CHAPTER 1

Introduction
Introduction

The use of energy-dense high-concentrate low-roughage feeding systems is widespread in many areas of animal production. Whilst such feeding strategies have been successful in allowing increased production per unit of area and increased production per unit of feed, physiological complications in the animal associated with the intake of concentrated foodstuffs still occur.

Rumen acidosis remains a problem associated with high-concentrate low-roughage feeding systems in ruminants, particularly during the adjustment phase from high-roughage to high-concentrate rations. If the supply of readily fermentable carbohydrate exceeds the capacity of the rumen microbes to metabolise it, uncontrolled fermentation can proceed and lead to changes in the balance of the microbial population (Allison et al., 1975).

Sodium bentonite as a feed additive has been used in high-concentrate low-roughage ruminant feeding systems and the benefits resulting from its inclusion, such as protection from acidosis (Dunn, Emerick and Embry, 1979) and increase in productivity in terms of daily weight gain and feed efficiency, have been well documented (Martin, Clifford and Tillman, 1969; and Colling et al., 1979).

Bentonite has also been used in monogastric species where high-energy feeding strategies are employed, such as poultry and pig production. Dietary bentonite has been associated with increased body weight gains and improved feed conversion ratios in chickens (Kurnick and Reid, 1960; and Van'ke, 1976); increased egg size and production in layers, as well as reduced incidence of diarrhoea (Quisenberry and Bradley, 1964; and Somaiah, 1969). In pigs, body weight gain in starter pigs increased (Collings et al., 1980), while red and white blood cell parameters
improved with the addition of bentonite to the diet (Tmenov and Dzagurov, 1978; and Dzagurov, 1978).

High-energy feeding systems are regularly employed in two other monogastric species of note, the horse and the dog. These feeding strategies are more common when requirements for a concentrated source of energy are high, such as reproduction, growth and competition. In most situations, these high-concentrate low-roughage diets are well tolerated.

The potential for these diets to induce disturbance of the gastrointestinal microbial population is recognised. In the horse, intestinal crises, endotoxaemia, systemic lactic acidosis and laminitis are well documented (Moore et al, 1979; and Garner et al, 1978). In the dog, dietary alteration of the microbial population has been reported to cause clinical signs ranging from flatulence and minor changes to faecal consistency through to severe diarrhoea (Meyer et al, 1980).

Extrapolation from studies undertaken in other species suggested that bentonite may be beneficial in the horse and the dog receiving high-concentrate low-roughage diets, particularly in the changeover period from high-roughage to high-concentrate. Some of these benefits have only recently been recognised.

Field trials of a bentonite feed additive (Nature Vet Thrive P™ for Horses) incorporated in the rations of horses receiving high-grain diets was found to confer a degree of protection from commonly observed side effects, including depraved appetite, lower leg oedema, skin rashes, excitability and scours (Whatmore, 1986). Studies undertaken at Hawkesbury Agricultural College (Sriskandarajah and Woog, 1987) provided evidence that the product Nature Vet Thrive P™ for Horses improved feed digestibility in yearling horses fed a concentrate diet,
and lessened the deleterious effect of a strangles outbreak on body weight changes in the face of reduced feed consumption.

Dogs receiving high-energy diets have also benefited from the inclusion of a similar bentonite feed additive (Nature Vet Thrive D™ for Dogs) in their feed. The changes observed included a reduction in diet-related scouring, improved growth and body weight gain, and reductions in behavioural excitability and in the incidence of skin disorders (Pemberton, 1986; and Sadler, 1986). However, no formalised trial work had been undertaken in the dog to determine the efficacy of the product.

The experimental studies reported in Part A of this thesis were conducted with the following aims:

1. to determine the effects of the commercial product Nature Vet Thrive P™ for Horses on blood parameters (including lactic acid and endotoxin concentrations) when included in equine diets of varied grain to roughage ratio. Results were anticipated to allow the development of a better understanding of the action of the product on food digestion and gastrointestinal physiology and thereby allow more accurate recommendations on the prevention of feed-related gastrointestinal dysfunction and related systemic effects. In particular, the establishment of a beneficial association between the product and levels of blood lactic acid and endotoxin may have important implications in the prevention of acidosis and endotoxaemia when concentrate feeding strategies are used.

2. to quantify the effect of the product Nature Vet Thrive D™ for Dogs on feed digestibility and faecal parameters (formation and smell) when included in canine diets based on a mixture of dry kibble and mince. Results of this study were anticipated to assist in the registration of the
product for commercial distribution and sale, and to provide data suitable for marketing purposes.

The literature review presented in Chapter 2 incorporates a relevant overview of bentonite and its application to feeding systems. It provides background on bentonites, the properties they possess and the various areas of animal production where they have been used. Also included is a review of relevant aspects of both equine and canine digestive physiology, the effects of different feeding systems on local and systemic physiology and consideration as to the potential benefits that may arise from the inclusion of bentonite feed additives in high-concentrate low-roughage (energy-dense) diets in these species.

Chapters 3 to 6 provide an account of the experimental work undertaken. Chapter 3 details the materials and methods used, while Chapter 4 documents the results of the equine studies and Chapter 5, the results of the canine study. A discussion of future directions in research are presented in Chapter 6.
CHAPTER 2

Literature Review
2.1 Introduction

Bentonite clays are montmorillonite type clays and have been used in the production of commercial animal feeds as a pellet binding agent. However, the beneficial effects of the dietary inclusion of bentonite on production has been observed in a variety of animals in many situations.

There are two principal areas where bentonite has been found to be nutritionally beneficial. Firstly in the improvement of nutrient utilisation, and secondly, in minimising the side effects of high-grain rations. The mechanisms by which these effects are brought about are largely unknown, however, the unusual structure and physicochemical properties of bentonite are considered to be intimately involved.

The following review of the literature will detail aspects of sodium bentonite and its use in animal feeding and the structure and properties of sodium bentonite that are involved in its beneficial effects when used in animal feeding systems. In addition, aspects of the digestive physiology of the horse and the dog, and the influence of diet in each species will be presented. Following this review, the objectives behind the investigations documented in this thesis will be clarified.

2.2 Sodium Bentonite and Animal Nutrition

2.2.1 Pelleting

Bentonite is frequently used in the preparation of pelleted feeds at an inclusion level of 1.5 to 2.0% (Douglas, 1984). The properties that make bentonite beneficial in pelleting are its ability to disperse small particles evenly throughout a mixture and its ability to adsorb water
and therefore confer lubricant properties as feeds are passed through pelleting dies.

2.2.2 High-Concentrate Low-Roughage Feeding

Rumen acidosis is an important problem in ruminant production systems, particularly in the adjustment phase from high-roughage to high-concentrate rations. The rate at which high-concentrate feeds can be introduced without disturbing ruminal microbial populations and causing acidosis appears dependent on the rate at which protozoa can increase their numbers in response to the availability of starches or sugars (Mackie et al., 1978; Allison, Bucklin and Dougherty, 1964).

If the supply of readily fermentable carbohydrate exceeds the capacity of protozoa to metabolize it, uncontrolled fermentation can occur resulting in changes in the balance of the microbial population. Normal bacterial flora and ciliate fauna are depressed, while the numbers of *Streptococcus bovis* grow rapidly. As rumen pH declines, lactobacilli species become predominant, which is accompanied by a further lowering of ruminal pH, increased levels of lactic acid, and an increase in propionic acid level relative to acetic and butyric acids (Allison et al., 1975; Schwartz and Gilchrist, 1975).

2.2.3 Beef Cattle

Studies carried out by Erwin, Elam and Dyer (1957) found slightly higher weight gains in steers when 3% sodium bentonite was included in a 75% concentrate diet. However, other studies have failed to demonstrate any beneficial effect of bentonite when included in steer finishing rations (Martin et al., 1969; Colling et al., 1979).

Sodium bentonite has shown most benefit in beef cattle when used during the changeover period from high-roughage to high-grain
rations. Dunn et al (1979) found that the inclusion of 2% sodium bicarbonate and 2% sodium bentonite in the diet prevented stock from going off their feed between days 5 and 6, and led to improved daily intake and daily live weight gain particularly in the early grain feeding phase (days 1-21). In later feeding (days 22-93), bentonite tended to lower feed intake and live weight gains.

These observations have been supported by other studies (Erwin et al, 1957; Burkitt, 1969; Marshall and Van Horn, 1973). Dietary sodium bentonite (2-3.7%) in high-concentrate diets for beef cattle was seen to be beneficial in terms of overall feedlot performance, although benefits were seen primarily in the early feeding period and ration adjustment phase (Ashwood, 1982).

2.2.4 Sheep

Martin et al (1969) found that the addition of 2% sodium bentonite to concentrate diets had no significant effect on feed consumption and growth. However, a later study demonstrated that the inclusion of sodium bentonite in the high-concentrate feeds of lambs was found to result in more constant feed intakes, increase live weight gains and less scouring in the first 28 days of the concentrate diet (Huntington, Emerick and Embry, 1977).

Dunn et al (1979) subsequently demonstrated that in lambs rapidly adjusted to high-concentrate diets, the inclusion of 2% bentonite was associated with a drop in acidosis-related deaths from 19% to 3%. The inclusion of sodium bentonite has been shown to result in increased ruminal acetate and butyrate levels relative to propionate in lambs receiving concentrate diets, but to have no overall effect on ruminal pH or lactate levels (Colling et al, 1979).
2.2.5 Dairy Cattle

Lactating dairy cattle are frequently fed high-concentrate and low-roughage diets, which can result in the depression of milk fat percentage along with a reduction in roughage intake. The factors related to reduced milk fat content have been described as decreased acetic acid and increased propionic acid levels in the rumen, along with reduced plasma acetic acid and acetate uptake, and an increase in body weight (Rindsig, Schultz and Shook, 1969). The decline in ruminal pH and the resulting impairment of cellulolytic activity of microorganisms brings about the reduction in roughage intake.

Bringe and Schultz (1969) found that the inclusion of 5% sodium bentonite in experimental rations having a grain to dry-roughage ratio of 3:1 improved milk production and milk fat per cent in early to mid lactation cows. This finding was supported by Rindsig et al (1969), who found that 5% or 10% sodium bentonite resulted in improved milk fat per cent and milk production, no change in protein and solids non fats, maintained roughage intake, while the ruminal acetate:propionate ratio and the blood acetate level both increased.

In contrast, a later study by Rindsig and Schultz (1970) found there to be no significant difference between dry matter digestibility at 5 and 10% sodium bentonite inclusion levels. It was suggested that the beneficial effects (improved milk production and milk fat per cent) were due to either a change in rate of food passage, a small increase in rumen pH, ion exchange properties or a combination of these.

2.2.6 Pigs

Dietary bentonite at an inclusion level of 2 and 3% increased the rate and efficiency of weight gain in the starter period in pigs fed
concentrate diets, but not in the growing and finishing periods (Collings et al., 1980). Neither feed intakes nor carcass measurements were significantly affected by the level of sodium bentonite.

Bentonite at inclusion levels of 1, 1.5 and 2% were associated with significant weight gains of 20.1, 12.9 and 16% respectively in Large White piglets weighing 1.6 to 2kg (Tmenov and Dzagurov, 1978). Erythrocyte and haemoglobin values were also observed to be greater in the treatment groups, while weight gains associated with 1 and 2% bentonite were significantly greater than control animals.

Dzagurov (1978) showed subsequently that 5 to 60 day old pigs receiving a concentrate diet with 1% bentonite had similar weight gains at 5 days but significantly greater weight gains at 60 days compared to the control group. Diarrhoea was not observed in the treatment group, while haemoglobin, erythrocyte and leukocyte values were greater at 1 month and 2 months respectively in the treatment groups.

### 2.2.7 Poultry

The inclusion of 2.5% bentonite in low-energy diets produced a highly significant improvement in growth rate, a slightly delayed feed passage time and an increased feed intake in chicks (Kurnick and Reid, 1960). Van’ke (1976) found, that in Plymouth Rock chickens fed a concentrate diet, that the inclusion of bentonite at 1, 1.5, 2 and 2.5% resulted in significantly greater weight gains and significantly improved feed conversion ratios as inclusion levels rose.

Boza, Pintor and Varela (1972) found that 2% bentonite incorporated as a binding/pelleting agent in pellets fed to month-old chickens was associated with a nitrogen retention of 60.5%, as compared to 55.0% for pellets with no bentonite. A similar finding was reported by
Almquist, Christensen and Maurer (1967), where the inclusion of bentonite in the diet of turkeys improved the digestibility of protein and energy.

The inclusion of 2.5 or 5% bentonite in the diet of layers significantly reduced the water content of the droppings, and was associated with increased body weights and egg size, and improvements in the feed efficiency of egg production (Quisenberry and Bradley, 1964). A similar finding was reported by Somaiah (1969) where 5% bentonite in the diet of commercial egg-type pullets resulted in reduced water content and higher egg production.

2.2.8 Horses

Sodium bentonite has also seen use in horse feeding systems where high-concentrate restricted-roughage diets are fed. Its use has been largely due to the incorporation of the commercial feed additive Nature Vet Thrive P™ for Horses in grain-based diets. The formulation of this product contains approximately 67% sodium hydrogen montmorillonite, the principal constituent of bentonite. Details of the product formulation are provided in Chapter 3.

Initial studies and anecdotal evidence indicated that Nature Vet Thrive P™ was capable of preventing the decline in caecal pH at 6, 8 and 12 hours, following the administration of a corn starch/wheat flour meal (10g/kg) by stomach tube (Whatmore, 1985). Subsequent studies demonstrated that a 1% inclusion rate of Nature Vet Thrive P™ in the low-volume concentrate ration fed to Thoroughbred yearlings prevented many of the untoward effects frequently seen with such diets, such as lower leg oedema, flatulent colic and diarrhoea (Whatmore, 1986).
Horses that received a bentonite feed additive were observed to spend more time eating, lying down or standing, and less time drinking or playing with water, chewing wood, searching or eating manure (Whatmore, 1986). The quieter nature of the horses receiving the additive was reflected in shorter catching times and less veterinary attention for trauma suffered during the trial. Mean daily weight gains were also found to be 175g greater in the treatment group, which was interpreted to be due to more efficient nutrient utilisation in these animals.

The ability of Nature Vet Thrive P™ to influence digestion was subsequently demonstrated by Sriskandarajah and Woog (1987). The daily inclusion of 90g of Nature Vet Thrive P™ (montmorillonite inclusion level of 1%) in the high-grain low-roughage diet fed to Standardbred yearlings, resulted in a difference of 6.6% in overall dry matter digestibility. This higher digestibility was reflected in higher digestibilities of organic matter, nitrogen and fibre of 7.3, 5.2 and 10.5% respectively. Such improvements were considered to arise as a result of the effects of the product on microbial fermentation in the caecum and large colon.

Endurance horses fed heavy grain diets containing Nature Vet Thrive P™ (90g/day) passed less whole grains in their manure compared to control animals (Whatmore, undated). While all horses were observed to lose weight over the same period, those receiving the additive were found to lose significantly less weight than control animals, despite the fact that the control animals received 33% more feed.

Nature Vet Thrive P™ has been used specifically in a number of horses with high serum levels of muscle enzymes (serum glutamic oxaloacetic transaminase, SGOT; and serum creatine phosphokinase,
CPK) and was found to prevent exertional myopathy and reduce post-exercise serum muscle enzyme levels (Whatmore, undated). It was felt that the regulation of acid production, particularly lactic acid, by the product in the large intestine may have been involved. Montmorillonite has also been shown to increase the feeding times of young horses from 13 to 36 minutes (Whatmore, undated), a finding consistent with other research. Such changes were evident following a 6-12 day adaptation period, and montmorillonite inclusion rate of 2%.

Nature Vet Thrive P™ has also been shown to increase the rate of hoof growth in 5 polo ponies over two 5 week periods (Whatmore, undated). Earlier research had demonstrated that high-grain diets are known to inhibit the growth of the hoof, presumably as a result of the effects of vasoactive regulatory amines producing digital and coronary band vasoconstriction (Robinson et al, 1975). In the extreme form, this vasoconstriction can lead to carbohydrate overload laminitis. The inclusion of the bentonite feed additive was believed to regulate the production of these vasoactive amines, and so minimise the associated risks of high-grain diets.

2.2.9 Dogs

Through its inclusion in the product Nature Vet Thrive D™ for Dogs, bentonite has also seen use in dog feeding systems. The formulation of this product contains approximately 72% sodium hydrogen montmorillonite and is detailed in Chapter 3.

Pemberton (1986) demonstrated that the inclusion of Nature Vet Thrive D™ in commercially prepared kibble and tinned food diets produced increased growth rates and improved skeletal development and maturation in pups. In all cases, puppies had improved quality,
quantity and colour of coats, and as well appeared to have quieter temperaments and increased learning ability. In older dogs, Nature Vet Thrive D™ was associated with improved skin health and dogs with refractory chronic bacterial folliculitis responded completely within 3 weeks of commencing treatment and maintained healthy follicles.

Subsequent clinical trials have shown further benefits associated with the inclusion of Nature Vet Thrive D™ in the diet (Sadler, 1986). Greyhounds with a history of recurrent bowel upsets and those that were difficult to maintain condition were found to respond in 10 to 14 days with less bowel upsets, particularly in the more nervous dogs. These dogs also appeared calmer during travelling and prior to racing, with less tendency to fight. The observed beneficial effects were considered to be associated with improved digestion, reduced toxin production in the gut and subsequently reduced toxin absorption.

Sadler (1986) also reported improvement in 12 dogs suffering from Exocrine Pancreatic Insufficiency when diets were supplemented with 5 to 10g of Nature Vet Thrive D™ daily. Dogs recovering from bowel surgery were observed to have more rapid return of normal bowel motions, while in dogs suffering from chronic flatulence and chronic anal gland impaction, Nature Vet Thrive D™ was found to have important deodourising and bulking action.

Nature Vet Thrive D™ was also observed to reduce the incidence of parvoviral diarrhoea in Greyhounds (McKee, 1986). When parvoviral diarrhoea was widespread in 1986, 43% of the 37 pups born in one breeding establishment died. Following the introduction of Nature Vet Thrive D™ to the diet fed to bitches 2 weeks before whelping and with continued treatment while the pups nursed, and
then inclusion in the pup's diet until 6 months of age, no mortalities were observed.

2.2.10 Adverse Effects of Bentonite in the Diet

2.2.10.1 Mineral Adsorption

Martin et al (1969) found that with increasing levels of sodium bentonite, calcium was adsorbed at the 8% inclusion level. Rindsig and Schultz (1970) observed that faecal excretion of calcium in cows fed sodium bentonite was higher, resulting in an overall decline in calcium balance. Reductions were also apparent with magnesium and phosphorus retention in the faeces, however, the level of decline of mineral availability was higher at 10% than at 5% inclusion levels.

Such a reduction in mineral balances may be detrimental if the ration is low in a mineral - for example, grain is low in calcium and molasses is low in phosphorus. The inclusion of sodium bentonite in a marginally balanced ration may therefore require mineral supplementation, particularly with calcium and phosphorus where maintenance of an adequate Ca:P ratio is important.

2.2.10.2 Vitamin Adsorption

Bentonite strongly adsorbs vitamin A, riboflavin and choline (Ashwood, 1982). When diets are low in these vitamins, deficiencies may potentially arise, however, microbial synthesis of B vitamins in the gastrointestinal tract of ruminants and hind gut fermenters may well reduce this risk.

Erwin et al (1957) demonstrated strong adsorption of carotene pigments and vitamins in vitro, however, whether a similar adsorption in vivo occurs to a degree that may lead to a vitamin
deficiency was not examined. Laughland and Phillips (1954) found in rats that sodium bentonite could result in a potential vitamin A deficiency, while Briggs and Fox (1956) and Laughland and Phillips (1956) found a similar situation in poultry. Wheeler (1980) later demonstrated that sodium bentonite could result in problems of vitamin A utilisation in ruminants.

2.2.10.3 Retention in the Gastrointestinal Tract

Marker studies and feeding trials have indicated that bentonite was not retained in the rumen (Slanina, 1974). Likewise, samples taken of horse digestive tracts at postmortem failed to detect retention of the bentonite product Nature Vet Thrive P™ (Whatmore, 1985).

2.2.10.4 Inhalation of Dust

Analysis of lung tissue of lambs slaughtered after being fed bentonite at 2, 4, 8 or 12% showed no significant differences in lung silica content from those lambs fed a control diet (Huntington et al., 1977). It was concluded that the extreme dustiness of the high-bentonite diets did not cause an accumulation of silica in the respiratory tracts of these lambs.

2.3 Structure of Bentonite

Most bentonites are composed largely of the smectite clay mineral montmorillonite, and are derived as a result of weathering and chemical alteration of igneous material. Whilst bentonites may also contain other components, including kaolinite, mica, calcite, dolomite, quartz, feldspar and pyrite, it is the structure of the montmorillonite which determines the unique physical and chemical properties of bentonites (Grim and Guven, 1978).
The structure of montmorillonite was determined by Hofmann, Endell and Wilm (cited in Theng, 1979) and was found to be similar to pyrophyllite. The structure of pyrophyllite is comprised of three layers: an inner layer of octahedral aluminium oxide hydrate, and two outer layers themselves comprised of tetrahedral silicon oxide. The resulting structure is electrically neutral and non-expansive.

Montmorillonite retains a three layer structure, however it differs from pyrophyllite by virtue of isomorphc cation substitution - in the octahedral sites, Al$^{3+}$ can be exchanged for Mg$^{2+}$ or Fe$^{3+}$, while in the tetrahedral sites, Si$^{4+}$ can be replaced by Al$^{3+}$. These substitutions result in a structure possessing a net negative charge, which is balanced by interlayer cations which in natural bentonites are normally Na$^+$ or Ca$^{2+}$ (Grim and Guven, 1978; Theng, 1979).

The intercalation of water in the interlayer spaces of montmorillonite causes bentonite to swell. This ability to swell is influenced by the amount and site of isomorphc substitution, by the particle size and by the nature of the saturating cation (Theng, 1979). For example, sodium montmorillonite is able to swell to a greater degree than calcium montmorillonite, which is believed to be due to the smaller electrostatic attraction that exists between the sodium cation and the layer, as compared to the calcium cation and the layer in calcium montmorillonite (Norrish, 1954).

The ability of bentonite to adsorb water and undergo interlayer expansion is due to the montmorillonite component. It is this capacity to expand and expose a large surface area (700 - 800m$^2$/g) that gives bentonite its useful properties in water systems, and allows it to adsorb compounds as diverse as H$^+$ through to proteins and carbohydrates (Grim and Guven, 1978 and Theng, 1979). However,
such properties will vary between bentonites according to the nature of the saturating cation, the amount and site of isomorphous substitution, the presence of non-smectite components and whether the bentonite has been physically or chemically treated (Grim and Guven, 1978).

2.4 Properties of Bentonite

The mechanisms whereby sodium bentonite has produced alterations to the utilisation of food in animal nutrition are not fully understood. The properties that would appear to be important are bentonite's ability to swell, its capacity to adsorb polymeric molecules, and its ability to interact with microorganisms and so modify their behaviour. As bentonites vary in their makeup, and because bentonites are largely comprised of montmorillonite, the latter has been used in the majority of studies. A comprehensive review of the properties of bentonite has been published by Douglas (1984). Only relevant areas will be reviewed in this document.

2.4.1 Swelling

When dry montmorillonite is immersed in water, it swells in three stages to give an expanded lattice structure (Norrish, 1954). Stage 1 is due primarily to the hydration of the interlayer cations, while Stage 2 is associated with the uptake of between 10 - 20 times the dry weight in water resulting in a clay-water system with the consistency of a thick gel. Stage 3 is where the system has become a dilute solution or colloidal suspension.

The extent of swelling is affected by the type of saturating cations, type of water and pH. Montmorillonite containing small monovalent ions such as Li⁺ or Na⁺ is capable of swelling to stages 2 and 3, while montmorillonite containing polyvalent cations such as Ca²⁺ usually
does not expand beyond stage 1 because of greater electrostatic attraction between cations. Maximum swelling is achieved in distilled water, while the presence of salts and low pH tends to decrease the extent of expansion by reducing the level of like-charge repulsion on crystal lattice faces (Douglas, 1984).

2.4.2 Adsorption

When placed in water or dilute electrolyte solutions, montmorillonites are able to intercollate a variety of guest molecules.

2.4.2.1 Cations

Greenland and Hayes (1978) determined the order of preference for ammonium and other monovalent cations and their ability to be adsorbed to montmorillonite as \( \text{Cs}^+ > \text{Rb}^+ > \text{K}^+ = \text{NH}_4^+ > \text{Na}^+ > \text{Li}^+ \). This order indicates a greater attraction of the surfaces of the montmorillonite for the less hydrated cations.

However, the order of preference for cations is not only determined by the charge and degree of hydration, but as well by the efficiency with which the cations can exchange with each other, which is in turn controlled by the concentrations of the ions present. Therefore the degree to which any bentonite can adsorb cations is dependent on its cation exchange capacity, which may vary from 30 - 120 mEq/100g (Grim and Guven, 1978).

2.4.2.2 Carbohydrates

Greenland (1956a and 1956b) and Lynch, Wright and Cotnoir (1956) demonstrated that carbohydrates employed in nutrition can be intercollated into montmorillonite, a process that is believed to be partially dependent on hydrogen bonding. Table 2.1 presents the
results of the carbohydrates studied, and as can be seen, the amounts adsorbed are generally low (less than 10%). Two exceptions are corn starch at 21.6% and 22.8% and ethylcellulose at 20.0% and 20.5% for calcium and hydrogen montmorillonite respectively. Such adsorption may partly explain the benefits seen when montmorillonite is fed in association with high-concentrate low-roughage diets.

<table>
<thead>
<tr>
<th>Table 2.1. The amounts of carbohydrate and related compounds required to produce a maximum adsorption on 1g of clay in 20mL of water and the per cent of added compound which was adsorbed (from Douglas, 1984)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Calcium montmorillonite</strong></td>
</tr>
<tr>
<td>amount added</td>
</tr>
<tr>
<td>mg</td>
</tr>
<tr>
<td>Ethylcellulose</td>
</tr>
<tr>
<td>Methylcellulose 15cps</td>
</tr>
<tr>
<td>Cellulose dextrin</td>
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<tr>
<td>Starch dextrin</td>
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<tr>
<td>Inulin</td>
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<td>Glycogen</td>
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<td>Corn polysaccharide</td>
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<tr>
<td>Hydroxyethylcellulose</td>
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<td>Carboxymethylcellulose</td>
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<td>Corn starch</td>
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<tr>
<td>Sucrose</td>
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</table>

2.4.2.3 Proteins

Theng (1979) has reviewed the work carried out on the formation of protein-montmorillonite complexes. X-ray diffraction studies have shown that proteins that can be adsorbed to montmorillonite are divisible into two broad groups (Talibudeen, 1955). Firstly, those which can be incorporated either directly or after modification into the inter-lamellar spaces of montmorillonoids, for example salmine
and pepsin respectively. Secondly, those which cannot be incorporated into the inter-lamellar spaces, such as those proteins exhibiting a high degree of crystalline order, for example viral proteins.

However, for interlayer adsorption to take place, the montmorillonite must first swell which is influenced by the saturating cation. Harter and Stotzky (1971) have demonstrated subsequently that this adsorption of proteins to montmorillonite decreases in the order of $H^+ > Na^+ > Ca^{2+} > Al^{3+} > La^{3+} > Th^{4+}$.

Whilst protein adsorption is related to cation exchange capacity, electrostatic interaction alone provides an incomplete explanation of the nature of the interaction (Chiang and Chao, 1964). If adsorption was due to cationic effects alone, protein uptake should increase as a solution pH decreases away from the pI and little adsorption should occur above pH levels greater than pI. McLaren, Petersen and Barshad (1958) found that protein adsorption to montmorillonite generally attains a maximum at or near the isoelectric point of the particular protein, and that initial sorption of proteins is rapid - 75% of the maximum uptake occurs in the first few minutes in suspension.

Proteins adsorbed to montmorillonite cannot be desorbed by addition of salts, which indicates that other forces, possibly Van der Waals interaction are involved. However, proteins may be desorbed from clays by changing the pH of the suspending solution (Morgan and Corke, 1976) and by decreasing the amount of neutral electrolyte (Douglas, 1984).

2.4.2.4 Amino Acids

The structure of amino acids varies according to the functional group present, which may include an acid (carboxyl group) or a basic (amino
group), or both groups in the one structure. Amino acids can therefore exist as a cation, an anion or a zwitterion (equal numbers of positive amino groups and negative carboxylic groups), with the pH of the local system dictating which species predominates (McGilvery, 1979). When pH levels approximate neutral, monoamino monocarboxyl amino acids are electrically neutral, dicarboxylic amino acids are anionic, while dibasic amino acids are cationic.

The adsorption of amino acids to montmorillonite is sensitive to pH - adsorption and intercollation of amino acids above neutral pH is low, but increases steeply as pH decreases (Sieskind and Wey, 1959 - cited in Douglas, 1984). Most research has focused on cationic and zwitterionic forms because the anionic form of amino acids is relatively inactive towards the negative charge of the montmorillonite, (Greenland, Laby and Quirk, 1965a and 1965b).

Whilst the cationic nature of the amino acid is an essential component involved in its adsorption to montmorillonite, the size and also the shape of the amino acid may also influence uptake (Seiskind and Wey, 1959 - cited in Douglas, 1984). These latter two factors affect the extent of physical (Van der Waals) adsorption of the amino acid to the montmorillonite, and also its ability to gain access to the interlayers of the lamellar structure. Studies carried out by Greenland, Laby and Quirk (1962) and Greenland et al (1965a) have determined the extent to which various amino acids and peptides can be intercollated.

Amino acids may also be adsorbed to montmorillonite by the formation of stable coordination complexes involving the cations copper, cobalt or zinc. However, this interaction appears to be favoured by pH values 6-7, that is, near the isoelectric point for the amino acid (Bodenheimer and Heller, 1967)
2.4.2.5 Bacteria

Marshall (1971) has reviewed the interactions of montmorillonite with bacteria. The interactions between clay and bacteria are complex and it is possible that several mechanisms are involved in the adsorption process. Clay-bacteria interactions are pH sensitive and strengthen as pH declines (Santoro and Stotzky, 1967), yet there is no obvious relationship between the surface charge density of the cell and the amount of clay adsorbed (Marshall, 1971). Electrostatic attraction is important but fails to completely explain this interaction.

Whilst size, motility and Gram stain reaction of bacteria have no bearing on the clay-bacteria interaction, variations do exist between microorganisms and their ability to be adsorbed to clay and these remain quite specific (Santoro and Stotzky, 1968). The adhesion of bacteria to montmorillonite was also found to be greatest when the cation present in the clay was polyvalent (Santoro and Stotzky, 1967). Such selectivity is believed to influence the balance of populations of microorganisms in the soil (Marshall, 1971).

McLaren (1960) found that enzymes complexed to clay minerals operate in a structurally restricted system and therefore their behaviour towards substrate was different as compared to enzymes in aqueous solution. The negative charge of the montmorillonite brings about a shift to higher pH values in the pH-activity profile for the adsorbed enzyme. Estermann, Petersen and McLaren (1959) demonstrated that when substrate such as protein was bound to montmorillonite, enzyme activity on this substrate was decreased.

In some instances, the sorption of bacteria, enzymes, substrates and products onto the montmorillonite may depress activity by preventing contact between enzymes and substrate. Lynch and Cotnoir (1956) found that bentonite retarded the breakdown of many
of the substrates in crop residues of chickens. In other cases, bacterial activity and growth may be enhanced by bentonite improving aeration, removing toxic substances and or by acting as a concentrating centre in a dilute media. Stotzky and Rem (1966) found that montmorillonite was capable of stimulating respiration in a wide range of bacterial species, a response believed to be due primarily to the maintenance of pH at levels adequate for sustained growth.

Regardless of the exact mechanism, montmorillonite possesses the ability to influence microbial growth and activity, the ability to alter the balance of the reactions the microbes carry out and the ability to affect the composition of microorganic populations. It is believed that these properties of montmorillonite are also important in accounting for the beneficial effects seen when bentonite is fed in association with concentrate diets.

2.4.2.6 Organic Toxins

Carson and Smith (1983) found that bentonite was effective in preventing the feed refusal and growth retardation associated with the ingestion of T-2 aflatoxin in rats. Whilst some anionic exchange was believed likely to be involved, it was considered that the more important property was the capacity of bentonite to physically entrap the toxin in the polymeric gel matrix that develops when bentonite is admixed with water. Complementing this physical antitoxin effect was the observation that the inclusion of bentonite in the rats food was associated with a reduction in transit time of digesta through the digestive tract, which would be expected to result in further faecal loss of toxin.

Smith (1984) subsequently demonstrated that feeding spent canola oil bleaching clays to immature pigs overcame the growth depression
and feed refusal caused by T-2 toxin. The effects of spent bentonite in overcoming T-2 toxicosis in pigs was similar to that observed in rats, and it was believed that these spent colloidal hydrated aluminium silicates effectively bound the toxin in the gastrointestinal tract and so prevented the toxin from contacting the intestinal mucosa.

Hydrated sodium calcium aluminosilicates, when included in the diets of broiler leghorn chicks at a concentration of 0.5%, was found to be 55 to 100% effective in preventing the adverse effects of dietary aflatoxin (Phillips et al., 1988). Subsequent in vivo studies indicated that hydrated sodium calcium aluminosilicates binds aflatoxin and so reduces its bioavailability in the chicken (Davidson et al., 1987).

Harvey et al. (1989) demonstrated that hydrated sodium calcium aluminosilicate at a dietary inclusion rate of 0.5% or 2.0% was capable of protecting growing barrows from the deleterious effects of dietary aflatoxin. Such results are consistent with those found in poultry and it was concluded that hydrated sodium calcium aluminosilicate is a high-affinity sorbent for aflatoxin, both in vivo and in vitro, effective for the prevention of aflatoxicosis in growing barrows.

Bentonite, along with other adsorbents, was investigated using the murine model for its ability to bind bacterial endotoxin (Ditter, Urbaschek and Urbaschek, 1983). The hydrated sodium calcium aluminosilicate bentonite was found to be more effective in binding endotoxin in vitro than charcoal particles, kapectate, kaolin/pectin mixtures, kaolin, pectin and lactulose.

This efficacy was reflected in vivo, where the decreased frequency of endotoxaemia was correlated with the increased amount of endotoxin bound to bentonite in the intestine. The ability of bentonite to bind endotoxin was greatest at pH 3 as compared to pH 7.4, and was termed by the researchers as "striking". These results
appeared consistent with an earlier study by Novakova (1968), who demonstrated that bentonite was adsorbed onto the surface of gram-negative bacteria with a subsequent effect upon their growth.

Bentonite has also been found to be effective in the treatment of Lantana camara poisoning in cattle (McKenzie, 1991). The beneficial effect of bentonite was not considered to be due to cation exchange but more to the physical sequestration of the triterpene acid toxins and the stimulation of ruminal motility by swelling the ruminal contents. However, as increased ruminal activity may be expected to result in further uptake of toxin, the author considered that adsorption of toxin to the bentonite may also be involved as no such worsening of condition was seen.

2.5 Aspects of Equine Gastrointestinal Physiology

The physiology of digestion, which involves the physical and chemical breakdown of ingested food into a form that may be absorbed through the wall of the gastrointestinal tract, has been well documented (Alexander, 1963; Stevens, 1977 and Frate, 1986). Enzymes secreted by the host are involved with the conversion of carbohydrates into glucose and other simple sugars, proteins into amino acids and small peptide molecules, and fats into fatty acids and glycerol. Fibre is converted to the volatile fatty acid end-products acetate, propionate and butyrate by the microorganisms that primarily inhabit the large intestine.

In the natural environment, the wild horse selected succulent forages, which contained relatively large amounts of water, soluble proteins, lipids, sugars and structural carbohydrates, such as cellulose, hemicellulose and lignin. Starch was a non-essential component of the diet. Grazing periods were short and frequent, and whilst
predominantly occurring during the day, they stretched into the night (Frape, 1986).

The domesticated horse, however, may consume a variety of feeds ranging from forages high in moisture through to cereals high in starch. Feeding intervals have been altered to suit the management system as opposed to the animal, and many horses receive two or three meals per day. As well, novel feed materials such as cereals, protein concentrates and dried forages have been introduced.

Whilst the horse is capable of adapting to different substrates and feeding routines, digestive disturbances remain a potential hazard of modern feeding practices (Frape, 1986). The most common and potentially the most serious problems that can arise are generally associated with a disturbance in the normal balance of the microorganisms which exist in the digestive tract. The influence of diet on gastrointestinal microbial ecology will be reviewed, along with aspects of the functional anatomy and physiology of the digestive system.

2.5.1 Functional Anatomy of the Digestive Tract

2.5.1.1 Oral Cavity

Alexander (1972) demonstrated that the physical presence of food in the mouth is the trigger for the profuse flow of saliva in the masticating horse, with the parotid salivary gland contributing up to 50mL/min. The stimulus for this flow was found to be the chewing movement itself, which results in intermittent salivary flow greatest on the side that is chewed on.

The intermittent flow and hypotonic composition of the horses parotid saliva distinguishes it from the continuous and almost
isotonic parotid secretion of the ruminant (Alexander, 1972). The total daily secretion of the parotid salivary gland is in the order of 10-12L, containing approximately 50mEq/L of bicarbonate (Alexander, 1972).

The profuse secretion of a bicarbonate-rich saliva is believed to provide important buffering properties to support the extensive amounts of bacterial fermentation and lactic acid production that takes place in the stomach (Alexander and Davies, 1963; Argenzio, Southworth and Stevens, 1974b). This lactic acid is believed to be absorbed in the small intestine whereby it contributes to the overall nutrition of the horse. Lactic acid that fails to be absorbed in the small intestine passes to the large intestine where it becomes available for further bacterial fermentation and conversion to fatty acids (Alexander and Davies, 1963).

2.5.1.2 Stomach

The stomach of the horse is comparatively small and comprises about 8.5% by volume of the adult gastrointestinal system (Stevens, 1977). This small size makes it necessary for the horse to eat and chew food almost continuously, which ensures a steady supply of bicarbonate enriched substrate to the microorganisms of the stomach. The amount of lactate produced at any one time would therefore be small, however, because eating is almost continuous, optimal conditions are maintained for continuous production of lactic acid at a rate which may significantly contribute to the animals nutrition (Alexander, 1972).

The acid environment of the stomach is also important in initiating the enzymic breakdown of protein through the proteolytic activity of pepsin. The activity of pepsin is 15 to 20 times greater in the pylorus than the fundus, however, because delay time in the stomach is only
short, protein digestion is only slight (Frape, 1986). Liquids have been reported to leave the stomach within 30 minutes, with less than 5% retained at 2 hours and can reach the caecum in less than 2 hours from the time of administration (Argenzio, Southworth and Stevens, 1974a). The gastric emptying process has been found to be faster in the foal than in the adult horse (Stevens, 1977).

2.5.1.3 Pancreas

Pancreatic secretions are reportedly profuse and apparently constant in the horse at rest, but increase to very high flow rates (4-5 times greater) when food is present in the stomach (Alexander and Hickson, 1969; Frape, 1986). Secretion volumes of 5 to 12L in 24 hours have been recorded (Alexander, 1972). Bicarbonate levels rise only slightly when flow rates increase and chloride remains the predominant inorganic anion, in contrast to the cat, dog, man and pig. The amount of bicarbonate present in pancreatic juice may be sufficient to raise the pH of the duodenal contents to values approximating neutrality, but it is felt that the real importance of this secretion is to provide a medium for further anion exchange in the distal ileum (Alexander, 1972; Argenzio and Stevens, 1975).

In a number of species, bicarbonate is secreted in the distal small intestine (distal ileal section) and the colon, possibly in exchange for chloride (Alexander, 1972). The same is believed to occur in the horse and may function as a mechanism to provide sufficient bicarbonate to act as a buffer for the acid products of fermentation in the large intestine (Argenzio et al., 1977).

The output of pancreatic digestive enzymes in the pancreatic fluid is extremely small (Roberts, 1975a). Under normal feeding conditions, the level of amylase is considered to be sufficient to initiate polysaccharide digestion, which can then be continued by the small
intestinal brush border disaccharidase enzymes to yield hexose products which are capable of absorption (Roberts, 1975b).

2.5.1.4 Small Intestine

The small intestine is the primary site for digestion and absorption of dietary fat and long chain fatty acids. Fat digestion is very efficient in the horse, and the inclusion of edible fat in the diet has been shown to be beneficial to the endurance horse (Kane, Baker and Bulls, 1979). Protein hydrolysis is about three times that of the stomach, and appears heavily dependent on brush border peptidase activity (Frape, 1986).

Whilst the small intestine is about 22m long and accounts for 30% of the gastrointestinal tract volume, digesta remains in it for only a short period of time. The small intestine is a highly muscular structure and in the adult horse, can move food to the caecum within 45 minutes of feeding (Frape, 1986). The fact that digesta may pass at speeds of up to 30cm/minute is believed to play an important role in the nutrition and well-being of the horse, particularly when concentrate diets are fed.

2.5.1.5 Large Intestine

The soluble carbohydrate content of hay and pasture is about 5% dry matter, and is generally digested and absorbed precaecally. The remaining insoluble carbohydrates (cellulose, lignin and fibrous plant material) pass to the caecum where they undergo microbial fermentation into the volatile fatty acids (VFA's) acetic, propionic and butyric acid (Roberts, 1975b).

