COMPARISON OF THE PASSIVE PROPHYLACTIC EFFECT OF BOVINE MILK IMMUNOGLOBULIN FED EITHER AS A BOLUS OR CONTINUOUSLY AGAINST DIARRHOEA CAUSED BY *ESCHERICHIA COLI* K88 USING PIGLETS AS MODELS

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ABSTRACT

The overall aim of the study was to determine the passive prophylatic effect of bovine milk immunoglobulin administered orally either as a bolus (once a day) or as continuously (three times a day) against diarrhoea caused by *Escherichia coli* K 88.

As the piglets have gastrointestinal structural and physiological similarities to humans they were assumed to be an appropriate model for the study. The first part of the study was done to evaluate the quantity of undigested immunoglobulin G in the gastrointestinal tract of the piglet. This was conducted in two experiments. One was a pilot study and the other was to estimate the quantity of undigested immunoglobulin G in the gut.

A pilot study was conducted to evaluate the rate of movement and the quantities of digesta that can be collected in the gastrointestinal tract regions at varying time intervals. Ten piglets were randomly divided into five groups of two in each. One from each group was selected, fed with an experimental diet which contained blue colour glass beads and dye and slaughtered at either 1, 5, 9, 16 and 24 hrs after feeding. Digesta was collected from various regions of the gastrointestinal tract. Faeces were collected using ostomy bags from those slaughtered at 9, 16 and 24 hrs only. The dye movement and the glass beads recovered were monitored. The movement of the dye was observed up to the small intestine at 1 hr, the caecum at 5 hrs, the beginning of the colon at 9 hrs, the two third of the colon at 16 hrs, and in the faeces at 24 hrs. Most of the beads were found in the stomach between 1 and 5 hrs, spread throughout the small intestine at 9 hrs, in the caecum at 16 hrs and in the colon at 24 hrs. The results confirmed that a sufficient amount of digesta could be collected from the various regions over a 24 hr period. The data facilitated the planning of the immunoglobulin digestibility trial which is the second part of the experiment.

To measure the IgG digestibility, the piglets were fed on a large dose of an experimental diet (10% of their metabolic body weight $kg^{0.75}$ contain 30% immunoglobulin) and the digesta, faeces and blood were collected. On the slaughter day, a group of five animals were fed on an experimental diet and digesta and blood were collected 1, 5, 9, 16, and 24 hours after feeding. Faeces were collected from those killed at 16 and 24 hours. Blood was analysed for immunoglobulin G for all piglets. Digesta and faeces were

analysed for chromium and immunoglobulin G. There was no evidence of the presence of immunoglobulin G in the blood and faeces. A larger quantity of immunoglobulin G was found in the stomach ($p<0.001$) with a less in the first and second third of the small intestine ($p<0.05$) 24 hours post-prandially. This demonstrated that immunoglobulin G could resist digestion in the gut.

The second part of the study was conducted to compare the effect of feeding bovine immunoglobulin as a bolus versus continuous against *Escherichia coli* K88 diarrhoea. Twenty-four piglets, four-week-old, were randomly allocated to three treatment groups, namely continuous (fed a diet containing 10% immunoglobulins three times a day), control (fed an immunoglobulins free diet), and bolus (fed a 30% immunoglobulins diet in the morning and control diet the other two feeds). On Day 9, 30 minutes before the morning feed, the piglets were inoculated with 1×10^9 cfu *Escherichia coli* by a syringe into their throat, and observed for nine days. On day 17, all piglets were fed on control diet (no Ig) to evaluate if *Escherichia coli* K88 would have any effect on recolonisation and diarrhoea and observed for further three days. Finally they were all treated with antibiotics, Biosol M and Tylan 200. The observations include faecal culture, faecal consistency, the percent of free water content in the faeces, weight and feed intake. Faecal culture was done twice before inoculation, three times during treatment, and once after all were fed on control diet and once after the antibiotics treatment. The free liquid content in the faeces was highest in the control group (37.5%) and lower in the continuous immunoglobulin group (25.0%) and least in the bolus immunoglobulin group (17.5%). Bolus immunoglobulin feeding (11.25%) lessened the severity of diarrhoea (classified by consistency) compared with the control group (26.2%) and continuous group (25.0%). Hence bolus immunoglobulin feeding had a better effect in controlling water loss and the severity of diarrhoea. A higher dosage of immunoglobulin in bolus feeding may also have prevented bacterial shedding. From this study, it can be concluded that feeding immunoglobulin as a bolus could be used as a prophylactic treatment for diarrhoea.

