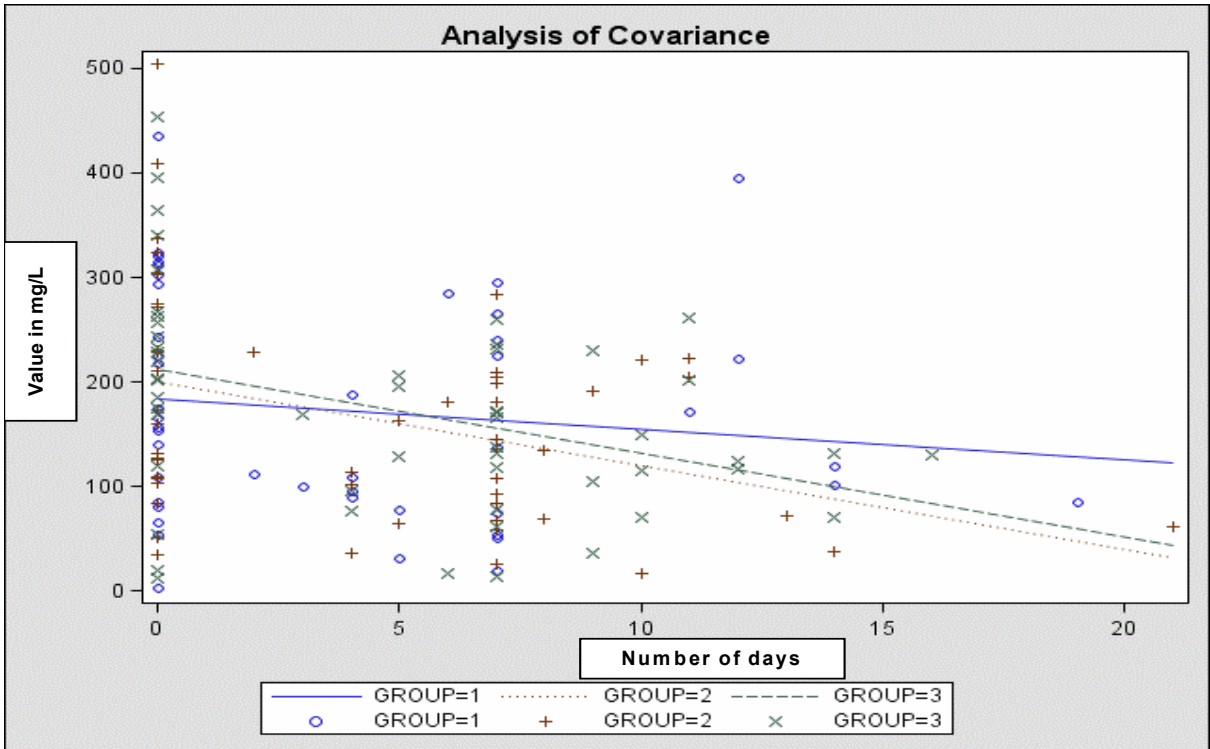


Figure 14 ANCOVA for CRP per day in the different feeding groups during the trial period