Diets high in insoluble carbohydrates are generally consumed more slowly and pass through the digestive system more slowly. However,
pelletising or chopping roughage effectively reduces its particle size and increases the speed at which the digesta passes from the stomach into the large intestine (Hintz, 1975; Argenzio et al., 1974a). Similarly, diets high in soluble carbohydrate with less roughage pass more quickly through the small intestine, allowing less time for nutrient digestion and absorption and thereby increasing the availability of substrate for large intestinal microbial fermentation (Argenzio, 1975).

Studies in ponies found that about 50% of the soluble carbohydrate and nearly all the insoluble carbohydrate escapes the small intestine (Hintz et al., 1971). Argenzio et al. (1974a) found that even on a high-grain diet, only about 25% of basal energy requirements of a horse is supplied by glucose oxidation. The carbohydrate reaching the large intestine is converted to lactic acid and volatile fatty acids, which are estimated to provide up to 25% of the animals basal energy requirements (Argenzio et al., 1974a; Annison and Armstrong, 1969).

Other nutrients are also digested and absorbed in the hindgut. While 60-70% of dietary protein may be digested and absorbed before reaching the large intestine and so provide the bulk of the required amino acids, nonprotein nitrogen can be converted to bacterial protein which also may provide variable amounts of amino acids to the host animal (Hintz, 1975; Rerat, 1978; Hintz, Schryver and Stevens, 1978). Digestion, fermentation and absorption from the large intestine is therefore believed to account for 30% of dietary protein, 15-30% of dietary soluble carbohydrate, 75-85% of insoluble carbohydrate and up to 80% of nitrogen compounds (Frape, 1986).

The large bowel is also intimately involved in the reclamation of the large amounts of water that shift into the gut during the process of digestion, including saliva, pancreatic secretions and other intestinal secretions. Some 19-20L of water enters the hindgut from the small intestine, and a further 10L of water passes out of the plasma into the
lumen of the hindgut. The large intestine therefore must recover a volume approximately equal to the extracellular volume (i.e. 30L) every 24 hours (Argenzio, 1975; Argenzio et al, 1974a).

2.5.2 Nutrition and the Effect of Diet on Microbial Digestion

2.5.2.1 Nutrient Requirements of the Horse

Nutrition of the horse has been reviewed by many authors (Frape, 1986; and Kerrigan, 1986). However, the most recent and complete documentation of the nutritional requirements and feeding strategies for the horse have been published by the National Research Council (NRC, 1989). Whilst all components of nutrition are important, the energy and roughage components of the diet are considered most important and form the major focus of interest in the research reported in this thesis.

The adult horse can generally sustain sufficient energy, protein, vitamins and minerals, from good quality pasture to satisfy maintenance requirements and allow the horse to perform moderate exercise. Supplementary feeding such as good quality hay may be necessary when pasture is poor. However, growing horses, breeding horses and horses stabled for competition have increased requirements and must receive supplementation which is generally in the form of cereal grains and concentrates.

The diet selected for the horse will therefore depend upon the individual nutrient requirements of that animal, and the combination of the ingredients selected to provide these requirements. Horses should receive in the order of 1kg roughage or dry matter (DM)/100kg body weight to maintain healthy alimentary function, and the remaining energy needs should be met with concentrates. Once energy and roughage requirements have been
satisfied, then the remaining dietary requirements for proteins, minerals and vitamins can be balanced (NRC, 1989).

Energy can be supplied by soluble and insoluble carbohydrates, fats and proteins. Volatile fatty acids produced as a result of microbial digestion of fibre in the large intestine is considered to provide the horse with up to 25% of its energy needs. The energy levels in pasture are generally adequate to maintain the adult horse. However, the performance animal requires additional readily digested energy supplementation in the form of concentrates (NRC, 1989; Fraps, 1986).

When concentrates such as grain are included in the diet, a corresponding reduction in the roughage component of the diet must occur. This results in reduced gut fill associated with fibrous feed and reduced water retention in the large intestine, and therefore reduced weight the animal has to carry. Also, if the roughage component was not reduced, the excess energy would be deposited as fat. This frequently leads to the situation where the horse fails to receive 1kg DM/100kg body weight, which is believed to predispose the horse to alimentary dysfunction.

The grains most frequently used as energy supplements include oats, barley, corn and wheat. These provide high levels of oligosaccharides and disaccharides that are readily broken down to monosaccharides (glucose and galactose) in the small intestine where they are readily absorbed (Roberts, 1975b). In general, 65-75% of these soluble carbohydrates are digested precaecally and absorbed as glucose, while the remainder, as well as the insoluble carbohydrates pass to the large intestine to be converted into volatile fatty acids, and D- and L-lactic acid (Roberts, 1975a).
2.5.2.2 Volatile Fatty Acids

The bacteria that occupy the caecocolic unit are qualitatively very similar to those found in the rumen of the sheep and cow and have been shown to possess similar nutritive requirements (Hintz, 1975; Garner et al., 1978; Van Soest, 1982). The end products of caecal and large colon bacterial fermentation of soluble and insoluble carbohydrate digestion are also similar to those produced in the rumen of cattle and sheep in both type and proportions (Hintz et al., 1978).

These organic acids are absorbed through the gut wall, but their rate of absorption is inversely proportional to molecular weight and appears to be affected by the rate of VFA metabolism (Argenzio et al., 1974b). Lactic acid too may also be absorbed through the hindgut, although at a slower rate than in the small intestine (Rerat, 1978).

Acetate is the primary VFA produced in all species, and propionate levels are normally higher than that of butyrate in many species. Changing the grain:hay ratio causes alterations to caecal fluid levels of acetate and propionate (increased grain leads to increased propionate and reduced acetate levels) in a similar fashion to the ruminant, which is presumed to be due to larger amounts of starch reaching the hindgut (Hintz et al., 1978; Rerat, 1978).

These acids are produced by a carefully balanced ecosystem that exists in the caecum and colon. There are seven families of largely homofermentative bacteria that are maintained in a balanced population as a result of the very narrow post-prandial movement of caecal pH in the hay or forage fed horse. Shifts of pH wider than normally seen with horses fed a roughage diet, such as those where concentrates are fed, may bring about changes in the numbers of certain Enterobacteriaceae and thereby upset this balance. This may
result in a variety of pathologic conditions (Sprouse and Garner, 1982).

The normal requirements for microbial digestion in the hindgut of the horse are the same as the ruminant, and include substrate availability, prolonged retention of digesta, anaerobic conditions and optimal pH, achieved by neutralisation or removal of acidic fermentation products (Argenzio, 1975). Diets that are high in grain have been demonstrated to be associated with increased levels of soluble carbohydrate reaching the caecum (Hintz et al., 1971), which has been shown to result in a more rapid fermentation of substrate and a significant fall in caecal pH at 4-6 hours after feeding, as compared to feeding hay (Willard, et al., 1977). This drop in pH suppresses acetate producing bacteria and encourages propionate and lactate producers.

Sprouse and Garner (1982) demonstrated that associated with such pH changes was a large increase in lactic acid producing bacteria, including lactobacilli and certain strains of streptococci, such as Streptococcus bovis. The decline in caecal pH was compounded by the production of D- and L-lactic acid from such bacteria.

2.5.2.3 Lactic Acid

The metabolism of lactic acid produced by ruminal bacteria has been described by Giesecke and Stangassinger (1979). Lactic acid production and fermentation is common amongst the monogastric animals, especially the young, and in general consists of both the D- and L-isomer forms although the D- form normally represents less than 50%. Lactic acid production is well known to increase as the levels of soluble carbohydrates in the diet is increased (Giesecke and Stangassinger, 1979).
In ruminants, the absorption of the D-isomer appears faster than for the L-form, and appears to occur mainly in the small intestine. L-lactate is an important precursor for fatty acid synthesis, is about twice as active as D-lactate in gluconeogenesis and glycerogenesis and 2.5-4 times more active than D-lactate in oxidation reactions (Giesecke and Stangassinger, 1979). Both forms of lactate may also be excreted by the kidneys.

Lactic acidosis that is seen in the ruminant overloaded with starch/sugars is believed to be due to D-lactate accumulation in the blood (Dunlop and Hammond, 1965; Prior, 1983). This situation appears to arise when the normal process that converts more than 80% of D-/L-lactate to volatile fatty acids in the rumen is overloaded (Counotte, Lankhorst and Prins, 1983).

The significance of plasma L-lactate levels in gastrointestinal pathology in the equine has been recognised for some time, but only recently have the levels of D-lactate and other acids been examined to determine their possible role in the pathogenesis of various conditions. In a retrospective study on horses with intestinal disorders, Gossett et al. (1987) noted that plasma D-lactate levels increased. As mammalian enzyme systems did not produce the D-isomer in any quantity, it was considered that this increase resulted from absorption of bacterial products from the gut. Whilst the increased plasma D-lactate levels were not considered to be common in intestinal dysfunction in the horse, it was believed that increased levels took on significance in certain types of bacterial infection or grain overload.

Other studies have confirmed this increase in bacteria, with 99.6% of the total viable count at 24 hours following change over to concentrate diet being starch utilisers, and a corresponding fall in the levels of protozoa (Goodson et al, 1985). Such changes are similar to
those that take place in the ruminant challenged with an overload of grain, and is believed to be due to an increase in lactic acid production.

2.5.2.4 Systemic Effects of Changes in Diet

Changes to the end products of fermentation associated with changes to feed substrate have been observed to have systemic effects on the horse, including alterations to feeding behaviour. Young horses fed concentrate diets were found to be coprophagic, chewed wood and other horses manes and tails, while ponies fed a completely pelletised diet were observed to become extremely nervous and to chew wood (Haenlein, Holdren and Yoon, 1966).

Such changes were further demonstrated by Willard et al, (1977) who established correlations between caecal propionate and lactate levels (both of which increased) and the time spent eating soil, faeces and chewing wood. Further research demonstrated that intracaecal infusions of propionate resulted in an increased total feed intake in horses of 7.5% compared to controls, and that higher doses of propionate and acetate reduced feed intake by prolonging the first intermeal interval by 143% and 71 to 74% for propionate and acetate respectively (Ralston, Freeman and Baile, 1983).

The condition Exertional Myopathy also appears to have links to dietary factors. Horses that suffer from this debilitating condition invariably are maintained on high-energy and high-protein diets, and have variations in work load, high muscle glycogen and increased levels of muscle waste products, including lactic acid (White, 1983). High-energy diets result in increases in caecal propionate production, which upon absorption, produces an insulin response leading to its storage as muscle glycogen. As well, high-grain diets produce
increased amounts of lactic acid, which is readily absorbed from the gut and adds to the background lactate levels.

Research has shown that even on high-grain diets, only 25% of horses energy needs are met by glucose oxidation (Argenzio, 1975; Argenzio and Hintz, 1972) and only at high speeds is it increased (Whatmore, undated). Acetate is the main energy source for low rates of exercise and maintenance needs (Harper, 1968).

The high-energy concentrations in the muscle, poor vascular perfusion, increased lactate levels in muscles and a rise in vasoactive amine concentrations (particularly serotonin) appear to interact to bring on exertional myopathy (Carlson, 1985). In addition, certain strains of oats have been reportedly associated with high blood serotonin levels (Whatmore, undated).

Grain filling or lower leg oedema of horses is a well documented condition that responds quickly to the removal of grain from the diet, along with mineral oil and saline laxatives to remove colonic contents. The lymph concentrations in the leg and foot of the horse are higher than in other animals, and any factor which affects lymph production or drainage may readily result in oedema (Robinson et al, 1975).

Laminitis is another metabolic disease that is demonstrated to be intimately linked with the diet. Various studies have demonstrated the pathogenesis of carbohydrate-induced laminitis and its association with caecal endotoxin and lactic acid production (Sprouse, Garner and Green, 1987; Garner et al, 1978; and Moore et al, 1979). The horse is known for its sensitivity to endotoxin and the vasoactive amines that are released as a result of platelet-toxin interaction (Kunze, 1983).
Diets high in soluble carbohydrates are also associated with other conditions such as gas colic and diarrhoea. Increased rate of fermentation of unaccustomed substrate by bacteria is believed to give rise to excessive gas production, and the toxin produced by *Clostridium perfringens* can induce severe diarrhoea (Frape, 1986). Also, the large increase in acid by-products act osmotically and may play a role in water movement out of the body (Argenzio, 1975; Argenzio *et al.*, 1974a).

### 2.5.2.5 Endotoxins and Endotoxaemia

Endotoxins are non-protein fragments of the cell wall of gram-negative bacteria, and must be absorbed into the systemic circulation to produce disease (Burrows, 1981a; and Semrad and Moore, 1987). They are less toxic than the proteinaceous exotoxins secreted from either gram-positive or gram-negative bacteria.

The Lipid A component of the endotoxin complex is associated with most of the toxic and lethal effects, and is considered to be functionally identical regardless of bacterial source (Burrows, 1981a). The similarity of systemic effects associated with endotoxins is thought to be due to the similarity of the endotoxin between bacteria.

It is postulated that small amounts of endotoxin are absorbed continuously from the gut and are detoxified by the liver. Any disease process which increases absorption of endotoxin, such as septic foci, peritonitis, alimentary tract disease, abscesses or large burn areas, or which reduces hepatic clearance may precipitate complications due to systemic actions of endotoxins (Burrows, 1981a). In other words, endotoxin must gain entry to the general circulation by one of two methods - either entering damaged mucosal barrier or overwhelming the Kupffer cells of the reticuloendothelial system (Semrad and Moore, 1987).
The effects brought about by endotoxin following entrance to the systemic circulation are extensive. Changes include cardiovascular compromise, pulmonary hypertension, damage to respiratory vascular endothelium, leukopaenia, thrombocytopenia, interference with carbohydrate, lipid and protein metabolism, lactic acidosis, hepatic, renal and nervous system dysfunction, gastrointestinal disturbances and coagulopathies (Semrad and Moore, 1987; and Burrows, 1981a).

The pathophysiology of endotoxin-induced damage is complex and not clearly defined (Semrad and Moore, 1987; and Morris and Moore, 1987). The involvement of the cyclooxygenase pathway in producing the metabolites that are integral in the pathogenesis of endotoxaemia, has resulted in extensive research into the use of cyclooxygenase inhibitors, such as the non-steroidal antiinflammatory drugs, to treat clinical endotoxaemia (Moore, 1981; Burrows, 1981b; Moore et al., 1981; and Semrad et al., 1987). The most commonly used and beneficial drugs in the treatment of endotoxaemia include flunixin meglumine, dipyrone and phenylbutazone (Semrad and Moore, 1987).

A recent study by Sprouse et al. (1987) demonstrated the effect of carbohydrate overload on the levels of plasma endotoxin. This study demonstrated that gram-negative endotoxaemia can develop following carbohydrate overload, which can be associated with laminitis. It also proposed the theory that if endotoxin could be blocked or neutralised, serious laminitis and lameness caused by carbohydrate overload could be attenuated or prevented and death caused by endotoxaemia could be prevented.

The mean plasma concentrations of endotoxin in race horses was shown to be significantly increased, while concentrations of plasma
anti-lipopolysaccharide antibody were significantly decreased following races of 1000m, 2000m, 2800m (Baker et al., 1988). The researchers considered that training-induced stress leads to lipopolysaccharide leakage into the systemic circulation and resulted in self-immunisation.

Gaffin and colleagues (1982) described how immunisation with antibodies specific to endotoxins had significantly reduced the morbidity and mortality rates due to endotoxaemia in laboratory animals and man. They also described the usefulness of these antibodies in treating Colitis X (a condition believed to be an acute form of endotoxaemia), peritonitis, and foals with peracute enteritis and epidemic gastroenteritis.

2.6 Aspects of Canine Gastrointestinal Physiology

The wild dog hunted its prey in packs, and generally ate the stomach, intestinal contents and viscera before the meat and bones (Commins, 1991; Kronfeld, 1983). The gastrointestinal tract of the prey invariably contained vegetative matter, so whilst dogs were and are carnivores, and indeed belong to the order Carnivora (as do cats, wolves, pandas etc), they are not true carnivores like cats. Instead, they are omnivores and for that part are more like humans and pigs.

Domestication of the dog has not only modified the behaviour of the animal, but has left the dog dependent on the human owner for a diet that will allow health and longevity. Whilst largely successful, modern feeding practices have been associated with side effects when physiological limits are exceeded (Meyer et al., 1980).

The physiology of digestion and nutrient absorption in the dog is detailed in many publications (NRC 1985; Lewis, Morris and Hand, 1987; Ettinger, 1983; and Stevens and Sellers, 1977). Only those aspects
of gastrointestinal tract anatomy and physiology, and feeding strategies relevant to the research reported in this thesis will be detailed.

2.6.1 Functional Anatomy of the Digestive Tract

The digestive tract of the dog is relatively short and simple, possessing a non-compartmentalised stomach and a short small intestine (Stevens, 1977). The length of the digestive tract beyond the stomach is 4.82m, as compared to the 29.91m in the horse. Whilst comparisons of digestive tract anatomy between species are meaningless because of dietary differences, comparisons can be made through the use of the ratio of body length to intestinal length. This ratio in the dog is 1:6 and in the horse is 1:12. Only the cat has less at 1:4 - all other domesticated animals have much longer intestinal tracts resulting in smaller ratios, for example - cow 1:20, sheep and goat 1:27, and the pig 1:14.

2.6.1.1 Stomach

The dog stomach represents 62.3% (4.33L) of the volume capacity of the alimentary tract, where it serves as a reservoir for food and is the initial site for protein and fat digestion (Stevens, 1977). The comparatively large size of the stomach allows the dog to consume large meals and to regulate the flow of digesta into the intestinal tract which in turn affects the efficiency of nutrient digestion.

Gastric emptying is determined by the volume, composition, osmolality, and physical consistency of the meal (Stevens, 1977). The stomach empties liquids more quickly than solids, and carbohydrates more quickly than fats. However, the nutritive density (kJ/mL) of the meal is also involved in the determination of the rate of gastric emptying (Twedt and Wingfield, 1983).
Gastric emptying of fluid and particulate markers was shown to be slower in the dog than in the pony - 1.5 hours compared to 0.3 hours respectively (Stevens, 1977). However, passage of particulate matter through the digestive tract of the dog was quite rapid, with both fluid and particulate markers passing through the large intestine at the same rate. At 12 hours, 60% of the fluid marker and 30% of particulate marker was found in the colon, with 10% of the fluid marker seen in the faeces. At 24 hours, 40% of the fluid marker and 30% of the particulate marker was seen in the colon, while 50% of the fluid marker and 30% of the particulate marker was seen in the faeces.

2.6.1.2 Small Intestine

The small intestine of the dog is 4.14m long and accounts for 23.3% (1.62L) of the volume capacity of the alimentary tract. Enzymes (amylases, proteases and lipases) along with bicarbonate are secreted by the pancreas, which together with the mucosal brush border enzymes, allows the digestion of proteins, carbohydrates and fats into their respective amino acids, monosaccharides and fatty acids (Sherding, 1983).

The rate of food passage through the small intestine is rapid, compared to the stomach and the large intestine. This rapid transit is believed to be responsible for the distribution of bacteria in the digestive tract (Hirsh, 1980).

2.6.1.3 Large Intestine

The hindgut is made up of the caecum, colon and rectum, which together are approximately 68cm long and account for 14.4% (1.0L) of the volume capacity of the gastrointestinal tract. Its primary function
is the absorption of water and electrolytes of dietary and secretory origin, but it also contains many bacteria which process substrate that escapes digestion and absorption in the anterior gut (Stevens, 1977).

Whilst sacculations are not an important feature in the dog digestive tract, it is believed that digesta passage is slowed by the small amount that does exist, as well as by areas of constriction, such as the ileocaecal valve (Stevens, 1977). Prolongation of digesta passage not only allows greater time for digestion and absorption, it also increases the time available for microbial digestion.

2.6.2 Microbial Digestion

The canine gastrointestinal tract is sterile at birth, but is quickly colonised by bacteria from the dam and the immediate environment. The microbial flora is very stable with each particular strain of microbe occupying a particular niche. In most instances, bacteria fed to a normal animal will be eliminated in 24-48 hours (Hirsh, 1980).

The greatest numbers of bacteria are found in the stomach, ileum and large intestine, and it is thought that the slow passage of food through these organs is responsible for this distribution. These bacteria are believed to serve a variety of functions, including a protective role in preventing pathogens establishing, and assisting in the digestion of poor quality feedstuffs (Stevens, 1977; Hirsh, 1980).

Microbial digestion of carbohydrates similar to that seen in the ruminant stomach occurs in the stomach of a wide range of mammals and in the large intestine of a wider range of mammals (Stevens, 1977). Volatile Fatty Acid (VFA) concentrations in the dog stomach are generally lower than in the horse, while VFA levels in the large intestine of the dog are approximately twice the levels seen in the horse implying active microbial digestion (Stevens, 1977).
The presence of VFA's in the stomach contents indicates that microorganisms survive the low gastric pH. While the higher pH at the lumenal surface of the gastric contents is believed to offer some protection, the pH of gastric contents is acknowledged to be high enough to support microbial digestion between meals (Stevens, 1977).

The highest concentration of VFA's and lowest concentration of lactic acid are found in the large intestine, as they are in the horse (Stevens, 1977). The ability of the dog to maintain the pH of caecal contents within a relatively narrow range of 5.5-7.0 suggests that secretion of buffers by the large bowel takes place to neutralise acids produced in this location. Low values of lactic acid in large intestinal contents may be due to a reduced availability of soluble carbohydrates in the large bowel.

Studies have found differences in gastrointestinal pH and VFA concentrations between dogs fed an all meat diet and a concentrate diet (dry chow) containing more soluble carbohydrate and fibre than meat (Stevens, 1977). Gastric and small intestinal pH were slightly lower in the dogs receiving the all meat diet, while caecal and large intestinal pH was lower in the dry chow-fed dogs. Volatile fatty acid levels in the stomach and small intestine of the all meat diet were higher, while the VFA levels in the concentrate diet were low in the stomach and small intestine and sharply increased in the large intestine.

2.6.3 Diet-Related Alimentary Dysfunction

Alimentary dysfunction in a healthy dog may occur as a result of incorrect food composition and feeding techniques. The associated clinical signs can range from flatulence and minor changes in faecal consistency, through to severe diarrhoea (Meyer et al, 1980).
Malassimilation, or incomplete digestion and/or absorption of food, generally occurs in the cranial region of the digestive tract. Unabsorbed nutrients may then be broken down by the intestinal flora with the release of catabolic products and bacterial toxins, which along with undigested nutrients can influence alimentary peristalsis and water secretion or absorption, resulting in diarrhoea.

Incomplete digestion in healthy dogs generally occurs as a result of the type of food fed, its treatment and to a certain extent, the feeding technique. Normal or excessive levels of carbohydrates, proteins and fats in the diet can disturb the process of digestion (Lewis et al, 1987). The pathogenesis of alimentary disorders is summarised in Figure 2.1.

2.6.3.1 Carbohydrate

Malassimilation of carbohydrates may result in increased gut sounds and flatulence and if severe, can lead to weight loss and diarrhoea. Diarrhoea results from colonic fermentation of unassimilated carbohydrate into osmotically active short chain carbon metabolites, as well as the presence of increased levels of osmotically active, unassimilated, non-fermented carbohydrates in the bowel (Washabau, Buffington and Strombeck, 1986). The observed flatulence may be a result of gas production, including hydrogen, carbon dioxide and methane as well as swallowed air (Washabau et al, 1986).

The digestibility of soluble carbohydrate in commercial feeds is about 85%, and is largely dependent upon the ability of the host to digest starch into dextrins (Lewis et al, 1987). Modern feeding strategies frequently incorporate large quantities of starch (over 60% in some food substances), which may overwhelm the dog’s digestive capacity (Lewis et al, 1987).
<table>
<thead>
<tr>
<th>I  Incomplete digestion and absorption in the proximal part of the intestinal tract</th>
<th>II Increased bacterial breakdown in the distal region of the intestinal tract as a result of dependent, amongst other things, on</th>
</tr>
</thead>
<tbody>
<tr>
<td>- failure to break down contents (sucrose, natural starch, veg. protein)</td>
<td>- the quantity and type of undigested nutrients in the proximal section of the intestine</td>
</tr>
<tr>
<td>- occurrence of enzyme-resistant compounds (overheated proteins)</td>
<td>- passage speed of the chyme</td>
</tr>
<tr>
<td>- trypsin inhibitors (raw egg white)</td>
<td>- other chemical and physiological conditions (pH, water bonding)</td>
</tr>
<tr>
<td>- sudden change in diet or too large quantities of food.</td>
<td></td>
</tr>
</tbody>
</table>

**SMALL INTESTINE**

**LARGE INTESTINE**

<table>
<thead>
<tr>
<th>III Active mechanisms</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>- organic acids</td>
<td>- disorders in osmotic balance</td>
</tr>
<tr>
<td>- NH₃, H₂S</td>
<td>- stimulation of secretion and motility</td>
</tr>
<tr>
<td>- deconjugated bile acids (?)</td>
<td>- inhibition of Na and water absorption ↓</td>
</tr>
<tr>
<td>- enterotoxins</td>
<td>increased water content in faeces</td>
</tr>
</tbody>
</table>

*Figure 2.1. The pathogenesis of alimentary disorders in dogs (Meyer et al, 1980)*

**2.6.3.2 Protein**

The digestibility of fresh animal proteins, such as meat, blood and milk is high, and provided reasonable quantities are fed, no disturbance occurs (Meyer et al, 1980). The digestibility of protein in commercial foods is considered to be between 80-90% (Lewis et al, 1987).
Proteins generally represent a small part of most diets and the amounts of gastric and pancreatic enzymes available for their digestion is adequate (Washabau et al., 1986). Even with the loss of pancreatic enzymes in conditions such as Exocrine Pancreatic Insufficiency, gastric pepsin allows some protein digestion to take place and as dietary nitrogen can be efficiently absorbed as peptides, few problems are observed (Washabau et al., 1986).

Where the diet is grossly unbalanced for protein, passage of digesta through the large intestine may be slowed as a result of the small quantity of undigestible material, so that secondary fermentation, characterised by soft, unpleasant smelling faeces is seen (Goddard et al., 1970; and Morris, Teeter, and Collins, 1971). Vegetable proteins are generally less easily digested by dogs compared to animal proteins and where large amounts are fed, there is an increased risk of fermentation and indigestion.

Soya protein fed at amounts of 5g/kg body weight or more produces changes in faecal consistency through to diarrhoea (Meyer et al., 1980). Despite soya protein having a digestibility of 80.4% when included at levels of 65% of the ration, 12.7% is found unchanged in the faeces. This has been reported to result in precipitating soft motions in 28.7% of dogs and shapeless slimey to liquid motions in 71.3% of dogs (Meyer et al., 1980).

2.6.3.3 Fat

Under most circumstances, more than 95% of ingested fat is assimilated (Sherding, 1983). However, the digestion of fat is the most sensitive of all digestive processes, as it requires the closely integrated operation of the pancreas, small intestine, liver and the lymphatics.
Mild disturbance to any of these organs may result in fat malassimilation and steatorrhoea.

The type of fat also influences its digestibility. Puppies fed fat at levels as high as 10g/kg body weight and adults at levels of 16g/kg body weight had digestibilities over 95%, however, butter and caprionic acids in quantities of 2-4% in the dry food substance may cause diarrhoea (Meyer et al., 1980).

2.6.3.4 Feeding Techniques

The alimentary tract of the dog is designed to receive large meals that are initially stored in the stomach. However, the tolerance of food can be affected by rapid passage through the stomach, which is encouraged by fine particle size such as chopped food, and reduced fat percentage (Meyer et al., 1980). Feeding techniques, such as the quantity of food per day or meal, sudden changes in diet and temperature of food are also involved in the aetiology of alimentary dysfunction in the healthy dog (Meyer et al., 1980).

Differences have also been found between feeding offal and feeding carbohydrate-rich diets either as a daily meal or double the amount fed twice daily (Meyer et al., 1980). Digestibility of fat, protein, soluble carbohydrates (nitrogen free extract) and organic matter was less in the carbohydrate diet as compared to the offal diet. The digestibility of each component for each double meal size was also less than the normal meal size for both offal and carbohydrate-rich diet, and it was concluded that the concentrate diet was less efficiently digested than the offal diet.

Sudden changes in diet, particularly from one high in protein and fat to one high in carbohydrate is also known to induce changes in digestibility and tolerance (Meyer et al., 1980). Likewise, intake of very
cold food reduces digestibility and tolerance as a result of increased peristalsis. Microbial activity can also contribute to gastroenteric dysfunction, and it is particularly dependent on the quality of undigested material, its ability to be broken down by the bacteria and the transit time through the gut (Meyer et al, 1980).

The digestibility of glucose, sucrose, lactose, dextrin, and starch mixed with animal tissue in a properly cooked diet may be as high as 94% (Lewis et al, 1987). However, large quantities of easily fermentable substances in the diet, such as sucrose, lactose, starch and protein are likely to disrupt the microbial balance in the gut. If the material is indigestible, such as cellulose, then disorders are less likely as microbial activity is not as vigorous (Meyer et al, 1980).

2.6.3.5 Digesta Passage and its Effect on Microbial Activity

Speed of digesta passage through the gut also influences intestinal microbial activity - easily digestible rations pass through the alimentary canal more slowly than foods containing indigestible components. In one study, a highly digestible basic food containing little fibre took 60-70 hours to pass through the alimentary tract, while a diet with 7-8% raw fibre took approximately 35 hours (Meyer et al, 1980).

The low-fibre diet was associated with an increased production of a broader range of organic acids compared to the high-fibre diet. Increased fibre content resulted in reduced levels of N-valeric acid, iso-valeric acid, N-butyric acid and iso-butyric acid, a large decrease in propionic acid levels and a large increase in acetic acid levels. The changes seen in the high-fibre diet are indicative of a diet that has passed through the gut more quickly and has undergone less bacterial metabolism (Meyer et al, 1980).
Small quantities of structural, undigestible substances have been demonstrated to improve the tolerance of diarrhoea-inducing foods and result in firmer faecal consistency and less water content (Meyer et al., 1980; Lewis et al., 1987). The slightly faster passage is believed to result in less bacterial digestion compared to the concentrate diet, resulting in less acid production and less osmosis into the lumen. The presence of some fibre also allows some water binding, and may have a bacterial population stabilising effect, along with pH stabilising effect that would be lacking with a carbohydrate-rich diet (Meyer et al., 1980).

The metabolism of undigested carbohydrates by microorganisms would therefore result in the production of large amounts of volatile fatty acids which is presumed to result in osmosis of water into the lumen. Absorption of these volatile fatty acids is thought to be slow, while the volatile fatty acids butyric and caproic acid may directly encourage fluid secretion (Meyer et al., 1980).

Other products of microbial digestion are also known to contribute towards water shifts into the lumen. Ammonia produced by the microbial decomposition of proteins is recognised in pigs to be capable of inducing diarrhoea by impairing sodium and water absorption (Drochner and Meyer, 1975 - cited in Meyer et al., 1980). Fats may also result in the production of short chain fatty acids and add to this osmotic process, while bile salt metabolites are known to inhibit sodium and water absorption (Mekhjian and Phillips, 1970 - cited in Meyer et al., 1980). The resulting disturbance in the bacterial flora results in the production of enterotoxins and endotoxins which exacerbate the condition (Meyer et al., 1980).
2.6.4 Feeding Strategies of the Domesticated Dog

There are two basic types of pet food used in feeding dogs - the homemade and the commercially produced. Most owners prefer the commercial foods, because they are convenient, cheap and generally problem-free when it comes to nutritional imbalances. Homemade diets are often associated with nutritional imbalances because the owner will feed foods suitable and balanced for humans but not for dogs, or the pet develops a preference for certain foods which if fed exclusively can result in nutritional imbalances. A survey of dog and cat owners in the United States found that 92% utilised commercially produced diets (Lewis et al, 1987).

There are three major forms of commercial pet foods available - dry (6-10% moisture), semi-moist (23-40% moisture) and canned (68-78% moisture). An American survey in 1985 found that on energy basis, 81% of pet foods sold was in the dry form, 11% in the canned form, and 4% as semi-moist. The remaining 4% was classified as snack foods or treats (Lewis et al, 1987).

2.6.4.1 Dry Foods

The most common types of dry foods are the kibble and expanded foods. Kibbled food is baked on a sheet, and then broken in to small pieces called kibbles, while expanded pet foods are cooked in an extruder and forced through a die, which results in expansion (Lewis et al, 1987). Expanded products have largely replaced kibble food, although the name kibble is still used by the pet food industry to describe dry dog feed.

The major advantages of dry foods are that they are 33-50% the cost of feeding a canned or semi-moist food of equal quality, they can be fed free choice, and their abrasive effect reduces the accumulation of
dental tartar. The reduction of tartar accumulation helps reduce gum inflammation, periodontal disease and halitosis, and as a result, promotes healthier gums and teeth.

The disadvantages of dry foods are that they are less palatable and may require moistening to encourage animals to eat. This moisture will cancel the beneficial abrasive effect on the teeth, and if left in the bowl, will turn food mouldy. Dry foods also require dry ingredients in their formulation, and the harsh drying used in the manufacture process can reduce the nutrient content.

Fresh animal tissues cannot be used in the manufacture of dry foods, and because of the packaging and processing requirements, the amount of fat that can be included in the diet is limited. Dry foods also tend to have lower digestibility than the canned or semi-moist types, as well as a shorter shelf-life (Lewis et al, 1987).

Dry foods therefore tend to be the lowest in essential fatty acids, a factor compounded by the use of beef tallow. Oxidation of fat also occurs readily, which turns the food rancid and further lowers the nutritional value of the fat (Lewis et al, 1987).

The consumption of dry foods, with their lower digestibility, lower fat content and higher fibre content, generally results in elevations in faecal water levels and reductions in urinary water excretion. This has the effect of increasing the incidence of dietary-related diarrhoea and increasing the risk of urolithiasis (Lewis et al, 1987).

2.6.4.2 Canned Foods

Canned foods are generally more palatable and digestible than dry foods but more expensive per unit weight of dry matter. They have
the advantage of being able to utilise either wet or dry food ingredients.

There are two types of canned pet foods - the ration type and the gourmet or meat type. The former are usually formulated from a variety of ingredients including animal tissues, soy products and cereals. They are less expensive, good nutritionally and do prevent development of food preferences. The gourmet canned food appears to contain a large proportion of muscle, but in fact contains a variety of animal by-products and textured vegetable protein, which is primarily soy flour coloured red to look like meat (Lewis et al, 1987).

The high-protein content of gourmet-type canned food requires animals to use it as an energy source, which because of increased protein catabolism brings about renal hyperperfusion resulting in renal damage. However, as these foods are so palatable, they are frequently used in the anorexic animal to encourage them to eat (Lewis et al, 1987).

2.6.4.3 Semi-Moist Foods

Semi-moist foods have a fairly long shelf life, do not require refrigeration, may be fed free-choice, and on the whole are quite palatable. Of the three forms, the semi-moist foods often have the highest energy digestibility. This is due to the high digestibility of the carbohydrate portion which is comprised primarily of corn syrup and polyhydric alcohols (propylene glycol), which provide antibacterial, antifungal, and moisture retention properties (Lewis et al, 1987).

Many soft foods contain acids such as phosphoric, hydrochloric and malic acid which not only aids in the manufacturing process but through low pH, helps retard bacterial growth. Sugars, corn syrup and salts elevate the soluble solids in the products and bind water to
further inhibit microbial growth. Propylene glycol also binds water and helps keep the food moist and pliable.

The major advantage of the semi-moist foods is that more of the ingredients can be fresh animal tissues. However, semi-moist foods frequently contain increased amounts of dry product ingredients to reduce production costs (Lewis et al., 1987).

2.7 Conclusions

Bentonite has been shown to be beneficial in a number of domesticated production animals receiving a high-concentrate low-roughage diet. More recently, the benefits of dietary inclusion of bentonite have been observed in domesticated companion animals receiving a similar diet, such as the horse and the dog.

The association of high-concentrate restricted-roughage diets with disturbances in gastrointestinal microbial flora has been well established (Mackie et al., 1978; Allison et al., 1964). The resulting changes in end products of microbial fermentation, along with the local and systemic physiologic effects of such changes have been widely documented (Allison et al., 1975; Schwartz and Gilchrist, 1975).

It is believed that the beneficial effects of bentonite inclusion in the diet arise largely as a result of its effects on microbial activity. The mechanisms by which these benefits are elicited are not fully understood. However, it is considered that bentonite's ability to swell when in contact with water, its ability to adsorb polymeric molecules (largely through cation exchange) and its ability to interact with microorganisms are an integral part of its action (Douglas, 1984).

High-concentrate low-roughage diets are recognised in the horse to be associated with many serious systemic effects, including lactic acidosis
(White, 1983) and the life threatening condition endotoxaemia (Sprouse et al, 1987). Research into these conditions has primarily been associated with establishing the systemic physiological effects of lactic acid and endotoxins, the association of diet and exercise with the development of lactic acidosis and endotoxaemia and the development of suitable treatment strategies to combat them. Whilst work has been undertaken on dietary prevention of lactic acidosis, little work has focussed on manipulation of the diet to prevent endotoxaemia.

Bentonite has been demonstrated to be the most effective of various adsorbents in binding bacterial endotoxin in vitro, and was subsequently shown to be the most effective adsorbent in vivo (murine model) in the prevention of endotoxaemia (Ditter et al, 1983). As a bentonite (montmorillonite) feed additive had been reported to result in improved nutrient digestibility in the horse (Sriskandarajah and Woog, 1987), it was logical that investigation of the effects of this feed additive on blood levels of endotoxin in the horse receiving a high-concentrate low-roughage diet should follow. At the same time, assessment of the effect of diet and the bentonite feed additive on blood lactic acid levels would be undertaken through the examination of the L-lactate isomer.

The dog is seemingly the least well equipped of the domestic animals to tolerate dietary perturbations and has few effective mechanisms to prevent bacterial upsets (Meyer et al, 1980). The range and frequency of gastrointestinal problems evident in the dog is possibly due to its low body length to intestinal length ratio, which may predispose the dog to dietary related gastroenteric disturbance.

The wild dog that consumed the whole carcass ate a diet high in digestible proteins and fats, with only small amounts of indigestible components such as vegetable fibres or keratin. Such a diet seemingly
resulted in the passage of food through the gut at a critical speed to limit the risk of intestinal microbial flora-linked dysfunction (Meyer et al, 1980).

Present day commercial feeding strategies for the domesticated dog involves the use of diets high in vegetable protein and starches. This has effectively removed the few safety factors present to guard against disturbance of the microbial flora. The sequelae of alimentary dysfunction associated with diet in the healthy dog, namely flatulence and diarrhoea, are widely documented (Washabau et al, 1986; Meyer et al, 1980).

A bentonite (montmorillonite) feed additive had been reportedly shown to be beneficial in dogs with diet-related alimentary dysfunction. Other benefits had also been observed in young growing dogs, dogs with chronic skin conditions and dogs recovering from bowel surgery.

However, no research had been undertaken to quantify the effects of this bentonite feed additive on nutrient digestion and on the common indicators of alimentary dysfunction, flatulence and diarrhoea. In addition, studies undertaken to date had provided insufficient data to enable registration of the product in Australia.

Part A of this thesis therefore reports on the findings of the investigations stimulated as a result of this review of the literature. These include firstly, the evaluation of the effect of the bentonite feed additive Nature Vet Thrive P™ for Horses on blood levels of endotoxin and lactic acid in horses receiving high-concentrate low-roughage diets.

Secondly, the assessment of the effect of a bentonite feed additive Nature Vet Thrive D™ for Dogs on nutrient digestion in dogs fed a
commercial diet and the ability of the product to prevent diet-related disorders, such as flatulence and diarrhoea. It was envisaged that the results of the latter investigation would facilitate the registration of Nature Vet Thrive D™ for commercial sale.
CHAPTER 3

Materials and Methods
3.1 Stock

3.1.1 Horses

Five healthy geldings (1 Standardbred, 1 Arabian-Draft cross and 3 Stock horses) were used in the investigations. The horses were resident animals of the Hawkesbury Agricultural College Horse Facility. All animals were mature (age range 8 to 14 years) and in good paddock condition (body weight range of 475 to 525kg - average weight 500kg). Vaccination histories were current.

3.1.2 Dogs

Ten healthy male crossbred dogs were used in the investigations. They were housed at Meadow Mist Boarding Kennels and Cattery Pty Ltd (Marsden Park, NSW, Australia). All dogs were mature (age range 4 to 6 years) and in good condition (body weight range 27 to 43kg - average weight 31.7kg). Vaccination and worming histories for all dogs were current.

3.2 Facilities and Management

3.2.1 Hawkesbury Agricultural College Beef Feedlot

Experiment 1 was undertaken at the Hawkesbury Agricultural College Horse Facility where the two horses were housed in individual stables (approximately 3.5m²). Each horse had access to its own exercise yard during the day (approximately 10 x 3.5m). Experiments 2 and 3 were undertaken simultaneously at the Hawkesbury Agricultural College Beef Feedlot, where each horse was housed in individual stalls (approximately 4m²). Each horse had access to its own exercise yard during the day (approximately 10 x 4m). Each horse had access to separate automatic waterers and feed bins. Manure was
collected and removed daily. Feet were examined and cleaned every morning.

3.2.2 Meadow Mist Boarding Kennels and Cattery Pty Ltd

The dogs were housed in individual, concrete-floored runs within an environmentally controlled room. Each run (approximately 4 x 1m) was constructed of floor to ceiling steel mesh and was equipped with a raised hessian bed, and separate water and feed bowls. Manure was collected twice daily and the runs hosed clean once daily. Dogs were examined daily to monitor health.