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ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
CCM	Caecum
COL	Colon
Cr	chromium
Cr FD	chromium in freeze dried sample
DICP	Di calcium phosphate
E.coli	Escherichia coli
FD	Freeze dried
Fab	Fragment antigen binding
Fc	Fragment crystalline
Ig	Immunoglobulin
Igs	Immunoglobulins
IgA	Immunoglobulin A
IgD	Immunoglobulin D
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgG FD	IgG in freeze dried sample
IgM	Immunoglobulin M
IgY	Immunoglobulin Y
IM	Intramuscular
IV	Intravenous
N	Negative
NF	Not fed
NS	No samples
P	Positive
PBS	Phosphate buffer solution
RID	Radial immuno diffusion
SI1	Small intestine 1
SI2	Small intestine 2
SI3	Small intestine 3
SIgA	Secretory immunoglobulin A
SIgGCr	Sample IgG/chromium
SMP	skim milk powder
STM	Stomach
TNBS	Trinitrobenzenesulfonic acid
WGC	Whey globulin concentrate

]INTRODUCTION TO EXPERIMENTS

Diarrhoea continues to be a major problem of morbidity and mortality in infants and young children in developing countries. In developed nations, enteric infections are an important health problem as individuals who are immuno-compromised, attend day-care centres, or travel to less developed countries are at risk. Although progress in immunization has been reported, safe and effective vaccines for many common enteric pathogens are not yet available. In addition, widespread use of prophylactic antimicrobial agents is not encouraged because of the possible side effects. Bovine milk immunoglobulin concentrates have been investigated as a safe and effective alternative against enteric pathogens. The rationale for this approach is supported by results from epidemiologic studies which suggest that disease-specific antibodies in human milk provide protection against diarrhoea in breast fed infants. Orally ingested immunoglobulins must resist denaturation and proteolysis by the intestinal secretions to be effective against pathogens that reside primarily in the gastrointestinal tract. The mode of administration of immunoglobulin may have an effect on protection against infection.

The first aim of the study was to determine the digestibility of orally ingested immunoglobulins in the gastrointestinal tract of piglets. The second aim was to compare the passive prophylactic effect of bovine milk immunoglobulins fed either as a bolus (feeding once a day) or continuously (feeding three times a day) against diarrhoea caused by *Escherichia coli* K88.

CHAPTER 1

REVIEW OF LITERATURE

1.1 IMMUNITY

Living organisms have a precarious existence as they are constantly threatened by the environmental agents, such as micro-pathogens and their products, foods, chemicals, drugs, pollen or animal hair. To guard against such threats, they develop an immune system within their body. The term immunity refers to all those physiologic mechanisms that endow the animal with the capacity to recognize materials as foreign to itself and to neutralize, eliminate or metabolize them with or without injury to its own tissues (Bellanti, 1971). Immunity can be classified as innate or natural, and acquired or specific (Table 1.1, Redmond et al., 1996).