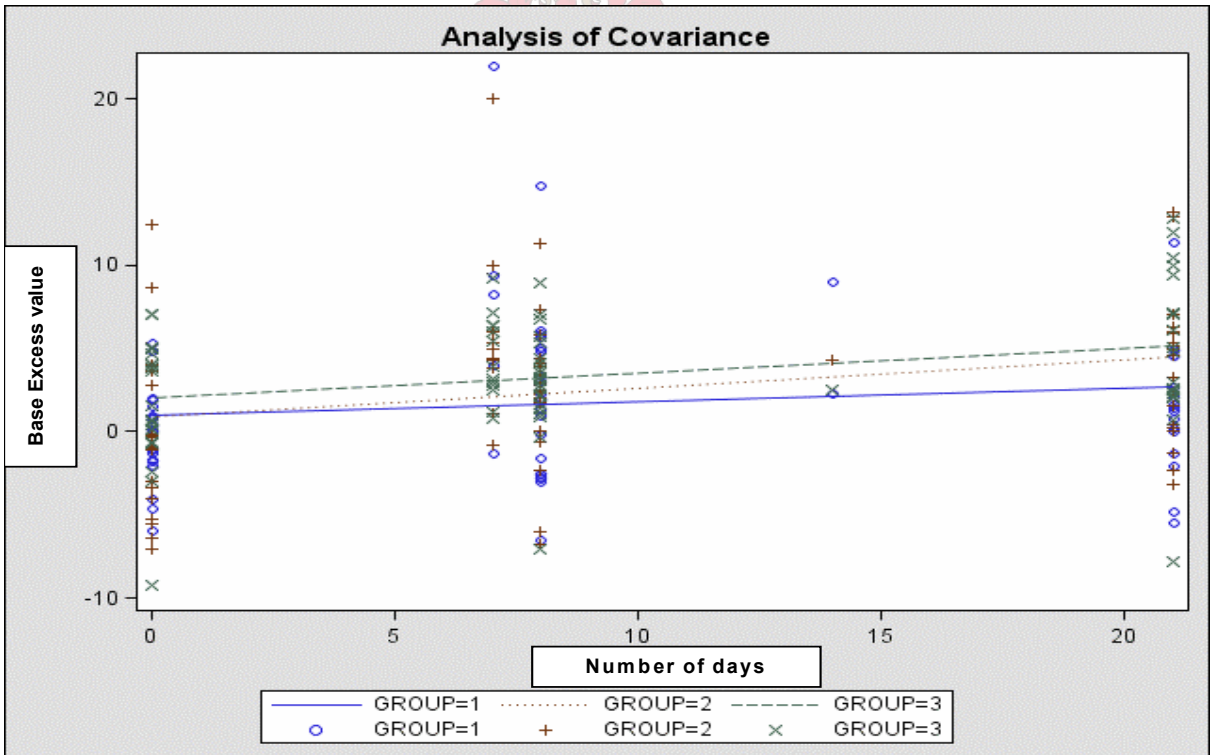


Figure 15 ANCOVA for base excess per day in the different feeding groups during the trial period

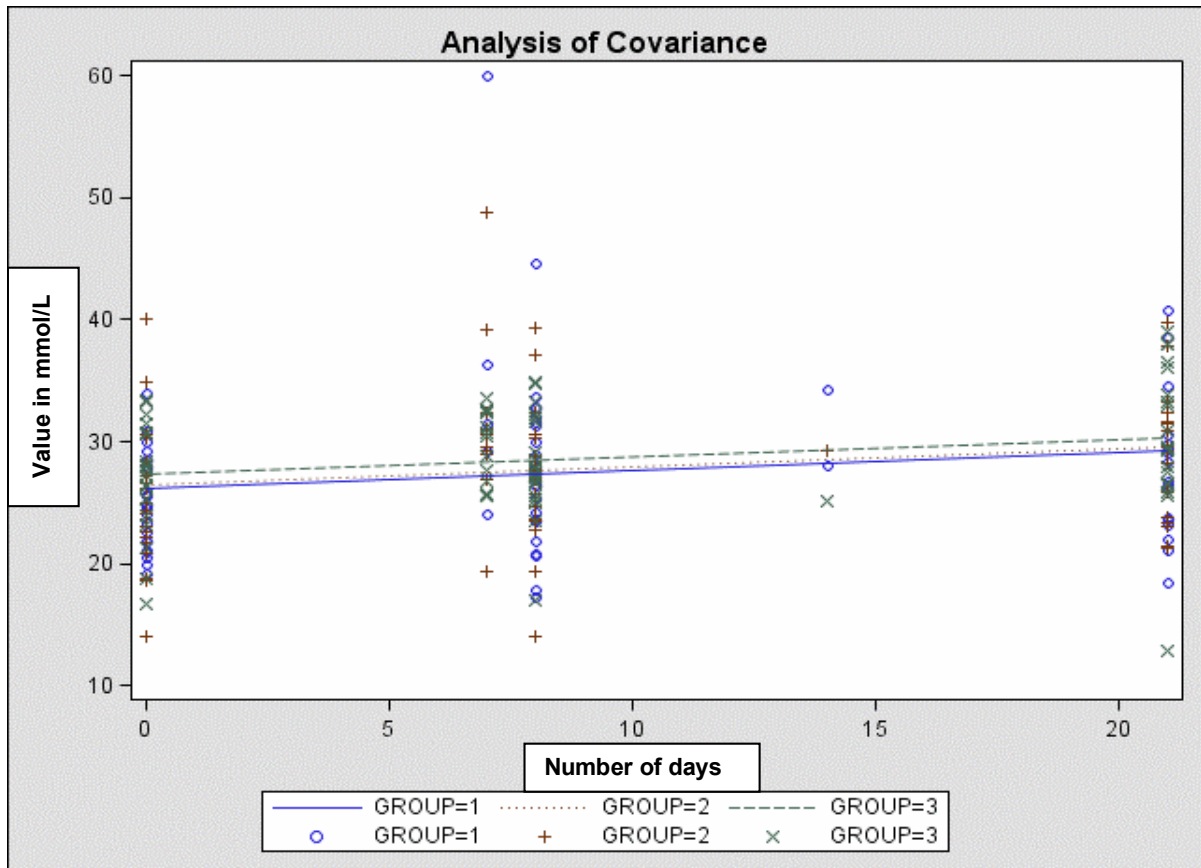
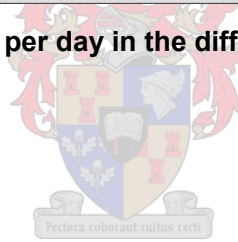