3.3 Food Materials

3.3.1 Horses

The chemical analysis of the lucerne chaff used in the trial is detailed in Table 3.1. The values for the oats in this table are averages of analysis results from similar oats used in nutrition trials at Hawkesbury Agricultural College.

<table>
<thead>
<tr>
<th>Table 3.1. Chemical Analyses of Lucerne Chaff and Oats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Matter (DM) %</td>
</tr>
<tr>
<td>Gross Energy (GE)</td>
</tr>
<tr>
<td>Organic Matter (OM)</td>
</tr>
<tr>
<td>Neutral Detergent Fibre (NDF)</td>
</tr>
<tr>
<td>Crude Protein (CP)</td>
</tr>
</tbody>
</table>

*Values provided by Dr. P. Trevor-Jones (Hawkesbury Agricultural College)

3.3.2 Dogs

The dry dog food used was Harper’s Dog Chow™ (Friskies Pet Care Pty Ltd, Bankstown, NSW, Australia). It was selected as being representative of the many brands of dry dog food commonly used in
domestic feeding systems. Mince meat made from ox cheek and ox tongue was also included in the diet to simulate the feeding of fresh meat in domestic feeding systems. The chemical analyses of the two feed components is detailed in Table 3.2, while the guaranteed analysis printed on the packaging for Harper's Dog Chow™ is presented in Table 3.3.

<table>
<thead>
<tr>
<th>Table 3.2. Chemical Analysis of Harper's Dog Chow™ and Mince Meat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>Mince Meat</td>
</tr>
<tr>
<td>Harper's Dog Chow</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3.3. Guaranteed Analysis of Harper's Dog Chow™</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum Crude Protein</td>
</tr>
<tr>
<td>Crude Fat</td>
</tr>
<tr>
<td>Maximum Crude Fibre</td>
</tr>
<tr>
<td>Salt</td>
</tr>
</tbody>
</table>

3.4 Bentonite Feed Additives - Formulations

3.4.1 Horses

The registered feed additive Nature Vet Thrive P™ for Horses (Nature Vet Pty Ltd, Agnes Banks, NSW, Australia) was utilised in the investigations undertaken in horses to determine the effect of bentonite inclusion in a high-concentrate low-roughage diet on blood parameters. The formulation of this product is detailed in Table 3.4.

3.4.2 Dogs

The feed additive Nature Vet Thrive D™ for Dogs (Nature Vet Pty Ltd, Agnes Banks, NSW, Australia) was utilised in the investigation undertaken in dogs to determine the effect of bentonite inclusion in a combination commercial dog food and mince meat diet on nutrient
digestion and diet-related disorders, such as flatulence and diarrhoea. The formulation of this product is detailed in Table 3.5.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hydrogen montmorillonite</td>
<td>674.25g/kg</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>75.00g/kg</td>
</tr>
<tr>
<td>Methionine</td>
<td>150.00g/kg</td>
</tr>
<tr>
<td>Lysine</td>
<td>100.00g/kg</td>
</tr>
<tr>
<td>Maltase 700*</td>
<td>0.25g/kg</td>
</tr>
<tr>
<td>Protease N7*</td>
<td>0.25g/kg</td>
</tr>
<tr>
<td>Cellulase T2*</td>
<td>0.25g/kg</td>
</tr>
</tbody>
</table>

* - Pfizer Chemicals

Table 3.5. The formulation of Nature Vet Thrive D™ for Dogs (Label claim)

<table>
<thead>
<tr>
<th>Composition</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hydrogen montmorillonite</td>
<td>718.9g/kg</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>196.0g/kg</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>58.0g/kg</td>
</tr>
<tr>
<td>Maltase 700*</td>
<td>2.3g/kg</td>
</tr>
<tr>
<td>Protease N7*</td>
<td>2.3g/kg</td>
</tr>
<tr>
<td>Lipase AP*</td>
<td>2.6g/kg</td>
</tr>
<tr>
<td>Keletron Trace Mineral Polysaccharide Complex**</td>
<td>20.0g/kg</td>
</tr>
</tbody>
</table>

* - Pfizer Chemicals

** - Keletron Trace Mineral Mix (machined dehydrated kelp and zinc, iron, and copper polysaccharide complexes. Guaranteed analysis - zinc, not less than 10.00%; iron, not less than 5.00%; manganese, not less than 1.5%; and copper, not less than 0.75%).

3.5 Analytical Methods

3.5.1 Endotoxins

Blood endotoxin levels were measured in batches using a commercially available Limulus Amoebocyte Lysate (LAL) chromogenic substrate kit COATEST® Endotoxin (KabiVitrum AB, Stockholm, Sweden; distributed by Johnson and Johnson Medical Pty Ltd, North Ryde, NSW, Australia).
Venous blood samples were collected by sterile jugular venipuncture at 0, 2, 4, 6, 8, 10 and 12 hours postprandially into sterile Vacutainers® (Becton Dickinson, Rutherford, NJ, USA) containing the anticoagulant lithium heparin. The skin at venipuncture sites was initially clipped, and then decontaminated with alcohol and then povidone iodine solutions. Clean handling was employed at all stages of collection to minimise contamination of samples with background endotoxin.

Blood samples were placed on ice for transport to the laboratory facilities at the Faculty of Agriculture and centrifuged at 200 x gravity for 10 minutes. The platelet/endotoxin-rich plasma was then drawn off using a sterile pipette and stored at -20°C in sterile tissue culture tubes for analysis within 2 weeks.

Stored plasma samples were analysed using the microplate method. The microplate procedure was performed in accordance with the materials and methods described in the COATEST® Endotoxin - Practical Advice and Trouble Shooting technical sheet (Appendix 1). A water bath customised to maintain an operating temperature of 37°C was utilised instead of a block heater. Sterile endotoxin-free 96 well microtitre plates and a multichannel pipette (8 channel) equipped with sterile disposable pipettes were used to minimise contamination with endotoxin. The assay was carried out in a laminar flow cabinet.

After the enzyme reaction was stopped with acetic acid, measurement of changes in colour (absorbance) reflecting changes in the level of endotoxin in solution were determined using a Vitatron automated photometer (Behring Diagnostics Pty Ltd, Kinsgrove, NSW, Australia) set at 405nm. Endotoxin levels were then calculated from standardised absorption/endotoxin graphs.
3.5.2 Lactate

Blood L-lactate levels were measured using a commercially available chromogenic kit, Stat-Pack™ Rapid Lactate Test (Behring Diagnostics Inc, Somerville, NJ, USA).

Blood samples were collected by the sterile technique described previously into sterile Vacutainers® containing the anticoagulant fluoride oxalate. Following collection, these samples were placed on ice and transported to the laboratory at the Faculty of Agriculture, where they were centrifuged and the plasma drawn off. 1.0mL of cold plasma was then deproteinated by the addition of 2.0mL ice cold perchloric acid (0.6M) and centrifugation at 200 x gravity for 5 minutes. The supernatant was drawn off and stored at -20°C for later analysis.

Stored deproteinated samples were processed according to the directions detailed in the Stat-Pack™ Rapid Lactate test kit insert (Appendix 2). Changes in absorbance of samples reflecting L-lactate levels were then determined using the Vitatron photometer at 340nm.

3.5.3 Haematology

Blood samples were collected by the sterile technique described previously into sterile Vacutainers® containing the anticoagulant ethylene diamine tetraacetic acid (EDTA). These samples were analysed in the Haematology Laboratory at Faculty of Agriculture using standard methods for packed cell volume (PCV), total plasma protein (TPP), electronic Coulter Counter (Coulter Electronics, England) for total red blood cell (RBC) and total white blood cell
(WBC) counts, and differential white blood cell analysis using the Diff-Quik staining technique (Bacto Laboratories Pty Ltd, Liverpool, NSW, Australia). Platelet counts were determined using electronic equipment at Macquarie Vetsotics Pty Ltd (Leichhardt, NSW, Australia) except in Experiment 1 where estimations from the slides used in the differential white blood cell determination were used.

3.5.4 Food and Faecal Analyses

Moisture, ash, nitrogen, energy, fat and fibre levels in dog food and faecal material were determined according to standard methods of the Association of Analytical Chemists (AOAC, 1980).

3.5.5 Faecal Smell and Faecal Formation

All daily manure samples collected during the dog trial were weighed, and rated according to smell and degree of faecal or stool formation. The rating system used is outlined in Table 3.6.

<table>
<thead>
<tr>
<th>Smell</th>
<th>Degree of Stool Formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mild</td>
</tr>
<tr>
<td>2</td>
<td>Moderate</td>
</tr>
<tr>
<td>3</td>
<td>Strong</td>
</tr>
<tr>
<td>4</td>
<td>Pungent</td>
</tr>
<tr>
<td></td>
<td>1 Dry</td>
</tr>
<tr>
<td></td>
<td>2 Moist</td>
</tr>
<tr>
<td></td>
<td>3 Wet</td>
</tr>
<tr>
<td></td>
<td>4 Sloppy (unformed)</td>
</tr>
</tbody>
</table>

3.5.6 Body Weight

The body weight of each horse was determined at the commencement and conclusion of each experiment using a Toledo weigh scale at the Hawkesbury College Beef Cattle facility. Body weight determinations for each dog were undertaken at the commencement of the trial, and at the end of each collection period using a digital walk-on scale.
3.6 Statistical Methods

Analysis of data from the horse investigations were carried out using the Biomedical Date Analysis Package (BMDP) statistical package (BMDP Statistical Software, Inc, Los Angeles, CA, USA). A two way analysis of variance was used to determine the significance of any differences observed in the trial.

Data was first grouped according to the individual horse to determine the effect of the individual animal on the results, assuming that all other factors were equal. The data was then regrouped according to diet, assuming there was no difference between horses and that other influences were equal, and then analysed. Application of the least significant difference (P=0.005) for the average of each variable was then employed to provide an indication of the degree of difference between diets.

Analysis of data from the dog study was carried out using the BMDP statistical program. A two way analysis of variance was utilised to determine the significance of differences observed in the trial. The data was initially examined for differences between dogs, between treatments, between collection periods and for any treatment-week interaction. The data was then grouped according to treatment to assess the significance of differences between control and treatment diets.
CHAPTER 4

Equine Studies
The Effect of Diet and Bentonite Feed Additive on Blood Parameters
4.1 Introduction

The levels of soluble dietary carbohydrate required to bring about changes in caecal pH, lactate and endotoxin levels in the horse are well documented (Garner et al, 1975; Garner et al, 1977; Garner et al, 1978; Sprouse et al, 1987). A ration consisting of 85% corn starch and 15% wood cellulose flour in the form of a gruel has been used as a standard laminitis-inducing ration (Garner et al, 1975).

 Whilst the equine research undertaken was concerned with feed-related laminitis, the objective was not to produce laminitis but rather to establish the effects of feeding diets of varying concentrate and roughage levels on the parameters known to change dramatically with this condition. Haematology, blood lactate and blood endotoxin levels were used to monitor these effects, and were also used to assess the effect of the bentonite feed additive Nature Vet Thrive P™ for Horses when included in a high-concentrate low-roughage diet.

The hypothesis was that the inclusion of the bentonite additive to a high-concentrate low-roughage diet would alter its digestion, and so bring about associated changes in the blood parameters measured. If such changes could be established, predictions about the role of the bentonite product in performance horse feeding systems, where individuals may be vulnerable to lactic acidosis, endotoxaemia and laminitis may be possible.

Three studies were undertaken, with the latter two being run in parallel. Experiment 1 involved a pilot study to assess the relative safety and suitability of a high-concentrate low-roughage feed comprised of 30% lucerne chaff and 70% oats. The pilot study therefore set out to ensure that this diet produced no clinical ill-effect (no laminitis or other adverse effects) and to determine whether such a grain-based diet was sufficient to produce changes in haematology,
blood lactate and endotoxin levels that were detectable using the techniques available.

Experiment 2 was an expansion on the first study, and involved a comparison of the effects of diets of differing concentrate-roughage levels (all equivalent in energy level) on the blood parameters measured. The ratio of roughage to concentrate (lucerne chaff:oats) in the diets was 100%:0%, 70%:30%, 50%:50%, and 30%:70%. Experiment 3 involved a pilot study to assess the effects of dietary inclusion of a bentonite feed additive in the 30%:70% diet on the blood parameters described, and was carried out at the same time as Experiment 2.

4.2 Materials and Methods

4.2.1 Horses

The horses used in these experiments were mature geldings (8 to 14 years old) and were in good paddock condition (475 to 525kg body weight). One Standardbred, one Arabian-Draft cross and three Stock horses were used. Vaccination histories were current.

Experiment 1 required two horses (one Standardbred and one Arabian-Draft cross), while experiments 2 and 3 required four horses (one Standardbred, one Arabian-Draft cross and two Stock horses) and one horse (Stock horse) respectively.

4.2.2 Facilities

Experiment 1 was undertaken at the Hawkesbury Agricultural College Horse Facility where the two horses were housed in individual stables (approximately 3.5m²). Each horse had access to its own exercise yard during the day (approximately 10 x 3.5m). Experiments 2 and 3 were undertaken simultaneously at the Hawkesbury
Agricultural College Beef Feedlot, where each horse was housed in individual stalls (approximately 4m²). Each horse had access to its own exercise yard during the day (approximately 10 x 4m).

Each horse had access to separate automatic waterers and feed bins. Manure was collected and removed daily. Feet were examined and cleaned every morning.

### 4.2.3 Diets

Table 4.1 lists the diets used in these experiments. The diets were formulated to provide daily maintenance energy requirements (68.84MJ DE) calculated by using an average body weight of 500kg and using figures provided for oats and lucerne chaff dry matter (DM%), digestible energy (MJ/kg DM), calcium (Ca%), phosphorus (P%) and crude protein (CP%) in the NRC (1978). Subsequent to the rations being designed, the NRC (1989) version was published. However, as the recommendations for the dietary constituents used to formulate the rations were not significantly different, the NRC (1978) values were retained. The ration was then evenly divided into morning and evening feeds, and 15g sodium chloride was added to each meal.

Table 4.2 demonstrates that each diet satisfied the minimum requirements of a 500kg body weight horse for protein, calcium and phosphorus as laid down by the NRC (1978), however diet D was slightly imbalanced in its Ca:P ratio. Given the short time each horse would receive diet D, supplementation of this diet with calcium was not undertaken.

Experiment 1 utilised diet D while experiment 2 utilised diets A, B, C, and D. Experiment 3 also utilised diet D but had 100g of the bentonite feed additive Nature Vet Thrive P™ for Horses included daily to establish the treatment diet.
Table 4.1. Crude protein (CP), calcium (Ca) and phosphorus (P) levels of the four diets (A, B, C & D) used

<table>
<thead>
<tr>
<th>DIET D</th>
<th>%</th>
<th>kg</th>
<th>CP(g)</th>
<th>Ca(g)</th>
<th>P(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lucerne Chaff</td>
<td>30</td>
<td>1.84</td>
<td>262.02</td>
<td>24.56</td>
<td>4.09</td>
</tr>
<tr>
<td>Oats</td>
<td>70</td>
<td>4.29</td>
<td>519.75</td>
<td>2.68</td>
<td>14.14</td>
</tr>
<tr>
<td>Total Weights</td>
<td>100</td>
<td>6.13</td>
<td>781.77</td>
<td>27.24</td>
<td>18.23</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DIET C</th>
<th>%</th>
<th>kg</th>
<th>CP(g)</th>
<th>Ca(g)</th>
<th>P(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lucerne Chaff</td>
<td>50</td>
<td>3.30</td>
<td>469.40</td>
<td>44.01</td>
<td>7.34</td>
</tr>
<tr>
<td>Oats</td>
<td>50</td>
<td>3.30</td>
<td>399.01</td>
<td>2.05</td>
<td>10.86</td>
</tr>
<tr>
<td>Total Weights</td>
<td>100</td>
<td>6.60</td>
<td>868.41</td>
<td>46.06</td>
<td>18.20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DIET B</th>
<th>%</th>
<th>kg</th>
<th>CP(g)</th>
<th>Ca(g)</th>
<th>P(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lucerne Chaff</td>
<td>70</td>
<td>4.99</td>
<td>710.40</td>
<td>66.60</td>
<td>11.10</td>
</tr>
<tr>
<td>Oats</td>
<td>30</td>
<td>2.14</td>
<td>259.76</td>
<td>1.34</td>
<td>7.07</td>
</tr>
<tr>
<td>Total Weights</td>
<td>100</td>
<td>7.13</td>
<td>970.16</td>
<td>67.94</td>
<td>18.17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DIET A</th>
<th>%</th>
<th>kg</th>
<th>CP(g)</th>
<th>Ca(g)</th>
<th>P(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lucerne Chaff</td>
<td>100</td>
<td>8.11</td>
<td>1,155.00</td>
<td>108.24</td>
<td>18.04</td>
</tr>
<tr>
<td>Oats</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Total Weights</td>
<td>100</td>
<td>8.11</td>
<td>1,155.00</td>
<td>108.24</td>
<td>18.04</td>
</tr>
</tbody>
</table>

Table 4.2. Crude protein (CP), calcium (Ca) and phosphorus (P) levels provided by the four respective diets (A, B, C & D) as compared to NRC (1978)

<table>
<thead>
<tr>
<th>Diet</th>
<th>CP(g)</th>
<th>Ca(g)</th>
<th>P(g)</th>
<th>CaP</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>781.77</td>
<td>27.24</td>
<td>18.23</td>
<td>1.49</td>
</tr>
<tr>
<td>C</td>
<td>868.41</td>
<td>46.06</td>
<td>18.20</td>
<td>2.53</td>
</tr>
<tr>
<td>B</td>
<td>970.16</td>
<td>67.94</td>
<td>18.17</td>
<td>3.74</td>
</tr>
<tr>
<td>A</td>
<td>1,155.00</td>
<td>108.24</td>
<td>18.04</td>
<td>6.00</td>
</tr>
<tr>
<td>NRC 500kg BW</td>
<td>630.00</td>
<td>23.00</td>
<td>14.00</td>
<td>1.64</td>
</tr>
</tbody>
</table>

4.2.4 Analytical Methods

Complete blood counts and measurements of blood lactate and endotoxin levels were carried out using the techniques described in the materials and methods, Chapter 3.

Analysis of results from Experiments 1 and 3 were not undertaken because of the limited number of observations. Analysis of results from Experiment 2 was undertaken as described in Chapter 3.
4.3 Procedures

Experiment 1: Two horses (horse 1 and 2) were brought into the stable facilities at the horse unit, examined, administered ivermectin (IVOMEC® Injection, Merck & Co, Inc, Whitehouse Station, NJ, USA) at 200μg/kg body weight by intramuscular injection to control parasites and given a one week acclimatisation period. During this time, both horses were gradually introduced to the concentrate ration by increasing the grain component while at the same time reducing the lucerne component. The horses were then given one week on this high-grain diet (diet D) before sampling commenced. The feeding regime described can be found in Table 4.3.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Lucerne Chaff</th>
<th>Oats</th>
<th>Total Feed</th>
<th>AM†</th>
<th>PM†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>kg</td>
<td></td>
<td>kg</td>
</tr>
<tr>
<td>A</td>
<td>100</td>
<td>0</td>
<td>0.00</td>
<td>8.11</td>
<td>4.05</td>
</tr>
<tr>
<td>B</td>
<td>70</td>
<td>30</td>
<td>2.14</td>
<td>7.13</td>
<td>3.56</td>
</tr>
<tr>
<td>C</td>
<td>50</td>
<td>50</td>
<td>3.30</td>
<td>6.60</td>
<td>3.30</td>
</tr>
<tr>
<td>D</td>
<td>30</td>
<td>70</td>
<td>4.29</td>
<td>6.13</td>
<td>3.06</td>
</tr>
</tbody>
</table>

† 15g sodium chloride added

<table>
<thead>
<tr>
<th>Diet</th>
<th>Lucerne Chaff</th>
<th>Oats</th>
<th>Total Feed</th>
<th>AM†</th>
<th>PM†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>kg</td>
<td></td>
<td>kg</td>
</tr>
<tr>
<td>A</td>
<td>100</td>
<td>0</td>
<td>0.00</td>
<td>8.11</td>
<td>4.05</td>
</tr>
<tr>
<td>B</td>
<td>70</td>
<td>30</td>
<td>2.14</td>
<td>7.13</td>
<td>3.56</td>
</tr>
<tr>
<td>C</td>
<td>50</td>
<td>50</td>
<td>3.30</td>
<td>6.60</td>
<td>3.30</td>
</tr>
<tr>
<td>D</td>
<td>30</td>
<td>70</td>
<td>4.29</td>
<td>6.13</td>
<td>3.06</td>
</tr>
</tbody>
</table>

Table 4.3.
(a) Diets used during the adaptation and collection period

(b) The procedure for diet changeover during the adaptation period

<table>
<thead>
<tr>
<th>Sunday</th>
<th>Monday</th>
<th>Tuesday</th>
<th>Wednesday</th>
<th>Thursday</th>
<th>Friday</th>
<th>Saturday</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>C</td>
<td>C</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>D</td>
<td>D</td>
<td>D</td>
<td>D†</td>
<td>End</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† - Collection day

Blood samples were collected every 2 hours for a 10 hour period on the collection day. The pre-morning feed blood collection was taken at 0 hours and continued throughout the day until the final 10 hour collection, following which the second half of the daily diet was provided.
Normal stable and horse husbandry practices were carried out and horses examined for any adverse effects related to the diet or to the frequent jugular venipuncture. The horses were then turned out to pasture the next day following a final physical examination.

Experiment 2: Four horses were introduced to the Beef Feedlot, and were examined, weighed and treated for parasites with ivermectin. A two week acclimatisation period was provided to allow adjustments to the facilities, frequent handling and the change in diet from pasture to lucerne chaff and oats.

The four horses were then randomly assigned to eventually receive one of the four diets outlined earlier. The level of oats was progressively increased over one week to achieve a 50% lucerne chaff:50% oats diet. On day 8, the respective experimental diets were introduced to each horse and a one week period was given before sampling commenced. The sequence of diets and collection periods are indicated in Table 4.4.

<table>
<thead>
<tr>
<th>Week</th>
<th>Sun</th>
<th>Mon</th>
<th>Tues</th>
<th>Wed</th>
<th>Thurs</th>
<th>Fri</th>
<th>Sat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>B</td>
<td>B</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>2</td>
<td>C</td>
<td>V</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>3</td>
<td>X</td>
<td>X</td>
<td>XO</td>
<td>X</td>
<td>XO</td>
<td>V</td>
<td>X</td>
</tr>
<tr>
<td>4</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>5</td>
<td>X</td>
<td>X</td>
<td>XO</td>
<td>X</td>
<td>XO</td>
<td>V</td>
<td>X</td>
</tr>
<tr>
<td>6</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>7</td>
<td>X</td>
<td>X</td>
<td>XO</td>
<td>X</td>
<td>XO</td>
<td>V</td>
<td>X</td>
</tr>
<tr>
<td>8</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>9</td>
<td>X</td>
<td>X</td>
<td>XO</td>
<td>X</td>
<td>XO</td>
<td>Z</td>
<td>A</td>
</tr>
<tr>
<td>10</td>
<td>A</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A - Diet A  
B - Diet B  
C - Diet C  
O - Blood collection days  
V - Change to required experimental diet  
X - Required experimental diet continues  
Z - Change diet back to Diet A

Blood samples were collected every 2 hours for a 12 hour period on two days (spaced one day apart). Collections commenced at 6 AM (0
hours) immediately prior to the morning feed, and then continued through until 6 PM, when the evening ration was provided.

Following the second collection, diets were rotated such that each horse received the diet that contained the next highest percentage of oats so as to avoid sudden diet changes and carbohydrate overload. The horse that received diet D reverted back to the diet A. A ten day adjustment period was allowed for the horses to become acclimatised to the new diet.

Experiment 3: The horse required for this study was introduced to the holding area at the same time as the four horses used in Experiment 2, and was examined, weighed, treated for parasites with ivermectin and placed in an individual stall. A two week acclimatisation period was provided to allow adjustments to the facilities, frequent handling and the change in diet from pasture to one of lucerne chaff and oats. The experimental diet was introduced over a one week period, and a further one week was given before sampling commenced.

Blood samples were collected every 2 hours for a 12 hour period on two days, as described in Experiment 2. Following the second collection, the control diet would be changed over to the experimental diet through the introduction of Nature Vet Thrive P™, 50g morning and 50g evening. A ten day adjustment period was then given to allow adaptation to the added supplement.

Normal stable and horse husbandry practices were then carried out and the horses in experiments 2 and 3 examined for any adverse effects relating to the diets, the 2 hourly jugular venipunctures, and the confinement for the 11 weeks of the experiments. The horses were then reweighed and returned to the usual holding facility at the conclusion of the trial.
4.4 Experiment 1

4.4.1 Results

Both horses tolerated the 30% lucerne chaff: 70% oats ration and the two hourly blood collection. Whilst the horses displayed behavioural alterations, including soil and manure ingestion and chewing stable doors, no evidence of colic, lower leg oedema, skin disorders or laminitis were observed.

Haematological changes were variable. The findings are presented as graphs in Figures 4.1 to 4.11. Full results are tabled and in Appendix 3. Despite the similarity in horse type, and that each horse received identical diets, the changes in the parameters throughout the day were different for each horse.

Figure 4.1. Changes in packed cell volume (PCV %) over time
Figure 4.2. Changes in total plasma protein (TPP g/dL) over time

Figure 4.3. Changes in red blood cells (RBC x10⁶/μL) over time
Figure 4.4. Changes in white blood cells (WBC x10^3/μL) over time

Figure 4.5. Changes in neutrophils (neutrophils x10^3/μL) over time
Figure 4.6. Changes in lymphocytes (lymphocytes x10^3/μL) over time

Figure 4.7. Changes in monocytes (monocytes x10^3/μL) over time
Figure 4.8. Changes in basophils (basophils $\times 10^3/\mu$L) over time

Figure 4.9. Changes in eosinophils (eosinophils $\times 10^3/\mu$L) over time
Figure 4.10. Changes in platelets (platelets x10^4/μL) over time

Figure 4.11. Changes in blood L-lactate (L-lactate mg/dL) over time
During a 10 hour period following feeding, the PCV of horse 2 increased from 35 to 37%, while horse 1 decreased from 40 to 37% and returned to the pre-feeding or 0 hour value of 40% at 10 hours (Figure 4.1). TPP in both horses fluctuated around the pre-feeding value of 75g/L in horse 1 and 77g/L in horse 2 (Figure 4.2).

RBC values in horse 2 rose from 6.80 x10⁶/µl at 0 hours to 7.5 x10⁶/µL at 10 hours, while they fluctuated in horse 1 around the 0 hour value of 8.04 x10⁶/µL (Figure 4.3). WBC values in both horses followed a similar trend, falling initially from 10.17 and 9.55 x10⁹/µL to 9.63 and 8.65 x10⁹/µL at 2 hours for horse 1 and 2 respectively, and then increasing to a maximum of 11.33 (8 hours) and 10.09 x10⁹/µL (6 hours) respectively. At 10 hours, WBC values had returned toward pre-feeding values (Figure 4.4).

Figures 4.5 to 4.9 show the changes that occurred in the differential WBC count of both horses. Neutrophil numbers dropped from 6.10 and 5.54 x10⁹/µL to 5.79 and 5.38 x10⁹/µL at 4 hours (horse 1 and 2 respectively), and then rose to a peak of 7.58 (6 hours) and 6.14 x10⁹/µL (8 hours) respectively before returning to near pre-feeding levels at 10 hours.

Changes in lymphocyte numbers were variable, with values fluctuating around the 0 hour values of 3.25 and 3.06 x10⁹/µL for horse 1 and 2 respectively. Changes in monocyte, basophil and eosinophil values were also variable, while platelet numbers (Figure 4.10) fluctuated around the 0 hour values of 7.25 and 12.00 x10⁶/µL for horse 1 and 2 respectively.

Changes to blood lactate levels over the 10 hour collection period were also variable (Figure 4.11). Horse 1 showed a rise in plasma lactate from a pre-feeding value of 3.00 to 6.00mg/dL at 4 hours, and then fluctuated between 3.00 and 6.00mg/dL until the completion of
sampling. Horse 2 had a higher resting lactate level of 6.00mg/dL which declined after 6 hours to reach a 10 hour low of 1.50mg/dL.

There were no results that could be reported for the endotoxin assay. A misunderstanding of an instruction from the distributor of the COATEST® Endotoxin kit meant that plasma samples were inappropriately heat-treated before making a 1:10 dilution. As a result of coagulation, the test was unable to be performed.

4.4.2 Discussion

Due to the limited numbers of samples in this pilot study, it was difficult to do anything more than pass comment on how some of the observed changes fit with present knowledge. Further studies and multiple samples would be necessary to remove the effects of individual horse variation on the results.

The failure to generate any worthwhile results from the endotoxin component of this study proved both frustrating and costly in time and money. However, the facilities available for processing these samples were appraised and appeared appropriate.

PCV and TPP both increase markedly when hay is fed to the horse, however, the changes associated with concentrate feeding are almost undetectable (Kerr and Snow, 1982; Clarke, Roberts and Argenzio, 1990). The results of the pilot study showed great variation with one horse displaying increased values while the other horse showed decreased values. It is possible that a diurnal variation overlies these results (Cardinet, Littrell and Schalm, 1964).

There is little specific published material documenting the changes to leukocyte numbers as they relate to feeding, however the study by Cardinet et al (1964) does provide some background knowledge. The
decrease in total WBC count at 2 hours may be due to margination of leukocytes in the peripheral circulation and subsequent removal from the circulating pool. It may also be due to a concentrating of WBC's in the splanchnic circulation in physiologic preparation for processing of ingested pathogens or as a defence against the local microflora that will increase in numbers as food substrate is provided.

The increase and subsequent peak of WBC's at around 6-8 hours after feeding may indicate mobilisation of leukocytes from the gastrointestinal site back into the peripheral circulation, or the demargination of WBC's. Such changes may also be diurnal, but it contradicts the diurnal changes documented by Cardinet et al (1964) in which total leukocyte numbers increased at 2 hours, decreased at 4 hours and then oscillated back towards pre-feeding values over 10 hours.

A leukopaenia at 2 to 4 hours may also be explained by the presence of endotoxins in the blood. Leukopaenia is a very sensitive diagnostic signal of endotoxaemia and may be present even when other clinical signs are absent (Semrad and Moore, 1987). Leukopaenia is mainly due to neutropaenia, which is often an important early sign of gram-negative bacterial sepsis. Such changes in neutrophil numbers were seen in this trial and it is possible that the changes in the WBC profile may have been due to exposure to endotoxin as a result of eating, food digestion, and perhaps the type of diet.

The other change frequently seen following exposure to endotoxin is a secondary leukocytosis, characterised by increased release of immature leukocytes from the bone marrow (Semrad and Moore, 1987). This may explain the neutrophil dominated rise in leukocytes seen at 4 to 6 hours post feeding. However, this area requires further research to establish diurnal patterns of WBC's and their associations with feeding, along with positive identification of endotoxin.
Endotoxin can also lead to a lowering of platelet numbers in the peripheral blood (Semrad and Moore, 1987). This may be due to aggregation precipitated by endotoxin, aggregation and adhesion to damaged endothelium, sequestration of platelet aggregates in the lungs and stimulation of the extrinsic complement system. Platelet numbers as estimated from blood smears were quite variable and as such, did not allow comment. However, their quantification was considered worthwhile in further work.

It is possible that the adaptation of the horses to the high-grain diet may have reduced the impact of the diet itself. The production of endotoxaemia in the horse related to diet has been observed when horses are challenged with a high soluble carbohydrate load, however, such a challenge was not used in this situation. It may be necessary in further work to consider such a challenge, however, the endotoxin assay would need to be standardised.

The variable changes in blood L-lactate make comment difficult. Horse 1 demonstrated an increase in lactate at 4 hours, followed by a decrease and then a second rise. The first rise may indicate lactic acid production in the stomach and absorption in the small intestine, while the second rise may relate to large bowel fermentation and lactate production. Similar results have been produced in earlier studies in horses fed concentrate meals (Argenzio et al, 1974b). However, horse 2 had contradictory changes in L-lactate levels, with higher initial readings falling away to lower readings at 4-6 hours.

Such changes in plasma lactate may also reflect lactate production associated with ingestion and digestion of food and have little to with the diet and microbial lactate production. These observed changes require further investigation.
4.4.3 Conclusions

The results of Experiment 1 supported expansion into a larger trial to test the same parameters. It appeared that sampling every 2 hours was suitable for the production of meaningful results and had no adverse effect on the animals’ health. Likewise, the proportion of grain fed had no detrimental effect on the horses.

The failure of the endotoxin assay technique meant that more development work was necessary. However, the changes in WBC’s suggested the presence of endotoxins, and supported further work with the assay. More accurate quantification of the platelet levels in blood was also considered worthwhile as a further tool in supporting the presence of endotoxin.

4.5 Experiment 2

4.5.1 Results

The trial was completed with no major diet-related health problems, however loose motions, lower leg oedema and increased numbers of whole grains passed in the manure were seen in the horses receiving diet D. Such findings are not unusual with this level of grain feeding, as has been discussed earlier.

Two unexpected health problems did arise during the experiment. Firstly, horse 2 suffered a sterile reaction to the intramuscular use of ivermectin for parasite control administered at the beginning of the trial. Drainage and normal wound treatment resolved this reaction before the trial was commenced.

Secondly, the unusually wet and warm summer provided ideal conditions for mosquito and midge activity which caused
pronounced cutaneous reactions in horse 2. This horse was monitored closely and treated conservatively with mild skin cleansers. All horses were then sprayed with repellents (citronella) to prevent further insect distress.

The results of the trial are presented in two forms. Graphical representation of average values for each parameter according to diet are depicted in Figures 4.12 to 4.23. Full results are presented in table form in Appendix 3.

Statistical analysis allowed two conclusions to be drawn. When the results were grouped according to horse and analysed (Table 4.5), significant differences existed (p<0.01) between each horse for the average of every variable. Application of the least significant difference (p=0.005) for the average of each variable indicated the degree of difference between horses.

When the results were grouped according to diet (Table 4.6), and when the influence of horse, collection period, time of collection and week of collection were considered equal, significant differences existed between average RBC levels for the different diets (p<0.05) and L-lactate levels (p<0.01). Application of the least significant difference (p=0.005) for the average of each variable indicated the degree of difference between diets.

Whilst it is apparent that significant differences existed between the way certain horses behaved on certain diets, the design of the trial does not permit further comment. Graphical presentation of results allows insight into changes according to time for each diet.
<table>
<thead>
<tr>
<th>Horse</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>39.054(a)*</td>
<td>39.429(a)</td>
<td>37.232(b)</td>
<td>32.411(c)</td>
<td>0.0000</td>
</tr>
<tr>
<td>TPP (mg/dL)</td>
<td>68.518(d)</td>
<td>80.696(a)</td>
<td>73.657(b)</td>
<td>72.214(c)</td>
<td>0.0000</td>
</tr>
<tr>
<td>RBC (x10^6/μL)</td>
<td>7.150(a)</td>
<td>7.181(a)</td>
<td>7.126(a)</td>
<td>6.862(b)</td>
<td>0.0034</td>
</tr>
<tr>
<td>WBC (x10^3/μL)</td>
<td>6.203(d)</td>
<td>9.642(b)</td>
<td>10.209(a)</td>
<td>6.771(c)</td>
<td>0.0000</td>
</tr>
<tr>
<td>Neutrophils (x10^3/μL)</td>
<td>3.617(c)</td>
<td>5.879(a)</td>
<td>6.205(a)</td>
<td>4.165(b)</td>
<td>0.0000</td>
</tr>
<tr>
<td>Band Neutrophils (x10^3/μL)</td>
<td>0.008(ab)</td>
<td>0.009(ab)</td>
<td>0.027(a)</td>
<td>0.002(b)</td>
<td>0.0165</td>
</tr>
<tr>
<td>Lymphocytes (x10^3/μL)</td>
<td>2.422(b)</td>
<td>3.318(a)</td>
<td>3.431(a)</td>
<td>2.332(b)</td>
<td>0.0000</td>
</tr>
<tr>
<td>Monocytes (x10^3/μL)</td>
<td>0.053(b)</td>
<td>0.078(ab)</td>
<td>0.111(a)</td>
<td>0.046(b)</td>
<td>0.0007</td>
</tr>
<tr>
<td>Eosinophils (x10^3/μL)</td>
<td>0.095(c)</td>
<td>0.365(a)</td>
<td>0.414(a)</td>
<td>0.237(b)</td>
<td>0.0000</td>
</tr>
<tr>
<td>Basophils (x10^3/μL)</td>
<td>0.005(b)</td>
<td>0.044(a)</td>
<td>0.026(ab)</td>
<td>0.018(b)</td>
<td>0.0010</td>
</tr>
<tr>
<td>Platelets (x10^4/μL)</td>
<td>1.049(b)</td>
<td>1.755(a)</td>
<td>0.727(d)</td>
<td>0.913(c)</td>
<td>0.0000</td>
</tr>
<tr>
<td>L-lactate (mg/dL)</td>
<td>6.919(b)</td>
<td>8.250(a)</td>
<td>7.152(ab)</td>
<td>8.036(ab)</td>
<td>0.0029</td>
</tr>
</tbody>
</table>

* Average values with common identifying letters (a,b,c,d) are not significantly different (p<0.005)
Table 4.6. Average values for measured variables grouped by diet

<table>
<thead>
<tr>
<th>Diet</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>36.839(ab)</td>
<td>37.357(a)*</td>
<td>36.357(b)</td>
<td>37.571(a)</td>
<td>0.2818</td>
</tr>
<tr>
<td>TPP (mg/dL)</td>
<td>73.750(a)</td>
<td>73.679(a)</td>
<td>73.750(a)</td>
<td>73.607(a)</td>
<td>0.9988</td>
</tr>
<tr>
<td>RBC (x10^6/μL)</td>
<td>7.071(ab)</td>
<td>7.096(ab)</td>
<td>6.929(b)</td>
<td>7.225(a)</td>
<td>0.0261</td>
</tr>
<tr>
<td>WBC (x10^3/μL)</td>
<td>8.583(a)</td>
<td>8.186(ab)</td>
<td>8.028(b)</td>
<td>8.098(b)</td>
<td>0.4869</td>
</tr>
<tr>
<td>Neutrophils (x10^3/μL)</td>
<td>5.275(a)</td>
<td>4.941(ab)</td>
<td>4.944(ab)</td>
<td>4.728(b)</td>
<td>0.2748</td>
</tr>
<tr>
<td>Band Neutrophils (x10^3/μL)</td>
<td>0.012(a)</td>
<td>0.009(a)</td>
<td>0.005(a)</td>
<td>0.020(a)</td>
<td>0.3203</td>
</tr>
<tr>
<td>Lymphocytes (x10^3/μL)</td>
<td>2.945(a)</td>
<td>2.889(a)</td>
<td>2.754(a)</td>
<td>2.927(a)</td>
<td>0.5124</td>
</tr>
<tr>
<td>Monocytes (x10^3/μL)</td>
<td>0.077(a)</td>
<td>0.067(a)</td>
<td>0.083(a)</td>
<td>0.062(a)</td>
<td>0.6511</td>
</tr>
<tr>
<td>Eosinophils (x10^3/μL)</td>
<td>0.293(a)</td>
<td>0.258(a)</td>
<td>0.263(a)</td>
<td>0.300(a)</td>
<td>0.6783</td>
</tr>
<tr>
<td>Basophils (x10^3/μL)</td>
<td>0.033(a)</td>
<td>0.020(a)</td>
<td>0.009(a)</td>
<td>0.031(a)</td>
<td>0.0556</td>
</tr>
<tr>
<td>Platelets (x10^4/μL)</td>
<td>1.119(ab)</td>
<td>1.173(a)</td>
<td>1.076(b)</td>
<td>1.083(b)</td>
<td>0.6261</td>
</tr>
<tr>
<td>L-lactate (mg/dL)</td>
<td>9.432(a)</td>
<td>7.500(b)</td>
<td>6.562(b)</td>
<td>6.884(b)</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

* Average values with common identifying letters (a,b,c,d) are not significantly different (p<0.005)
Figure 4.12. Changes in average packed cell volume (PCV %) over time

Figure 4.13. Changes in average total plasma protein (TPP g/dL) over time
Figure 4.14. Changes in average red blood cells (RBC x10^6/μL) over time

Figure 4.15. Changes in average white blood cells (WBC x10^3/μL) over time
Figure 4.16. Changes in average neutrophils (neutrophils $\times 10^3/\mu L$) over time

Figure 4.17. Changes in average band neutrophils (band neutrophils $\times 10^3/\mu L$) over time
Figure 4.18. Changes in average lymphocytes (lymphocytes $\times 10^3/\mu$L) over time

Figure 4.19. Changes in average monocytes (monocytes $\times 10^3/\mu$L) over time
Figure 4.20. Changes in average basophils (basophils x10³/µL) over time

Figure 4.21. Changes in average eosinophils (eosinophils x10³/µL) over time
Figure 4.22. Changes in average platelets (platelets $\times 10^4/\mu$L) over time

Figure 4.23. Changes in average blood L-lactate (L-lactate mg/dL) over time
PCV was seen to rise at 2 hours and then fall at 4 hours to values below the pre-feeding value (Figure 4.12). The degree of change was greatest for diet A and least for diet D, and only diet D showed any evidence of return towards pre-feeding values by 12 hours after feeding.