Table 1.1
Components of the immune system♣

	System	
Component	Innate	Acquired
Physical	Skin, mucus, cilia, gastric, hydrochloric acid	--------
Biochemical	Acute phase proteins, lysozyme, C-reactive proteins, complement	Antibodies
Cellular	Phagocytes, natural killer cells	lymphocytes, B lymphocytes

♣ Redmond et al., 1996

Innate immunity is conferred by all the elements with which an individual is born. They are always present and available at short notice to protect the individual from challenges by "foreign" invaders. These elements include body surfaces such as the skin and the mucus membranes, as well as internal components, like the cough reflex, all of which present an effective barrier to environmental agents (Tizard, 1992a).

Acquired immunity is induced by exposure to foreign material (antigens) and can be achieved in different ways, i.e. active, adaptive or passive immunization. Active immunization refers to stimulation of the immune system of an individual by administration of an antigen (Barrett, 1976). Adaptive immunization refers to the transfer of immunity by the transfer of immune cells (Barrett, 1976). Passive immunity can be defined as protection acquired through the transfer of specific antibodies or

immune cells from an immunized individual to an unprotected individual (Barrett, 1976).

The cells composing the immune system are distributed throughout the body mainly found in the lymphoreticular organs, such as, lymph nodes, spleen, bone marrow, thymus, and the mucosa-associated lymphoid tissues of the gastrointestinal tract. Immune cells of the extravascular tissues occupy the interstices of a network of interlocking reticular cells and fibres with a supporting framework of reticular cells. The predominant immunocytes are lymphocytes but monocyte-macrophages, endothelial cells, and rarely eosinophils and mast cells also play a role in the immune system. The cells responsible for the immune system are developed from pluripotent, self-renewing stem cells in the bone marrow (Kamini & Douglas, 1991).

Fundamental features of the immune system are; to be able to discriminate "self" from "nonself", selective or specific reactivity, cytotoxic or antagonistic reaction following sensitization, memory, and systemic dissemination. An ability to discriminate "self" from "nonself" implies that the cells of the immune system can recognize whether a molecular configuration is foreign and therefore requires a response, or host specific in which case an antagonistic response would be inappropriate (Roitt, 1988). Selective or specific reactivity means the immune system is able to recognize and distinguish one antigen from another (Roitt, 1988). Cytotoxic or antagonistic reactions following sensitization refers to the production of effector molecules or antibodies which are produced to promote neutralization or destruction of the antigen source or infectious agent (Roitt, 1988). Memory refers to the ability to selectively alter the immune response on secondary contact (Kamini & Douglas, 1991). Usually this property leads to more vigorous and accelerated response against an antigen previously encountered (Roitt, 1988). Systemic dissemination means that a specific immune response at one site yields immunity to that antigen throughout the body (Tizard, 1992a).

Specific immune responses depend upon prior exposure to a foreign configuration and the subsequent recognition and reaction to it. Natural immunity responses occur following initial exposure to a foreign configuration and, while selective in

differentiating "self" from "nonself", are not dependent upon specific recognition. (Roitt, 1988).

The immune response is comprised of two arms namely, cell mediated immunity, responsible for delayed hypersensitivity, and humoral immunity responsible for antibody production (Barrett, 1976). The lymphoid precursor cells from the bone marrow develop in the central lymphoid organ, giving rise to T and B cells as well as K cells. These cells migrate to the peripheral lymphoid organs and develop their common and/or specific functional capabilities (Roitt, 1988). The interaction between these cells leads to immunologic phenomena such as antibody (immunoglobulin) production, cellular immunity, and tolerance.

1.2 IMMUNOGLOBULINS: CLASSES, STRUCTURE AND FUNCTIONS

Immunoglobulins (Igs) contain both a structural and a functional element. Structurally, all Igs can be related to prototypical heavy chain (H chain) and light chain (L chain) formula (H_2L_2 formula), linked by disulphide bridges (Roitt, 1988). Classes of Igs are distinguished solely by structural differences in the heavy chains. The two major forms of light chain occur in all of the immunoglobulin (Ig) classes, although they are all complex glycoproteins (Figure 1.1 – 1.5). These structural differences are of course reflected in distinctive antigenic properties (Tizard, 1992a). Functionally Igs demonstrate an ability to interact in a selective, specific way with the particular antigen used to induce their production.