Figure 16 ANCOVA for TCO₂ per day in the different feeding groups during the trial period



APPENDIX I

Study title	Efficacy and Safety of Acidified Enteral Formulae in Tube Fed-Patients in an Intensive Care Unit	Trial Number:	00.19 CLI
Center :	Dept.of Human Nutrition, University of Stellenbosch, Tygerberg,. S.AFRICA	Investigator :	Prof. D. Labadarios
		Date of visit :	March 3-4, 2004

I. List of persons participating

1	Prof. Labadarios (Principal Investigator)
2	Mrs. Jeanne-Marie Kruger (Co-Investigator)
3	Sr. Kinnear
4	Dr. Choo Hoffmann (monitor)
5	Dr. Jean-Claude Maire (NRC)

I. General

		Yes	No	N/A	Comments
1.	Are there any significant changes at the study site since		X		
2.	Has new study personnel been appointed since last visit?		X		
3.	Are there any specific problems in this center?		X		
4.	Have protocol deviations / violations been identified?		X		
5.	Is the trial conducted according to GCP and legislation?	X			

II. Subjects

1	Number of subjects enrolled since last visit	1
---	--	---

Enrollment Table (number of subjects)

	Pink	White	Blue	Total
Enrolled	21	23	23	67
Dropout	3	5	8	16
Active	0	0	0	0
Completed	18	18	15	51
SAE	1	1	2	4

		Ye	No	N/A	Comments
--	--	----	----	-----	----------

2	Is informed consent regularly obtained from subjects?	X			
3	Have subjects been randomized correctly?	X			

III. Adverse events

		Yes	No	N/A	Comments
1	Review of all AEs occurring since last monitoring visit?	X			
2	Did any SAE occur since last visit?		X		

IV. Product accountability – inventory

		Yes	No	N/A	Comments
1	Is the product dispensed in accordance with the protocol?	X			
2	Is the product adequately stored?			X	Study completed: balance of product on site retrieved

Date of product		Nestle Enteral Formula**		Product	Presentation	Quantity per	Total received to date	Total at study
Sent	Received	Batch no.	exp. date	Label Code 480	(powder, milk, etc.)	Container	case	tins
02/02	02/02	1	09/03	PINK	Powder	400g	28	
		1	09/03	WHITE	"	"	28	
		1	09/03	BLUE	"	"	28	
Total product shipped to this center (at the time of this monitoring visit)							N/A: study completed	
Total product dispensed to patients (product dispensing log)..tins							97 Pink, 110 White, 91 Blue	
Total unused product remaining at investigator's site							None	
Total product returned (empty containers)							N/A	

V. Case report forms

		Ye	No	N/A	Comments
1	Was source data verification performed?	X			
2	Are CRFs regularly and satisfactorily completed?	X			
3	Are laboratory reports enclosed with CRFs?	X			
4	CRF numbers collected at this visit.	Completed checking of CRFs #63-67. Collected balance of completed CRFs (Pats # 51-67).			