The changes in TPP were similar (Figure 4.13). The values at 2 hours post-feeding were all elevated, with the greatest change being seen with diet A. Values were then shown to return to near pre-feeding levels at 4 hours and showed minor fluctuations thereafter for diets B and C.

Total RBC changes closely mimicked the changes seen in the PCV, with an increase at 2 hours in all diets, the greatest being for diet A and least for diet D (Figure 4.14). All values declined after 2 hours, however diet A showed the greatest downward movement, followed by diet B. Only diets' B and D produced values approximating pre-feeding values at 12 hours.

WBC changes were less pronounced (Figure 4.15). All diets, with the exception of diet B, were associated with a fall in total WBC count at 2 hours. The WBC numbers in those horses receiving diet D showed a further decline in WBC numbers at 4 hours, while those receiving diet B also showed a drop. The general trend was then towards increased total WBC counts towards pre-feeding values at 12 hours. Only diets A and B failed to show any return towards 0 hour values.

The differential WBC counts demonstrated that the neutrophil was the principle cell responsible for the post-prandial changes in total WBC (Figures 4.16 to 4.21). Neutrophil numbers decreased at 2 hours post-feeding, with the exception of diet B, and returned to pre-feeding values at 12 hours (Figure 4.16). Band neutrophil numbers were
relatively unchanged, with the exception of diet A which peaked higher than other diets at 4 hours (Figure 4.17).

The lymphocyte responses (Figure 4.18) were variable and generally showed increased numbers at 2 hours post-feeding with diets A and D, despite an overall fall in WBC’s and neutrophils. Monocyte changes were also variable (Figure 4.19), but in the main showed reductions at 2 hours post-feeding for all diets except diet C. The latter showed large increases at 2-4 hours, before approximating pre-feeding values at 12 hours.

Basophil numbers varied widely, which may have accounted for the probability (p=0.0556) from statistical analysis of the average basophil value for each diet suggesting a dietary influence on this cellular component (Figure 4.20). With the exception of diet B, eosinophil values for all diets decreased and then increased back to pre-feeding values by 12 hours (Figure 4.21). Diet B showed an increase in eosinophil value at 2 hours, then a small decline after to peak at 10 hours and then return to 0 hour value at 12 hours.

Platelet values showed a general increase at 2 hours and then a decrease at 4 hours for all diets (Figure 4.22). Diets A and D were then associated with an overall decline in platelet numbers to a value approximating the pre-feeding value. Diets B and C showed marked oscillations after 4 hours and finished at levels above the pre-feeding values.

Plasma L-lactate values increased at 2 to 4 hours post-feeding, and then showed a gradual decline towards pre-feeding values at 12 hours in all diets (Figure 4.23). The greatest increases in lactate levels were seen in diet A, followed by diets B and C. Values for these diets did not peak until 4 hours, as compared diet D in which plasma levels
peaked at 2 hours. The single greatest rise in plasma L-lactate was evident in diets C and D.

Plasma L-lactate therefore rose more quickly with those diets having a higher oats contents, and then declined more quickly in comparison with diets with a higher roughage content. The changes seen in L-lactate levels with diets A and B were slowest to occur but showed the greatest increase at 4 hours and beyond.

Results of the endotoxin assay were not forthcoming, and difficulties were encountered when attempting to develop standard curves to allow measurements of samples and controls. Figures 4.24 to 4.32 are included as examples of the results obtained after four attempts to develop standard curves to be used for the determination of plasma endotoxin levels. Tabulated results are presented in Appendix 3.

![Graph](image)

**Figure 4.24.** Plasma standard graph from collection 1 (R² = 1.000; Absorbance at 405nm = 0.263 + 0.606 x Endotoxin Concentration)
Figure 4.25. Plasma standard graph from collection 2 \( (R^2=0.987; \text{Absorbance at } 405\text{nm} = 0.293 + 1.889 \times \text{Endotoxin Concentration}) \)

Figure 4.26. Water standard graph from collection 2 \( (R^2=0.965; \text{Absorbance at } 405\text{nm} = 0.113 + 0.592 \times \text{Endotoxin Concentration}) \)
Figure 4.27. Plasma standard graph from collection 3 ($R^2$=0.815; Absorbance at 405 nm = 0.155 + 0.758 x Endotoxin Concentration)

Figure 4.28. Water standard graph from collection 3 ($R^2$=0.161; Absorbance at 405 nm = 0.140 + 0.038 x Endotoxin Concentration)
Figure 4.29. Plasma standard graph from collection 7 (R^2=0.501; Absorbance at 405nm = 0.188 + 0.656 x Endotoxin Concentration)

Figure 4.30. Water standard graph from collection 7 (R^2=0.972; Absorbance at 405nm = 0.072 + 0.083 x Endotoxin Concentration)
Figure 4.31. Final plasma standard graph ($R^2=0.952$; Absorbance at 405nm = 0.650 + 0.608 x Endotoxin Concentration)

Figure 4.32. Final water standard graph ($R^2=0.955$; Absorbance at 405nm = 0.305 + 0.651 x Endotoxin Concentration)
Two problems were evident with these standard curves. Firstly, the slope of the standard curves (Figures 4.24 to 4.30 inclusive) were variable and relatively flat and contrast to the standards attained in Figures 4.31 and 4.32, which were obtained after many adjustments to incubation times. A flat graph would not provide the accuracy needed for endotoxin determination.

Secondly, large variations existed between replicates, a fact highlighted by the control samples studied. Such variation was also apparent in the trial samples where from one collection, 35ng endotoxin/mL may have been detected, yet in the replicate, the value was 110ng/mL (1ng = 12EU). Because of such variations and the inaccuracy of the standard graphs, these results have not been included here. Rather, the standard graphs only have been documented and comments about the problems encountered and the measures taken to resolve them will be included in the discussion section.

4.5.2 Discussion

This study confirms earlier studies that found when horses commence eating, there was a loss of water from the plasma sufficient to cause alterations in PCV and TPP (Kerr and Snow, 1982; Clarke et al, 1990; and Argenzio et al, 1974a). The finding that TPP and PCV both increased by approximately the same percentage at 2 hours after ingestion of a meal indicates the effect is due to haemoconcentration, as opposed to mobilisation of splenic erythrocyte stores.

The changes in RBC levels correspond well with the changes to PCV, and support the theory that the changes were due to water loss rather than splenic contraction. The volumes of parotid saliva and pancreatic fluid secreted in association with food ingestion would
explain largely where and why the greatest fluid shifts out of the blood occur with diet A at 2 hours, and that as roughage is substituted by oats, so the fluid shift is reduced. Diets requiring greater mastication, such as diet A correspondingly result in more chewing and mixing with saliva than a diet containing more grain, such as diet D.

Clarke et al (1990) found that plasma volume was normalised at 2 hours after eating. Such differences probably relate to the pelleted hay/grain ration used in their experiment. Such a diet would allow faster food consumption, and therefore faster fluid fluxes compared to the less concentrated oats and lucerne chaff mix which would conceivably take longer to ingest.

The fall in TPP below baseline levels in all diets may be a reflection of absorption of ingested water, in addition to the absorption of water with the movement of osmotically active nutrients, such as lactate, VFA's and glucose into the bloodstream (Argenzio et al, 1974b). The greatest absorption of water in the roughage fed animal occurred between 2 and 8 hours postfeeding. Such a pattern appeared evident in the graphs from this trial.

The increase in TPP at 8 to 10 hours postfeeding may be due to an outflow of ileal and colonic secretions in support of microbial fermentation (Argenzio et al, 1974b; and Clarke et al, 1990). In this study TPP levels increased at about this 8 hour mark for diets A, B and C. This may coincide with the commencement of bacterial fermentation in the caecum of the horse.

Diet D however, did not show such an increase in TPP until 10-12 hours, and as well, did not show a greater fall in TPP at 4-6 hours. It is possible that the higher amount of soluble carbohydrate present in the grain is more accessible to breakdown and earlier absorption in
the anterior gut (within 4-6 hours) and that unabsorbed material passes through to the hindgut and causes an earlier initiation of microbial digestion (hence a flatter line between 6 to 10 hours). The sudden increase in TPP at 10-12 hours may be due to increased fermentative activity with diet D, above and beyond the increases seen with the other diets, resulting in an outpouring of ileal contents to buffer caecal pH changes (Alexander, 1972; and Argenzio et al, 1977).

The general fall in plasma WBC numbers 2 hours after feeding may be due to increased blood flow to the gut and a corresponding increase of WBC movement into the portal system. It may also be due to margination and removal of WBC's from the circulating pool. However, diet B produced a rise in WBC at this 2 hour point, similar to the change documented by Cardinet et al (1964). Why such a contradiction exists is difficult to explain and would require further studies to clarify.

The respective fall in WBC level at 2 hours was reduced in diets A, C and D. Coincidently, the fluid shift out of the blood was reduced as grain level increased. It may be that the responses are connected - the physiologic effect of eating an all roughage diet results in a more rapid disappearance of leukocytes from the circulating leukocyte pool than a more concentrated meal.

Whilst diet D was associated with its lowest WBC count at 4 hours, diet A did not achieve minimum levels until 10 hours. From 4 hours to 10 hours, all diets except diet A had WBC values that increased. While WBC values for diets B and C approached pre-feeding values at 12 hours, the WBC count associated with diet A was suppressed, while that associated with diet B was elevated.
It is difficult to comment much on the relative degree of change of total leucocyte count for each diet, as the variations are reasonably small. There may be no significant dietary related change, despite the results of the statistical analysis.

When the WBC values change, it is primarily as a result of changes to the levels of the most common leucocyte, the neutrophil. This is particularly obvious at 2 hours, but is apparent throughout the 12 hour period. A drop in leucocyte numbers, particularly in neutrophils, may also be due to increased levels of blood endotoxin seen shortly after feeding. If this were to be the case, and the earlier hypothesis correct, one would expect a greater drop with diet D. Instead, diet A shows the greatest response, so it appears unlikely that endotoxin is primarily involved with this decline in neutrophils.

Changes to lymphocyte counts are variable and are of relatively small magnitude. Monocyte levels fluctuate about baseline values, while in nearly all diets, eosinophil changes were small and trended down to 6 hours before returning to pre-feeding values at 12 hours. Changes in basophil numbers were larger and suggested a possible relationship with diet. However, due to the normal scarcity of this cell type in blood, more observations would be necessary to determine the relative importance of the changes seen in this trial.

Platelets generally increased at 2 hours and then decreased. Such a change may be related to fluid shifts out of the blood creating a relative hypovolaemia. However, platelet numbers are generally lowered with the presence of endotoxins. While platelet counts were seen to decrease with diets C and D, they also declined for the diet A, which suggests that diet may have little effect on platelet count besides a haemoconcentrating effect.
The gastrointestinal tract of the horse has been shown to contain bacteria capable of fermentative activity in the stomach and the small intestine, in addition to the bacterial population that exists in the caecum and large intestine (Mackie and Wilkins, 1988; Argenzio et al., 1974b; and Alexander and Davies, 1963). Anaerobic digestion of a diet low in rapidly fermentable sugars was found to produce acetate in the small intestine. However, major lactate producing bacteria such as Lactobacillus and Streptococcus spp, together with Clostridium spp were also shown to be abundant in the duodenum and increased in numbers towards the caecum and colon (Clarke et al., 1990).

The predominance of anaerobic gram-negative bacilli throughout the small intestine (increasing aborally) suggests that significant fermentative activity may develop in the stomach and the small intestine following the introduction of concentrate feed. The production of lactic acid and volatile fatty acids in the stomach and small intestine have been well documented (Argenzio et al., 1974b; and Alexander and Davies, 1963).

Argenzio et al. (1974b) demonstrated that lactic acid is produced in quite large amounts very quickly (2 to 4 hours), following the feeding of concentrate diets to ponies. The feeding of a roughage diet however did not produce as significant increase in levels in the stomach/small intestine and the increases were seen to occur more in the small intestine.

The changes in blood levels of L-lactate in this trial appear to correlate well with other reports. Diets C and D produced the most rapid and single largest rise in the blood lactate levels at 2 hours, while values for diets A and B were lower. It was also apparent that as the roughage component was increased, so did the total rise of blood lactate in the 12 hour period become greater. Associated with this prolonged rise as roughage component increased was a prolonged fall.
The rapid rise and fall of blood lactate associated with diet D is probably due to the greater availability of soluble carbohydrate for fermentative bacteria in the anterior gut. This carbohydrate could be fermented to lactate and then readily absorbed by the lower small intestinal surfaces. Such a situation has been likened to a subclinical carbohydrate overload (Clarke et al., 1990).

In this study, blood lactate remained elevated after feeding over the 12 hour sampling period, and approached pre-feeding values at 12 hours. The elevation of lactate at 4, 6 and 8 hours for diet D may be associated with large intestinal fermentation of soluble carbohydrate that has escaped the small intestine. The small particle size of a concentrate diet allows less gastric retention and faster progression through the small intestine (Argenzio et al., 1974a) and as well, a high caloric meal appears to increase propulsive motility in the small intestine (Clarke et al., 1990). To support this, caecal lactate levels have been demonstrated to be 25 times greater on a concentrate diet, even following the adjustment of bacteria to the diet (Willard et al., 1977).

In considering the results, it must be kept in mind that part of the lactate levels seen in the blood may be of animal origin. Eating a roughage diet is associated with more chewing and therefore more work, which may result in increased metabolic waste production. However, it is more likely that aerobic production of energy is involved with mastication and that lactate production associated with eating is small.

The persistent high lactate levels seen in diet A may be as a result of more controlled fermentation of roughage in the anterior gut giving rise to a gradual change in blood values. Particle size will influence the passage of roughage through to the large intestine, which may explain the gentle decline in plasma lactate value. It can also be seen
that acid production in the caecum/large colon is not significant for roughage diets (Argenzio et al., 1974b).

The results of the endotoxin assay raised two concerns. Firstly, the lack of repeatability between values for replicates and secondly, the accuracy of these values. To resolve these issues, research was undertaken to obtain more information about the assay method.

Scientific publications that supported the suitability of the methodology in the determination of plasma endotoxins were obtained (Binder and Mortensen, 1985; Hurley, Louis and Tosolini, 1988; Hakogi et al., 1984/85; Sturk et al., 1985; Mortensen and Binder, 1985; Pearson et al., 1985; and Webster, 1980).

Researchers familiar with the use of the assay confirmed the accuracy of the LAL chromogenic assay method (COATEST® Endotoxin) for endotoxin detection in water, but expressed concerns over its accuracy for endotoxin determination in plasma because of the presence of enzyme activators and inhibitors in the plasma. Whilst the technique incorporated dilution and heat treatment procedures for their removal, such activators and inhibitors remained an area of concern for these researchers.

Dr Desmond Leadon (1989 pers comm.), a respected veterinary surgeon at the Irish Equine Centre, considered the method to be "fiddly". Lyn Spencer (1989 pers comm.), a National Company Teaching Scheme (NCTS) associate working in Adelaide described similar frustrations to those documented in this thesis when using the COATEST® Endotoxin kit. After 6 months effort in attempting to develop reliable standard curves, this part of her research was abandoned.
Yvette Segal (1989 pers comm.) at The Prince Henry Hospital was experienced with the assay technique with water but not with plasma. John Mayer (1989 pers comm.), at CSL Ltd felt that the COATEST® Endotoxin was excellent for endotoxin testing of water-based products, but was less reliable for plasma-based products. Also, the microtitre plate technique produced problems with time and temperature control compared to the tube technique.

Kevin Healy (1989 pers comm.), also of CSL Ltd, described the reliability of the COATEST® Endotoxin for testing water products as being superior, but its use with plasma was less reliable because of clotting and enzyme interference. It was considered too sensitive and too readily inhibited by factors in the plasma. Dr Ray Pritchard (1989 pers comm.) at Royal North Shore Hospital Microbiology section believed that the COATEST® Endotoxin was not well suited to plasma and preferred the MA Bioproducts (CSL Ltd, Parkville, Victoria, Australia) version of the LAL assay which utilised the gel clot principle (now the Multi-Test Limulus Amoeocyte Lysate PYROGEN®, BioWhittaker, Inc, Walkersville, MD, USA).

Consideration was given to changing to the MA Bioproducts assay, however this would have meant further delay and increase set up costs. The decision to remain with the chromogenic method for plasma endotoxin determination was made on the basis that published data supported the accuracy of the chromogenic method in plasma and because the sensitivity of the chromogenic assay was greater.

Further studies were carried out on the equipment used in performing the COATEST® Endotoxin assay. An automated microplate reader (Bio-Tek Instruments Pty Ltd, Auburn, NSW, Australia) was used to confirm the accuracy of the Vitatron
spectrophotometer and the problems experienced with the assay were therefore concluded to remain within the equipment used to carry out the procedure.

Timing and temperature control were emphasised by many of the researchers spoken to as being critical to the successful operation of the assay. Whilst the timing component with our facilities was adequate, temperature control appeared deficient. An inspection by a representative of Johnson and Johnson Medical Pty Ltd suggested that the problem lay in the waterbath heating system and it was recommended to change to a block heater (Johnston, 1989 pers comm.).

A block heater was not obtained at this point, and attempts to standardise the technique using the waterbath by adjusting the incubation times continued. However, this task proved increasingly more difficult as temperature variation from day to day at the same setting was very large.

Virginia Ellis (1989 pers comm.) of Cyto Systems Pty Ltd confirmed the effect of a heating deficit with the microplate method, describing the significant temperature gradients that can arise between wells on the microtitre plate, even when using a block heater. Following her recommendation, a thermocouple was obtained and the temperature variation observed between wells was considered responsible for the variable results seen with the COATEST® Endotoxin assay. The evidence supported the immediate acquisition of a block heater.

The incubation times that produced adequate standard curves turned out to be different to those recommended by KabiVitrum. With the block heater set to 41°C, the perimeter well temperatures were 36°C, while all others were 37°C. The best protocol for water standards required incubating LAL (after being freshly made up and allowed to
stand for 10 minutes at room temperature) with samples for 25 minutes. Substrate/buffer mix was preheated to 37°C 10 minutes before it was required, and then added to the samples for 5.5 minutes. The reaction was then terminated with 20% acetic acid. The best plasma standards were developed using incubation times of 30 minutes (LAL) and 5 minutes (substrate/buffer). Figures 4.31 and 4.32 demonstrate the improvement in the standard curves obtained over earlier attempts (Figures 4.24 to 4.30).

Plasma endotoxin determination from experimental samples was not achieved, however, the review of the literature did provide insight into the levels that may be expected. Normal healthy human blood has a mean endotoxin level of 0.02 to 1.57pg/mL, while those patients with gram-negative bacteraemia had values ranging from 120-850pg/mL (Pearson et al, 1985). Research using cattle and swine plasma demonstrated that positive LAL reaction to endotoxin, were only obtained in close proximity to endotoxin injection, indicating that clearance to a level below test sensitivity was rapid (Binder and Mortensen, 1985).

Horses at rest have been shown to have a normal level of plasma endotoxin ranging from 0.0126 to 0.14117ng/mL (average 0.0554ng/mL), while endotoxin levels in the racehorse rose from 0.062 to 0.094ng/mL (Baker et al, 1988). It was concluded in this study that the severe stress of racing brought about this elevation, which may have been due to a reduction in splanchnic blood flow as a result of increased core temperature and increased adrenergic activity.

Plasma endotoxin levels in horses subjected to carbohydrate-induced laminitis were shown to change from values of less than 0.1ng/L (<0.0001ng/mL) in controls, to values ranging from 2.40 to 81.53ng/L (0.0024 to 0.08153ng/mL) in 85% of horses suffering onset of Obel
Grade 3 lameness (Sprouse et al., 1987). This corresponds with caecal levels of approximately 500ug/mL (500,000ng/mL) (Moore et al., 1979).

The values obtained for clinically normal horses in these trials using inaccurate standard curves ranged from 0 to 70ng/L (0 to 0.07ng/mL). The significance of such results in clinically normal horses, given the variation between replicates, is difficult to assess. Further research is therefore necessary to confirm the reproducibility of the assay, and to reassess the blood levels in association with diet and ingestion.

4.5.3 Conclusions

Whilst Experiment 2 failed to provide further knowledge about plasma endotoxin levels in the horse, the COATEST® Endotoxin assay was refined to a point where it appeared suitable for further use. The expense of the assay could be countered by the greater accuracy of this method.

As a result of this study and the literature review, quantitation of plasma D-lactate in further studies appeared to be appropriate. If plasma L-lactate levels changed with the diet, it was conceivable that there would be associated changes in D-lactate levels, as this form of lactate is frequently produced by bacterial activity.

The importance of D-/L-lactate as an intermediate in rumen metabolism in hay-fed animals has been reported (Giesecke and Stangassinger, 1979). The increased production of D-/L-lactate by bacteria in cattle suddenly introduced to a soluble carbohydrate-rich diet is believed to be a protective device to prevent the accumulation of intracellular reduced equivalents (Counotte et al., 1983). In addition, 16% of L-lactate was fermented to propionic acid, while 75% of D-lactate was converted to propionic acid.
Whilst D-lactic acid is formed primarily in the rumen of animals suffering lactic acidosis, the proportion of D-lactate produced in animals engorging grain is variable (Prior, 1983). Absorption studies of L- and D-lactate in steers demonstrated that despite ruminal levels of D-/L-lactate increasing with concentrate feed or glucose, absorption for L-lactate increased only 70% whereas D-lactate absorption increased sixfold (Harmon et al., 1985).

Most mammalian tissue has a greater capacity to utilise the L- isomer, however, both D- and L-lactic acid are eliminated by similar routes, including oxidation to carbon dioxide, gluconeogenesis and renal excretion (Giesecke and Stangassinger, 1979). D-lactate is removed by oxidation (45%), gluconeogenesis (14%) and renal excretion (12-15%) (Giesecke and Stangassinger, 1979). At higher rates of D-lactate entry, oxidation decreases and renal excretion increases. However, if gastrointestinal lactate levels are high, resulting in body fluid shift into the gut, then urinary excretion would fall and leave oxidation and gluconeogenesis as the possible sole routes of D-lactate elimination (Giesecke and Stangassinger, 1979).

D-lactic acid has also been shown to be produced by S. bovis and S. equinus in the equine caecum, where the production of lactic acid in the caecum increased in animals fed high-grain diets (Kern et al., 1973). The importance of bacterial fermentation in both the hindgut and the foregut has been well documented (Stevens, 1977). Concentrate diets have been shown to increase caecal lactate levels (Willard et al., 1977) and be associated with profuse changes to bacterial population (Goodson et al., 1985). It is feasible that D- and L-lactate are produced and utilised in similar fashion to the ruminant system and that closer examination of this would be justified.

A better understanding of the associations of diet and D- and L-lactate production would be beneficial in understanding the pathophysiology
of gastrointestinal disease in the horse. Much work has been done on
the association of lactic acidosis/endotoxaemia associated with
carbohydrate overload and laminitis in the horse (Sprouse et al. 1987),
but the relationship of plasma levels of L-lactate and D-lactate and
various diets requires further studies to improve the overall
understanding.

The assay used for the determination of plasma D-lactic acid is
available in kit form as D-/L-Lactic Acid Test-Combination
(Boehringer Mannheim GmbH, Mannheim, Germany) and the
requirements and operation are almost identical to the Stat-Pack™
Rapid Lactate assay used to measure L-lactate levels. This kit would
provide a useful streamlining to plasma lactate measurements as the
one kit and procedure was capable of measuring both isomers of
lactate, which would result in reductions in time and cost.

4.6 Experiment 3

4.6.1 Results

No significant diet-related health problems were observed in this
study, although droppings were infrequently loose and the fetlock
areas oedematous. The droppings appeared firmer when the horse
received the bentonite feed additive, however the fetlocks remained
swollen. Frequent venipuncture did not adversely affect the horse
and insect bite allergy was not a problem.

The results for this study are presented graphically in Figures 4.33 to
4.43. Full results are tabled in Appendix 3.

PCV increased at 2 hours and decreased to near pre-feeding value at 4
hours with the treatment diet, while the control was associated with a
gradual decrease in PCV until 4 hours (Figure 4.33). From 4 hours,
PCV increased and then decreased towards pre-feeding values, with the treatment diet PCV dropping below pre-feeding value at 12 hours.

TPP for both diets was relatively unaltered at 2 hours, but at 4 hours, the TPP in both diets showed an equal distinct fall (Figure 4.34). The control diet TPP showed a gradual increase back towards pre-feeding value at 12 hours, while the treatment diet TPP oscillated with rises and falls until 10 hours before approaching the 0 hour value.

![Graph showing changes in PCV over time](image)

**Figure 4.33. Changes in average packed cell volume (PCV %) over time**
Figure 4.34. Changes in average total plasma protein (TPP g/dL) over time

Figure 4.35. Changes in average red blood cells (RBC x10⁶/µL) over time
Figure 4.36. Changes in average white blood cells (WBC $\times 10^3/\mu$L) over time

Figure 4.37. Changes in average neutrophils (neutrophils $\times 10^3/\mu$L) over time
Figure 4.38. Changes in average lymphocytes (lymphocytes \(x10^3/\mu L\)) over time

Figure 4.39. Changes in average monocytes (monocytes \(x10^3/\mu L\)) over time
Figure 4.40. Changes in average basophils (basophils $\times 10^3/\mu$L) over time

Figure 4.41. Changes in average eosinophils (eosinophils $\times 10^3/\mu$L) over time
Figure 4.42. Changes in average platelets (platelets x10⁴/µL) over time

Figure 4.43. Changes in average blood L-lactate (L-lactate mg/dL) over time
The RBC values showed a similar increase and decrease at 2 and 4 hours in both control and treatment diets (Figure 4.35). From 4 hours, the treatment diet was associated with a gradual increase in RBC values to peak at 10 hours, while the control diet showed a small peak at 6 hours, and then a steady decline to fall below pre-feeding value at 12 hours.

WBC alterations were also different between control and treatment (Figure 4.36). A decline in total WBC count was seen at 2 hours with the control diet, while the treatment diet showed only a small reduction. At 4 hours, the treatment diet was associated with a slightly larger fall before rising to 6 hours. The control diet however, showed a small increase at 4 hours followed by a large rise at 6 hours before steadying and falling below pre-feeding value at 12 hours. The treatment diet however oscillated but increased to 10 hours before finishing below 0 hour value at 12 hours.

The differential WBC count showed that the major cell responsible for the changes in total WBC counts was the neutrophil, with a curve that tightly corresponded with the total WBC curve (Figure 4.37). Lymphocyte alterations were similar to 4 hours, increasing gradually until the treatment diet was associated with oscillating changes to finish above 0 hour values, while the control diet showed a steady increase to an above 0 hour value. Monocyte changes were highly variable (Figure 4.39) and basophil counts were variable (Figure 4.40). Eosinophils declined in both diets until 4 hours, before increasing to a peak at 6 hours (Figure 4.41). Values then generally rose until 12 hours, but finished below pre-feeding value.

Platelets showed a steady decline until 10 hours in the control diet, before peaking at 12 hours (Figure 4.42). The treatment diet was
associated with a fall at 2 hours, a peak at 4 hours before a steady fall through to below pre-feeding value at 12 hours.

Plasma L-lactate values both increased at 2 hours, however the control diet was associated with the greater increase in this period (Figure 4.43). At 4 hours, the control diet lactate value had fallen to approximately 0 hour value, whereas the treatment value rose again to reach its peak. Plasma lactate values fell away quickly in the control diet to reach its lowest level at 10 hours before rising to near pre-feeding levels at 12 hours. Treatment lactate values fell at 6 hours and then rose slightly at 8 hours before falling to below 0 hour value at 12 hours.

4.6.2 Discussion

The lack of replicates in this trial does not permit detailed discussion about the relative differences between the control and treatment diets. However, some comparisons are possible with those diets (diets A, B, C and D) used in Experiment 2.

PCV changes for the control diet are similar to those seen for diet D, while PCV changes in the treatment diet are more similar to diet A. This suggests that Nature Vet Thrive P™ has had some effect. TPP changes for the control diet were again similar to diet D, while the changes in the treatment diet were dissimilar to diet A. However, there were peaks and troughs of TPP suggesting possible water fluxes. Increased water intake at 4 hours and 8 hours compared to the control diet may explain the drops seen in TPP. Such a drop was also seen at 8 hours with diet A in Experiment 2, which may be associated with fluid resorption in the large colon.

The inclusion of the bentonite feed additive into a diet identical to diet D of Experiment 2 may therefore influence fluid shifts into and
out of the gut, producing values of PCV and TPP that are more similar to diet A rather than diet D.

The minor RBC changes associated with the control diet mirrored the changes seen in diet D, while the treatment diet was similar to diet A. Additional values would allow accurate comment to be made, but it appeared that Nature Vet Thrive P™ had little effect on RBC numbers, except at the 10 hour mark.

The changes in WBC pattern for the control diet was similar to diet D, although levels of changes may have been slightly larger. The change in WBC pattern for the treatment diet was different and appeared similar to diet B. It is possible that such changes in WBC patterns may be associated with Nature Vet Thrive P™, however more samples would need to be taken to confirm this.

As most of the WBC changes were due to neutrophil changes, it is not surprising that the changes seen with the control diet are similar to those seen in diet D. Neutrophil changes in the treatment resembled more closely diet A than diet B. Lymphocyte changes in the control diet resembled those of diet D, while values for the treatment diet oscillated in a manner similar to diets A and B. It is possible that there may be some systemic influence of Nature Vet Thrive P™ on the neutrophil and lymphocyte changes, however further studies would be necessary to enable comment. Monocyte, eosinophil and basophil changes were variable.

The fall in platelet levels at 2 hours on the control diet, contrasts to the increase seen in diet D. Following this, platelet numbers appeared to go through a similar decline. The treatment diet, however, appeared to result in oscillation pattern similar to those seen with diets B and C. However, it is difficult to make anything of this as the
variations are slightly inconsistent and the results were influenced by clotting in some samples.

Plasma L-lactate levels, however, showed a clear distinction between control and treatment group. The rise and fall in plasma lactate level associated with the control diet is similar to diet D in Experiment 2, while the treatment diet is similar to diet A.

It is apparent that dietary inclusion of Nature Vet Thrive P™ produced an alteration to the normal pattern of changes in blood lactate, however this requires validation through further research. The changes seen may be due to the effect of the bentonite feed additive on the process of digestion. Slower feed ingestion and increased salivation could result in a regulation of substrate availability and buffer necessary for bacterial fermentation in the stomach. It could also be that Nature Vet Thrive P™ itself acts to directly regulate fermentative activity in the stomach and small intestine, and also regulate absorption in the small intestine. It is most likely a combination of these and perhaps other factors that are involved.

The similarity of the lactate curve in the treatment diet and diet A, particularly from 4 hours onwards, may be due to the slowing of digesta passage through the small intestine to a level similar to an all-roughage diet. The inclusion of Nature Vet Thrive P™ in a concentrate diet may have an 'evening-out' effect on both the production and absorption of lactate produced in the gut compared to an additive-free concentrate meal.

Sudden changes in the gut physiology are extremely dangerous to the well-being of the horse. The inclusion of Nature Vet Thrive P™ may help to moderate such changes and reduce the risks of overactive
fermentation and lactic acid production which is known to be associated with many serious pathologic states. Nature Vet Thrive P™ appeared to normalise plasma lactate levels in high-grain fed horse towards those that may be seen with an all-roughage diet. However, this trial would have to be repeated on a larger scale and results analysed before any conclusions could be drawn. Also, measurements of D-lactate levels in addition to L-lactate would allow comment on bacterial fermentation taking place in the gut of horses fed a high-concentrate low-roughage diet.

The failure of the endotoxin assay, as described earlier in this chapter, prevents any reporting or discussion of results in this trial. However, it is possible that differences in blood endotoxin levels may also arise with the addition of Nature Vet Thrive P™ to the diet.

4.6.3 Conclusions

Experiment 3 suggested that there were differences in the measured parameters between the control diet and the treatment diet containing the feed additive Nature Vet Thrive P™ for Horses. Whilst the parameters measured (PCV, TPP, RBC, WBC, neutrophils, lymphocytes, monocytes, basophils, eosinophils, platelets, and L-lactate) when the feed additive was included reflected patterns and levels similar to an all-roughage diet, the change to plasma L-lactate levels was most noticeable.

Strong conclusions about the influence of the bentonite feed additive on the blood parameters measured cannot be made until further studies provide an increased number of observations at each collection time. There is sufficient evidence to support expansion of the trial and in particular, to explore the quantitative changes in plasma D- and L-lactic acid levels. With the refinement of the assay
for the determination of plasma endotoxin levels discussed in Experiment 2, there is also need for assessment of endotoxin levels and examination for correlation of these endotoxin levels between diet and plasma lactate levels.

4.7 Conclusions of Equine Research

The results of Experiment 1 supported the expansion of the equine study into a larger trial to test the nominated blood parameters. It also confirmed the safety and suitability of the high-concentrate low-roughage diet, along with the safety and suitability of jugular venipuncture every 2 hours.

Whilst Experiment 2 failed to provide knowledge about plasma endotoxin levels in the horse, the COATEST® Endotoxin assay was refined to a point where it appeared suitable for further use. As a result of this study and further review of the literature, measurement of plasma D-lactate in addition to L-lactate is an appropriate addition to the parameters to be examined.

D-lactic acid has also been shown to be produced by S. bovis and S. equinus in the equine caecum and the production of lactic acid in the caecum increased in animals fed high-grain diets (Kern et al. 1973). An understanding of the associations of diet, D- and L-lactate production and the resulting plasma levels would be beneficial in understanding the pathophysiology of gastrointestinal disease in the horse.

The results of Experiment 3 suggested that the inclusion of the bentonite feed additive Nature Vet Thrive P™ for Horses to a high-concentrate low-roughage diet brought about changes in the blood parameters measured. Whilst the parameters measured reflected
levels similar to an all-roughage diet when Nature Vet Thrive P™ was incorporated into a high-concentrate low-roughage diet, the changes to plasma lactate levels were most noticeable.

The results of these investigations indicated that feeding and formulation of diet influenced parameters measured in the blood. The results also lend support to the hypothesis that the inclusion of the bentonite additive Nature Vet Thrive P™ for Horses to a high-concentrate low-roughage diet alters the digestion of such a diet. The changes to systemic L-lactate levels may reflect changes in the gastrointestinal levels of lactic acid brought about through modification of the digestive activity of gastrointestinal microorganisms by the bentonite-based formulation.

The ability of bentonite to modify microbial metabolic activity has been reviewed earlier. Such activity in the gastrointestinal system of the horse may prevent the decline in pH and consequent changes to microfloral balance associated with the ingestion of high-concentrate diets. It may also influence the base levels of endotoxin in the gut and the blood, however, such activity was not able to be demonstrated in these studies due to technical difficulties.

The results of this research suggests an important role of Nature Vet Thrive P™ for Horses receiving high-concentrate low-roughage diets to prevent alterations in gastrointestinal physiology that can be measured indirectly by the examination of certain blood parameters. The potential of this bentonite-based additive to influence both endotoxin production in the gastrointestinal tract and blood levels of endotoxins remains to be determined. The results of this research suggests that an effect can be anticipated.
CHAPTER 5

Canine Study
The Effect of Bentonite Feed Additive on Digestive Function in the Dog
5.1 Introduction

The bentonite feed additive Nature Vet Thrive D™ for Dogs had been found in clinical use to be beneficial to dogs. The aim of this research was to quantify the effect of Nature Vet Thrive D™ for Dogs on feed digestibility and faecal parameters when included in canine diets based on a mixture of dry kibble and mince meat.

A controlled intensive digestibility trial was undertaken to develop this knowledge about Nature Vet Thrive D™. Results that could be statistically analysed and thereby facilitate the registration of the product were also a desired outcome.

5.2 Materials and Methods

5.2.1 Dogs

Ten healthy male crossbred dogs were used in the investigations. They were housed at Meadow Mist Boarding Kennels and Cattery Pty Ltd, Marsden Park, NSW, Australia. All dogs were mature (age range 4 to 6 years) and in good condition (body weight range 27 to 43kg - average weight 31.7kg). Vaccination and worming histories for all dogs were current. All individuals were examined for physical health and were wormed prior to inclusion.

5.2.2 Facilities

The dogs were housed in individual, concrete-floored runs within an environmentally controlled room. Each run (approximately 4 x 1m) was constructed of floor to ceiling steel mesh and was equipped with a raised hessian bed, and separate water and feed bowls. Manure was
collected twice daily and the runs hosed clean once daily. Dogs were examined daily to monitor health.

5.2.3 Feeds

Each dog received a mix of dry food (Harper’s Dog Chow™) and a meat mince formulated at the kennels. The chemical analysis of the meat mix and the dry food have been described in Chapter 3.

The manufacturer’s recommendation for daily intake of dry feed was 500g, however as these dogs were to receive 150g mince per day, the kibble ration was adjusted to 325g per day. Such a feeding level was selected as being reflective of the quantity an owner might choose. This ration satisfied maintenance requirements for protein and fat but not energy (NRC, 1985). The treatment diet differed from the control diet through the daily addition of 12 g of Nature Vet Thrive D™.

5.2.4 Analytical Methods

The analytical methods used in this study have been previously described in Chapter 3.

5.3 Procedure

The trial employed a partial cross-over design with dogs being randomly allocated to treatments and pens. The dogs were fed once daily in the morning following the removal of faeces and cleaning of the runs. A ten day period of dietary adjustment was provided before the first collection period was commenced.

The trial was divided into two 2 week periods, each containing a five day collection period. During the first five day collection period, faeces
were collected twice daily and stored in a sealed bag for weighing. Faeces were then rated for smell and consistency and then representative samples from each were removed and frozen for later analysis.

At the end of the first period, three dogs from each group were randomly chosen and crossed over. Collections were then continued until the completion of the trial. Representative feed samples were also taken and frozen for later processing.

5.4 Results

The trial was completed with some complications. As dog 9 failed to eat variable portions of its ration during both collection periods, the amount of Nature Vet Thrive D™ consumed when this dog was receiving the treatment diet could not accurately be established. Consequently, its results were not included in the statistical analysis.

On day 4 of the first collection period, dog 10 became unavailable and a replacement dog (dog 11) of similar type, age and weight was used to complete the trial. During the second collection period, dogs 2 and 5 (control and treatment group respectively) became unavailable for final faecal collection and weighing on day 5.

In addition, dog 8 was observed during the first 2 week period (control group) to exhibit a weight loss that prompted an increase in its daily dietary intake for the second 2 week period (treatment group) to 250g mince and 542g dry feed. This dog was observed to be more nervous than the other dogs in the trial.

Statistical analysis of the results demonstrated there to be no significant differences between dogs, collection periods, treatments and found no treatment/week interaction. As no significant
differences were evident, the data was grouped according to treatment
and analysed. Mean and standard error of the mean (SEM) results are
documented in Table 5.1 and Figures 5.1 to 5.5. Complete results are
presented in Appendix 4.

<table>
<thead>
<tr>
<th>Table 5.1. Mean Digestibility Values (%) of Feed Dry Matter, Organic Matter, Crude Protein and Fat, and Mean Digestible Energy Content (MJ DE/kg DM) of Control (C) and Treatment (T) Diets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Matter</td>
</tr>
<tr>
<td>Mean</td>
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<tr>
<td>C</td>
</tr>
<tr>
<td>T</td>
</tr>
</tbody>
</table>

** - p<0.01
* - p<0.05
# - not significant

Figure 5.1. Dry matter digestibility (DMD %) in control and treatment
groups. Note - 50% of the values fall within a box (interquartile
range), which has the median value indicated by a line
- whisker lines indicate the spread of data to a maximum of 1.5 times
the interquartile range. Values lying beyond this are indicated by *
Figure 5.2. Organic matter digestibility (OMD %) in control and treatment groups

Figure 5.3. Protein digestibility (PD %) in control and treatment groups
Figure 5.4. Fat digestibility (FD %) in control and treatment groups

Figure 5.5. Energy digestibility (ED: MJ DE/kg DM) in control and treatment groups
The digestibility of dry matter and organic matter was significantly increased in the treatment group (p=0.0375 and p=0.0249 respectively), while the digestibility of protein and energy increased but not significantly (p=0.2712 and p=0.1066 respectively). Fat digestibility decreased within the treatment group (p=0.3019) however, the difference was not significant.

The treatment group also demonstrated a significant reduction in the degree of faecal smell (p=0.0017) and improvement in the degree of faecal formation (p=0.0322). Faecal water content was less in the treatment animals, however the difference was not significant (p=0.1283). These results are presented in Table 5.2 and Figures 5.6 to 5.8.