1.2.1 Immunoglobulin G (IgG)

Immunoglobulin G is composed of two identical polypeptide chains (the γ Chains) of 55000 daltons (heavy or H chains) as well as two identical chains (either λ or κ) of 22000 daltons (light or L chains). The chains are linked by disulfide bonds to give a total molecular weight of approximately 150,000 daltons and a sedimentation coefficient of 7S (Tizard, 1992a). They are cleaved by proteolytic enzymes to give two fragments: the fragment antigen binding (Fab, antigen binding) and the fragment crystalline (Fc, c' fixing) (Ballabriga et al., 1974/75; Tizard, 1992a). The IgG class is

divided into four subclasses IgG_1, IgG_2, IgG_3, and IgG_4 based on differences in their H chain (Tizard, 1992a).

All IgG subclasses except IgG_2 can pass through the placenta, enabling the mother to transfer her immunity to the foetus in human (Catty et al., 1979; Johnson & Matre, 1979). Their half-life in serum is approximately 23 days except for IgG_3 which has a half-life of seven days (Benjamini & Leskowitz, 1991). Half-life persistence in the serum makes IgG most suitable for passive immunization by transfer of antibodies. Immunoglobulin G is an opsonizing antibody (Hilpert et al., 1977; Rivier & Sobotka, 1978; Bortolussi & Fischer, 1986). It reacts with epitopes on micro-organisms via its Fab portion, but it is the Fc region that confers the opsonizing property. Many phagocytic cells, including macrophages and polymorphonuclear phagocytes, have receptors for the Fc portion of the IgG molecule (Tizard, 1992a). These cells will adhere to the antibody-coated bacteria by virtue of their receptors for Fc. The net effect is a zipper-like closure of the surface membrane of the phagocytic cell around the organism, as receptors for Fc and the Fc regions on the antibodies continue to combine, leading to the final engulfing and destruction of the micro-organism (Porter & Hill, 1970; Brandenburg & Wilson, 1973; Thoman et al., 1981).

The IgG molecule plays an important role in antibody-dependent, cell mediated cytotoxicity (Tizard, 1992a). In this form of cytotoxicity, the Fab portion binds with the target cell (for example a micro-organism or a tumor cell), and the Fc portion binds to specific receptors on certain large granular lymphocytic cells called natural killer cells (Roitt, 1988). By this mechanism, the IgG molecule focuses the killer cells on their target, and the killer cells destroy the target (Benjamini & Leskowitz, 1991; Dwek et al., 1995).

Immunoglobulin G molecules in sufficient numbers can activate the complement system, which results in the release of several important biologically active molecules (the complement cascade) (Janeway & Travers, 1994). Some of these components are chemotactic, i.e. they attract phagocytic cells while others act as opsonins, binding to the target antigen and thereby enhancing phagocytosis (Benjamini & Leskowitz, 1991;

Bricker et al., 1991). Activation of complement may also lead to cell lysis if the antibody binds to antigens on the cell surface.

Immunoglobulin G molecules are efficient in immobilizing various motile bacteria. Antibodies specific for the flagella and cilia of certain micro-organisms cause the bacteria to clump, thereby arresting their movement and preventing their ability to spread or invade tissues (McGuire et al., 1979; Dalhoff, 1985). Inhibition of viral attachment effectively arrests infection (Husu et al., 1993). Other antibodies are thought to inhibit viral penetration or shedding of the viral coat required for release of the viral DNA and RNA needed to induce infection (McGuire & Musoke, 1984).

The IgG molecule is an excellent antibody for the neutralization of toxins (Brandenburg & Wilson, 1973; Ebina et al., 1992; Lissner et al., 1996; Kelly et al., 1996). The reaction of IgG with soluble, multivalent antigens can generate precipitates of insoluble antigen body complexes that are easily phagocytised and destroyed by phagocytic cells (Benjamini & Leskowitz, 1991).