VI. Trial documentation (investigator's file)

		Ye	No	N/	Comments
1	Is the investigator's file complete & updated?	X			Copy of 4 SAEs + Notification of SAE for pat #58 to be inserted.
2	Have any code envelopes been opened?		X		

VII. Miscellaneous

		Ye	No	N/	Comments
1	Biological sample: shipped			X	
2	Monitoring Log: complete	X			

Prepared by : C.C.Hoffmann **Signature :** **Date:** March 19, 2004

J-Cl. Maire

Signature:

NARRATIVE:**Other Issues:**

There appears to be a significant notable presence of Candida in the following:

1. Patient # 65: Note: On day 7: presence of Candida spp in feeding bottles, Candida spp + Candida albicans in delivery system. Both were present in nasogastric aspirate. On day 8: Candida spp was present in feeding bottles & delivery system. Both were also detected in nasogastric aspirate. On day 10: Candida spp was present in feeding bottles and delivery system.
2. Patient # 66: Note: Day 1: Candida spp present in both feeding bottle & delivery system at 24 hrs. On day 2: Candida albicans in feeding bottles, and both Candida spp + Candida albicans in delivery system. Candida albicans was detected in tracheal aspirate.
3. Patient # 67: On Days 1-2: Candida spp. in feeding bottles and delivery system and again on days 6 and 10. On days 3,5: Candida spp. detected in nasogastric aspirates, in addition to the presence of Actinobacter baumannii on both days. Actinobacter spp. was also detected in tracheal aspirates on day 2, and Actinobacter baumannii was detected on days 4, 6, 8 and in subsequent tracheal aspirates until patient completed the study on day 11. On day 10, E.Coli, Citrobacter freundii and Klebsiella pneumonia were detected in the reconstituted powder.

Summary:

This study has been very well organised and conducted, and team work has been excellent. Site will be closed on this visit.

APPENDIX J

Completed serious adverse event forms



Trial No: 00.19.CLI Subject No: 008 Subject initials: DEDO **SAE**

Form For Registration of Serious Adverse Event #1 (SAE)

(Page 1 of 2)

Subject data

Date of birth: 29 JUN 02 Current weight (kg): 93 . 10 Sex: M F

Date of 1st study product administration: 03 JUL 02

Date of last administration before onset of SAE: 22 JUL 02 Time: 13 : 00

Last dose/amount received before onset of SAE (mg/kg/day): 080 or not applicable

Information about the event

Date of onset of SAE: 22 JUL 02 Time at onset: 13 : 00

Description of event: Pt. deceased, on 22 Jul 02 (Day 20) at 13h00. Study formula was being administered at time of death.

Time between onset of SAE and last administration of study formula: 00 : 00

Duration of symptom: : Not applicable

Date of resolution: Time of resolution: : or continuing

Differential diagnosis considered: _____

Seriousness		Clinical course		Causal relationship (investigator's opinion)	
Subject died	<input checked="" type="checkbox"/>	Persistent event	<input type="checkbox"/>	Unrelated	<input checked="" type="checkbox"/>
Hospitalisation needed/prolonged	<input type="checkbox"/>	Improving	<input type="checkbox"/>	Unlikely	<input type="checkbox"/>
Persistent or significant disability	<input type="checkbox"/>	Recovered	<input type="checkbox"/>	Probable	<input type="checkbox"/>
Congenital anomaly/birth defect	<input type="checkbox"/>	Recovered with sequelae	<input type="checkbox"/>	Certain	<input type="checkbox"/>
Medically relevant event	<input type="checkbox"/>	Worsening	<input type="checkbox"/>		
		Death	<input checked="" type="checkbox"/>		
		Unknown	<input type="checkbox"/>		