<table>
<thead>
<tr>
<th></th>
<th>Smell</th>
<th>Degree of Stool Formation</th>
<th>Moisture Content</th>
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<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
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<tr>
<td>C</td>
<td>2.69</td>
<td>0.10**</td>
<td>2.51</td>
</tr>
<tr>
<td>T</td>
<td>1.73</td>
<td>0.22</td>
<td>1.85</td>
</tr>
</tbody>
</table>

** - p<0.01  
* - p<0.05  
# - not significant

Average body weight changes over the trial were negative, however less weight loss was observed in the treatment animals, although the difference was not significant (p=0.9394). The changes for body weight are presented in Table 5.3.
Figure 5.6. Faecal smell in control and treatment groups (1 = mild; 2 = moderate; 3 = strong; and 4 = pungent)

Figure 5.7. Degree of stool formation in control and treatment group (1 = dry; 2 = moist; 3 = wet; and 4 = unformed)
Table 5.3. Average Weight Changes (kg) over the Experimental Period

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SEM</th>
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<tbody>
<tr>
<td>C</td>
<td>-0.30</td>
<td>0.39#</td>
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<tr>
<td>T</td>
<td>-0.17</td>
<td>0.26</td>
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</table>

# - not significant

5.5 Discussion

The complications that arose during this study were accepted as being one of the risks involved when intensive digestibility trials are undertaken. The trial demonstrated the digestive aid properties of the product Nature Vet Thrive D™ for Dogs, and assisted in its registration in Australia. The improvement in dry matter, organic matter, protein and energy digestibilities are consistent with those changes seen with the use of a bentonite feed additive in the horse (Sriskandarajah and Woog, 1987).
The digestibility of an offal diet has been shown to be very high - fat 96.7% and protein 93.3% (Meyer et al., 1980). This compares to the digestibilities of respective components of the carbohydrate-rich diet 92.8%, 87.6% and the soluble energy component or nitrogen-free extractives (NFE) 88.5%. The digestibility figures obtained in this trial are apparently lower for respective components, but are in close correlation with NRC (1985) recommendations.

The increase in digestibility of all components (except fat) may explain why dogs receiving Nature Vet Thrive D™ lost less weight than those dogs that were not receiving the additive despite a negative energy balance. Fat digestion is the most vulnerable of the nutrients to disturbance, and it is possible that the bentonite feed additive may have induced a mild degree of fat malassimilation.

A fat digestibility of 91% is adequate as the digestibility of fat in average commercial dog foods is approximately 90% (Lewis et al., 1987). The result appeared to be influenced by one dog that displayed a greater reduction in fat digestibility than any other animal when receiving the feed additive. This dog may have possessed an alimentary dysfunction that precipitated a relative fat malassimilation which was exacerbated by the feed additive.

The results of the trial suggest that the type of diet does affect the rate of passage through the digestive tract. The kibble component of the meal is quite finely prepared and together with the fine mince (despite its high fat content), may promote shorter gastric retention. The crude fibre content of 5% would contribute to a further shortening of gastric retention time.

The rapid passage of nutrients through the small intestine to the large intestine may result in extensive microbial fermentation due to
increased availability of readily fermentable carbohydrate substrate. These products of fermentation are considered active in the aetiology of the loose motions that are seen and the pungent odours smelt in diet-related alimentary dysfunction (Meyer et al, 1980).

It is possible that the physical rate-limiting benefits of the montmorillonite slows ingestion, gastric emptying and passage of food through the gut, thereby allowing increased opportunity for assimilation of nutrients in the small intestine. This effect is probably enhanced by the incorporation of acid stable digestive enzymes in Nature Vet Thrive D™ which would assist the body's own enzymes in breaking down the food substrate into component molecules for absorption. Many of the benefits of this product may be a result of its action in the anterior gut.

As a consequence of this, less substrate would be available for microbial fermentation in the large bowel. The reduced liquid component of the faeces may be related to reduced levels of osmotically active bacterial volatile fatty acids and other waste products that may evoke a fluid shift into the lumen. The reduction in smell may also be explained by more controlled fermentation, and the binding of certain products of bacterial metabolism (including ammonia) and their removal from effect.

The ingredients of Harper's Dog Chow™ included meat by-products from beef and mutton, wheat or maize, wheat by-products including wheat germ, soybean meal, stabilised animal fat, whey, skimmed milk and oat flour. To this is added ferrous sulphate, copper oxide, cobalt sulphate, potassium iodide, zinc iodide, manganese oxide, vitamins A, D3, E, B1, B6, B12, calcium pantothenate, niacin, and colouring and flavouring agents.
Diets high in carbohydrates, particularly soluble starches, and diets containing soya meal protein are recognised for their potential in causing alimentary dysfunction (Meyer et al, 1980). The diet used in this study was such a diet, and these factors along with others not known may have been involved in the production of the loose, malodorous faeces in the control animals.

There are many possible areas that could cause alimentary dysfunction in the healthy dog fed a dry dog food. This study demonstrated that Nature Vet Thrive D™ was able to alter the gastrointestinal physiology of the dog to a degree that resulted in improved nutrient digestibility and reduced incidence of certain side effects frequently seen with the feeding of concentrate diets.

5.6 Conclusions of Canine Research

The results of this trial revealed several aspects about the usefulness of the product Nature Vet Thrive D™ for Dogs. The daily inclusion of 12g of Nature Vet Thrive D™ in a diet comprised of Harper's Dog Chow™ and mince meat resulted in a significant improvement in both the digestibility of dry matter (p<0.05) and organic matter (p<0.05); an improvement in both the digestibility of energy and protein; and a reduction in fat digestibility. A significant reduction in faecal smell (p<0.01) and significant improvement in the degree of stool formation (p<0.05) was also observed in those dogs receiving the bentonite feed additive. Faecal moisture content was also lower in those dogs receiving the feed additive, however the difference was not significant (p=0.1283).

The improvement in digestibility of diet was also reflected in the fact that those dogs receiving Nature Vet Thrive D™ did not lose as much weight as control dogs in the face of negative caloric intake. The
monitoring of body weight changes was shown to be important to enable the manufacturer's feeding recommendations to be adjusted to the individual animal's requirements.

It was concluded that the product Nature Vet Thrive D™ for Dogs brought about an improvement in nutrient digestibility of canine diets comprised of a mix of dry kibble and mince meat. As well, the product demonstrated useful activity in deodourising faeces and conferring more solid consistency to the stools.
CHAPTER 6

Conclusions
6.1 Equine Studies

The studies relating to Nature Vet Thrive P™ for Horses and equine blood physiology reported in this thesis were complicated by three factors. Firstly, there was a shortage of money for this type of research due to a general shortage of funds available through the Australian Research Council (ARC), the Australian Equine Research Foundation (AERF), and the ARC Small Grants Scheme. To complicate this, competition for this limited research funding was significant.

Secondly, there was a time shortage, which was compounded by the fact that procedures such as plasma endotoxin testing were not commonplace in industry which resulted in time delays associated with the learning process. Progress was further slowed by concerns about the expense of the assay and the costs of the associated failures, and by the unknown biologic factors that inherently occur when undertaking experiments involving animals.

And finally, the technical knowledge base being drawn on at the research facility was inadequate in regard endotoxins and their assay. However, the fact that the studies touched on unfamiliar ground provided a challenge and an incentive for the future development of this research.

For further work to be carried out successfully in this field, these three issues must be addressed. Sufficient funds must be available. Sufficient time must be allocated. And finally, the knowledge base to support such work should be expanded.

Future research should focus on the area of equine feeding with emphasis on the relationship of diets suitable for performance and caecal/large intestinal physiology. The Australian horse industry demands accurate and locally relevant information on the nutritive
value of feeds and nutrient requirements of different classes of horses.

Scientific information developed overseas is still largely used to make decisions regarding the most suitable feedstuffs to feed local horses. Only limited Australian data regarding nutritive values of common feed materials as well as nutrient requirements of different classes of horses exists. Most of the local feeding strategies have developed around the guidelines laid out by the National Research Council. Only recently (NRC, 1989) have these recommendations been updated from those published in 1978. Whilst the value of these resources is unequivocal, the reliance upon these recommendations under Australian conditions remains equivocal.

Scientific feeding of the horse requires an understanding of the interaction between the level of activity of the horse, the type of feed offered to the horse and the process of digestion. Whilst present feeding strategies appear successful, there still exists scope for greater knowledge about the process of digestion in the horse and in particular, how this process can be improved through better understanding of what happens to the feedstuffs provided.

There are therefore three key areas of concern in regard equine nutrition. Firstly, the lack of Australian data on nutrient requirements of various classes of horses, nutrient composition of common and some new feedstuffs, and nutrient interactions between feeds. Secondly, the lack of Australian data on nutrient requirements of performance horses and the interaction between nutrition and exercise. And finally, the lack of understanding of the problems associated with high-concentrate low-roughage (high-grain) feeding strategies in the horse.
The lack of understanding of problems associated with feeding high levels of grain to horses is possibly the most important area to consider as the logical consequence of the studies reported in this thesis. High-grain feeding is common practice in the horse industry as a means of providing the large amounts of energy in a concentrated form required for athletic performance.

Associated with a high-concentrate low-roughage feeding routine is the potential for many problems, ranging from digestive disturbances and colic through to more systemic problems, such as endotoxaemia, lactic acidosis and laminitis. Such problems remain an important loss in the horse industry despite significant amounts of research.

With the development of technology, researchers have been able to more accurately predict the nutritional requirements of the performance horse, and with an improved knowledge about equine exercise physiology, more effectively train these horses. However, the area in which research may improve understanding is how the performance diet can affect performance in ways other than by simply providing essential nutrients.

For example, knowledge about the effects of diet on the large intestinal physiology and in particular, the production of normal metabolites and end products such as lactic acid and endotoxin, may prove useful in allowing safer high-energy diets to be formulated. Overproduction of lactic acid and endotoxin have been demonstrated to play an important role in the pathophysiology of laminitis and endotoxaemia, and have attracted recent attention (Baker et al, 1988; Morris and Moore, 1987; Moore et al, 1979: Moore et al, 1981; Sprouse et al, 1987; Gossett et al, 1987; Willard et al, 1977; Garner et al, 1977; Garner et al, 1978).
Future research should be aimed at the development of the technique of the in vitro caecal fermentation procedure, which could be used alongside caecally fistulated horses to develop the information required to build such a database of nutritional information. This research would also allow clarification of certain aspects associated with high-grain feeding practices, such as lactic acidosis and endotoxaemia.

The development of the in vitro caecal fermentation technique to examine feedstuff digestibility would not replace conventional in vivo digestibility trials. It would provide an insight into caecal digestion and physiology as it relates to different feeding strategies, and would also provide a convenient and reliable mechanism to test hypotheses regarding feeding strategies before more expensive and time consuming traditional digestibility trials on stall-fed animals were carried out. In a time when sound animal care and ethics in animal experimentation is paramount, the development of the in vitro technique to study digestion in the horse is logical.

Two levels of future investigation are proposed. Specific digestibility investigations using the in vitro caecal fermentation technique (Applegate and Hershberger, 1969; Koller et al., 1978; and Stott et al., 1983) would be conducted to generate information about common feedstuffs. Caecal samples used to establish this technique would be obtained from euthanased horses at an abattoir.

The development of this technique would enable large numbers of tests on differing feed materials to be undertaken with the development of large amounts of data in a short space of time. To carry out such tests on caecal-fistulated animals would not only be more expensive but would be far slower. Findings from these in vitro trials would then be used to develop feeding trials to be carried out on other abattoir horses, and the resident experimental horse population
at the university. Table 6.1 details the proposed methodology for future studies.

<table>
<thead>
<tr>
<th>Type of Study</th>
<th>Type of Horse</th>
<th>Proposed Measurements</th>
<th>Expected Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Development of digestibility</td>
<td>In vitro caecal fermentation</td>
<td>Caecal digestive products</td>
<td>Information on nutritive value/chemical composition and digestibility of feeds.</td>
</tr>
<tr>
<td>techniques</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simple digestibility</td>
<td>Stall-fed university horses</td>
<td>Feed intake &amp; blood-borne</td>
<td>Information on nutritive value/chemical composition and digestibility of feeds.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>products of digestion</td>
<td></td>
</tr>
<tr>
<td>Simple digestibility</td>
<td>Knackery horses</td>
<td>Blood and caecal digestive</td>
<td>Information on nutritive value/chemical composition and digestibility of feeds.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>products</td>
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</tr>
</tbody>
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Future studies would focus on the following:

1. Routine chemical and digestibility evaluation of a range of feedstuffs in the major categories of forages, cereal grains, legume grains and protein supplements.

2. Examination of key parameters of digestion such as blood glucose, blood volatile fatty acid levels, and blood D- and L-lactic acid levels under different dietary regimes.

3. Examination of key parameters of digestion such as caecal pH, caecal volatile fatty acid levels, and caecal D- and L-lactic acid levels under different dietary regimes.

4. Examination of blood and caecal endotoxin levels under these different dietary regimes.

5. The findings of the work undertaken in the above areas would facilitate the design of experiments for the examination of the interrelationship of exercise and nutrition in the horse.

To support this future direction in research, funding must be secured. Some funding was forthcoming following successful applications to the ARC and the AERF in 1990 regarding a proposal to establish an in vitro caecal fermentation model at Hawkesbury, but came through
following the conclusion of my contract. Some of these funds may allow future endotoxin studies to be carried out.

These research funds would enable the standardisation of a practical model to allow intensive assessment of digestibilities of feed materials for horses by laboratory means, with the objective of rapidly and economically testing substrate digestibilities. Results from these in vitro studies would enable improved planning of in vivo trials. There would be two main benefits arising out of the in vitro research - limited use of animals wherever possible and as a result, conservation of money thereby maintaining its availability for in vivo digestibility trials.

With the establishment of a standardised model, changes in caecal fluid samples of D-/L-lactate and endotoxin as they relate to different feed substrates could be measured. Examination of plasma levels in animals on rations created from these substrates would then follow.

Whilst assays for D-/L-lactate in caecal fluid and plasma are established and appear reliable, it would be necessary to perform more tests with the endotoxin assay to determine its reliability. From the studies reported in this thesis, it was apparent that determination of plasma endotoxin levels using the COATEST® Endotoxin kit was possible. However, further work would be necessary to confirm this, and to determine what level of dietary grain would be necessary to raise plasma endotoxin to a level where the feed additive Nature Vet Thrive P™ for Horses had a measurable effect. Higher levels of grain inclusion may need to be evaluated.

It would also be necessary to determine whether the COATEST® Endotoxin kit would be the appropriate assay for measuring caecal endotoxin levels. It is clear that the high levels of endotoxin normally
found in the caecal fluid would need substantial dilution to prevent the assay from being overwhelmed. Theoretically, the assay should work, however, it would need verification in the in vitro model.

6.2 Canine Study

The study undertaken with Nature Vet Thrive D™ for Dogs successfully established the effects of the bentonite feed additive and ultimately facilitated the successful national registration of this product. The improvements in food digestion, along with the improvement in stool formation and reduction in faecal water content and faecal odour were consistent with published reports describing the use of bentonite as a feed additive in other monogastric animals.

The research findings suggest that the bentonite-based feed additive is capable of modifying the local gastrointestinal environment, and thereby alter the processes of digestion and assimilation. Exactly where and how this modification occurs remains to be elucidated. It is likely that as in the horse, the bentonite feed additive is regulating the availability of substrate to the gastrointestinal microorganisms and thereby influencing the balance of microfloral waste products, as well as improving the overall availability of nutrients to the host. The details of such activities has been reviewed earlier in this thesis.

There are several areas where further studies on the use of the bentonite feed additive in canine feeding systems is recommended.

The study reported in this thesis used a feeding strategy based on a mixture of a dry food and fresh meat. A digestibility study should be undertaken to determine nutrient digestibility of the four major feeding systems utilised - dry food, semi-moist, tinned food and fresh
food (that is, meat and vegetables). The same parameters, including
degree of stool formation and faecal odour, should be assessed.

This study would highlight the similarities and differences that may
exist in how such diets are utilised by the dog. The anticipated benefits
of the inclusion of the bentonite feed additive Nature Vet Thrive D™
in different feeding strategies could then be more accurately predicted
and tested by further digestibility studies.

The research findings would provide more insight into diet-related
gastrointestinal dysfunction, and in particular, highlight the
difference between commercially prepared and more natural based
feeding strategies. In addition, the research would establish whether
the digestive benefits of the bentonite feed additive demonstrated in a
dry food diet exist in other commercially prepared dog foods and in a
more natural meat and vegetable diet.

Such research findings would contribute substantially to the scientific
literature on the use of bentonite as a feed additive in canine feeding
systems.

6.3 Summary

The equine studies demonstrated that alteration in the ratio of
concentrate (oats) to roughage (lucerne chaff) in the diet produced
significant changes in the red blood cell (p<0.05) and L-lactate (p<0.01)
blood parameters. Other blood parameters measured, including
packed cell volume, total plasma protein, white blood cell count,
differential white blood cell count and platelet count were not
significantly different between diets. Blood endotoxin levels were not
determined due to technical difficulties.
The inclusion of the bentonite feed additive Nature Vet Thrive P™ for Horses to a high-concentrate low-roughage diet was associated with a movement of values for the same blood parameters towards those expected to be seen when a high-roughage diet was fed. The change in blood L-lactate levels was the most apparent. Such findings suggest an influence of the additive on the processes of digestion and assimilation. However, given the limited number of replicates and the concomitant lack of statistical analysis, further research would be necessary to clarify such changes and to determine the potential benefit of the dietary inclusion of Nature Vet Thrive P™ for Horses.

The canine study demonstrated that the inclusion of Nature Vet Thrive D™ for Dogs into a mince meat and dry food diet significantly increased the digestion of both dry matter and organic matter components of the diet (p<0.05). The digestion of the protein and energy components of the diet were also increased, although the difference was not significant (p=0.2712 and p=0.1066 respectively). The digestion of dietary fat was decreased, however the difference was not significant (p=0.3019). Associated with these changes was less body weight loss in dogs receiving the additive when subjected to negative caloric intake, although the difference was not significant (p=0.9394).

The study also demonstrated that the dietary inclusion of Nature Vet Thrive D™ significantly reduced the degree of smell (p<0.01) and improved the degree of faecal formation (p<0.05). Faecal water content was markedly less in those dogs receiving Nature Vet Thrive D™, however the difference was not significant (p=0.1283).

The inclusion of the bentonite feed additive Nature Vet Thrive D™ for Dogs resulted in improvements in feed digestibility and the faecal parameters, smell and stool formation. The study demonstrated that the bentonite feed additive can be considered a digestive aid.
PART B
CHAPTER 7

Introduction
Introduction

Science remains today the principle research approach for acquiring knowledge about nature, reality and the world in which we live. Scientific knowledge is considered reliable knowledge because it is objectively proven and is therefore free of personal opinion, preferences and speculation (Graziano and Raulin, 1989).

The success of the scientific approach in knowledge acquisition and the development of technology is evident in virtually all aspects of our lives. Whether it be farm production, building construction or transportation, to name but a few areas, we have all benefited from the knowledge gained from science.

However, we are increasingly confronted with reports of 'problems' that appear to have arisen as a spin-off from the knowledge attained by the scientific approach. Current topical examples include global warming associated with increased atmospheric carbon dioxide levels arising from destruction of forests and the burning of fossil fuels; the 'non-biodegradability' of plastics used as packaging material; and soil degradation and ground water contamination associated with modern agricultural practices. The question could be asked "If the methodology of science is so good, how is it that we are left with such devastating reminders of its application?"

Issues such as those just described have stimulated moves away from science as 'the way' of acquiring knowledge. Whilst the reductionism, refutation and repeatability of science may still be appropriate in many situations, it is apparent that the environment within which the methodology is to be used must be recognised more than it has been.
A living example of such a shift in perception away from science is found within the Faculty of Agriculture and Rural Development at the University of Western Sydney, Hawkesbury (formerly the Faculty of Agriculture, Hawkesbury Agricultural College). The hard science and technology base that was employed by the Faculty has given way to a softer, less objective approach to finding out about the world and taking action within it.

Part B of this thesis has developed as a result of my desire to better understand the philosophy of my practice. This thesis therefore reports on the research task (Part A) and my personal learning experience associated with this research (Part B).

Of immediate interest was the difference between the methodology utilised in the research work undertaken and the methodology utilised by the Faculty of Agriculture for the acquisition of knowledge. The studies undertaken utilised the three R's of science (reductionism, refutation and repeatability) to achieve true knowledge that was free of personal opinion, while the Faculty utilised systems thinking and action research to achieve true knowledge. By virtue of design, the Faculty's approach relied heavily on personal opinion.

Three issues were of concern. Firstly, did I understand the philosophy of my own practice, let alone that of the Faculty of Agriculture? Secondly, was it possible to carry out reductionist scientific work within an environment espousing alternative philosophies? And finally, was it possible that as a result of being associated with two opposing philosophies that an improved awareness of both would be forthcoming?

The material documented in Part B is comprised of two main parts. Chapter 8 is a review of the literature, documenting relevant aspects
of science, systems thinking, experiential learning and the Hawkesbury approach. This review has been written from a science-based perspective, in which little knowledge about philosophies and paradigm development existed.

The review enabled reflection and documentation of insights that arose from experiences whilst operating within the Hawkesbury environment and are presented in Chapter 9. The increased awareness of learning styles (particularly experiential learning), systems thinking and different levels of knowledge are detailed in this chapter, along with how these have influenced my views on the research undertaken.

Chapter 10 restates the learnings that have been documented throughout the thesis. As such, it incorporates learning outcomes from both Part A and Part B, and reflections on how I believe I have developed as a learner and researcher as a result of these experiences.
CHAPTER 8

Literature Review
8.1 Introduction

Science as a way of knowing and acquiring knowledge continues to play an important role in the modern world. However, science is no longer the only mechanism whereby true and meaningful knowledge can be attained. The recent development of systems thinking and systems methodologies has allowed research to be carried out in areas where the traditional scientific approach has faltered.

The following review touches on the key elements that is science and systems thinking, and highlights the important differences between the methodologies. It is not intended to be a complete review of published literature, but rather a summary of relevant readings. An overview of experiential learning is also presented, along with its relationship with systems thinking and the Hawkesbury approach.

8.2 Science - What is it?

The development of science from the early Greek Ionian period through to the Scientific Revolution and beyond is described in many texts, and will not be covered in this thesis. Whilst these accounts may be written from differing world views, they do highlight the major shifts that occurred in the evolution of this human activity, and provide insights into the fact that science is not only a product of our civilisation, but also a creator of it (Chalmers, 1976; Checkland, 1981; Graziano and Raulin, 1989; Hull, 1959). Only those components of the human activity system that is science (Checkland, 1981) which directly relate to the studies undertaken will be detailed in this chapter.

Graziano and Raulin (1989 p.2) describe science as being about "carefully composing questions and systematically seeking their answers to gain a better understanding of nature. Science involves a
process of inquiry, a particular way of thinking". It is driven by an urge to find out "how and why the world is" (Checkland, 1981 p.24).

A popular meaning that many people appear to give modern science is outlined by Chalmers (1976) and is similar to Bacon's concept of science (Charlesworth, 1982). Scientific knowledge is about proven knowledge - whereby scientific theories are extracted from the facts of experience acquired as a result of experimentation and observation.

Scientific knowledge is considered to be reliable knowledge because it is objectively proven, or in other words, it is free of personal opinion, preferences and speculation. The essence of science is therefore in its systematic, disciplined way of thinking aimed at gaining knowledge about nature (Graziano and Raulin, 1989).

However, three points should be made about modern science as we know it today. Firstly, the results of scientific work are never absolute - instead they may be replaced by improved conceptual models. Secondly, scientifically acquired and tested knowledge is not knowledge of reality. It is more accurately spoken of as "knowledge of the best description of reality that we have at that moment in time" (Checkland, 1981 p.50). And finally, the rules that govern the acquisition of scientific knowledge are human constructions, which suggests that personal opinion, preferences and speculation are inextricably interwoven (Guba and Lincoln, 1988).

Wheatley (1992, p.17) described in her writings on New Sciences how the modern world's need for order to enable the comprehension and definition of complex structures in nature has arisen from the "fuzzy understanding of concepts that originated with seventeenth-century science". She refers to autopoeitic (self-producing) structures, where each structure within the greater environment has a unique identity
with defined boundaries, while at the same time it is inextricably linked to the larger environment and to other autopoeitic structures.

Prigogine (1983, cited in Wheatley, 1992) developed the term "dissipative structures" in an effort to describe how disorder can be the source of new order in chemistry. Dissipative activity, processes by which energy is slowly lost, was discovered not to lead to the demise of a system. Rather, it was part of the process by which a system let go of its current form and could then reemerge in a form better suited to the demands of the present environment.

Wheatley (1992) commented that if the system pays attention to new information entering its boundaries, the information grows in strength as it interacts with the system and is fed back on itself. The disturbance that follows grows to a level so great the system in its current form falls apart, but rather than being the death of the system, a new system can reconfigure at a higher level of complexity. This new system is better able to deal with the new environment.

Science is therefore a process concerned with the acquisition of knowledge. However, there are various methods of acquiring knowledge which differ according to increasing demands for information.

Such methods, in order of increasing requirement for information include (a) tenacity - a willingness to accept an idea as valid as the idea has been accepted for a long period of time; (b) intuition - knowledge achieved directly without any intellectual effort, or without any involvement of sensory processes; (c) authority - a method of acquiring knowledge following the acceptance of an idea as valid because its origin was in a respected source; (d) rationalism - a way of thinking in which knowledge is developed through reasoning only; and (e) empiricism - a way of gaining knowledge through the
observation of real events, or in other words, knowing by experiencing through our senses (Helmstadter, 1970 cited in Graziano and Raulin, 1989).

Tenacity, intuition and authority, as methods of knowledge acquisition, make few demands on their information and processes. Something is known to be true with these methods because it has always been that way (tenacity), it is felt to be that way (intuition) or because a higher authority says it is that way (authority).

A rationalistic way of thinking involves information which is carefully stated, and where logical rules are adhered to in order to reach acceptable conclusions. However, knowledge that arises from this approach is not only dependent on the reasoning process, but also on the accuracy and correctness of the assumptions used in the reasoning.

Empiricism, on the other hand, is a method of gaining knowledge through the observation of real events. It is not sufficient to know through reason, tenacity, intuition or authority - it is obligatory to experience events through the five senses.

Science, as a way of knowing and acquiring knowledge, brings together the two latter methods - rationalism and empiricism. The scientist, that is the person seeking knowledge and understanding through the process of science, utilises both rational logic and empirical observation to check each step. There is a shuttling between empirical observation of facts and abstract rational thoughts about these facts. It was this movement between empirical observation and rational thinking that marked the emergence of modern science in the 16th and 17th centuries.
8.2.1 Basic Assumptions of Science

Science is considered to be a consciously recognised, organised human activity system and as such is an inquiring or learning system. It is a system concerned with finding out or coming to know about the world we inhabit (Checkland, 1981; Guba and Lincoln, 1990).

Fundamental to this activity system are the beliefs or assumptions that underpin it. There are three primary interrelated questions that are relevant to the process of knowing and include - What is there that can be known?; What is the relationship between the knower and the known?; and, How can one go about finding out? (Guba and Lincoln, 1990).

These questions may be respectively termed ontological, epistemological and methodological questions, and the answers to these form the premises (basic belief systems) that determine what the inquiry is and how it should be practised. As already mentioned, the questions are interrelated - so that a given epistemology implies a parallel ontology, leading to an appropriate methodology. Therefore the entire belief system, including ontology, epistemology and methodology, constitutes a way of knowledge pursuit, an ethic and a way of living.

Only since the 17th century work of Descartes can science be considered approximate to its modern form. Descartes was preoccupied by the fear of believing something that was not certainly true (Guba and Lincoln, 1990). This concern has led to the development of the term "Cartesian anxiety" - a deep concern with foundational knowledge that is entrenched in scientists and society today. That is to say, the question that really drives research is along the lines of "how does it work?"
This preoccupation has contributed to the development of the belief system that is and has been modern science for the last 200 years - the belief that there is a single objective reality (capable of meaningful division into subparts) that exists out there. This reality is driven by natural and unchangeable laws, irrespective of the notice that humans may take. The ontology of modern science is therefore realist - a true physical universe does exist, and while there is randomness and unpredictability, it is a primarily orderly system (Guba and Lincoln, 1990; Graziano and Raulin, 1989).

Modern science sets out to discover the true nature of reality and how it works, on the basis that there is truth out there. The success or otherwise of science is assessed according to its ability to predict and control nature, which implies knowledge about supposed causal mechanisms or a machine-like image of nature. The realist ontology which underlies science implies underlying causal linkages operate in nature, which are time and context-free (Guba and Lincoln, 1990).

The operation of a realist ontology restricts the scientist to an epistemology that is objective, independent and essentially value-free. That is, the knower is wary of his or her relationship with the known. This distancing has two effects - firstly, to avoid any influence of the inquirer on Nature's operations, and secondly, to prevent the inquirer's observations and subsequent interpretations from being affected by Nature's confused ways or by the inquirer's own bias. Objectivity therefore implies that the inquirer can discover "how things really are" or "how things really work" (Guba and Lincoln, 1988).

Therefore, to find out how things "really are" given the realist ontology and objectivist epistemology that underpins conventional science, an appropriate methodology must be employed. This
methodology is that of experimentalism or manipulation, in which propositions or statements are put forward based on what is already known or hypothesised. This was the approach used and described in Part A of this thesis.

These propositions are then subjected to empirical testing according to experimentation and observation, which in modern science entails falsification. These tests require manipulation of the environmental conditions to achieve the desired treatments and so enable the researcher to acquire a higher level of truth or to serve some social benefit.

The belief system or paradigm of conventional science (positivism) is therefore based on a realist ontology, an objectivist epistemology and an experimentalism or manipulative methodology (Guba and Lincoln, 1990).

8.2.2 Observations vs Inference/ Facts vs Constructs

Scientific research, as a general rule, involves posing a question, determining how to go about answering the question, planning and making appropriate empirical observations, and rationalising these observations to make sense of them. Science therefore involves carefully observing events, attempting to reason why they occurred and then attempting to make predictions based on ideas developed during the reasoning process.

The facts in science are based on those events that can be directly empirically observed (Graziano and Raulin, 1989). Observation therefore is the empirical process of using our senses to recognise factual events to allow them to be recorded.
An extension of facts and observations is inference, which applies to properties which cannot be observed. For example, a person's intelligence cannot be observed directly, however, through the observation of behaviour (facts), it can be inferred or deduced. Inference, when used in science, therefore defines the process in which conclusions are reached from carefully observed empirical facts or other ideas. The inference therefore is part of the researcher not the subject.

Non-observable inferred events, such as gravity or electricity are rational ideas that have been constructed by the researcher, and as such are not facts. Such ideas created by the observer are therefore called constructs, and are used as if they exist in fact. Fact and construct terminologies are sometimes erroneously used as synonymous terms - constructs are related to observed facts by being derived from observations, and serving as a basis for the prediction of future observations and facts. Construction itself has been the subject of careful study (Kelly, 1955).

8.2.3 Conceptual Models in Science

The process of knowledge acquisition in the scientific approach involves the to and fro movement of empirical observation and rational abstraction by the observer. The results of rational abstractions, or constructs, are continually refined by further rationalisation of further empirical observations. It is as a result of this interchange that explanations about the relationship of facts and constructs are slowly created. In other words, a model to represent reality is born.

In science, a model is used to depict a miniature representation of reality or phenomenon that exists, and generally assists us in
understanding something that is frequently very complex. It is not meant to duplicate reality.

Models can be developed to represent any aspect of knowledge about which further knowledge is desired. Through the use of models, hypotheses can be tested and relationships between component parts observed, with the outflow usually being new ideas about how reality operates. Models can be physical in their construction, such as in model boats in a wave tank, or can be abstract, such as conceptual models, constructed of ideas and expressed verbally or mathematically. To be useful, models do not necessarily have to be real or true, provided they make accurate predictions about relationships between observed events (Graziano and Raulin, 1989).

All models therefore share four important features (a) they are constructed representations of the real world, having close correlation with some aspects of the reality being represented; (b) they are convenient and manageable (compact) representations of a larger, complex and mainly unknown reality; (c) they are incomplete, tentative and analogical, capable of being redefined in the event of new knowledge; and (d) as a result of information organisation, models help us illustrate relationships, create new ideas and predict new observations (Graziano and Raulin, 1989).

Possibly the fundamental major goal for science is the development of solid theory that will allow organisation, prediction and explanation of natural phenomena. By theory, it is meant a "formalised set of concepts that organises observations and inferences and predicts or explains phenomena" (Graziano and Raulin, 1989 p.31). Theories must therefore be carefully constructed from empirical observations, constructs, and deductive and inductive argument. They result from a bringing together or integration of what has been learnt about a particular phenomenon.
Theory and model are sometimes used interchangeably, but according to Marx (1963, cited in Graziano and Raulin, 1989), a model usually does not do well in predicting new observations or in explaining phenomena as it is only a representation of reality. A model can be referred to as a minithesis and is thus a stage in the development of a theory.

8.2.4 Induction vs Deduction

Science is therefore a combination of two markedly opposite kinds of thinking (Chalmers, 1976). Empirical observation of signs or relationships are inducted, inferred or concluded to produce general laws and theories that cannot directly be observed. From these laws and theories, predictions and explanations can then be made about future observations by the process of deduction. Deduction is therefore the opposite to induction and involves inferring from the general to the specific.

Inductive and deductive thinking are rational processes used routinely in science. The researcher generally begins with empirical observations from which constructs are induced by the process inductive reasoning. Induction therefore allows partial conclusions and alternatives to be appreciated.

These constructs form the base upon which predictions are made about new and specific observations by deductive reasoning. Deduction therefore allows the conclusions to be assessed as entirely conclusive or entirely inconclusive.

Whilst such thought processing occurs naturally in every day life, science requires unequivocal precision in the process. The systematic inductive-deductive process of science therefore uses the best
empirically observed facts to carry out research that will result in fact production that is scientifically sound and therefore acceptable. The making of accurate empirical observations is therefore the crux of the inductive-deductive scientific approach.

8.3 The Method of Science

The method employed in the human activity of science is dependent upon three important features, the origin of which can be traced back to the history of the development of science (Checkland, 1981). These features are reductionism, repeatability and refutation or falsification.

8.3.1 Reductionism

Science acknowledges the world to be so complex and varied that in order to carry out investigations that do not fall apart, it is necessary to simplify this world. This is achieved by selecting the items of interest so that experimentation can be carried out on a reduction of the world.

Such an approach relies heavily on the ontological view of science that there is a singular objective view of the world that can be meaningfully divided into subparts without altering the reduced component. This outlook has evolved largely from the work of Descartes, who believed that problems should be broken down and analysed component by component. In other words, the thinking behind science is analytical (Checkland, 1981).

The experiments involved in science are a unique kind of observation, where the experimenter, by means of reduction aims for complete control over the investigation. Such a requirement therefore allows any changes which may occur in the experiment to be seen to be a result of his actions, rather than the result of complex
interactions about which he is unaware. Only after such control can questions be asked and theories laid down about nature.

The reductionism of science is considered valuable in explanation, and Ockham’s examination of inductionism highlighted the importance of observation as being the key to the discovery of facts about the world (Checkland, 1981). He also wrote that entities should not be multiplied unless necessary and that faced with alternative explanations, accept the simpler one. These thoughts have been termed Ockham’s Razor and are fundamental in the framing of scientific explanation.

The reductionism of explanation in science involves the explanation of complex phenomena in terms of simpler ones. This extension of Ockham’s Razor is highlighted by the view that biology can be explained in terms of chemistry, and chemistry in terms of physics. Therefore, reductionism is made up of a hierarchy of sciences, which implies various levels of complexity of higher or lower order. Reductionism therefore is associated with examining lower orders in an effort to gain knowledge about higher orders.

8.3.2 Repeatability

The repeatability of experiments in science separates scientific knowledge from other types of knowledge. As the knowledge of science is public, it must be accepted if it can be shown to be repeatable. This distinguishes it from knowledge about music or religion, which is private and varies from person to person, as we have the choice whether to accept the knowledge or not.

Closely linked to the repeatability criterion for science is the importance of measurement - measured values can be recorded and repeated more easily than subjective or qualitative values.
However, it is the "happenings of the experiment" that is the aspect that "has to be accepted" (Checkland, 1981 p.53). The theory that makes the experiment meaningful and the interpretation of the results can be disputed, but so long as disinterested parties can repeat the experimental happenings, then they count as scientific. As Checkland (1981 p.53) writes "It is the repeatability of the experimental facts which places this knowledge in a different category to opinion, preference and speculation. It gives the activity of science a solid core which is unaffected by the irrationality, the emotionalism and the foolishness of human beings - including scientists, who are not less human than any other group."

8.3.3 Refutation

The third major criterion which defines science is that of refutation or falsification. In science, proposed speculative theories are rigorously and ruthlessly tested by observation and experiment. Those theories that fail to stand up to this, that is they are falsified or refuted, are discarded and further theories developed. These can in turn be tested by others and refuted, so that only the fittest theory survives. And significantly, whilst a theory can never be said to be true, it stands until another better theory is available (Checkland, 1981).

The method of science therefore involves the selection of a section of the world the scientist wishes to examine; making a reduction and designing an artificial situation to examine certain variables while keeping others constant (the experimental design will make sense in terms of a particular view or theory about the part of the world being investigated); and describing, analysing and interpreting results before being critiqued by fellow scientists.
Hull (1959) writes that for this scientific method to be useful, certain qualities are required in the researcher. These include a deep understanding of previous science, alertness, power of observation, ability to see connections between disconnected facts, imagination in forming the hypothesis, experimental ingenuity and mechanical skill to implement it.

8.4 The Methodology of the Science Paradigm

The method of science (reductionism, repeatability and refutation) is what is used in order to gain understanding and knowledge, whilst the methodology of science concerns how the method is applied and for what purposes. Methodology therefore partly deals with abstraction and reflection on past experiences, while method deals in concrete and deals with present and future.

Method is an orderly way of proceeding that we know will result in a solution, while methodology is a structured approach incorporating methods, but with no guarantee that it will actually work. The relationship between methodology and method is hierarchial, with methodology bringing a higher level of abstraction than method (Bolke, 1989).

The complex methodology that is science is diagrammatically represented in Figure 8.1, which shows the conventional or scientific approach as described by Guba and Lincoln (1988).

There are effectively two separate parts to the methodology detailed in Figure 8.1- discovery and verification. Discovery is loosely defined as the elements of history, contextual press, activities and insights that lead an inquirer to posit a theory and its concomitant deductive hypothesis. Verification can be defined as the set of activities or processes by which the hypotheses arrived at through discovery are
tested empirically through reduction, repetition and refutation (Guba and Lincoln, 1988).

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**Figure 8.1. The methodology of conventional inquiry (Guba and Lincoln, 1988)**

For the positivist, that is the scientist who is interested in only positive facts and observable phenomena, only the verification area of the methodology is considered real science. Discovery in the conventional paradigm is considered as a precursor to inquiry. As a
result, theories and their associated hypotheses need not necessarily be derived from facts, but can instead be derived from tacit knowledge, intuition or hunches. The important matter is whether or not the hypotheses can be sustained when they are referred to Nature for judgement (Guba and Lincoln, 1988).

Verification can be seen to specify a certain pathway. The theory that comes out of the discovery phase which directs the inquiry may give rise to a large number of hypotheses or questions. A selection must then be made from the various hypotheses and is based upon the inquirer’s interest, heuristic value, the possibility of posing critical tests and many other factors. A design is then detailed that will test out the hypotheses selected.

As the hypotheses are stated deductively (a priori) and are usually expressible in quantitative form, the design selected is invariably statistical in nature. The design is also selected on its relative ability to delineate true and false hypotheses with the smallest sample size, and for the inquirer’s ability to manage the conditions of the inquiry in a way that the assumptions underlying the statistical tests will be satisfied.

8.5 The Research Process of the Science Methodology

The research process of the science methodology involves a trio of specifications which must be closely adhered to if the outcomes of the study are to be meaningful. The first specification is that the inquiry process must be carried out under controlled conditions. This evolved from the idea of reducing the study to a level that can be "taken home" by the researcher and studied in isolation (Checkland, 1981). In general, this is achieved through the use of a laboratory or controlled environment facility, for anything less may allow true
experimental design to become quasi-experimental (Guba and Lincoln, 1988).

Secondly, the instruments used to objectify the measurements must be beyond manipulation or misinterpretation. Such instruments must be independently standardised and normed to assure accuracy.

The third and final requirement of the inquiry process is that sufficient samples are used so that adequate representation of the real world situation is achieved. Random samples are normally used, however, where specific areas are being examined, random samples must be drawn from within this specific area. Control and treatment groups are then selected randomly, as the integrity of the statistical techniques employed rely on random selection of the object material.

The inquiry process must closely follow the experimental plan, and where deviations occur, the experimental design is such that remedial measures can be made to allow for these, or at worst, the error may be enlarged to a point where "real" effects become undetectable (Guba and Lincoln, 1988). Following the completion of data collection, and generally not before, data analysis may commence, according to the procedures (usually statistical) outlined in the experimental design. These analyses are intended to determine the degree of correspondence of the findings of the sample or model to the real world that it represents.

The results of these analyses then allow comment on the hypotheses by way of falsification rather than verification. That is, the null hypothesis is accepted or rejected and conclusions relevant to the hypothesis and the guiding theory are drawn. However, as Guba and Lincoln (1988) point out, the rejection of the null hypothesis in science does not assure that the alternative hypothesis is correct.
Following the completion of data analysis, a technical report or communication is then developed, detailing the findings, the competence of the investigator, and as well, the implications for the theory. The theory is reputedly altered to take into account what the data shows if indeed the data is beyond challenge.

In general, the same or similar studies are carried out through a process of recursive self-correction until a stable body of evidence exists which supports the refined theory. This pathway is intended to assure the validity of the theory and because it is internal, is in essence closed to new data that may suggest that a different theory is valid. When this process is carried out sufficiently so that theory and its implications may be considered established, its propositions may then be regarded as having the form of universal laws (Guba and Lincoln, 1988). These laws will have application to the population from which the samples were drawn.

The central activity of the methodology, that is the making of empirical observations within a systematic rational process, characterises science as a method of acquiring knowledge that is different to others. It is a systematic, cyclical process through rational thinking and so on that makes science unique.