1.2.2 Immunoglobulin M (IgM)

Immunoglobulin M has molecular weight of 900000daltons and a sedimentation coefficient of 19S, and consists of five structural subunits, (each consisting of two L and two H chains), all joined together by additional disulfide bonds between their Fc portions, and by a polypeptide chain termed the J chain. All chains (L, H and J) are synthesized in the B cell or plasma cell (Ballabriga et al., 1974/75; Tizard, 1992a).

Immunoglobulin M molecules are efficient agglutinating antibodies (Martinsson, 1970). Immunoglobulin M can form macromolecular bridges between epitopes that may be too far from each other to be bridged by the smaller IgG antibodies because of its pentameric form. They are, therefore, particularly well suited to combine with antigens that contain repeated patterns of the same antigenic determinant, as in the case of polysaccharide antigens or cellular antigens, which have multiple expression on cell surfaces (Janeway & Travers, 1994).

Immunoglobulin M is an excellent complement-fixing or complement-activating antibody. Unlike other classes of Igs, a single molecule of IgM, upon binding to antigen with at least two of its Fab arms, can initiate the complement cascade, making it the most efficient Ig as an initiator of the complement-mediated lysis of micro-organisms and other cells (Benjamini & Leskowitz, 1991). This ability makes IgM antibodies very important in early neonatal life for immunological defence against bacterial infections. Immunoglobulin M antibodies are not, however, very versatile; they are poor toxin neutralizing antibodies, and they are not efficient in the neutralization of viruses (Janeway & Travers, 1994).

1.2.3 Immunoglobulin A (IgA)

The IgA molecule consists of either two λ chains or two κ chains and two H α chains. The molecular weight of monomeric IgA is approximately 165000 daltons, and its sedimentation coefficient is 7S. IgA, present in mucus secretions, exists as a dimer consisting of two monomeric subunits linked by the same joining (J) chain found in the IgM molecule. In addition, mucosal IgA has another attached glycoprotein, the secretory component, or S piece (Tizard, 1992a). Plasma cells make only the basic IgA molecules and the J chain, which form the dimers. When these dimeric molecules are released from plasma cells, they are bound to the basal membranes of adjacent epithelial cells by a receptor on these cells, which is the secretory component itself. This receptor transports the molecules through the epithelial cells and releases them into the extracellular fluid as dimeric IgA, with the secretory piece attached (Ballabriga et al., 1974/75; Tizard, 1992a). The IgA class of Igs contain two subclasses: IgA_1 (93%) and IgA_2 (7%) (Benjamini & Leskowitz, 1991). IgA_2 contains no disulfide bonds linking the heavy and light chains (Shearman et al., 1972; Ballabriga et al., 1974/75).

The IgA present in serum is predominantly monomeric and has presumably been released before dimerization so that it fails to bind to the secretory component. Little is known of any function for serum IgA (Benjamini & Leskowitz, 1991). The IgA present in external secretions, however, is important in the primary immunological defence against local infections in, for example, the mammary gland, and the respiratory and gastrointestinal tracts (Shearman et al., 1972). Immunoglobulin A acts by preventing