Previous adverse reaction to similar treatment: Yes No Unknown

If yes, give details: _____

Measures taken concerning the study product:

None Withdrawal of study product → Date of withdrawal:

Dose reduction specify: NOT APPLICABLE

Reintroduced and positive Reintroduced and negative

Other, specify: NONE

(continued next page) →

Trial No: 00.19.CLI	Subject No: <u>008</u>	Subject initials: <u>D E D O</u>	SAE
---------------------	------------------------	----------------------------------	-----

SAE #1 Report (Page 2 of 2)

Corrective therapy: NONE

Code breaking: Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> Not applicable <input type="checkbox"/>
If yes, product code or name: <u>N/A</u> Batch No: <u>N/A</u>

Concomitant medication and SAE treatment:

Name	Indication	Route of administration	Dose (units)	Dates	
				Started	Ended

If further examinations are required to assess causality, note findings and attach results of examinations (lab reports, X-rays, etc): _____

Other medical comments: Patient known with ischemic heart disease with CAD stud in situ. Post aorticostomy there was an episode of decalculation of stentostomy tube with aortic branch injury. Contributing to the above was a possible coronary infarct leading to death.

Principal Investigator (or Co-Investigator):
DLABADARIOS Demetrio Labadarios 29/7/02
 Printed name Signature Date

Please fax this SAE form within 48 hours of onset of event (pages 1 and 2) to:
 Nestec (Denis Barclay, fax +41 21 924 4529)

Monitor check (date, initials)

Trial No: 00.19.CLI Subject No: 0491 Subject initials: UCDE **SAE**

Form For Registration of Serious Adverse Event #1 (SAE)

(Page 1 of 2)

Subject data

Date of birth: 11 | 02 | MAR | 52 | Current weight (kg): 62 | . | 10 Sex: M F
day month year
 Date of 1st study product administration: 02 | JUL | 03 |
day month year
 Date of last administration before onset of SAE: 10 | JUL | 03 | Time: 14 : 00 |
day month year hrs mins
 Last dose/amount received before onset of SAE (ml/kg): 0.8 | 0 | or not applicable

Information about the event

Date of onset of SAE: 11 | 07 | 03 | Time at onset: 14 : 30 |
day month year hrs mins
 Description of event:
Severe haemoptysis with (cardiorespiratory arrest + death)
 Time between onset of SAE and last administration of study formula: | | : | | |
hrs mins
 Duration of symptom: | | : | | | *Not applicable*
hrs mins
 Date of resolution: | | | | Time of resolution: | | : | | | or continuing
day month year hrs mins
 Differential diagnosis considered:

Seriousness		Clinical course		Causal relationship (investigator's opinion)	
Subject died	<input checked="" type="checkbox"/>	Persistent event	<input type="checkbox"/>	Unrelated	<input checked="" type="checkbox"/>
Hospitalisation needed/prolonged	<input type="checkbox"/>	Improving	<input type="checkbox"/>	Unlikely	<input type="checkbox"/>
Persistent or significant disability	<input type="checkbox"/>	Recovered	<input type="checkbox"/>	Probable	<input type="checkbox"/>
Congenital anomaly/birth defect	<input type="checkbox"/>	Recovered with sequelae	<input type="checkbox"/>	Certain	<input type="checkbox"/>
Medically relevant event	<input type="checkbox"/>	Worsening	<input type="checkbox"/>		
		Death	<input checked="" type="checkbox"/>		
		Unknown	<input type="checkbox"/>		

Previous adverse reaction to similar treatment: Yes No Unknown -
 If yes, give details: _____

Measures taken concerning the study product:

None Withdrawal of study product → Date of withdrawal: | | | | | |
day month year

Dose reduction specify: _____

Reintroduced and positive Reintroduced and negative

Other, specify: _____

(continued next page) →

Demetris Labadaris 11/7/03

Trial No: 00.19.CLI Subject No: 049 Subject initials: G|C|D|E **SAE**

SAE #1 Report (Page 2 of 2)

Corrective therapy: _____

Code breaking: Yes No Not applicable *Kinger 10 Jul 03*
 If yes, product code or name: _____ Batch No: _____