8.6 Systems Thinking

The history and evolution of systems thinking is documented in several texts (Bertalanffy, 1972; Ackoff, 1973; Churchman, 1971 and Checkland, 1981). Wilson (1988) provides an introductory review of systems thinking and its relevance in the agricultural context. Only relevant areas of systems thinking will be presented, including the emergence of systems thinking and how it differs from science.
8.6.1 Emergence of Systems Thinking

An eagerness to understand and account for the workings of the real world, or in other words nature, has lead to the development of many processes for the acquisition of knowledge, ranging from the various religions to science (Graziano and Raulin, 1989; Checkland, 1981; and Hull, 1959). These various views differ from each other according to their particular world view or set of assumptions about the nature of reality (Open University Press, 1984).

Whilst some approaches require a rigid set of procedures or methodology to be adhered to, others allow a variety of approaches. It is however the underlying philosophies that directs the activities of a methodology used by researchers. This underlying philosophy is termed the "world view" because it represents the view of the world of the people using the methodology.

The German word weltenschauung is used in preference to world view in systems thinking. Literally translated, weltenschauung means a philosophical survey of the world as a whole, and so includes components of beliefs and attitudes that impose upon the people involved in the methodology. These components that affect the world view may go unnoticed as they are often part of a persons culture. However, weltenschauung demands them to be acknowledged.

The weltenschauung of science includes the idea of causality, objectivity, experimental testing and the underlying philosophy that things are knowable. Also included in this weltenschauung is the notion that science is 'neutral' or value-free, and can therefore be applied universally as an investigatory tool for any natural phenomenon (Open University Press, 1984).
Science is argued to be based on a hypothetico-deductive method. This involves the scientist starting by creating an hypothesis (a tentative idea that connects together known facts), which is then studied and from which deductions are made about some new fact which would have to be true if the hypothesis was true. The scientist then carries out experiments to make observations to test his theories. This process in science closely entails the idea of reductionism, whereby explanations of the whole are sought in terms of explanation of components at a 'lower level'.

8.6.2 The Notion of Complexity

"The crucial problem which science faces is its ability to cope with complexity" (Checkland, 1981 p.59). Complexity implies that something has a wide range of behaviours, or is unpredictable or both. Complexity is therefore related to predictability and to our understanding of phenomenon.

Science has coped with complexity through the division of a problem into separate parts, which makes the assumption that such a division will not distort the phenomenon being studied. In a restricted science such as physics or chemistry in which a limited range of phenomena are studied, it is possible to conduct well defined reductionist experiments in a laboratory and it is possible that hypotheses expressed mathematically can be tested in quantitative measurements (Checkland, 1981). In an unrestricted science such as biology or geology, the effects under study are so complex that designed experiments with controls are not possible.

Science also has problems in the unrestricted sciences termed social sciences, such as anthropology, economics, sociology and political science, which are concerned with the mutual relationships of people. The complexity of the subject matter and the nonavailability
of experimental objects in the social sciences makes it difficult to achieve a reduction required to allow meaningful controlled experiments.

The special nature of the phenomenon studied in social sciences also creates problems for the scientific approach. Checkland (1981) lists three key problem areas - (a) generalisations will be imprecise as the variety of possible viewpoints in the social science area is far greater than in natural sciences; (b) the subject of study for the social scientist is the human, who even if depersonalised, will still be an active participant in the phenomenon being investigated, attributing meaning to the investigation and modifying the situation in a potentially unique way; and (c) it is difficult to make predictions about social systems, because they can react to these predictions, whilst physical systems cannot.

8.6.3 The Problem of Choice

The final issue that science struggles with when applied to the social situation is that the real world problems it addresses are of a kind that involves decisions between options or choices. These problems are as Checkland (1981) puts it, problems of management, which means deciding to do or not to do something, planning, considering alternatives and so on.

The major "disabling characteristics" of the conventional positivist approach, as it is applied to social sciences were outlined by Guba and Lincoln (1990). These characteristics included the fact that the science approach was (a) absolutist in character - any proposition must be true if correspondence exists between the proposition and that aspect of reality which it is intended to describe (human judgement plays no role - the data speaks for itself); (b) objectivist character - all entities that may be studied are defined as objects, including humans, and
these objects obey certain natural laws and are determined by them; (c) disempowering character - it is solely the inquirer's province to decide about the propositions to be studied and the methodology to be studied, an approach that exploits the subjects (humans) and subverts their interests to those of the inquirer, therefore ignoring human input and tending to maintain the status quo; and (d) the belief in a final or ultimate truth may evoke unethical practices, while the manipulative character of the conventional positivist paradigm denies the rights of the individuals involved to choose their own fate.

The recognition of these areas, especially as they apply to complexity in biology and to the social sciences, led to the search for more effective methods of coping with this complexity. There are numerous ways of coping with complex situations in the various professions or crafts, with each tending to be linked with the subject matter. However, because of a lack of common language and intercommunication, basic similarities in approach have tended to be obscured.

The fact that similar methods of coping with complexity coexisted but in different language is due to the fact that the problems faced had essentially similar elements. The detection of these underlying features that were similar in many areas formed the basis of systems thinking, and is outlined by Checkland (1981). The relationship of these systems approaches and science is represented in Figure 8.2.

8.7 The Underlying Tenets of Systems Thinking

The weltenschauung of systems thinking is critical in this approach, and directs the attention of the inquirer towards wholes or systems, rather than components. The focus of inquiry is therefore on complete entities and on interacting systems, so that a system is not
studied in a laboratory but rather in its environment (Open University Press, 1984).

The study of wholes is part of the philosophical approach known as holism. Holism concerned itself with the notion that assemblies of component parts when organised together in special ways can reveal unique emergent properties that are not possessed by the parts alone. This idea is fundamental to the systems Weltenschaung. If a system or whole entity possessed a particular property when it was entire which disappeared when it was broken down or reorganised, then this property of the system can only be studied and understood by study of the whole system - not by analytical study of its parts.

Figure 8.2. Systems theory and approaches and their relationship with the sciences (Open University Press, 1984)
There are thus four fundamental tenets of systems thinking. Firstly, that systems only exist in the mind of the inquirer; secondly, these creations are only useful if they improve our chances of exploring how key elements interact with one another; thirdly, one must assume that these relationships are best understood as being non-linear; and finally, our imagination is used to define the boundary of the system. Systems thinking is therefore concerned with wholes, organicist ideas (that is, vital substance) and emergent properties, as compared to science which is concerned with parts, mechanistic concepts and properties of individual parts (Open University Press, 1984).

As Checkland (1981) puts it, the key concepts behind systems thinking are two pairs of ideas. Firstly, that of emergence and hierarchy - all systems contain subsystems, and are themselves subsystems of wider systems. There are different emergent properties at each level of such a hierarchy, properties that are not exhibited by any of the parts of that particular level. And secondly, communication and control - the how, who, why, what, where and when factors, or in other words, the processes taking place within the system.

A system therefore can be defined as an assembly of parts connected in an organised way that has been identified by a human being as of special interest and that behaves in some way (Open University Press, 1984). A system having emergent properties and a hierarchial structure survives in a continuously changing environment by means of communication and control, where the system and its environment are logically inseparable (Martin, 1987).
8.8 Systems Classification

Systems may be classified according to their behaviour and their outcome. Checkland (1981) has distinguished between types of systems according to the nature of their purposes, whilst acknowledging that there is a spectrum of activity from relatively hard to soft. Hard systems are characterised by easy to define purposes, clearly defined decision taking procedures and quantitative measures of performance. Soft systems are those in which purposes are difficult to define, decision taking uncertain, measures of performance at best qualitative and there is involvement of humans with irrational behaviour.

The approach for hard systems according to Checkland (1981) is to acknowledge a given system and ask how its performance can be improved. These hard systems are purposive or goal seeking and the methodology followed systematic, that is, logical and sequential, rather than systemic or holistic.

The approach of soft systems on the other hand is to acknowledge a complex situation and determine what relevant systems may be manipulated to improve the situation. The approach is therefore purposeful or goal setting and models are used as vehicles for debate about desirable and feasible change, rather than as indicators of performance.

8.9 The Methodology of Systems

According to Checkland (1981), hard systems methodology is the systems approach used for dealing with real world problems in which an objective or end-to-be-achieved can be taken as given. Hard systems thinking is about achieving a stated objective, or optimising. Conversely, soft systems methodology entails the systems approach
for tackling real world problems in which known-to-be-desirable ends cannot be taken as given. Soft systems methodology is based upon a phenomenological stance, that is, the mental processes of learning.

Checkland (1985) details how the work of Vickers helped stimulate the development of soft systems out of hard systems through the rehumanisation of the word systems in the concept of appreciation. A concept of appreciation rejects both the goal seeking model and the version of the cybernetic model in which standards are set from outside the system. Vickers conceived the idea of the appreciative system, a soft systems methodology with a cultural mechanism that maintains desirable relationships and eludes undesirable ones (Checkland and Casar, 1986).

Hard systems methodology therefore considers goal seeking to be an adequate model of human behaviour and involves the language of problems and solutions. It seeks to emulate the rules of science in a systemic context. Soft systems methodology, on the other hand, regards systems models as models relevant to arguing about the world, not models of the world, and replaces optimising with learning and talks the language of issues and accommodation rather than solutions. The relationship between the two methodologies is described in Checkland (1985).

Soft systems methodology was developed because the methodology of systems engineering did not work when applied to messy ill-structured world problems (Checkland, 1985). The seven stage process of analysis that is the Checkland Methodology, which evolved out of the soft systems methodology, is described in great detail in many texts (Checkland, 1981; and Wilson, 1984). The Checkland Methodology uses the concept of the human activity system as a mechanism of moving from finding out about a situation to taking
action, and is an integral part of the Hawkesbury approach of the Faculty of Agriculture.

8.10 The Hawkesbury Approach

The complex process of evolution that has taken place in the teaching paradigm at the Faculty of Agriculture is documented in many areas (Bawden et al., 1984; Bawden et al., 1985; Bawden and Valentine, 1984; Packham, Roberts, and Bawden, 1989; and Macadam, 1988). Central to this change towards the Hawkesbury approach was the concern that agricultural teaching practices created agriculturalists that were overly specialised in their knowledge base; were taking a restricted and static view of naturally complex and dynamic agriculture (Macadam, 1990); and were still focussing on the "desired end" of productivity growth rather than on land use patterns that were "ethically defensible" (Bawden and Packham, 1993).

Instead, a new type of graduate who would take a holistic approach to social, economic and production aspects of agriculture was desired (Packham et al., 1989). These agriculturalists would be developed through the Hawkesbury approach, which involved education by using knowledge gained from research into how people learn (in a learning environment designed to optimise such learning) using the systems approach.

Indeed, this was the environment in which the research reported in Part A of this thesis was carried out. This environment resulted in my reflection on both the philosophy on which my practice was based and on my own learning. This reflection is described in Part B of this thesis.

The basic assumptions of the Hawkesbury approach are its relativist ontology (reality is not out there and therefore cannot be known in a
time and context-free sense; nor can it be independent of the values we put on it); a systems epistemology (nothing can be viewed in isolation or in reduced form without potentially altering its meaning); and a methodology that utilises experiential learning.

8.10.1 Experiential Learning

In accordance with Reason and Rowan (1981), the Faculty of Agriculture considers it useful to distinguish between three interconnected ways of learning - propositional (learning for knowing), practical (learning for doing) and experiential (learning for being). A fourth type of learning, intuitive learning (learning for fitting in) was later added to the triad (Bawden, 1990). An effective learner is one who is able to utilise all four models, however modern teaching strategies have placed much emphasis on propositional and practical learning, while almost completely ignoring experiential learning.

Experiential learning can be "considered to be the learning which occurs while individuals are immersed in, and are having an effect on, complex real world or simulated situations. It involves the senses, emotions as well as the intellect" (Richards, 1984 p.1). Kolb (1988) defined experiential learning as a process whereby knowledge is created through the transformation of experience.

The role of experiential learning initially makes the learner more aware of the process of learning. Ultimately though, it allows the creation of a repertoire of learning and problem solving methodologies which are consciously controlled by and work for an individual. The experiential learner believes that what we experience, how we perceive these experiences and the meanings, values and theories we credit them, will determine the actions we
take. It is therefore a theory-informed practice or praxis that is involved in experiential learning.

Several models of experiential learning have been described (Richards, 1984), however, it is the model developed by Kolb (1988) that is used at the Faculty of Agriculture. Experiential learning is a dynamic process whereby there is a flux between sensory experiences of the world and the mental abstractions of them. The learning comes out of the experiencing and making sense of the experience, and both are highly personal and idiosyncratic.

The experiential learning model suggests that the starting point is the emersion of self in concrete experience. Each phase involves different talents of the learner. Firstly, the learner needs the ability to diverge or to spread the initial inquiry as broadly as the learner deems acceptable. Reflective observations must then be assimilated - that is, the divergent knowledge developed regarding the issue of concern, must be converted into patterns in the mind or constructs. These images are then used to generate models for testing through a process of convergence. And finally, the learner must accommodate the use of these models in ways which are useful to achieving action.

Importantly, such a model of learning is just that - it is a model. While in many situations, the starting point for experiential learning is concrete experience, it is not always the case. Likewise, movement between phases is not always sequential and there can be oscillations between stages.

The experiential learning model suggests that the effective learner will be open to new experiences and when faced with a problematic situation, will develop a rich picture of the problem context and so will avoid false or premature problem definition. New concepts are assessed after first going through a diverging and assimilating
process, and will then be assessed and utilised for practical value in developing plans for improving a situation (Bawden, 1990). The experiential learning model is therefore a powerful tool for communicating the need to combine theory and practice in improving problem situations (Macadam, 1990).

Learning is very personal. That is, the individual experience and the way sense is made of it, along with the actions deemed appropriate to take are unique to the individual. What is learnt is largely determined by the individuals weltenschauung, such that one's view of reality is dependent on one's 'window of the world' (Checkland and Davies, 1986).

To be more effective in learning, individual biases or preferences that exist in an individuals learning style must also be addressed (Kolb, 1988). Where certain techniques feel more comfortable, the individual will frequently spend additional time there to the detriment of the other learning areas. The addressing of such biases is critical to our effective learning and learning how to learn. Effective learning therefore occurs at two levels - firstly, the learning about the issue, and secondly, the learning about the learning process in understanding issues or metacognition (Sriskandarajah, Bawden and Packham, 1989; and Kitchener, 1983).

In Chapter 9, my experiences of learning about the issues reported in Part A of the thesis and my learning about the process of learning which paralleled these experiences are reviewed. In addition, the influence of my weltenschauung on the process is discussed.

8.10.2 Systems Thinking and Learning

The Faculty of Agriculture has recognised that to deal with the complex issues of contemporary agriculture and rural development,
and to focus on the inter-relationships between people and their natural and sociocultural environments, a methodology that could accommodate the wholeness of issues being studied is required. This systemic or holistic approach contrasts with the classical approach to agricultural problems based on reductionism, yet still allows reductionist methodologies to be utilised once the whole situation is perceived (Sriskandarajah et al. 1989).

The systems models utilised at Hawkesbury are not models that describe or predict the world, but are rather vehicles for debate about desirable and feasible changes in it (Sriskandarajah et al. 1989). The emphasis of research has been shifted away from an object out there to issues and ways of viewing these issues (Ulrich, 1988; Bawden and Packham, 1993). Additionally, the Faculty has developed the distinction between hard and soft systems first proposed by Checkland (Bawden et al., 1985) whereby the issue determines the system.

Holism and reductionism are two opposing ways of examining the same reality. When integrated into the experiential learning process, they add a third dimension or set of polar opposites. That is to say, the relationships of integration and separation is the same as sensory perception to mental abstraction. This has been detailed in several papers (Bawden, 1987; Bawden, 1990; and Sriskandarajah et al., 1989).

It is this synthesis of these two key notions, experiential learning and systems thinking, which provides the framework for the Hawkesbury approach to learning and researching.

8.10.3 The Hawkesbury Hierarchy

Researching is learning with the special intention of adding to public knowledge (Bawden, 1990; and Sriskandarajah et al., 1989). In carrying out research, therefore, an attempt is being made to understand the
nature of the world and to share its propositions. Whether it is problem solving using traditional reductionist methodologies or improving human situations through social sciences, research processes can be seen as a variation in method on the same basic theme of experiential learning (Bawden and Packham, 1993). This is well represented by Figure 8.3.

Figure 8.3. The interconnectiveness of learning subsystems (Sriskandarajah et al., 1989)
Figure 8.4. A systems hierarchy of learning in each system of inquiry (Bawden and Packham, 1993)

The connectiveness of the learning process through integration towards holism and separation towards reductionism, brings about a continuum of methodologies of inquiry processes stretching from holism to reductionism. This hierarchy of interconnected methodologies or Hawkesbury spiral is documented in many places (Packham et al, 1989). More recently, each inquiring system of the Hawkesbury spiral has been described to possess its own learning, metalearning and epistemic learning dimensions illustrated as loops but conceptualised as a hierarchy of systems and subsystems within the total suprasystem of human inquiry (Bawden and Packham, 1993). This is illustrated in Figure 8.4.

The level of inquiry and therefore the selection of appropriate methodology by the researcher (learner) is dependent on the nature of the problem situation. The hierarchical spiral depicts four levels of
learning/researching process that are appropriate to ranges of issues of decreasing complexity and uncertainty. The open ended model also indicates possibilities for new approaches to learning as this dynamic praxis generates new ideas (Sriskandarajah et al., 1989).

Each level of inquiry provides a perspective for the subsequent level, allowing clearer focus, while each lower level provides insights for the higher levels. The integration/separation relationship that connects these levels of inquiry methodology must remain consistent with the rules of systems. Movement down the methodologic hierarchy from whole to parts is possible (separation), while the reverse (integration) is not directly possible. Instead, reductionist science can only provide insights into the wider situation that can be studied by a more systemic approach.

8.10.4 Action Researching Systems

Learning systems or action researching systems are integral to the Hawkesbury approach. Action research involves learning with the special intention of achieving social action whilst at the same time adding to public knowledge. Central to it is the initiation of an activity based on available information with the built in flexibility to modify the activity through continual testing, monitoring and adding new information (Grundy, 1986).

Action research is about the improvement of social conditions of existence, and where the improvements are not to be imposed upon the participants, but rather the participants themselves are to be the controllers of the improvement process. The epistemology therefore of action research is in improvement and involvement (Grundy, 1986).
The action research methodology deals with immediate practical problems. It takes into account peoples values, attitudes and beliefs; it combines thinking with doing; it enables reflection, self-growth and learning as you go; the answer is not paramount; it empowers people to learn how to make their own changes; it is specific to a particular problem, not a generalised solution to a problem; and it is emancipatory and critical (Pinn, 1989 pers comm.). In other words, action research takes into account the values of the participants in the soft situation and allows better understanding and knowledge to be developed.

The process of action in action research consists of a number of moments which are reciprocally related to one another - action and reflection; and through organisational moments - planning and observation (Grundy, 1986). The similarities therefore to the experiential (action) learning methodology are apparent.

For an inquiry to be classified as action research, the project must firstly take social practice as its subject matter, with strategic action susceptible to improvement. Secondly, the project proceeds through a spiral of cycles of planning, acting, observing, reflecting - each activity being systemically and self-critically implemented and interrelated. And thirdly, the project involves those responsible (Grundy, 1988).

Additionally, there are three modes of action research - technical, practical and emancipatory (Grundy, 1988). Technical action research involves making action, while practical action research involves doing action and emancipatory action research involves freeing up the action following the release from critical intent.

It is through this process of participatory action research that the Hawkesbury approach allows integration of research, education and extension functions (Macadam, 1990). Participatory action research is
based upon mutual respect and collaboration in gathering, processing and sharing the relevant information and upon initiating activities in a more timely manner with participation of all concerned.

The Faculty believes that ways of researching need to be developed which combine finding out about complex and dynamic situations with taking action to improve them in such a way that the actors and beneficiaries of the action research are intimately involved as participants in the whole process. In other words, research is undertaken with the farmers and rural communities rather than for or on them. The action research must therefore be participatory (Sriskandarajah et al., 1989; and Bawden, 1990).

8.10.5 The Ten Tenets of the Hawkesbury Approach

The espoused working philosophy at Hawkesbury can be summarised in the form of ten tenets that underpin the learning environment:

1. Self-directed learning as a basic human competence;
2. Learning as a life-long process of 'meaning making' involving the whole person;
3. Problem solving as a creative learning process;
4. Experientialism as the base of effective learning;
5. Facilitation of learning as a most desirable role for the academic;
6. Integrating perspectives as crucial elements in effective curricula;
7. A clear intellectual map of agriculture as the production interface between people and their environments;
8. A systems approach as a vital perspective for making sense out of complex realities;
9. Farming as a human activity system or agroecosystem;
10. Definable competencies as appropriate characteristics of life roles.
CHAPTER 9

Reflections - Hawkesbury's Influence on Learning and Researching
9.1 Introduction

Whilst some of the influences of the Hawkesbury approach have been realised following exposure to the teaching paradigm, there are likely others yet to be appreciated. Those effects that have impacted at a level to be consciously recognised will be reflected upon, while those that remain unrecognised await later discovery.

Exposure to the learning approach at the Faculty has led to the appreciation of three important concepts. Firstly, that knowledge acquired from reductionism or science must be accepted with an appreciation of the limitations inherent in the methodology. Secondly, the reductionist science methodology is not suitable for all areas of research and that other more appropriate mechanisms of knowledge acquisition can be used in its place. And finally, the Hawkesbury approach has allowed the development of a better understanding of where science and scientific knowledge 'fit' within the lived in world.

Some of the thoughts stimulated as a result of undertaking scientific reductionist research within the framework of the Hawkesbury environment have been put into writing in the next few pages. The contrast in ontology, epistemology and methodology of the reductionist approach used in the equine and canine studies and the phenomenological approach used by the Faculty for educational growth generated confusion and apprehension. However, the contrast stimulated learnings which enabled the development of an appreciation of the advantages and disadvantages of the two approaches, along with an appreciation of the broader setting in which the reductionist research was actually founded.

In the chapter that follows, the major learnings arising out of the Masters program will be detailed. In particular, the increased
knowledge of the learning process, the understanding of different levels of knowledge and the appreciation of systems thinking will be examined in reference to how they have assisted the development of a clearer appreciation of reductionist research and science. In addition, familiarity with the systems methodology and the experiential learning process has enabled modelling of an improved methodology for undertaking biologic research, which increases the power of the reductionist approach.

9.2 Changes in Learning Style

Perhaps the most important learning outcome to arise from reflections on the studies undertaken was an improved understanding of the learning process. The realisation of the simplicity of the learning process, following introduction to the Kolb cycle for the first time after some seventeen years of education, was overwhelming.

Initial reaction to the simplicity of the Kolb cycle was scepticism, as knowledge gained through authority, rationalism and empiricism had appeared adequate. This scepticism stimulated the completion of the Learning Style Inventory, as developed by Kolb (1988) on several occasions during the Masters program.

Recognising that this inventory is designed to describe how an individual learns, rather than their ability to learn, the results would be valuable indicators of what is happening in the subconscious. With learning and growth, responses to questions would change as views and feelings change.

The learning inventory was completed on first arrival at Hawkesbury and then at one year intervals thereafter, which included the time of
involvement in the Masters program. The results of these four assessments can be seen in Table 9.1 and Figure 9.1.

| Table 9.1. Learning Style Inventory results (adapted from Kolb, 1988) |
|------------------|------|------|------|------|
| CE (concrete experience) | 17     | 13     | 17     | 12     |
| RO (reflective observation) | 13     | 19     | 7      | 9      |
| AC (abstract conceptualisation) | 14     | 15     | 10     | 16     |
| AE (active experimentation) | 16     | 15     | 21     | 17     |
| AC-CE (extent to which abstractness emphasised over concreteness) | -3     | 2      | -7     | 4      |
| AE-RO (extent to which active experimentation emphasised over reflection) | 3      | -4     | 14     | 8      |

Figure 9.1. Learning Style Inventory results (adapted from Kolb, 1988)
In July 1988, the learning style was borderline diverger/accommodator, indicating a preference for dealing with people. The diverger is sensitive to peoples feeling and values, listens with an open mind, gathers information and can imagine the implications of ambiguous situations. The accommodator on the other hand commits himself to objectives, seeks and exploits opportunities, influences and leads others, is personally involved and deals with people (Packham and Bawden, 1989).

The diverger has great strength in being able to view concrete situations from many perspectives, and is skilled at generating ideas. The accommodator is skilled at carrying out plans and experiments and being involved in new experiences. The accommodator is adaptive to the needs of specific immediate circumstances. This description of learning style appeared quite accurate, and so the credibility of the Kolb learning cycle was increased.

One year after joining Hawkesbury and 6 months after getting involved in the Masters program (July 1989), the inventory signalled a change towards strong divergence. Whilst active experimentation was favoured over reflective observation, abstraction was only weakly favoured over concrete experience. The emphasis in the learning approach had shifted dramatically to one concerned more with abstract conceptualisation where logical thinking and rational evaluation were heavily relied upon. Associated with this, reflective observation had a greater involvement in the learning style than active experimentation.

Such a learning approach corresponded with a time of apprehension about the Faculty and its approach to education, and frustration about the problems encountered with the equine and canine studies. The increase in reflective observation score indicated a tentative,
impartial and reflective approach to learning, relying on careful observation before making decisions. It signified a development of introversion in the learning style inventory.

The July 1989 score appeared to reflect a negative effect arising from involvement in the Faculty paradigm, along with the mechanical problems experienced in the equine and canine studies. This promoted a rebound stimulatory response to undertake a more positive approach and improve the other components of learning (accommodating, converging and assimilating). A change in personal attitude appeared necessary and to achieve this, the Hawkesbury approach had to be perceived in a positive and non-threatening way.

Reassessment of the learning inventory one year later (July 1990) indicated positive changes, which were interpreted as being signs of successful modification of learning style. The learning preferences clearly fell in the accommodator section. Gone was the tentative, impartial and reflective approach that surfaced in the previous year. Reflective observation had reverted from a high level back to a low level, while concrete experience and active experimentation had resumed importance.

The strong improvement in the active experimentation component was considered to be a result of positive response to new experiences, plans and experiments. Underlying this however, was a greater acceptance to take risks, to be more personally involved, and to influence and lead others.

The actions that led to this change in learning style involved broader reading of relevant research papers relating to learning and systems. Developing out of this was better communication about concerns and improved understanding of where the science-based studies being undertaken lay within the Hawkesbury approach, given the different
epistemology, ontology and methodology of the Faculty of Agriculture. This stimulated a new-found confidence in the Masters studies.

Adverse personal life developments resulted in a disenchantment with the Faculty and the Hawkesbury approach following the July 1990 learning style assessment. During this time, the emotional negativity engendered had adverse effects on the post-graduate studies and personal development. This period occurred around July 1991 and examination of Figure 9.1 shows that learning style had changed again to that of a converger. Abstract conceptualisation had developed while concrete experience dropped from the 1990 levels to one equivalent to the 1989 level.

Active experimentation was still an integral learning style, as it was in 1990, however more emphasis was now placed on abstraction and less on concrete experience. Convergers are recognised to be creative with new ways of thinking and doing and experimenting with new ideas. They are apt at choosing the best solutions, making decisions and setting goals. The knowledge of a converger is organised in such a way, that through hypothetico-deductive reasoning the person can focus on specific problems. This would appear to be a new development in contradiction to the earlier preference for dealing more with people than with things.

This review of learning style over the period 1988 to 1991 highlights that learning preferences changed as experience and world view changed. It is apparent that learning preferences can be modified through increased awareness of learning strengths and weaknesses.

The key to effective learning is being competent in each mode when it is appropriate, and that a totally balanced profile is not necessarily the best. The closer the Learning Style Inventory score is to the point
where the grid lines cross, the more balanced the learning style. Conversely, the location of the inventory score toward any one of the four 'corners', indicates learning style with a heavy dependence on one facet.

In summary, the learning style was initially balanced between experimentation and observation, but relied heavily on concrete experience. The learning style switched in 1989 to a balance between abstract conceptualising and concrete experience, but became heavily reflective and very short on action as a result of apprehension about the Hawkesbury approach.

With conscious effort to change this, the 1990 learning style became strongly based in concrete experience and active experimentation. In 1991, restructuring of all facets of life brought about a shift in learning style to one that still relied significantly on active experimentation but was more balanced between concrete experience and abstract conceptualisation.

It is apparent that learning strategies changed according to circumstance. While most of these changes occurred in the subconscious, these changes can be positively influenced by forces from without. Acceptance of the Kolb cycle and the undertaking of the Learning Style Inventory at appropriate times has been beneficial in allowing an appreciation and understanding of changes in approach to learning over time that may not have been appreciated otherwise. Such knowledge about learning is beneficial to both personal and business life as it allows improved problem solving.

9.3 From Didactic to Autonomous Learning

Reflection upon these changes in learning styles encouraged questioning of the type of knowledge that would be developed during
the association with Hawkesbury. It also prompted reflection about learning patterns prior to Hawkesbury.

The epistemologies of learning approaches have been described as a progression from teacher directed (didactic) through to learner directed (autonomous) (Bawden and Packham, 1983). The early learning style of primary and secondary schooling was very much based on teacher directed goal seeking, in which teachers provided knowledge and resources and then tested your ability. A similar learning style was carried through into tertiary education as a veterinary surgeon, but was extended to a level where experiences were provided and knowledge had to be found to solve the problem with the assistance of the teacher. Abilities of the student to solve these problems would then be assessed. By the end of tertiary education, the level of autonomous learning had been increased substantially, but there was still a strong emphasis on teacher direction.

Only after graduating did full autonomous learning abilities become realised following emersion into real-life situations. Identification of the problem, acquisition of knowledge to solve the problem and then self-evaluation of the developed problem solving ability became critical. Whilst didactic learning was still available through an employer-employee relationship, it was very much a secondary process.

This self-directed process has continued up until now, but only recently has the awareness been heightened to a point that allows identification with the process and acknowledgement of the merits of the various stages. The Hawkesbury experience has allowed realisation of the benefits to having access to all learning stages, and has reinforced the importance of autonomous learning.
Exposure to the Hawkesbury approach highlighted a one-sidedness of previous learning experiences. Knowledge levels had been consciously highly developed through propositional and practical learning, but they had not been consciously developed in the experiential and intuitive areas. The association with the Faculty allowed increased awareness about the range and benefits of learning experiences and that regardless of teaching paradigm, a balance of didactic and autonomous learning was vital.

This recognition of different learning approaches and particularly the learning for doing (praxis) approach that the Faculty espoused stimulated exploration beyond the conventional didactic approach to learning.

9.4 Epistemic Cognition and Contextual Relativism

Knowledge is recognised to be a multi-level phenomenon and has been described by Kitchener (1983) as a three level model suitable to account for the complex monitoring of ill-structured problems encountered by individuals. A similar three level cognition model has been described within the Hawkesbury hierarchy (Bawden and Packham, 1993).

Both models use the following structure. The first level is termed cognition and involves basic information processing tasks, such as perceiving, reading, speaking, computing and memorising. The second level, known as metacognition is where processes are employed to monitor the cognitive progress when an individual is engaged in level 1 cognitive tasks. This includes knowledge about cognitive tasks, such as how to memorise a list of words; about strategies that may be employed to solve the task; of when and how the strategies should be applied and about the success and failure of these processes.
Level 3 cognition is known as epistemic cognition and is characterised by the processes an individual uses to monitor the epistemic nature of problems and the truth value of alternative solutions. This includes the individual knowledge about the limits of what can and cannot be known, the certainty of knowing and the criteria for knowing. It also includes the strategies used to identify and choose between the type of solutions required for different problems.

Whilst levels 1 and 2 are developed from an early age in our educational process, level 3 is believed to surface in adult life around the time of leaving school and beyond. It is this level 3 cognition that has been further developed as a result of exposure to the Hawkesbury approach.

The Hawkesbury experience has allowed the development of epistemic knowledge about the areas of scientific research documented in this thesis and the approaches used to study them. The Hawkesbury approach is concerned with the development of epistemic cognitive processes in its students, a fact supported by faculty epistemology that incorporates the experiential learning process as a central theme behind the Hawkesbury spiral, which links the various methodologies used to approach a problem situation.

There are nine developmental positions, which can be summarised into three important stages in epistemic development in the student (Salner, 1986). These changes correspond closely with the three levels defined by Kitchener (1983). The first stage dualism involves an epistemology in which the student makes a clear distinction between self, the 'outside' world and assumes that knowledge exists out there. The learning process associated with this is authoritative and didactic.
The second stage in epistemological development is multiplicity, which arises as the dualist thinker confronts the pluralist (different points of view) social experiences. Black and white 'facts' are less attainable and make way to 'grey' areas and oneself takes on more importance as a knowledge source. Gone are single absolute truths, which are replaced by multiple truths.

The final stage is contextual relativism which arises out of frustrations encountered with the second stage. It represents the students increased awareness of the importance of contexts in defining truth and value. It seeks truth neither in the world (out there), nor in the self, but rather in the interaction between self and the world. It is this final epistemological stage, the realisation of the significance of context in defining truth and value in the scientific research, that has been appreciated as a result of the association with the Hawkesbury approach.

9.5 Experiential Learning at Work

Experiential learning was beneficial in several areas during the research and postgraduate studies. In particular, exposure to the theory of experiential learning assisted in the adjustment phase of the associate in the National Company Teaching Scheme (NCTS) by hastening the development of interpersonal relationships and the ability to deal with differing priorities and work ethics existing between the company (Nature Vet Pty Ltd) and the teaching institution (Hawkesbury Agricultural College). This eased the feeling of failing to belong anywhere, and allowed the more efficient pursuit of the work focus.

Increased awareness of the experiential learning process also engendered confidence to resolve concerns regarding relative deficiencies in knowledge of the horse industry that may have
impaired progress with the research focus. It promoted an open and more positive outlook about this short-coming, and stimulated the implementation of actions to improve the knowledge base. These included exposure to local horse breeding and spelling enterprises through a veterinary consultant of Nature Vet Pty Ltd, participating in farm visits with horse management students to studs in the Hunter Valley and general reading of literature relating to horses.

Perhaps the most important area where experiential learning was necessary was in the equine research area, including the development of the endotoxin assay and the development of knowledge about the association of endotoxaemia and diet. Knowledge was obtained from related studies in horses in which laminitis was induced from carbohydrate overload and blood endotoxin levels measured using a commercially available chromogenic LAL technique.

However, the study undertaken and presented in this thesis was not concerned with laminitic levels of blood endotoxin, but rather levels that exist in association with normal feeding practices, particularly those utilising high-grain diets to support performance activity. Additionally, the focus of the study was about the control and prevention of such changes through the use of the bentonite feed additive Nature Vet Thrive P™ for Horses.

The concerns over the lack of expertise and knowledge regarding measurement of blood endotoxin levels was countered by the optimism engendered by the commercial availability of an endotoxin assay technique. It seemed possible that the availability of such a kit would make it possible for people of limited experience to quantify blood endotoxin levels.

The COATEST® Endotoxin kit (Johnson & Johnson Medical Products Pty Ltd) was determined to be most suitable as it used minimum
volumes of expensive reagent and was highly sensitive, detecting endotoxin levels as low as 1 picogram. A highly sensitive test was considered essential because the endotoxin levels envisaged in the blood of clinically normal, non-laminic horses was expected to be very low or undetectable. Likewise, the endotoxin level in the horse receiving a high-grain ration was also expected be quite low (nanograms) and present for only a short period of time due to destruction by normal body defences.

The mechanics of the assay were assessed and fine-tuned through a number of 'dry runs' that utilised all necessary materials in the assay, except for actual reagents. No major problems were encountered in these 'dry runs', however, a decision was made to purchase a multichannel pipette to ensure better use of the microplate and to improve the accuracy of the incubation times for each step.

The assay was then tested in the 'real' world situation using plasma samples from experimental horses. More importantly, it was hoped that the results would provide a clue to the levels of plasma endotoxin expected and the timing of their appearance following feeding.

Whilst the high-grain low-roughage diet and two hourly blood sampling were well accepted by the horses and concluded to be suitable for use in a trial, the efficacy of the endotoxin assay was unable to be established. A misunderstanding had led to an inappropriately timed heat treatment of plasma which coagulated the protein and rendered the samples unsuitable for endotoxin determination. The other blood samples taken for plasma L-lactate and full blood counts were all successfully processed.

On reflection, the knowledge gained by authority in order to carry out the lactic acid assay was adequate to successfully process the samples
taken. Conversely, the experiential learning associated with my early attempts with the endotoxin testing were inadequate on a first passage through the learning cycle.

However, valuable lessons had been learnt regarding the assay procedure and the feasibility of frequent blood sampling, and the coresearching team (Woog, Whatmore and Hannon) decided it was appropriate to initiate a 12 week study using 5 horses on the basis that the procedure for the endotoxin assay would be readily learnt. Four of these horses were for Experiment 2, which was designed to determine the effects of feeding diets containing increasing quantities of oats (0%, 30%, 50% & 70%) on blood parameters including plasma lactate and endotoxin levels, while the fifth horse was used to examine the effect of Nature Vet Thrive P™ for Horses, when included in a ration containing 70% oats, on the same parameters (Experiment 3). Results from this horse would then assist the design of future feeding trials.

Blood samples were taken, and following a second passage through the experiential learning cycle, all assays other than the endotoxin assay worked extremely well. The results for plasma L-lactate supported the establishment of the assay for assessment of D-lactate, the isomer produced by microbial organisms in the gut. However, the endotoxin assay still failed to show any reproducibility and the results appeared unusually high. Plasma endotoxin readings of 70ng/L were recorded in horses that appeared clinically normal, yet in other published studies, such levels were associated with laminitis and obvious illness (Sprouse et al, 1987).

It was concluded that either the lessons learnt from the earlier failure with the endotoxin test kit had not been heeded, or that the assay technique was incapable of working accurately in horse plasma. The effect of these results were significant, and may well explain the 1989
learning style that was evident at the time - tentative, introverted, relying heavily on reflective observation.

With no reasonable explanation for the major variations in results, significant divergence was undertaken to obtain more information about the assay and its use in plasma. This involved literature searches and extensive networking with people familiar with both the LAL technique and the test kit itself. Such divergence has been dealt with in the discussion section of Chapter 4 (4.5.2).

The results of this divergence were useful. It reinforced concerns that inexperience with the assay technique was resulting in failure, but that such frustration and cost were unavoidable. In addition, the technique was widely accepted to be suitable for the determination of endotoxin levels in water but not in plasma, as there were deemed to be too many inhibitors and activators of the LAL enzyme system in plasma. And finally, that the assay was more sensitive to time and temperature variation than first thought.

These results were brought together to form a plan to resolve the difficulties. This plan included a visit by a Johnson and Johnson Medical Pty Ltd representative to assess laboratory facilities and techniques, and a check of all equipment used with the assay technique, including the photometer and the water bath used to incubate the microtitre plate.

No obvious faults were detected in the laboratory technique used with the assay procedure. The accuracy of the Vitatron photometer was verified against a Bio-Tek automated microplate photometer. Control of temperature levels in the microplate was assessed using a thermocouple and large variations in temperature between wells was established. It was concluded that this variation in temperature was responsible for the wide variation between replicate samples.
This situation could be improved through the use of a block heater, something that the assay had called for in the beginning but was discounted because of additional cost and questionable benefit (over a water bath). The learning outcome from the situation was that inexperience led to a necessary item being dismissed in an effort to conserve expense which ultimately resulted in further expense.

Following installation of a block heater and correct calibration of temperature, the assay technique was developed to a stage where some degree of confidence was achieved in measuring known endotoxin standards in water and plasma. Whilst the assay was never used in a trial situation to examine horse plasma levels, sufficient progress had been made to make further studies on establishing the technique for horse plasma worthwhile when further funding became available.

Use of experiential learning and adherence to the Kolb cycle assisted in the establishment of the endotoxin assay by providing a conscious course of action. Whilst the slowness of progress was frustrating, the rewards eventually came through perseverance and better understanding of the process used to improve the situation. That is to say, by observing and reflecting on concrete experience, forming concepts and generalisations and testing these implications, the endotoxin assay was developed to some level of accuracy and reproducibility.

9.6 Increasing Systems Awareness

Experiential learning has also played a large role in the development of an appreciation of systems thinking, which in turn has allowed better understanding of the broad environment in which the equine and canine studies were undertaken.
Veterinary science education involves an appreciable amount of didactic instruction about science-based subjects such as physics and chemistry in which the focus is quite narrow and reductionist scientific methodology the main process for knowledge acquisition. However, a number of subjects exist which are best 'taught' on the understanding that they operate around systems. Subjects like medicine and surgery are multisystemic, and require an understanding of many subsystems that work intimately with each other.

In the final years of veterinary science training, it is this appreciation of animals as being systems that is emphasised, particularly when looking at situation improvements such as treating injury or illness. Disorders of one system can't be viewed in isolation of other systems, because an incipient cause will bring about changes in many subsystems that must be appreciated for treatment to be effective.

A relevant example of this would be the disease laminitis that arises as a result of a horse eating a diet containing large amounts of soluble starch, such as grain. While simplistically the overconsumption of grain is the 'cause' of laminitis, in reality the situation is more complex. The intricate changes that occur in the population of gastrointestinal microflora as a result of grain ingestion are far reaching and include changes in volatile fatty acid production, lactic acid production, and as a result, increased concentrations of bacterial endotoxin. Alterations also take place in electrolyte and water movements across the gastrointestinal mucosa as volatile fatty acids, lactic acid and endotoxin are absorbed.