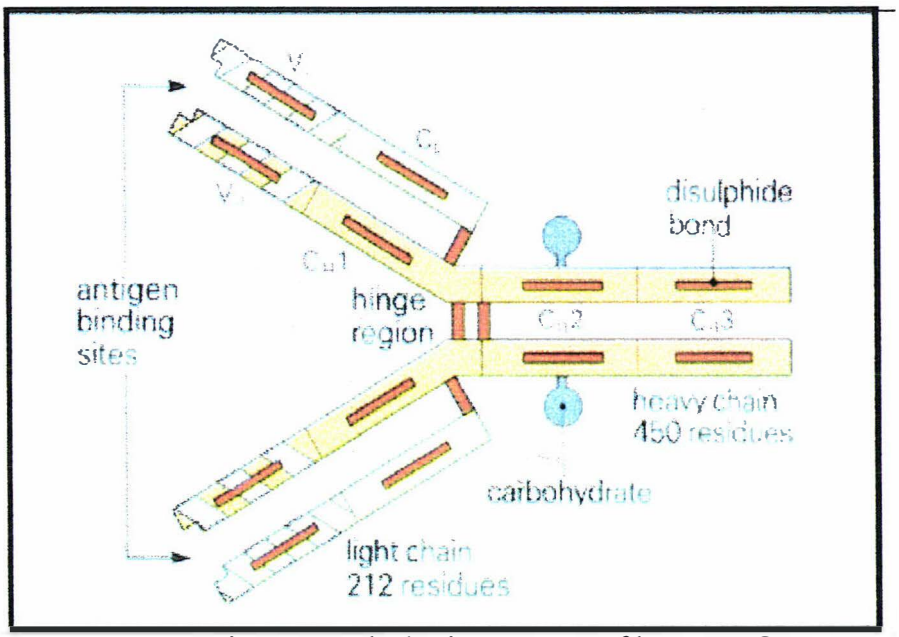

Figure 1.1 The basic structure of human IgG*

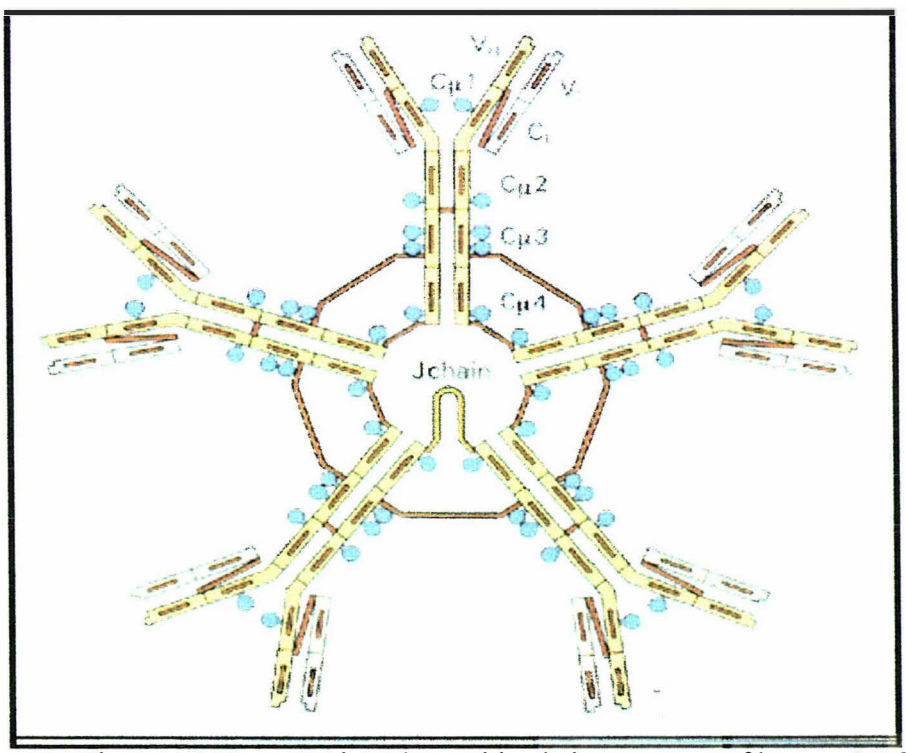

Figure 1.2 Pentameric polypeptide chain structure of human IgM*

* Adapted from Tizard, (1992b)