Concomitant medication and SAE treatment:

Name	Indication	Route of administration	Dose (units)	Dates	
				Started	Ended
<i>Adrenalin</i>	<i>(cardiorespiratory arrest)</i>	<i>IU</i>	<i>3 mg</i>	<i>10/7</i>	
<i>Atropine</i>	<i>(cardiorespiratory arrest)</i>	<i>IU</i>	<i>3 mg</i>	<i>10/7</i>	

If further examinations are required to assess causality, note findings and attach results of examinations (lab reports, X-rays, etc): _____

Other medical comments: *No causal relationship between study events and cardiorespiratory arrest. Patient died from underlying disease*

Principal Investigator (or Co-Investigator):
D. Plecha *[Signature]* *10/07/03*
 Printed name Signature Date

Please fax this SAE form within 48 hours of onset of event (pages 1 and 2) to:
 Nestec (Denis Barclay, fax +41 21 924 4529)
Demetre Kaladaris Monitor check (date, initials)

Trial No: 00.19.CLI Subject No: 0511 Subject initials: N | M | A **SAE**

Form For Registration of Serious Adverse Event #1 (SAE)
(Page 1 of 2)

Subject data

Date of birth: 04 | 10 | 54 Current weight (kg): 75 | . | 0 Sex: M F
day month year
 Date of 1st study product administration: 13 | JUL | 03
day month year
 Date of last administration before onset of SAE: 23 | JUL | 03 Time: 16 : 00
day month year hrs mins
 Last dose/amount received before onset of SAE (ml/kg/day): or not applicable

Information about the event

Date of onset of SAE: 23 | 07 | 03 Time at onset: 16 : 15
day month year hrs mins
 Description of event:
DEATH OF PATIENT
 Time between onset of SAE and last administration of study formula: 00 : 00
hrs mins
 Duration of symptom: : NOT APPLICABLE
hrs mins
 Date of resolution: | | Time of resolution: : or continuing
day month year hrs mins
 Differential diagnosis considered: _____

Seriousness	Clinical course	Causal relationship (investigator's opinion)
Subject died <input checked="" type="checkbox"/>	Persistent event <input type="checkbox"/>	Unrelated <input checked="" type="checkbox"/>
Hospitalisation needed/prolonged <input type="checkbox"/>	Improving <input type="checkbox"/>	Unlikely <input type="checkbox"/>
Persistent or significant disability <input type="checkbox"/>	Recovered <input type="checkbox"/>	Probable <input checked="" type="checkbox"/>
Congenital anomaly/birth defect <input type="checkbox"/>	Recovered with sequelae <input type="checkbox"/>	Certain <input type="checkbox"/>
Medically relevant event <input type="checkbox"/>	Worsening <input type="checkbox"/>	
	Death <input checked="" type="checkbox"/>	
	Unknown <input type="checkbox"/>	

Previous adverse reaction to similar treatment: Yes No Unknown

If yes, give details: _____

Measures taken concerning the study product:

None Withdrawal of study product → Date of withdrawal: | |
day month year

Dose reduction specify: _____

Reintroduced and positive Reintroduced and negative

Other, specify: _____

(continued next page) →

Trial No: 00.19.CLI Subject No: L0511 Subject initials: W1MA **SAE**

SAE #1 Report (Page 2 of 2)

Corrective therapy: NONE

Code breaking: Yes No Not applicable
 If yes, product code or name: _____ Batch No: _____

Concomitant medication and SAE treatment:

Name	Indication	Route of administration	Dose (units)	Dates	
				Started	Ended
Actrapid Insulin	Glucose control	IVI	2U/hr	11/07/03	23/07/03
Domicum	Sedation	IVI	6mg/hr	11/7/03	23/07/03
Morphine	Sedation	IVI	2mg/hr	23/7/03	23/7/03
Amiraplythe	Antiarrhythmia	IVI	0mg/hr	20/7/03	25/7/03
Piptaz	Antibiotic	IVI	4.5g daily	19/7/03	22/7/03
Solu-medrol	steroids	IVI	20mg 6hourly	19/7/03	23/7/03