These factors then interact with components of the circulatory and haematopoietic systems to bring about changes which ultimately lead to laminitis. It is the understanding of these transformation steps in
this complex interplay between subsystems that determines the most effective treatment and therefore prognosis.

The practice of veterinary science therefore requires a systems approach, as well as a systematic approach. The training environment of the veterinary school gradually scales up the level of systems involvement to reach peaks in the two final years. The importance of having a systems approach is carried over into the real world of veterinary science practice, where 'textbook cases' never appear to exist.

Exposure to the Hawkesbury approach has enabled improved understanding of systems thinking and its importance when dealing with problems and research at the biologic or animal level. In addition, it has engendered an appreciation of the operation of systems on larger scale, such as water catchment management and community planning. Increased awareness and understanding of systems thinking has therefore promoted the development of metacognition and epistemic cognition.

9.7 Modelling the National Company Teaching Scheme

Exposure to the Hawkesbury experiential learning approach stimulated questioning of the advantages and disadvantages of the systems approach to education, the suitability of the Hawkesbury educational environment for reductionist research, the limitations of reductionist research and how it could be improved, the ability to accurately quantify blood endotoxin levels and the relevance of the research and the learning experience to the objectives of the NCTS.

Exposure to systems thinking allowed the modelling of the research system in which Nature Vet Pty Ltd (Nature Vet or NV), Hawkesbury Agricultural College (HAC) and the writer operated in relation to the
outside world. This model is presented in Figure 9.2. The complex relationship that is the NCTS is a small point of intersection indicated in the centre of the diagram. Surrounding it are broader systems that include other activities of Nature Vet, Hawkesbury Agricultural College and the writer (RH), along with the horse and dog industries that Nature Vet is intimately associated. Beyond this is the agricultural system of which the described subsystem is a small part.

![Diagram](image)

**Key** -
- Agricultural System
- National Company Teaching Scheme (NCTS)

**Figure 9.2.** The NCTS and its relationship with the agricultural system.
Inputs
- Political - Funding cutbacks to education
- Industrial - Johnson & Johnson, Boehringer Mannheim, Behring Diagnostics
- Others - University of Sydney, overseas contacts, availability of hardware and software, funding

Department of Industry, Technology and Commerce

HAC

RH

NV

Researching

Resourcing

Documenting

Learning

PURPOSE

IMPACT

- Link between Nature Vet/HAC
- Improve knowledge of equine feeding
- Improve knowledge on Endotoxins and Lactic acid
- Better understanding of problem prevention
- Better understanding of the use of the HAC approach in science research
- Personal development of the associate
  * Experience in industry and academia
  * Masters program
  * NCTS, exposure to other associates
- Professional practice
  * Masters, better work approach
  * Experience in animal health pharmaceutical industry
  * Networking - professional and social

Figure 9.3. Transformation of the associate through the NCTS
The NCTS is a subsystem of the Department of Industry, Technology and Commerce (DITAC) and can be represented by the transformation seen in Figure 9.3. This model highlights the various functions the writer (RH) played as an associate within the scheme, the relevant transformations undertaken and the outcomes achieved.

9.8 Action Research as a Model

Exposure to experiential learning in the Hawkesbury approach was associated with exposure to the action research methodology, of which critical self-reflection is paramount (Kemmis and McTaggert, 1988). The research being undertaken was thought to be very similar to the action research model as it appeared to satisfy the two key elements of the action research process.

Firstly, it involved thinking through and consciously reflecting on what the researcher had done, what the researcher was doing and what the researcher intended to do so that knowledge from previous experiences may be continually built upon. Secondly, knowing and defining the problem was not necessary at the start of the research, as it was usually more clearly defined after passing through one or two loops of the spiral, and quite often the definition of the problem changed. The latter seemed particularly relevant in the case of the endotoxin studies where little was known. Figure 9.4 illustrates the 'action research' spiral proposed to describe the research.

Action research was originally designed as a methodology for teachers to improve their teaching skills, and was adopted by the Faculty as a teaching and research model because of its robustness and educational worth. The versatility of the model appeared to allow its adaptation to the approach used in the study of endotoxins and thereby facilitated understanding of the research process. Reductionist methodologies
would remain relevant and the action research spiral would provide improved clarity of understanding.

This view was put to the Faculty for debate and was rejected as it failed to satisfy the three minimal requirements for action research (Grundy, 1988). An action research project takes as its subject matter a social practice, regarding it as a strategic action susceptible to improvement. An action research project proceeds through a spiral of cycles of planning, acting, observing and reflecting, with each of these activities being systematically and self-critically implemented and interrelated. And finally, the action research project involves those responsible for the practice in each of the moments of activity, widening participation in the project gradually to include others affected by the practice and maintaining collaborative control of the process.

Whilst the collaborative endotoxin work being undertaken satisfied the second and third criteria, the first was not well accounted for and could be improved by greater involvement of DITAC. However, further involvement with DITAC was not feasible at the stage the project had reached and so the point was noted.

The other learning that came out of the Faculty input was the perception by the Faculty that action research was synonymous with emancipatory action research. This form of research is characterised by a critical focus and a willingness to encompass the social context within the field of investigation. It does not begin with theory and end with practice, rather it is informed by theory.

The two other forms of action research, namely technical and practical, appeared to be largely overlooked by the Faculty. Technical action research develops from action arising from a skill or craft (techne) and is product centred. That is, action is designed to produce
Literature Search - Endotoxins
  - Lactic acid
  - Haematology
  - Feeding horses

Consultation - Leigh Whatmore
  - Robert Woog
  - N. Sriskandarajah
  - John MacFarlane
  - Rosemary Johnston
  - Others

Plan - Conduct pilot study on two horses
  - Feed diet 30% lucerne chaff:70% oats
  - Blood samples to assess dietary effects on PCV, TPP, RBC, WBC, Platelets, Lactic acid and Endotoxin

Discuss explore assess negotiate plan

Conduct experiment

Process samples

Evaluate results
  - Failure of Endotoxin assay
  - Changes in WBC's

Results

Plan - Carry out full trial (four horses)
  - Four diets varying in oats %
  - Same samples as pilot study

Discuss explore assess negotiate plan

Conduct experiment

Process samples

Evaluate results
  - Difficulties with Endotoxin assay
  - Changes with TPP and L-Lactate marked

Results

Plan - Continue work on Endotoxin assay before starting new trial
  - Establish D-Lactate assay to include in new trial
  - New trial - to enable assessment of Nature Vet Thrive in diet containing 30% lucerne chaff:70% oats

Figure 9.4. 'Action researching' the effect of diet on blood parameters in horses
or make something. A project would be initiated by a particular person or people who through greater experience, would be regarded as expert(s). Practical action research, is more process centred and differs from technical action research by resulting in doing action or praxis. Practical action research seeks to improve practice through the application of the personal wisdom of the participants based upon the interaction of a personal 'idea' of 'the good' in a given situation (Grundy, 1988).

The Faculty agreed that there were benefits to be gained by adopting some of the thinking of action research in the investigation process, but that the process could not be considered to be action research. It was apparent that at the time, emancipatory action research was the stronger influence on the Faculty's thinking, to a point where many had seemingly forgotten about other research processes.

Whilst the research studies did appear to lie close to the technical action research process and perhaps practical action research, the position of the Faculty was accepted. Significantly, the thinking of the Faculty had been challenged, and the discussion that followed encouraged systemic thinking and efforts to model the reductionist research in a wider framework. A desire to develop an alternative methodology was stimulated.

9.9 Scientific Research as a Human Activity System

The description of science as a human activity system designed to assist development of understanding about the world we live in has been introduced earlier in this thesis. It involves idea flows and transformations of inputs and outputs. The human activity system within the Hawkesbury learning system centred on the Hawkesbury spiral of systems to reductionism and received two sets of inputs as shown in Figure 9.5.
Input of information occurred from the NCTS and the coresearchers, and from the post-graduate learnings. Both pathways passed through an intellectualising and conceptualising transformation process, which is analogous with the window through which the world is viewed and the bag of tricks needed to improve understanding of the situation. The NCTS cycle involves level 2 cognition or metacognition, which involves the processes used to monitor the cognitive progress being made in cognitive (level 1) tasks being carried out on the research focus.

The outer loop provides level 3 cognition, that is, epistemic cognition. The involvement in the post-graduate program and the learnings about the Hawkesbury approach facilitated the development of an understanding of the process used to monitor the epistemic nature of problems and the truth value of alternative solutions. The development of this thinking has enabled further modelling of the research process in an attempt to describe more accurately its association with values and other factors or standards.

Figure 9.6 illustrates the development of praxis from the proposed human activity system, and can be seen to borrow from the thoughts of Vickers (Checkland and Casar, 1986). It incorporates a comparison of standards of fact and value, good and bad, acceptable and unacceptable when assessing the outcomes of the human activity system. This provides a better informed idea of what an improved situation would be and there by ends with theory informed practice or praxis. This praxis then links back into the human activity system and the cycle repeats.
Figure 9.5. The Hawkesbury Human Activity System

Figure 9.6. Praxis - a result of the Human Activity System
This model stimulated exploration of the relationship between DITAC and the co-researching team and the study area. This is represented in Figure 9.7. The project is seen to be a subsystem of a much larger system and highlights the fact that DITAC does not play a close hands-on role in the research.

![Diagram](image)

**Figure 9.7. DITAC and the NCTS - a nest of Human Activity Systems (* - other NCTS Human Activity Systems overseen by DITAC)**

The NCTS subsystem is redrawn in Figure 9.8 to indicate the inputs and outputs and highlight the overall direction of this subsystem. From this model, the aims of the NCTS subsystem can also be seen.

When the system boundary is refined to that of the Hawkesbury/Nature Vet human activity system, the inputs and outputs and aims become focused to directly affect the research work, as compared to Figure 9.8, which showed them as relevant to DITAC's aims. This can be seen in Figure 9.9.
Inputs - Money
- Associate
- Concerns and desires of
  a Government
  b Community
  c Industry
  d Institution
- Commitment
- Conferences

National Company Teaching Scheme

Hawkesbury Agricultural College/
Nature Vet

RW
RH
LW

Focus

DITAC

Outputs - Skilled associate
- Company growth - products, personnel
- Institutional growth - research, publications, industry relevance
- Networking
- Improved knowledge for all parties

Aims of National Company Teaching Scheme
a Improve company performance
b Introduce appropriate technology/methodology
c Stimulate industry research and development
d Improve institution research relevance
e Training for company/academic staff
f Educate graduate for industry career
g Open employment doors
h Increase exports

Figure 9.8. The NCTS System as it relates to HAC and Nature Vet
Figure 9.9. The HAC/Nature Vet Human Activity System

Figure 9.9 can be seen to be evolutionary relative to Figure 9.5 with two primary differences. Firstly, the intellectualising and conceptualising transformations are accepted to be within the Hawkesbury/Nature Vet system just shown. Secondly and more importantly, the better appreciation of the idea flows and learning outcomes (inputs to outputs) of the transformation can be seen.
Such models are important because they acknowledge the larger system in which the research studies are undertaken. It supports the holistic belief that research undertaken does not happen 'out there' in isolation as a realist ontology would have you believe, but instead, through its systemic interconnectedness incorporates the researcher.

9.10 The Notion of Appreciation in Science

The improved knowledge about experiential learning and knowledge acquisition developed as a result of exposure to the Hawkesbury approach, allows modelling that more clearly shows where the reductionist science studies undertaken are located within the framework of the Faculty of Agriculture and the broader environment.

As Bawden (1990) described, because the world is very complex, we can only deal with certain issues at any one time. The learner must therefore take a portion of the issues of a size that they feel they can handle. In a sense, the learner is reducing his or her focus but still looking at the problem holistically. The portion of the world used in the studies reported in this document are defined as the feeding subsystems of horses and dogs.

The experiential learning cycle describes the relationship between the researcher and the ideas and events of the research focus within the feeding subsystem. The finding out process is affected by the world view and incorporates a synthesis of concrete and abstract, that is, facts and theories. In other words, it is value-dependent. The theories proposed can then be tested through the taking action process which likewise incorporates concrete and abstract contexts, and is dependent on the bag of tricks.
The involvement of the co-researchers in the human activity system is similar to Bawden's (1990) description of the facilitator and the co-researcher in an action researching system. Rather than the analyst being 'outside' the system being researched, as may be the case with hard systems research or even conventional science, the analyst or analysts (co-researchers) in this case were an involved part of the system under examination and effectively had a role equivalent to the facilitator in an action researching system.

The human activity system that is the Hawkesbury/Nature Vet relationship is directed by a 
\textit{weltenschauung} controlled by the values of the co-researchers. To enable modelling of where the system was located within its agricultural and social environments, the values of the co-researchers were taken into account using Vickers model of the appreciative system.

The process of appreciation as described by Vickers (Checkland and Casar, 1986) was considered to be synonymous with the intellectualising and conceptualising transformation of the Hawkesbury/Nature Vet system. The process of appreciation within the appreciative system can be substituted with the Hawkesbury/Nature Vet human activity system to allow the development of a model which accurately describes the ideas and action pathways utilised in the equine and canine studies. This model is presented in Figure 9.10.

Like in Vickers' model of the appreciative system, each passage through the Hawkesbury/Nature Vet human activity system results in an influence on the next cycle, in addition to providing feedback into the \textit{lebenswelt} (the flux of events and ideas). This is demonstrated in the expanded model detailed in Figure 9.11.
Figure 9.10. A model for the appreciation of science research undertaken within the environment of the Faculty of Agriculture

Figure 9.11. The appreciation of science influences the appreciation of science.
This model incorporates three important points. Firstly, the focus area of horse nutrition or for that matter, any biological study, is not 'out there' to be studied. Science researchers are inherently an associated part of the subject matter that they study. Secondly, values play an important role in the determination of what the important issues to be examined are, how they will be examined and how the results are to be used. The end product of this process is the notion of what an improved situation regarding the focus area is, which not only informs action (praxis) but also progressive work on the focus area.

And finally, recursive loops are intimately involved in the various processes, indicating an interconnection of knowledge from one moment in time to another. Ideas coming from the *lebenswelt* are processed and feed back into the ideas pool of the *lebenswelt*. As well and perhaps more importantly, the notion of what denotes an improved situation influences the next cycle.

The systemic nature of the learning subsystems incorporated within the Hawkesbury/Nature Vet human activity system can be represented by an adaptation of the Hawkesbury spiral, and is seen in Figure 9.12. The diagram serves as a reminder that the reductionist or hard areas of learning, in this case equine nutrition and endotoxin studies, have evolved out of broader areas of concern, or soft areas. In addition, each reduction from soft to hard leads to a subsequent hierarchy of learning subsystems, while the output from each hard (reductionist science) learning subsystem can only provide insight into the soft system that led to the research. The basic law of holism that one cannot build up a picture of a whole by assembling its components without changing the meaning of the whole is therefore upheld.
For research to be practically and theoretically useful, the methodology used should be open to recursive feedback and reflection of knowledge, as was described by Guba and Lincoln (1988) in their naturalist inquiry. However, does this mean that the conventional paradigm, with its lack of recursive feedback, should be dismissed as inadequate?

Whilst there is obvious value in the conventional reductionist approach, its limitations discussed earlier suggest that an approach that utilised both a conventional and alternative (naturalist) methodology at different stages would be an ideal paradigm to have operating at the Faculty of Agriculture. Guba and Lincoln (1988) considered that the two cannot be used together, however, a possible 'marriage' of these two approaches would be mutually beneficial in the production of knowledge.

The closed boundaries of the conventional approach prevent the feedback of findings from altering the positivist research path and so enforce the objectivity that really underlies this approach. However, the rejection of subjective data on the grounds that the investigator may bias the results is self-defeating. After all, the researcher has already biased the results by his or her opinion on what objectivity and experimental design is required for the production of the results and hence knowledge.

The trial which examined the effect of Nature Vet Thrive D™ on nutrient digestion by dogs is an example of where useful subjective data (smell and stool formation) can be incorporated into an otherwise strictly reductionist trial to provide important supportive data. The criticism of bias and accuracy in the interpretation of smell and appearance must be weighed up against the discovery of information and knowledge that may otherwise escape if strict adherence to the reductionist methodology was maintained.
Figure 9.12. The systemic nature of the research focus
The incorporation of a conventional approach within an alternative or softer approach would also allow more appropriate population sampling for experimentation. That is, a sample could be selected that is most representative of the population that the research is supposed to provide knowledge about. This may well avoid some of those studies that are undertaken where the results do not conclusively support or refute the null hypothesis.
The appreciative systems adaptation depicted in Figure 9.10 can be reworked to better define how a conventional reductionist approach may be integrated with the more naturalist approach utilised in the Hawkesbury/Nature Vet human activity system to provide a more complete methodology. Figure 9.13 illustrates this improved relationship of appreciation and reductionism.

Whilst the spiral of soft to hard still exists in the activity system, the output of its ideas where hard studies are required can be linked directly into the more conventional approach as shown. The same processing of ideas through standards and action, intellectualising and conceptualising would then take place before outflowing of results to influence the notion of an improved situation.

9.11 Conclusions

The scientific research undertaken at the Faculty of Agriculture was found to be inextricably linked to human activities and values and clearly did not exist in isolation. The realist ontology and the objective, essentially value-free epistemology of the reductionist approach was not flexible enough to take into account the beliefs and prejudices of the research parties and their differing agendas as the research path was journeyed.

Through heightened awareness of the process of learning, and in particular experiential learning, increased knowledge was developed about the science methodology, systems methodology, action research, and appreciative systems. Flowing out of this knowledge was a model which attempted to account for and make use of the obvious human inputs to the process of scientific research, in an attempt to create a more powerful mechanism for knowledge acquisition.
Whilst the model remains to be tested, it does provide a tool by which the relationship between the human element of scientific research and the research focus itself can be formalised. Recognition of this relationship therefore allows the inclusion of subjective measures of assessment, such as faecal smell and stool formation used in the canine study, without compromising the standards that underpin the scientific approach.

It also highlights the fact the results of research not only filter back to the \textit{lebenswelt} or area of published ideas and events, but that they also have a direct effect on the next cycle of the research path by influencing the standards (values) and actions component of the following research loop.

This model is symbolic of the epistemic cognition that has been developed as a result of the association with the Faculty of Agriculture. It provides a process by which the epistemic nature of problems can be monitored and the truth value of alternative solutions appreciated. In other words, it signifies a departure from the belief in single absolute truths to the belief in multiple truths, and recognises that scientific research is not devoid of human values.
CHAPTER 10

General Discussion and Conclusion
General Discussion and Conclusion

The studies reported in Part A were designed to investigate the effects of two bentonite feed additive products, manufactured by Nature Vet Pty Ltd, on target species. The aim of the studies was to improve the understanding of the effects of such products on digestion and gastrointestinal function and thereby assist in the development of the products. In so doing, the studies were in keeping with the guidelines of DITAC and the NCTS through which the research was made possible.

Part B of this thesis consists of the researcher’s extended learnings related to the studies reported in Part A, and to the researcher’s involvement in the post-graduate program. This involvement came about as a result of the DITAC aim for the research associate to receive further education whilst undertaking the nominated studies.

Three experiments were conducted to investigate the effects of high-concentrate low-roughage diets on blood parameters in the horse, including PCV, TPP, RBC and total WBC counts, differential WBC counts (neutrophils, lymphocytes, monocytes, basophils and eosinophils), platelets, L-lactate and endotoxin levels.

The first experiment was designed to assess the suitability of the high-concentrate low-roughage diet (70% oats:30% lucerne chaff) for measurement of the proposed parameters. The second experiment was carried out to determine the effect of diets of varying grain concentration (0%, 30%, 50% and 70% oats) on these parameters. The final experiment was undertaken to provide an indication as to whether the inclusion of the feed additive Nature Vet Thrive P™ for Horses in a diet containing
70% oats had any effect on the same parameters, and thereby encourage further investigation.

One experiment was undertaken to establish the effect of dietary inclusion of Nature Vet Thrive D™ for Dogs on food digestibility in the dog. The parameters measured in this experiment were mean digestibility values for dry matter, organic matter, protein, energy and fat, and in addition, faecal smell, degree of stool formation, faecal moisture content and body weight change were determined.

The work reported in Part A of this thesis suggested that the horse's diet does have some influence on certain blood parameters. RBC levels and L-lactate levels were found to vary significantly between diets, while other parameters did not show significant variation between diets. The difficulties experienced with the endotoxin assay did not allow quantification of plasma endotoxin levels, and as a result, no comment is possible in relation to dietary influence. However, the operation of the assay was developed to a stage where it could be implemented in future studies.

The inclusion of the bentonite feed additive Nature Vet Thrive P™ for Horses in a diet comprised of 70% oats had some influence on the blood parameters measured, however small sample size prevents any major conclusions being drawn. While PCV, TPP, RBC, WBC, differential WBC, platelet, and L-lactate values appeared to alter with the inclusion of the additive to values similar to those of an all-roughage diet seen in the earlier experiment, the change to plasma L-lactate was most noticeable.
The changes observed with the L-lactate time curve may be of significance in allowing a better understanding of how the dietary additive functions. Such results suggest that the inclusion of the montmorillonite product in a diet comprised of 70% oats altered the process of bacterial fermentation of the carbohydrate contained in the high-grain diet to a degree that the affect of the end products on the parameters measured are similar to those seen when a horse is receiving an all roughage diet.

The mechanisms through which this has occurred require further investigation, however it is likely that a number of the unique physicochemical properties of montmorillonite reviewed in Chapter 2 are involved. Should further studies (in vitro and in vivo) elucidate an influence of Nature Vet Thrive P™ on the post-prandial blood levels of D-/L-lactate and bacterial endotoxin, more accurate description of the function of the feed additive in the digestive tract may be possible. More importantly though, the potential of Nature Vet Thrive P™ in the prevention and possible treatment of disorders relating to elevated blood levels of lactic acid and endotoxin may be realised.

The inclusion of Nature Vet Thrive D™ for Dogs in the kibble/mince meat diet fed to dogs was demonstrated to bring about improvement in food digestibility. The significant improvements in the digestibility of dry matter and organic matter were associated with improvements in digestibility of protein and energy, however, a reduction in fat digestibility was observed. The process of fat digestion is vulnerable to disturbance, and whilst the results suggested a negative effect brought about by the feed additive, it could not be discounted that another factor(s) may have been involved in producing the reduction in fat digestibility.
Of almost equal importance to the products ability to improve nutrient digestibility was the highly significant reduction in faecal smell, the significant improvement in stool formation and the reduction in faecal moisture content associated with the use of Nature Vet Thrive D™. The ability of the bentonite feed additive to deodorise droppings and to reduce the incidence of sloppy motions in dogs receiving a kibble/mince meat diet was not an unexpected finding given the experience of bentonite inclusion in other animal feeding systems. This feature would present as a desirable benefit to certain dog owners.

The writings of Part B of this thesis, which have stemmed from the researcher's involvement in the Masters program, reflect the development of epistemic cognition that has essentially allowed him to stand back from the focus studies and consider more systemically the meaning of the work undertaken and the relevance and importance of the methodology employed. The importance of trial design and familiarity with experimental and laboratory techniques in reductionist research have been realised on a broader scale than would have been achieved without the benefits of the learnings of systems and experiential learning.

The researcher has, as a result of his sojourn within the Faculty and its post-graduate program, achieved a better appreciation of the three themes of experience detailed in the preface of this thesis. The interconnectedness of truth, reality and learning have been more firmly grasped through an improved understanding of science, and through an introduction to systems thinking and experiential learning.
The lived in world that the science methodology is a part cannot be viewed in isolation of the individual undertaking the research, nor from the complexity of the natural systems that the interest area is associated. The notion of appreciation, that is the values and ideals of the researcher, being inextricably interwoven into the fabric of the research, including reductionist studies, cannot be ignored.

Through increased awareness of the process of learning and knowledge acquisition and as a result of the realisation that science is a human activity system within a larger system, the researcher has progressed further down the evolutionary path towards autonomous learning. The appreciation of the importance of changes in learning styles over time and life experience has been of assistance in allowing the development of a more balanced approach to problem situations in and away from work, so that relationships other than cause-effect ones are considered.
APPENDIX 1

COATEST® Endotoxin -

- Reagent Specification and Manual Method - Insert
- Practical Advice and Trouble Shooting - Technical Sheet.

© COATEST is a Registered Trademark of KabiVitrum AB, Stockholm, Sweden.
The COATEST Endotoxin is a method specific for the quantitative determination of gram-negative bacterial endotoxins (1-3).

1. PRINCIPLE

1.1. Prenzyme Endotoxin

Enzyme

1.2. Substratum

Peptide + PNA (Fluorescein)

Gram-negative bacterial endotoxin catalyzes the activation of a preenzyme in the Limulus Amebocyte Lysate (LAL). The enzyme forming the PNA from the substratum S-2423. After stopping the reaction with acetic acid, the rate at which the PNA is released is measured photometrically at 405 nm. There is a linear correlation between the absorbance (A) and the amount of endotoxin in the 0.1-1.2 EU/ml range.

2. PRECAUTIONS

2.1. Aseptic technique must be used (4, 5). It is possible to perform the assay in a laminar flow bench.

2.2. All material coming into contact with the specimen and reagents must be endotoxin-free.

2.3. Use disposable glassware. These glassware can be rendered endotoxin-free by heating for 4 hours at 180°C.

2.4. Strict adherence to time and temperature of the test procedures is required.

Further information and recommendations of material (4) are available on request (KabiVitrum Diagnostics, or its representatives).

3. MATERIALS

Reagents on the kit

For in vitro diagnostic use.

The sealed reagents are stable at 2-8°C until the expiration date printed on the label.

Allow all reagents to attain room temperature before reconstitution. Tap the vials with lycopodium matter against the laboratory bench prior to opening.


1. S-2423

Chromogenic substrate (Ac-Ile-Glu-Arg-pNA HCl) 5 mg with mannitol added as a bulking agent. Reconstitute with 6.0 ml of sterile, endotoxin-free water to a concentration of 1.8 mmol/l. The solution is stable for one month at 2 to 8°C provided that no contamination occurs.

2. Limulus Amebocyte Lysate (LAL), 5 vials

LAL, manufactured by Whitaker M.A. Bioproducts, Walkersville, MD, USA. Reconstitute with 1.4 ml of sterile endotoxin-free water at room temperature and swivel gently to avoid foaming. To allow proper dissolution keep at 20-25°C for at least ten minutes. If the LAL is kept on ice it loses about 5% sensitivity per hour. Another alternative is to freeze the LAL in 100 ml aliquots immediately after the ten minutes dissolution period. The LAL is stable for 4 weeks at -20°C and should not be thawed until immediately before use.

3. Endotoxin (E. coli 0111:B4), 2 vials

Endotoxin (E. coli 0111:B4) in sodium, 24 EU (1.1-12 EU) compared to FDA standard EC-5-USP/uF. Reconstitute with sterile endotoxin-free water at room temperature using an appropriate volume (see batch certificate) to obtain a concentration of 1 EU/ml and shake vigorously for three minutes, preferably with a "Vortex" mixer. The shelf life of this stock solution is two weeks at 2-8°C provided that no contamination occurs. Before subsequent use, the solution must be warmed to room temperature and shaken vigorously for one minute. The loss in activity is usually less than 30% even after several months at 2 to 8°C or frozen.

4. Buffer

Sterile and endotoxin-free Tris 500 mmol/l pH 8.0. The solution is stable for one month at 2-8°C after breaking the seal, provided that no contamination occurs.

5. Sterile endotoxin-free water

Sterile and endotoxin-free water (European Pharmacopeia). Stable for one month at 2-8°C after breaking the seal, provided that no contamination occurs.

6. Substrate-buffer solution

For daily use mix one volume of S-2423 and one volume of buffer. The substrate-buffer solution is stable for 8 hours at 20°C to 25°C, provided that no contamination occurs.

For in vitro diagnostic use.
4. WATER METHOD

for the determination of endotoxin in water and solutions for parenteral admin-
istration.

Materials required but not provided:
1. Photometer, 405 nm.
2. Semi-micro cuvettes (1 cm).
3. Incubator, 37°C to ± 0.2°C.
4. Stop watch.
5. Test tubes. Disposable endotoxin-free (see Precautions) glass test tu-

bines, volumes 2 ml and 5 ml or 12 x 75 mm.
6. Pipettes. Endotoxin-free or at least sterile pipettes with an accuracy of ± 1%.
7. Acetic acid, 20%.

5. SPECIMEN COLLECTION

Samples must be collected and stored in such a way as to avoid bacterial con-

amination. Store the samples at 2 to 8°C. Before assay, the test sample

must be warmed to room temperature and shaken. Further information and

recommendations about sample handling (4) are available on request (Kab-

bivrum Diagnostica, or its representatives).

6. PROCEDURE – MANUAL TECHNIQUE

Calibration

In each series of tests use the standard dilutions 0.6 and 1.2 EU/ml, prepa-

red from the endotoxin stock solution and a blank.

<table>
<thead>
<tr>
<th>Standards</th>
<th>Endotoxin stock (EU)</th>
<th>Endotoxin-free water (µl)</th>
<th>Endotoxin-free solution prep. of the sample (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0</td>
<td>200</td>
<td>1800</td>
</tr>
<tr>
<td>0.6 EU/ml</td>
<td>100</td>
<td>100</td>
<td>1800</td>
</tr>
<tr>
<td>1.2 EU/ml</td>
<td>200</td>
<td>0</td>
<td>1800</td>
</tr>
</tbody>
</table>

These standards can be kept for one week at 2 to 8°C. Warm to room tem-

perature and shake vigorously for one minute before use.

Method

1. Dissolve the substrate in 6.5 ml endotoxin-free water.
2. Prepare the standards. Dissolve the endotoxin in endotoxin-free water to
do 12 EU/ml, mix for three minutes with a "Vortex" mixer. Dilute (see table above) and mix for one minute.
3. Mix substrate and buffer. Keep at 37°C.
4. Reconstitute the LAL in 1.4 ml endotoxin-free water (room tempera-
ture). Allow it to stand at room temperature for at least ten minutes.
5. Follow the table below.

Timing of the test may begin when the LAL is added to each tube and must
continue uninterrupted until the last step. It is suggested that in a series of
tests a new test should start every 15 seconds (that allows 12 determina-
tions in each series).

A sample blank (see table above) is then made as soon as possible after the
"12 determination series" using the 13th aliquot of LAL and the same proce-
dure, except that buffer is used instead of substrate-buffer solution. The
sample blank does not call for any incubation.

Add in a test tube

<table>
<thead>
<tr>
<th>Test sample of standard (20 to 23°C)</th>
<th>µl 100</th>
<th>Incubate at 37°C for 3-5 min</th>
<th>µl 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mix end incubate at 37°C exactly 10 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substrate-buffer solution (37°C)</td>
<td>µl 200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mix and incubate at 37°C exactly 3 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid 20%</td>
<td>µl 200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mix immediately</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6. Read the A of the sample and the standards against a blank (A =

0.10 to 0.15 for water) in a photometer at 405 nm. The colour is stable for at

least 4 hours.

Note 1

By extending the incubation time of LAL and sample to 20 — 30 min, depend-
ing on the LAL sensitivity, it is possible to measure concentrations of endo-
toxin between 0.01 and 0.2 EU/ml. The linear range must be verified from a
standard curve. This procedure can be used to determine endotoxin in diluted
samples (cf. the "plasma method").

Note 2

If the concentration of the endotoxin in the test sample is higher than 1.2 EU/
ml dilute the test sample 1:5 with sterile endotoxin-free water. Multiply the
finally calculated value by 5. When calculating the endotoxin concentration of the test sample observe
that the risk of losses of endotoxin to test tube surfaces increases with dilui-
tion in water. Addition of albumin (0.1%) will counteract such losses.

Note 3

By using a microtube plate technique (4) larger series of assays can be per-
formed. The amount of reagent per assay is also halved.

7. RESULTS

Endotoxin (EU/ml) = \frac{0.60}{A_{Ak} - A_{A0}} (A_{Ak} - 2A_{A0}) where

A_{Ak} is the absorbance for the 0.6 EU/ml standard
A_{A0} is the absorbance for the 1.2 EU/ml standard
A_{A0} is the absorbance for the test sample

Example

A_{Ak} = 0.490 A
A_{A0} = 0.980 A
A_{Ak} = 0.490 A
Endotoxin (EU/ml) = \frac{0.60}{0.980 - 0.490} \times (0.490 - 0.980 - 0.980) = 0.55

Samples other than water

The reaction conditions have been made optimal for water. Other samples may inhibit or enhance the enzymatic reaction. Such interference may be
decreased by diluting the sample with sterile endotoxin-free water and then
calculating using a dilution factor. If problems are still encountered, the met-
method can be varied (within certain limits). In respect of incubation times. Ad-
just the pit of the sample to 7.2 — 8.0 using endotoxin-free sodium hydrox-
ide or hydrochloric acid. Unbuffered solutions are adjusted automatically by
the LAL solution. Patterns of inhibition or enhancement different from those
of traditional LAL peltion test may occur.

For every new product to be tested, the linear relationship between en-
dotoxin concentration and absorbance should be confirmed by preparing a
complete standard curve using an endotoxin-free reference of the product.

To choose a reference for the product, use batches of good quality and fol-

low the method (See "initial quality control procedure" in the test "Practical
advice and trouble shooting" (4) available from Kabibvrum Diagnostica or

representatives).

If the sample is coloured or opaque a sample blank containing 100 µl of
sample, 300 µl of water and 200 µl of acetic acid 20% can also be used.

Limitations of procedure

1. Temperature control is crucial. During incubation both with the LAL and

with the substrate-buffer solution the temperature must be controlled
within ± 0.2°C. Note that an increase in incubation temperature of one
centigrade in the 30 to 35°C range results in an increase in the slope of
the standard curve of as much as 15% (3).

2. The incubation time of choice must be followed exactly since the reac-

tion is not completed within the incubation period.

3. Proper mixing is important to make sure that the reaction mixture is ho-

mogeneous. Use a "Vortex" type of mixer, but avoid contamination from
the neck of the tube and from the stopper.

4. If A_{Ak} - A_{A0} ≥ 0.2 absorbance units, the reagents are considered to be

contaminated, and not usable. If endotoxin contamination of the re-

agent is suspected, this can be checked by a procedure previously
described (3, 6).
8. PLASMA METHOD

The determination of endotoxin in plasma, plasma fractions, cerebrospinal fluid and urine.

Materials required but not provided
1-7 See respective No. of the material list of the WATER METHOD.
8. Centrifuge.
9. Endotoxin-free tubes for plasma sampling with endotoxin-free heparin of heparinized tubes, free of endotoxin (see note 2).
10. Platelet-rich normal human plasma free of endotoxin (for standardization).
11. Incubator (water bath), 75 ± 3°C.

Note 1
If possible, one set of automatic hand pipettes should be used for endotoxin-free material and one set for material containing endotoxin.

Note 2
The heparin solution or the heparinized tubes must be checked for endotoxin contamination. Dilute the heparin solution with sterile endotoxin-free water to a concentration of 25 IU/ml or add the volume of sterile endotoxin-free water corresponding to blood to the heparinized tube. In both cases, dilute further the heparin solution 1:10 with sterile endotoxin-free water. The "Procedure" below is then followed.

9. SPECIMEN COLLECTION

Sample blood with heparin added (approx. 25 IU/ml). Check each batch of anticoagulant or heparinized test tube for endotoxin contamination (see Note 2 above). Keep the blood on ice to avoid degradation or endotoxin. (7). Centrifuge the blood at 2000 g for 10 minutes at 4°C to obtain plasma rich in plasma. Platelet-rich plasma should be used as the platelelet may bind endotoxin (8). The plasma can be stored at ~20°C for 2 weeks (either before or after heat treatment). Dilute the plasma 1:10 with sterile endotoxin-free water and heat-treat for as long as possible 5 minutes in a waterbath at 75°C to destroy inhibitors which may interfere with the activation. This heat treatment also destroys proteolytic activities which may exist in plasma of e.g., septic patients as well as a system rapidly degrades endotoxin in native plasma (7). Store the sample for 15 minutes at room temperature and then shake vigorously before assay. The treated samples are stable at 2 to 8°C for 24 hours. By using serum instead of heparinized plasma, it is possible to avoid the contamination that often occurs in heparin but some endotoxin may be lost. Lymph may be treated in a similar manner before endotoxin assay. Cerebrospinal fluid (9) and urine are usually not heat-treated.

10. PROCEDURE – MANUAL TECHNIQUE

Calibration

In each series of tests use the endotoxin standards 0.9 and 1.2 EU/ml of undiluted sample. Dilute the endotoxin stock solution, 1.10 (1.2 EU/ml).

The standards must be heat-treated for 5 minutes in a waterbath at 75°C as soon as possible and will then be stable at 2 to 8°C for 24 hours (see "Specimen collection").

All-diluted solutions of endotoxin must be thoroughly shaken before use and kept at room temperature during the work.

Procedure:
1. Dissolve the substrate in 0.8 ml endotoxin-free water.
2. Prepare the standards. Dissolve the endotoxin in endotoxin-free water to obtain 1.2 EU/ml, mix for three minutes with a "Vortex" mixer. Dilute (see table above) and mix for one minute.
3. Mix substrate and buffer. Keep at 37°C.
4. Reconstitute the LAL in 1.4 ml endotoxin-free water (room temperature). Allow to stand at room temperature for at least ten minutes.
5. Follow the table below.

Timing of the test must begin when the LAL is added to each tube and must continue uninterrupted to the last step. It is suggested that in a series of tests, a new test should start every 15 seconds.

11. RESULTS

See under the same heading of the WATER METHOD.

Expected results

<table>
<thead>
<tr>
<th>Specimen (reference)</th>
<th>Normal range (EU/ml)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma* (1)</td>
<td>0.075-0.099</td>
<td>75</td>
<td>99</td>
</tr>
<tr>
<td>C-1</td>
<td>(10)</td>
<td>52</td>
<td>91</td>
</tr>
<tr>
<td>C-2</td>
<td>(11)</td>
<td>99</td>
<td>91</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>(9)</td>
<td>&lt;0.2</td>
<td>-</td>
</tr>
<tr>
<td>Urine</td>
<td>(12)</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>&gt;24</td>
<td>(13)</td>
<td>100</td>
<td>97</td>
</tr>
<tr>
<td>&lt;24</td>
<td>(14)</td>
<td>99</td>
<td>99</td>
</tr>
</tbody>
</table>

* The patient has to be followed by taking samples twice a day for several days.

12. PERFORMANCE CHARACTERISTICS

Precision

Duplicate samples should be run to establish good procedures and a low coefficient of variation. One point (e.g. 0.9 EU/ml) should be run say ten times in one sequence. The coefficient of variation of these ten absorbances should be less than 10%. After some time and experiences, values of 2-4% should be possible (4).

Sensitivity

By increasing the incubation times to 30 and 10 minutes respectively it is possible to measure an endotoxin concentration of 0.01 EU/ml plasma.

Accuracy

Since there is no other established method available the accuracy can not be confirmed.

Specificity

No lack of specificity has been reported. When 0.1-1.3 glycans which may interfere in the LAL assay, were tested in the present method, about 2000 times more of the compound, compared with endotoxin, was needed to obtain the same activity.

Protease inhibitors given to patients, Aprotinin and FOY are not denatured by heat and can thus interfere with the assay.
References
COATEST® ENDOTOXIN
- PRACTICAL ADVICE AND TROUBLE SHOOTING

Standard curve for water and preparations:
The standard curve is made by using the following dilution (Table 1) in sterile endotoxin-
free water or reference preparation of the sample (drug) to be tested.

<table>
<thead>
<tr>
<th>Endotoxin (EU/mL)</th>
<th>Endotoxin-free water or reference prep. (μl)</th>
<th>Endotoxin-free water (μl)</th>
<th>Endotoxin stock* solution, 1.2 EU/μl (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>1600</td>
<td>200</td>
<td>0</td>
</tr>
<tr>
<td>0.15</td>
<td>175</td>
<td>175</td>
<td>25</td>
</tr>
<tr>
<td>0.3</td>
<td>150</td>
<td>150</td>
<td>50</td>
</tr>
<tr>
<td>0.6</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.9</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>1.2</td>
<td>0</td>
<td>0</td>
<td>200</td>
</tr>
</tbody>
</table>

* This can be added to the first 1800 μl if water is used or if the reference preparation is not inhibitory.
** To be used as a blank.
*** 12 EU = 1 ng endotoxin.

Standard curve for plasma, plasma fractions and cerebrospinal fluid:
Dilute the endotoxin stock solution 1:10 with sterile endotoxin-free water to a concentration of 1.2 EU/mL. The standard curve is made by using the following dilution (Table 2) in endotoxin-free water and normal human plasma. Use endotoxin-free heparinized normal human plasma.

<table>
<thead>
<tr>
<th>Endotoxin (EU/mL plasma)</th>
<th>Endotoxin-free water (μl)</th>
<th>Normal human plasma (μl)</th>
<th>Endotoxin stock, 1.2 EU/μl (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>1800</td>
<td>200</td>
<td>0</td>
</tr>
<tr>
<td>0.15</td>
<td>1775</td>
<td>175</td>
<td>25</td>
</tr>
<tr>
<td>0.3</td>
<td>1750</td>
<td>150</td>
<td>50</td>
</tr>
<tr>
<td>0.6</td>
<td>1700</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.9</td>
<td>1650</td>
<td>50</td>
<td>150</td>
</tr>
<tr>
<td>1.2</td>
<td>1600</td>
<td>0</td>
<td>200</td>
</tr>
</tbody>
</table>

The standard with plasma must be treated immediately for 5 minutes at 70°C but will still be stable at 2°C to 24 hours.

All diluted solutions of endotoxin must be thoroughly shaken before use and kept at room temperature during the work. Perform the assay as indicated under "Procedure in the blue print or Figure 2. Plot the absorbance (A) for the standards against their concentrations of endotoxin on linear graph paper. The standard curves are linear in the range in question, the higher concentration giving an absorbance between 0.7 and 1.4 absorbance units.

LAL, 100 μl
10 mμl 37°C
Sample
10 mμl 37°C
Substrate 200 μl
Hac 20% 200 μl
25 and 3 min. for the plasma method

Figure 2. A schematic description of the chromogenic method for endotoxin determination, at the first stage the LAL reagent is activated by endotoxin in the sample. Then the enzyme obtained is measured with a chromogenic substrate. After the reaction is stopped by an addition of acid the colour developed is read on a photometer and the results are plotted in a standard curve giving the results in EU/mL (EU = Endotoxin Unit).

Sampling
It is important to take a representative sample and to ensure that no changes which influence the results appear before assay. Bacterial growth can be stopped by e.g., heating to about 100 degrees for five to ten minutes. Gram-negative bacteria treated in this way give a good response to the LAL assay. The amount of endotoxin is normally proportional to the number of Gram-negative bacteria in the log phase of bacterial growth. In the stationary phase no reaction should however be expected. Most samples have to be treated in different ways to avoid inhibition (see "Inhibition of inhibitory effects of"

Endotoxin in air is collected on Millipore 0.8 μm filter (dust) and Cambridge Absorbent filter (air). The filters are then shaken for 15-20 min. in 1% peptone
water in endotoxin in medical devices is performed by using the sample in endotoxin water according to a procedure recommended by the relevant authorities.

For clinical samples it is important that the patient's condition at the moment of sampling is registered. Usually it is necessary to take several samples over a certain time to obtain sufficient information to make a diagnosis.

Initial quality control
The performance of the chromogenic endotoxin test vary to a certain extent depend-
ing upon the specific technique used, the sensitivity of the LAL, and the nature of the product tested. A quality control procedure should therefore be employed in order to become acquainted with the procedure. A series of standard curves giving consistent results should be prepared (see below). The respective points should be on a straight line without curving either up or down. The slope of the line should always be the same. This procedure will provide a linear standard curve with a correlation coefficient of above 0.98. One point (e.g., 0.4 EU/mL) should be run about ten times in one sequence. The coefficient of variation of these ten absorbances should be less than 10%. After some time and experience, values of 2-4% should be obtained.

Always measure the pH of an aliquot of the bulk sample to avoid contamination by the pH electrode. pH adjustment may cause a high salt concentration which usually inhibits and thus has to be diluted with endotoxin-free water. If the sample cannot be chlorinated by using NaOCl, which even in some cases can be an effective disinfectant, the sample may be diluted 1:10 with distilled water and heated to 70-80°C for 1-5 minutes (Fig. 3). This gives a denaturation of interfering proteins without the formation of precipitate. The sample can then be analyzed directly without any centrifugation.

In order to eliminate inhibitory effects in biological samples containing protease inhibitors such as other proteins interfering with the assay, the sample can be diluted 1:10 with distilled water and heated to 70-80°C for 3 minutes (Fig. 3). This gives a denaturation of interfering proteins without the formation of precipitate. The sample can then be analyzed directly without any centrifugation. If neither dilution nor heating treatment is sufficient, ultracentrifugation is often the third choice. The materials needed (Sterilin 2002) are available from Sarstedt GmbH, D-3400 Göttingen, FRG.

Figure 3. Protease inhibitors present in plasma inhibit the enzyme activated in the LAL test. This leads to an underestimation of endotoxin in the sample. By heating the sample, the endotoxin-free water and including NaOH for 3 minutes at a temperature between 70°C and 80°C these inhibitors are denatured without any loss of endotoxin or precipitation. Also compounds interfering with endotoxin and non-specific protease activities are eliminated by this treatment.

Methodological parameters

A significant amount of work has been required to obtain optimal conditions for the LAL test. The kit reagents are, however, matched and controlled to function well in the kit procedure. The LAL reagent is slightly opaque and often contains some undissolved material. This is typical for many endotoxin reagents and is controlled. It is also important to have good standards to be able to guarantee reproducible results. LAL and endotoxin standards are from the US (MAI-Bioproducts) and thus controlled and licensed by the FDA.

By using a two-stage procedure it is easier to avoid contamination. The first stage is equivalent to the old procedure known and widely used for more than one decade with very few misinterpretations reported. After this first stage, which takes 10-30 minutes according to the chosen sensitivity, the reaction is stopped by dilution and change of pH. The second stage is then comparatively insensitive to contamination. The incubation time with the substrate can be chosen according to the sensitivity range. In order to obtain satisfactory reaction times the highest absorbance should not exceed 0.1.

The reaction temperature between samples can give rise to inaccuracy. One degree change can give rise to 10% error (9). The chromogenic substrate, the buffer and the endotoxin-free water are stable reagents (more than 6 months) but as there certainly is a risk for contamination using these reagents a shelf-life (2-8°C) of one month is more realistic.

The amount of endotoxin-free water in the kit may be insufficient for some purposes. It may be advisable to use a buffer that is fully diluted or to use a buffer that contains some endotoxin to prevent the test from being too sensitive. A careful calculation of the quantities required (e.g. 10x standard) is available from KabiVitrum and substituting the use of some microcuvettes with black side walls will contribute to the precision in the test.

Suitable material

It is important that the new user of the COATES® ENDOTOXIN kit begins with specific, endotoxin-free water. The kit tubes and other equipment which have been found suitable to avoid contamination. Once the assay procedure becomes routine, the material can be exchanged one by one in other material preferred at the laboratory in question. All areas of the installation must be kept in order to avoid contamination. The materials which will be in contact with the samples and reagents may be sterilized. All of the containers which come in contact with the samples and reagents may be sterilized. A lot of materials, which have been found useful, are given below. A careful calculation of the quantities required (e.g. 10x standard) is available from KabiVitrum and substituting the use of some microcuvettes with black side walls will contribute to the precision in the test.

Test tubes:
1. CORNING, Pyrex Disposable Culture Tubes borosilicate glass 10x75 mm, 12x75 mm Cat No 999445.
2. AMERICAN SCIENTIFIC PRODUCTS, Borosilicate Tubes 100x15 mm 1290-2.
3. GREiner, Polystyrene tubes with caps, 15.4x45 mm (4 x 5 ml) N° 70281.
4. FALCON, Tissue Culture Tubs with caps, 15x50 mm Cat No 3031.
5. BECTON DICKINSON, VERSATUB® Sarre Sodium Hepes, No 6840 for blood sampling.

Pipettes and tips:
1. EPPENDORF, Pipettes up to 1000 µl, N° 74700.
2. M.A. Automatic Pipette, N° 1021-1030 depending on volume. Disposable (endotoxin-free) tips, individually wrapped, 0.5 µl up to 200 µl (Cat No 21-541) and large 250-1000 µl (Cat No 2005).
3. FALCON Sarstedt measuring pipettes, 10 ml with plug, individually wrapped (Cat No 75511).

Additional material for the micro-plate method (see below):
1. STERILIN, 96-Well Flat Bottom Polystyrene, Cat No 429.
2. MA BIOPRODUCTS, 96-Wall Microtiter Plate, 4X4X96 Costar Cat No 3586 9 Channel Pipette 50 µl (Cat No 20-4005), 100 µl (Cat No 20-4000)
3. 9 Channel Pipette for use (Cat No 4581)
4. Reagent Resuscer, 10 pack Cat No 25-018.

FLOW LABORATORIES, Transkit Microtiter Pipettes, 4.8 or 12 channel fixed or variable volumes.

The assay in routine

There are two qualities that can make the present method almost obligatory for routine purposes in the pharmaceutical industry:

1. Acute tests. The assay can be arranged in such a manner that results are available within 15 to 20 minutes. This is very attractive for in-process controls, making it possible to save expensive starting material and intermediates in production as well as time for consigning a filling line for parameters.

2. A large number of tests. By using micropipette techniques or clinical chemistry automated equipment to carry out about 100 assays in 1 to 2 hours. Such tests can often be performed using less reagents which will reduce the price per test. Also the comparatively smaller number of standards and controls and the simplified handling of the test reduces the cost significantly.

The micropipette procedure (Table 4) is performed exactly as described in the kit for 24 tests with the exceptions that:

- Only half the volumes are used.
- The incubation time is prolonged by less efficient and faster tests.
- Multichannel pipettes are used at all reagents (not samples and standards). A special reagent reservoir is needed for the reagents. Repeating dispensers can also be used for the reagents.
- In some ELISA readers much of the calculation of the results can be automated.

Table 4. Micro-plate method

| Sample or standard (incubate at 37°C) | µl 50 |
| LAL reagent | µl 50 |
| Include 15 minutes (30 minutes for plasma) | µl 100 |
| Chromogenic substrate in buffer (37°C) | µl 100 |
| Include 2 minutes | µl 100 |

Acetic acid

Factors which can adversely affect the variability of the micropipette procedure include less efficient temperature regulation than in the test tube method and the fact that the micropipette tips are less efficient than the "Ibex" pipette tips. Less efficient mixing may also adversely affect the results.
Trouble shooting scheme
Bad reproducibility can be caused by:
- Not warming and mixing ("Vortex" mixer recommended) standards and samples properly before assay
- Using the wrong material or not using an aseptic technique (see above)
- Not reconstituting the LAL according to the insert
- Not keeping the substrate + buffer at 37°C before addition
- Bad mixing (minding can be done in the pipette tip - avoid contamination)
- Not using a proper semi-micro cuvette (blank side walls or all)
- Using an uncalibrated photometer
A flat standard curve can be caused by:
- Inhibitory effects of the sample. This can occasionally be tolerated.
- Keeping the plasma at room temperature before dilution and heat treatment
- Low temperature due to inefficient temperature regulation
- Using wrong material (see above)
- Not reconstituting the LAL according to the insert

Follow the instructions in the kit insert carefully! Further advice is available in this text.

References
APPENDIX 2

Stat-Pack™ Rapid Lactate Test

Lactate Reagents - Insert

™ Stat-Pack is a Trademark of Behring Diagnostics Inc, Somerville, NJ, USA
Stat-Pack™ Rapid
Lactate Test
Lactate Reagents

Cat. No. 869218: 10 x 15 mL

intended use

For the quantitative determination of lactate (lactic acid) in whole blood or plasma.

summary and explanation

The Rapid Lactate reagents are based on an original Behring Diagnostics' procedure. The addition of glutamate and alanine aminotransferase (ALAT) to the lactate dehydrogenase (LDH) reaction system forces the reaction to completion by removing pyruvate from the mixture. The assay requires a spectrophotometer capable of reading absorbance changes at 340 nm.

Lactate in blood is the intermediary product of carbohydrate metabolism, derived mainly from muscle and erythrocytes. Under normal conditions, it accounts for approximately one-half of the glucose delivered to the muscle by arterial circulation. Lactate produced in the muscle is either converted into glycogen or oxidized in the liver. It diffuses freely from the tissue into the blood stream with excess lactate being excreted in the urine.

Lactate levels are increased in conditions associated with anoxia. Thus, excessive quantities of lactate are produced during strenuous exercise. Relatively slight exertion produces abnormally high levels of blood lactate in pathological conditions associated with anoxia. Lactic acid acts as a vasodilator of the peripheral vessels, allowing a more generous supply of blood to active tissues. Measurement of the blood lactate levels has been proposed as an effective means of evaluating circulatory efficiency or myocardial reserve.

Friedman et al. have published a comprehensive list of conditions affecting lactate levels in serum and cerebrospinal fluid.

principles of the procedure

\[
\text{L-lactate} + \text{NAD} \rightarrow \text{Pyruvate} + \text{NADH} \\
\text{Pyruvate} \text{ Trapping Reaction} \\
\text{ALAT} \\
\text{Pyruvate} + \text{L-glutamate} \rightarrow \text{L- alanine} + \alpha\text{-KG}
\]

Abbreviations

\alpha\text{-KG} = \alpha\text{-Ketoglutarate}
ALAT = Alanine aminotransferase
LDH = Lactate dehydrogenase

Tris = Tris (hydroxymethyl) aminomethane
NAD = Nicotinamide-adenine dinucleotide
NADH = Nicotinamide-adenine dinucleotide, reduced

Lactate is oxidized to pyruvate in a reaction catalyzed by LDH and a molar equivalent of NAD is simultaneously required. The pyruvate formed in the reaction is converted to alanine in the ALAT reaction, thus forcing the LDH reaction to completion. The change in absorbance at 340 nm is proportional to the concentration of lactate in the sample.
reagent
Composition
Vial A (Reagent)
Tris Buffer $2.2 \times 10^{-1}$ moles/liter
Glutamate $2.1 \times 10^{-2}$ moles/liter
Alanine
aminotransferase
(animal) $2.4 \times 10^{-1}$ U/liter
Lactate dehydrogenase
(animal) $2.1 \times 10^{-1}$ U/liter
Stabilizers
pH 9.5
Vial B (Cofactor)
nicotinamide-
adename dinucleotide $3.1 \times 10^{-3}$ moles/liter
Stabilizer
Concentrations given are in the final reaction mixture exclusive of sample volume and may vary within manufacturing tolerances. These variations will not affect reagent performance.

Precautions
For In Vitro Diagnostic Use. Vent vials carefully, since contents of some vials are vacuum sealed. If there is any indication moisture has penetrated the seal, discard the vial in question. The toxicological properties of this reagent have not been determined; do not ingest.

Preparation
Cat. No. 869218: 15 mL
1. Pipet 15 mL of distilled water into one of the cofactor (B) vials. Cap and invert gently to dissolve.
2. Transfer contents of the cofactor (B) vial into one of the reagent (A) vials. Cap and immediately invert repeatedly to dissolve contents. Do not shake.

Storage and Stability
Store at 2° to 8°C. Prepared reagent is stable for at least 48 hours when stored in a glass container at 2° to 8°C.
If the well-mixed reagent shows an initial absorbance of more than 0.4 at 340 nm measured against distilled water or visible evidence of microbial contamination, consider the reagent unsuitable and discard.

specimen
Collection and Preparation
Venous blood should be drawn cleanly, without stasis, and with as little trauma as possible. Lactate and pyruvate concentrations change rapidly after blood is drawn unless collected in a suitable anticoagulant-preservative or immediately deproteinized.

1. Plasma
Use plasma from blood collected in a fluoride-oxalate tube. Whole blood must be kept cold prior to plasma separation. Plasma should be separated from the formed elements of blood without undue delay. These anticoagulants have no effect on the assay when the sample used is only 100 μL. Use 10 mg sodium fluoride and 10 mg potassium oxalate for 5 mL blood (for equivalent commercially available blood collection tube).

2. Deproteinized Sample
Add 1.0 mL of cold whole blood or plasma to 2.0 mL of ice-cold 0.6 M perchloric acid in a centrifuge tube. Mix thoroughly. Let stand for 5 minutes. Centrifuge at 3000 rpm for at least 5 minutes. Use the supernatant for the assay.
Interfering Substances
A number of drugs and substances affect the lactate assay. Young, et al. have published a comprehensive list of such substances.

Storage and Stability
If blood is cooled and centrifuged within 15 minutes of collection, the plasma is stable for 28 days at -20°C. Glycolysis is arrested for 24 hours at 4°C in blood collected in fluoride oxalate. Lactic acid has been reported to be stable in perchloric acid filtrates of oxalated blood for one week at 30°C.

procedure
Materials Provided
Vial A — Reagent
Vial B — Cofactor (NADH)
Materials Required But Not Provided
1. A photometer capable of reading absorbance changes at 340 nm and calibrated for theoretical response to the molar absorbance of NADH.
2. Waterbath, 30°C.
3. Cuvets, 1 cm pathlength.
4. Pipets, to deliver 2.9 mL.
5. Microspoons, 100 µL.
6. Timer.
7. Distilled or highly purified deionized water, free of particulate or microbial contamination.
8. 0.6 M perchloric acid. Dilute 5 mL of 70% reagent-grade perchloric acid to 100 mL with distilled water.
9. Sodium chloride, 0.9% (w/w). Dissolve 0.9 g of sodium chloride in sufficient distilled water to make 100 mL of solution.

Method

| Wavelength | 340 nm |
| Temperature | 30°C |
| Reagent Volume | 2.9 mL |
| Sample Volume | 100 µL |
| Reaction Time | 15 min. |
| Calculation Factor | 1.31 |
| Deproteinized Sample | 43.5 |

1. Make all absorbance measurements at 340 nm, using water as a blank.
2. Dispense 2.9 mL of prepared reagent into a clean, dry cuvet.
3. Warm reagent and sample to 30°C. Assay may be performed at 37°C if desired.
4. Measure initial absorbance (A₀) of the reagent alone.
5. Add 100 µL of plasma or deproteinized sample to cuvet. Mix quickly by gentle inversion. Avoid shaking as this can trap air bubbles in the solution. Incubate at 30°C.
6. Measure the final absorbance (Aₙ) 15 minutes after adding sample to the cuvet.
7. If the measured lactic acid concentration exceeds 50 mg/dL, repeat the test using a diluted sample.

Assay Procedure for Blank Values
A blank is not necessary when a deproteinized sample is used. When plasma is used, a blank determination is advisable, particularly if the sample is turbid or lipemic. For a blank determination, mix 2.9 mL of 0.9% sodium chloride with 100 µL plasma, incubate at 30°C for 15 minutes, and measure the absorbance (A₀) against distilled water, using optically matched cuvets.

Quality Control
Each day a sample is tested, a normal and abnormal control of known concentration should be analyzed. A lactic acid solution may be prepared by dissolving 10 to 20 mg of L-lactic acid, lithium salt in 100 mL distilled water.

Daily quality control results should fall within an established acceptable range. If the precision of the assay system does not correlate with this standard and repetition excludes errors in technique, check the following areas:
1. Cleanliness of glassware, especially cuvets.
2. Purity of water.
3. Instrument wavelength setting, light source and absorbance calibration.
4. Reaction temperature, including temperature control systems.
5. Pipetting and timing mechanisms.
6. Expiration date of reagent package and prepared reagents.
7. Storage conditions of prepared reagents.
results

A. Deproteinized Sample

Lactic acid (mg/dL) = \( \Delta A \times 131 \)

\( \Delta A = \) measured corrected absorbance change

131 = calculation factor

1. Multiply the initial absorbance by 0.967 to correct for change in volume due to the addition of 100 \( \mu \)L of sample:

\[ A_g \times 0.967 = A_c \]

2. To obtain \( \Delta A \), the change in absorbance, subtract the corrected initial absorbance \( A_c \) from the final absorbance \( A_f \):

\[ A_f - A_c = \Delta A \]

3. If the sample is too concentrated, make a suitable dilution of the sample with 0.9% sodium chloride and repeat the assay. The dilution factor, \( D \), is equal to the dilution made, i.e., \( D = 2 \) when a 1:1 dilution is made, etc. Multiply the answer by the dilution factor.

4. Calculation example (in mg/dL):

When \( A_g = 0.140 \) and \( A_f = 0.340 \) using 100 \( \mu \)L of protein-nixed sample, then:

\[ A_c = 0.140 \times 0.967 = 0.135 \]

\[ \Delta A = 0.340 - 0.135 = 0.205 \]

Lactic acid = 0.205 x 131

= 27 mg/dL

B. Plasma

Lactic acid (mg/dL) = \( \Delta A \times 43.5 \)

\( \Delta A = \) measured corrected absorbance change

43.5 = calculation factor

1. Multiply the initial absorbance by 0.967 to correct for change in volume due to the addition of 100 \( \mu \)L of sample:

\[ A_g \times 0.967 = A_c \]

2. To obtain \( \Delta A \), subtract the corrected initial absorbance \( A_c \) from the final absorbance \( A_f \):

\[ A_f - A_c = \Delta A \]

3. To obtain the change in absorbance, \( \Delta A \), subtract the blank absorbance, \( A_b \), from \( \Delta A \):

\[ \Delta A_b - \Delta A = \Delta A \]

4. If the sample is too concentrated, make a suitable dilution of the sample with 0.9% sodium chloride and repeat the assay. The dilution factor, \( D \), is equal to the dilution made, i.e., \( D = 2 \) when a 1:1 dilution is made, etc. Multiply the answer by the dilution factor.

5. Calculation example (in mg/dL):

When \( A_g = 0.140 \), \( A_b = 0.020 \), and \( A_f = 0.340 \) using 100 \( \mu \)L of plasma:

\[ \Delta A_c = 0.140 \times 0.967 = 0.135 \]

\[ \Delta A_f = 0.340 - 0.135 = 0.205 \]

\[ \Delta A = 0.205 - 0.020 = 0.185 \]

Lactic acid = 0.185 x 43.5

= 8 mg/dL

Derivation of the Factor

The following formula derives the factor for calculating lactic acid concentration in mg/dL sample:

\[ \Delta A = T.V. \times M.W. \times 100 \]

\[ \times S.V. \times P \]

\[ \times m \text{ lactic acid/dL sample} \]

\[ \times \text{measured corrected absorbance change} \]

\[ \times \text{molar absorptivity of NAOH} \]

\[ \text{L mole}^{-1} \text{ cm}^{-1} \]

\[ \times \text{S.V. \ sample volume (mL)} \]

\[ \times \text{T.V. \ total assay volume (mL)} \]

\[ \times \text{M.W. \ molecular weight of lactic acid = 90.1} \]

\[ \times \text{P \ lightpath (cm)} \]

\[ \times 100 \text{ \ factor to convert mL to dL} \]

Thus, the calculation for lactic acid concentration becomes:

\[ \Delta A \times 3.0 \times 90.1 \times 100 \]

\[ 8.22 \times 10^2 \times 0.1 \times 1 \]

\[ \times \Delta A \times 43.5 \times \text{mg lactic acid/dL sample} \]
A. Deproteinized sample
   The protein-free filtrate is a 1:3 dilution of the original sample. Thus, the calculation factor becomes:
   \[ \Delta A \times 43.5 \times 2 = \Delta A \times 131 \quad \text{mg lactic acid/dL sample}. \]

B. For Plasma:
   There is no dilution when plasma is used. Therefore, the calculation factor is:
   \[ \Delta A \times 43.5 = \text{mg lactic acid/dL sample}. \]

Limitations of the procedure
   Hemolyzed specimens should be deproteinized for assay.
   This reagent system is designed for 30°C, but assays may be performed at 37°C if desired. At lower temperatures (25°C), the reaction should be monitored to ensure completion within 15 minutes; the incubation time may be extended if desired.
   This procedure uses the known molar absorbance of NADH to calculate lactic acid concentration. If desired, a lactic acid standard solution may be used to calibrate the system.

Expected values
   Each laboratory should establish its own recovery values suitably representative of the range of expected values. The range of expected values determined for lactic acid in plasma samples from apparently healthy humans is 3 to 23 mg/dL. Based on a population of 200 males and females, the range was determined using a nonparametric analysis.\(^2\) Arterial blood levels vary between 3 and 7 mg/dL. The plasma level is 7% higher than the whole blood level.

Specific performance characteristics
   Assuming the minimum absorbance change which can be measured is 0.01, sensitivity of the method would be 1 mg/dL using deproteinized sample and 0.5 mg/dL using plasma.
   Other 2-hydroxy acids can act as substrates of LDH, but their concentration in serum is low.\(^7\) Replicate assays on an abnormal control serum yielded a coefficient of variation of less than 3%.
   When this reagent \( (y) \) was compared to that described by Rosenberg et al. \( (x) \) in assays on 31 samples (from 6 to 39 mg/dL), a correlation coefficient \( (r) \) of 0.998 was obtained with a regression formula of \( y = 1.01x + 1.0 \).

References

Warranty:

The product is warranted to perform as described in the labeling and in Behring Diagnostic's literature when using the procedure indicated herein. Any changes or modifications to the procedure may affect the results. In such event, Behring Diagnostics disclaims all warranties, expressed, implied or statutory, including any implied warranty of merchantability or fitness for use. In no event shall Behring Diagnostics be liable for any indirect or consequential damages arising out of the above mentioned express warranty.

Behring Diagnostics Inc.
17 Chubb Way
Somerville, NJ 08876
Published May 1, 1985

Doc. No. N90074
APPENDIX 3

Experiment Results - Equine

Experiment 1
Experiment 2
Experiment 3
## Experiment 1

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### Differential WBC

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### Collection 1. Plasma Standards
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Absorbance at 405nm = 0.263 + 0.606 x Endotoxin Concentration
### Collection 2. Water Standards

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Absorbance at 405nm = 0.113 + 0.592 x Endotoxin Concentration

### Collection 2. Plasma Standards

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Absorbance at 405nm = 0.293 + 1.889 x Endotoxin Concentration
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Absorbance at 405nm = 0.140 + 0.038 \times \text{Endotoxin Concentration}

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Absorbance at 405nm = 0.155 + 0.758 \times \text{Endotoxin Concentration}
### Collection 7. Water Standards

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Absorbance at 405nm = 0.072 + 0.083 x Endotoxin Concentration

### Collection 7. Plasma Standards

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Absorbance at 405nm = 0.188 + 0.656 x Endotoxin Concentration
### Final Water Endotoxin Standards

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Absorbance at 405nm = 0.305 + 0.651 \times \text{Endotoxin Concentration}

### Final Plasma Endotoxin Standards

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Absorbance at 405nm = 0.650 + 0.608 \times \text{Endotoxin Concentration}
Experiment 3

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APPENDIX 4

Experiment Results - Canine

Nutrient Digestibilities
Faecal Smell, Stool Formation and Water Content Assessment
Body Weights and Weight Changes
### Nutrient Digestibilities

| Digestibility Values (%) of Feed Dry Matter, Organic Matter, Fat and Crude Protein and Digestible Energy Content (MJ DE/kg DM) of Control (C) and Treatment (T) diets |
|---|---|---|---|---|
| | Dry Matter | Organic Matter | Fat | Protein | Energy |
| **Period 1** | | | | | |
| **Treatment C** | | | | | |
| Dog | 10 | 66.82 | 75.47 | 92.63 | 78.57 | 16.62 |
| | 8 | 76.34 | 81.44 | 94.44 | 83.26 | 17.85 |
| | 7 | 63.58 | 71.67 | 91.91 | 78.31 | 15.83 |
| | 6 | 61.30 | 70.95 | 93.61 | 78.45 | 16.03 |
| **Treatment T** | | | | | |
| Dog | 5 | 71.16 | 78.25 | 89.22 | 83.00 | 17.23 |
| | 4 | 74.26 | 81.13 | 96.98 | 84.31 | 17.71 |
| | 3 | 70.29 | 77.81 | 96.37 | 82.13 | 17.09 |
| | 2 | 73.82 | 79.99 | 96.70 | 81.44 | 17.43 |
| | 1 | 66.19 | 74.94 | 93.63 | 79.26 | 16.65 |
| **Period 2** | | | | | |
| **Treatment C** | | | | | |
| Dog | 6 | 69.34 | 76.82 | 97.06 | 82.69 | 16.87 |
| | 11 | 63.52 | 72.87 | 91.44 | 73.47 | 15.78 |
| | 4 | 66.88 | 76.04 | 89.93 | 78.86 | 16.67 |
| | 3 | 68.40 | 76.60 | 98.16 | 84.23 | 16.81 |
| | 2 | 65.63 | 76.04 | 96.93 | 78.60 | 16.65 |
| **Treatment T** | | | | | |
| Dog | 5 | 75.05 | 82.85 | 93.25 | 85.29 | 17.96 |
| | 1 | 76.71 | 82.71 | 92.30 | 87.47 | 18.10 |
| | 8 | 68.63 | 83.27 | 89.32 | 75.49 | 16.45 |
| | 7 | 65.72 | 72.92 | 71.77 | 75.97 | 15.67 |
Faecal Smell, Stool Formation and Water Content Assessments

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+ values according to the rating system outlined in Table 3.6
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AN EVALUATION OF BENTONITE FEED ADDITIVES IN HORSES AND DOGS AND A REFLECTION ON THE RESEARCH PROCESS

A thesis submitted for the degree of
Master of Science Honours -
Systems Agriculture

by

ROBERT LOGAN HANNON

1996

Faculty of Agriculture and Rural Development,
University of Western Sydney, Hawkesbury
Bourke Street,
RICHMOND NSW 2753
AUSTRALIA
PLEASE NOTE

The greatest amount of care has been taken while scanning this thesis,

and the best possible result has been obtained.
Declaration

The author certifies that the work recorded in this thesis was performed by the author, that all assistance received has been acknowledged and that the content has not been submitted for a higher degree at any other institution.

October 1996

Robert Logan Hannon
Summary

This thesis reports on investigations into the influence of diet and a bentonite feed additive on blood parameters in the horse, and the effect of a bentonite feed additive on the process of digestion in the dog. In addition, the methodology of scientific research is examined, and reflections of learning experiences arising from contact with the Hawkesbury approach described.

A high-concentrate low-roughage diet, consisting by weight of 70% oats and 30% lucerne chaff, when fed to horses was shown to influence blood red cell levels and blood L-lactate levels compared to three diets of equivalent energy content but reduced proportion of concentrate. The mean value for red blood cell level of $7.23 \times 10^6/\mu L$ and mean value for blood L-lactate of 6.88mg/dL observed with the high-concentrate diet were higher than those seen with the other diets. This difference was shown to be statistically significant ($p=0.0261$ and $p=0.0000$ respectively).

Other blood parameters measured included packed cell volume, total plasma protein, total and differential white blood cell count. These demonstrated no significant alteration with diet. Measurement of plasma endotoxin levels was attempted, however quantification of such values was not achieved.

Preliminary investigations examining the inclusion of a bentonite feed additive called Nature Vet Thrive P™ for Horses (Nature Vet Pty Ltd, Agnes Banks, NSW, Australia) in a diet containing 70% oats and 30% lucerne chaff did not significantly alter the measured blood parameters in the horse, except for plasma L-lactate levels which were observed to take on a pattern similar to that seen with a diet of 100% lucerne chaff.
The inclusion of a bentonite feed additive called Nature Vet Thrive D™ for Dogs (Nature Vet Pty Ltd, Agnes Banks, NSW, Australia) in a kibble and mince diet fed to dogs was demonstrated to improve the digestion of dry matter and organic matter components of the diet compared to the unmedicated control diet. Apparent mean digestibilities of dry matter and organic matter were 71.31% and 79.32% respectively. These improvements were statistically significant (p=0.0375 and p=0.0249 respectively).

Inclusion of the bentonite feed additive in the diet was also associated with an improvement in degree of stool formation and reduction in faecal odour. The mean rating for stool formation of 1.85 and faecal smell of 1.73 were significantly different from the control group (p=0.0322 and p=0.0017 respectively). Faecal moisture content in the treatment group was lower at 66.55%. This difference, however, was not statistically significant (p=0.1283).

The ability of the dog to digest dietary protein, energy and fat were also observed to be altered by the inclusion of the bentonite additive in the diet. Apparent mean digestibilities of protein and energy were seen to increase to 81.60% and 17.14MJ DE/kg dry matter respectively. These differences were not statistically significant (p=0.2712 and p=0.1066 respectively). The ability of the dog to digest fat was reduced in the treatment group. Apparent mean digestibility of fat was 91.06%, however, the difference was not significant (p=0.3019).

Participation in the Masters program whilst undertaking the investigations into the effects of bentonite feed additives in the horse and dog resulted in the acquisition of greater knowledge about science. It also enabled learning about systems, experiential learning and the Hawkesbury approach. This exposure and learning from the Masters program encouraged reflection on the research studies carried out and fostered the development of epistemic cognition. As an extension to
this, an improved appreciation of the reductionist methodology utilised in the investigations and the constructivist phenomenological learning paradigm of the Faculty of Agriculture were developed.

The methodology of science deals partly with abstraction and reflection on past experiences. It utilises the method of science, which deals in concrete and is focussed on present and future. This method involves selection of the world the scientist wishes to examine, making a reduction and designing an artificial situation to examine certain variables while keeping others constant. Results are then described, analysed and interpreted before being critiqued by fellow scientists.

Reflection on the method of science utilised in the studies reported in this thesis highlights the potential weakness of this process - its artificiality. Yet, this very reflection stimulated as a result of involvement in the Masters program, has enabled improved cognition of the methodology of science and thereby has improved the relevance of the results of the investigations for their application in the greater world.

The Hawkesbury paradigm, which is centred on experiential learning, initially makes the learner more aware of the process of learning, including the method of science. Ultimately though, it allows the creation of a repertoire of learning and problem solving methodologies which are consciously controlled by and work for an individual.

The experiential learning model suggests that the effective learner will be open to new experiences and when faced with a problematic situation, will develop a rich picture of the problem context and so will avoid false or premature problem definition. This model is a powerful tool for communicating the need to combine theory and practice in improving problem situations.
Indeed, this need to combine theory and practice stimulated the development of a methodology that incorporates the science method within a broader appreciative systems context. This methodology is described in this thesis. It involves the utilisation of the reductionist scientific approach within the framework of an appreciative systems methodology. Such an approach to scientific research, such as the research undertaken and reported in this thesis, would enable the use of 'subjective' data and researcher’s values within reductionist studies in a manner that did not detract from the 'objectivity' of the trial design, and would potentially improve the relevance of results and their application to real life situations.

This thesis is written in two sections. Part A reports on the investigation into the influence of diet on blood parameters in the horse, including the preliminary findings following the inclusion of a bentonite feed additive to a high-concentrate low-roughage diet. Also reported in this section are the findings from the investigation into the effect of a bentonite feed additive on the process of food digestion in the dog.

Part B of this thesis documents the learning experience developed from involvement in the Masters program and reflection on the research studies undertaken. A model for improved reductionist research is described.
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2.3 Structure of Bentonite  
2.4 Properties of Bentonite  
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<th>Abbreviation</th>
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<td>AC or C</td>
<td>abstract conceptualisation</td>
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<td>active experimentation</td>
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<td>AERF</td>
<td>Australian Equine Research Foundation</td>
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<td>Al&lt;sup&gt;3+&lt;/sup&gt;</td>
<td>aluminium ion</td>
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<tr>
<td>AM</td>
<td>morning</td>
</tr>
<tr>
<td>AOAC</td>
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<td>ARC</td>
<td>Australian Research Council</td>
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<td>BMDP</td>
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m  metre
M  molar
μg  microgram
μL  microlitre
mEq  milliequivalent
Mg²⁺  magnesium ion
mg  milligram
MJ  megajoule
mL  millilitre
n  number of observations
Na⁺  sodium ion
NCTS  National Company Teaching Scheme
NDF  neutral detergent fibre
ng  nanogram
NH₄⁺  ammonium ion
nm  nanometre
N/R  no reading
NRC  National Research Council
NV  Nature Vet Pty Ltd
OM  organic matter
OMD  organic matter digestibility
%  percentage
p  probability
P  phosphorus
PCV  packed cell volume
PD  protein digestibility
pg  picogram
pH  negative logarithm of the hydrogen ion concentration
pI  isoelectric point - the hydrogen ion concentration at which a molecule will fail to migrate in an electric field because it has no net charge
PM  afternoon
Rb⁺  rubidium ion
RBC  red blood cell
RH  Robert Hannon
RO or R  reflective observation
RW  Robert Woog, Hawkesbury Agricultural College
SEM  standard error of the mean
SGOT  serum glutamic oxaloacetic acid
Si⁴⁺  silicon ion
T  treatment group
Th⁴⁺  thorium ion
TPP  total plasma protein
VFA  volatile fatty acid
WBC  white blood cell
® or ™  registered trademark

To assist the reader’s understanding of this thesis, some background information is offered.

Who?

I am a Veterinary Scientist with formal training in veterinary medicine and surgery. Such training has equipped me with the skills to diagnose, treat and prognose disease states in animals.

In order to be able to perform such a functions, both a systematic and systemic approach to problem solving is necessary. Whilst these skills are possessed by most people and are used on a daily basis, formal veterinary training develops these to a level that enables understanding and interpretation of systems operations in non-human animals.

What?

Part A of this thesis reports on the investigations I undertook into the influence of diet and a bentonite feed additive on blood parameters in the horse, and the effect of a bentonite feed additive on the process of digestion in the dog.

Part B explores the method and the methodology of scientific research, systems thinking and the Hawkesbury approach to learning and research. It incorporates a reflection on these research approaches. There is also a reflection on the horse and dog studies undertaken from the phenomenological learning paradigm that is part of the Faculty of Agriculture, with a description of the enhancement in my understanding of the learning and researching processes resulting from this reflection.
When?

The investigations undertaken, the participation in the Masters program, the reflection on the learning experiences and the writing of this thesis came about as a result of my involvement as a research associate in the National Company Teaching Scheme (NCTS). This scheme was developed by the Commonwealth of Australia’s Department of Industry, Technology and Commerce (DITAC) and was designed to provide university graduates with simultaneous exposure to manufacturing industries and teaching institutions, and involve them in research studies that would benefit the company and the institution involved in the scheme.

As a result of the three-way working relationship between the company, the institution and the research associate, further professional development of the associate would be achieved. In addition to the learning arising out of the research, the associate would also further his education through involvement in post-graduate studies.

Where?

Hawkesbury Agricultural College (HAC), now the University of Western Sydney Hawkesbury since 1989, was chosen as the most appropriate location for undertaking post-graduate education for several reasons and will be recognised in this thesis by its former name. Firstly, this teaching facility had been successful (along with the private company Nature Vet Pty Ltd) in securing DITAC funding to allow a research associate to be employed.
Secondly, the location of the college was proximate to the company, the research facilities, and the technical and academic support necessary for the scientific investigations to be undertaken.

And finally, the Faculty of Agriculture, now the Faculty of Agriculture and Rural Development, had the necessary teaching paradigm to enable accommodation of the research studies and thesis within the post-graduate program. The constructivist approach espoused by the Faculty was seen as critical in allowing the achievement of optimal reflection and abstraction of the science method employed in the investigations.

Why?

The investigations reported in this thesis utilised the method of science - that is, reductionism, repeatability and refutation. This approach is widely considered to be standard practice for determining whether the alteration of one component in a biological system would result in measurable change to that system.

The method of science is recognised for its ability to improve knowledge about things unknown. It involves taking a piece of the world about which further knowledge is desired, finding that knowledge and then 'extrapolating' this new knowledge back into the greater world from which the sample was taken.

However, the phenomenological and constructivist learning paradigm of the Faculty of Agriculture challenged the notion as to whether the methodology of science was adequate to explain the complexities of the real world. In other words, can the results achieved at the conclusion of an enquiry undertaken using the method of science that examines a component of the real or greater world be referred back to the greater world with relevance?
The experiential learning practices of the Faculty of Agriculture and the post-graduate program stimulated critical reflection on the method used to achieve knowledge in the horse and dog investigations. Consequently, a drive to better understand the method of science and other approaches to knowledge acquisition, such as systems thinking which was used by the Faculty, was generated.

With this in mind, post-graduate education was pursued, enabling a higher level of understanding of the research process used in the investigation and the mechanisms by which people learn. As a result of the post-graduate experience, there was greater understanding of the relevance of the research findings and their suitability for referral back into the real world.

**How?**

Three themes of involvement were envisaged during the association with the Faculty of Agriculture and the Masters program. The first theme incorporated the search for truth and knowledge, which arose from the science component of the work. The second theme incorporated the understanding of reality and complexity which arose from being exposed to and operating within the world of systems. The increased appreciation that came from an association with the Masters program and the Faculty of Agriculture enabled improved realisation of where the science-based work undertaken lay within the broader context of science and knowledge. The final theme related to learning as an individual and the realisation of the significance of experiential learning in knowledge acquisition, particularly in the Action Research methodology utilised by the Faculty in many of its studies. The three themes taken together created the lived in world of constructivism and is summarised in Figure i.
In view of the diversity of subject material covered within this thesis, it is presented in two separate parts. Part A deals with the science-based work in which aspects of equine and canine nutrition and digestive physiology were investigated. Part B deals with the writer's association with the Faculty and its paradigm through involvement in the post-graduate program. It explores the notion of science as a method to acquire truth and knowledge about the world by examining its history and processes, and explores systems thinking and experiential learning as alternative methods of acquiring knowledge. Modelling of methodologies that may be more appropriate for biologic scientific research are explored.

Whilst Part A deals with the learnings and understandings that have come out of the science research, it is Part B that deals with the broad learnings that have come out of the biologic studies carried out and the association of the writer with the Masters program. Part B deals with the increased awareness of metacognition and epistemic cognition.

Outcome from Post-Graduate experience in the Masters Program

Figure 1. The three themes of experience at Hawkesbury
Acknowledgements

I would like to express my sincere appreciation to my supervisor, Dr Robert Woog, the late Leigh Whatmore (Nature Vet Pty Ltd) and Jack Wolfenden for their guidance and assistance during the course of this study. It is largely as a result of their support that this thesis ever came to fruition, and for that I am truly indebted.

I also wish to thank to Dr Nadarajah Sriskandarajah for his advice on equine nutrition and digestive physiology, and Olga Slezacek and June Stewart for their assistance with laboratory analyses of blood and faecal samples. I am also grateful to John MacFarlane for his advice on the statistical analysis of the results, to Joanne Derksen for her assistance with the preparation of this manuscript and to the members of the Faculty of Agriculture who supported this project.

And to my family and friends who kept me going during the tough times, thank you for being there.