If further examinations are required to assess causality, note findings and attach results of examinations (lab reports, X-rays, etc):
No

Other medical comments: patient had none regarding SAE as the background of COAD, grade 3 diarrhea, ischemic. Death due to rupture of ventricular aneurysm.
Dr. P. Barwick
Register internet medicine
ABW 25/July/2003

Principal Investigator (or Co-Investigator):
D. LABADARIS Demetris Labadarios 25/7/03
 Printed name Signature Date

Please fax this SAE form within 48 hours of onset of event (pages 1 and 2) to:
 Nestec (Denis Barclay, fax +41 21 924 4529)

Monitor check (date, initials)
19 Dec 03
LH

Trial No: 00.19.CLI Subject No: | 0 | 5 | 8 | Subject initials: | K | A | B | A | **SAE**

Form For Registration of Serious Adverse Event #1 (SAE)
(Page 1 of 2)

Subject data	Date of birth: 0 2 A P R 4 5 Current weight (kg): 5 5 . 0 Sex: M <input type="checkbox"/> F <input checked="" type="checkbox"/>
	Date of 1 st study product administration: 1 4 5 6 P 0 3
Information about the event	Date of last administration before onset of SAE: 1 5 5 6 P 0 3 Time: 0 1 : 0 0
	Last dose/amount received before onset of SAE (ml/kg/day): 0 2 0 or not applicable <input type="checkbox"/>
	Date of onset of SAE: 1 5 5 6 P 0 3 Time at onset: 0 1 : 0 0
	Description of event: <u>patient died</u>
	Time between onset of SAE and last administration of study formula: 0 0 : 0 0
	Duration of symptom: 0 0 : 0 0
	Date of resolution: Time of resolution: : or continuing <input type="checkbox"/>
	Differential diagnosis considered: <u>patient had a Brain Stem Haemorrhage</u>

Seriousness	Clinical course	Causal relationship (investigator's opinion)
Subject died <input checked="" type="checkbox"/>	Persistent event <input type="checkbox"/>	Unrelated <input checked="" type="checkbox"/>
Hospitalisation needed/prolonged <input type="checkbox"/>	Improving <input type="checkbox"/>	Unlikely <input type="checkbox"/>
Persistent or significant disability <input type="checkbox"/>	Recovered <input type="checkbox"/>	Probable <input type="checkbox"/>
Congenital anomaly/birth defect <input type="checkbox"/>	Recovered with sequelae <input type="checkbox"/>	Certain <input type="checkbox"/>
Medically relevant event <input type="checkbox"/>	Worsening <input type="checkbox"/>	
	Death <input checked="" type="checkbox"/>	
	Unknown <input type="checkbox"/>	

Previous adverse reaction to similar treatment: Yes No Unknown

If yes, give details: _____

Measures taken concerning the study product:

None Withdrawal of study product → Date of withdrawal: | | | | | | |
day month year

Dose reduction specify: no

Reintroduced and positive Reintroduced and negative

Other, specify: _____

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Trial No: 00.19.CLI	Subject No: C S E	Subject initials: K P M A	SAE
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SAE #1 Report (Page 2 of 2)

Corrective therapy: none given to patient

Code breaking: Yes No Not applicable
 If yes, product code or name: _____ Batch No: _____

Concomitant medication and SAE treatment:

Name	Indication	Route of administration	Dose (units)	Dates	
				Started	Ended
Adrenalin	hypotension	IVI	2mg/1hr	10/20/03	15/20/03
Granisetron	Constipation	PO	300mcg	10/20/03	15/20/03

If further examinations are required to assess causality, note findings and attach results of examinations (lab reports, X-rays, etc):

Other medical comments: I, D. GA depression, Department Neurology, feel that death unrelated to study

[Signature]
15/20/03

Principal Investigator (or Co-Investigator):
Denise Labadarios **DLABADARIUS** 15/19/03
 Printed name Signature Date

Please fax this SAE form within 48 hours of onset of event (pages 1 and 2) to:
Nestec (Denis Barclay, fax +41 21 924 4529)

Monitor check (date, initials)
10 Dec 03, JH