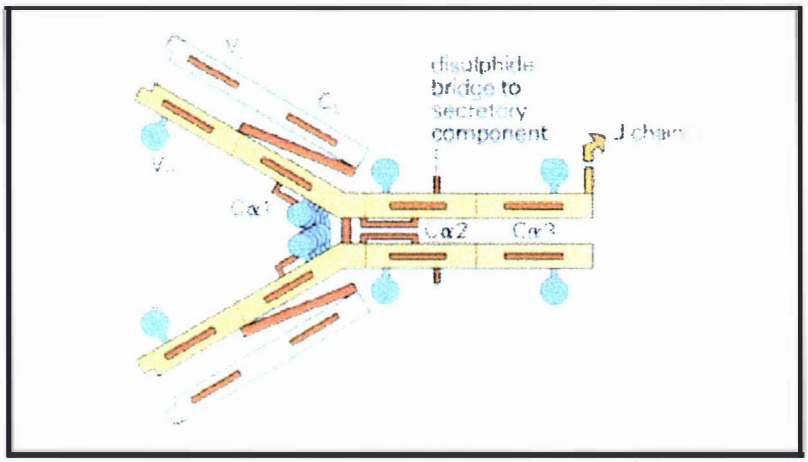

Figure 1.3 Pentameric polypeptide chain structure of human IgA*

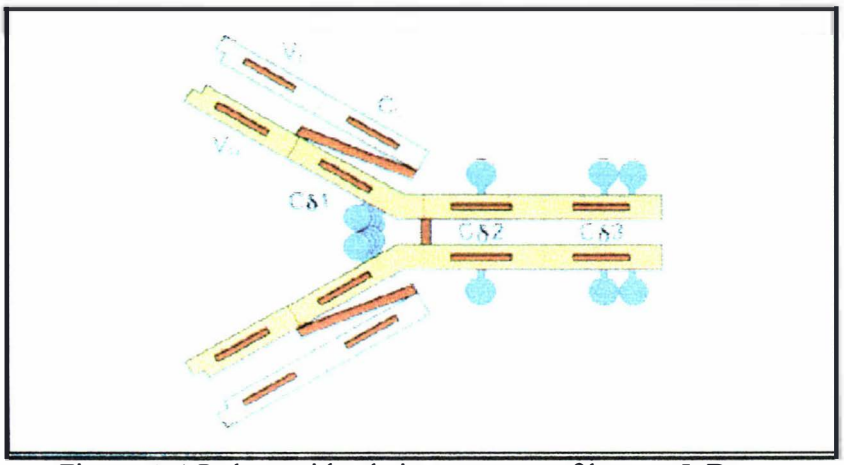

Figure 1.4 Polypeptide chain structure of human IgD*

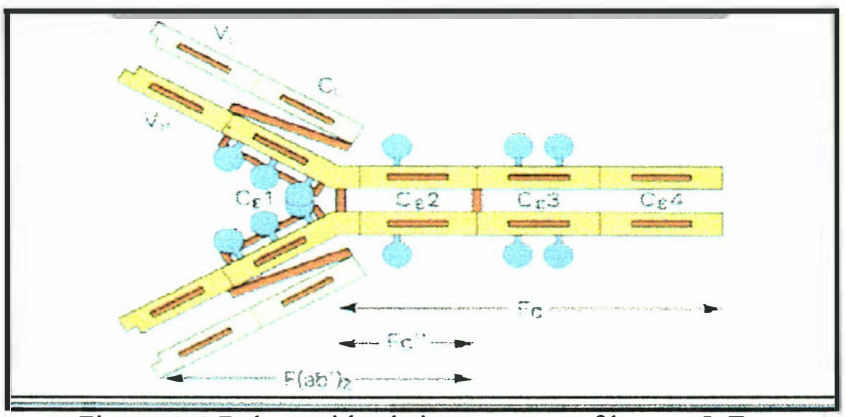

Figure 1.5 Polypeptide chain structure of human IgE*

* Adapted from Tizard, (1992b)

attachment of invading microorganisms to the host cells, thus providing protection from the pathogen (Wernet et al., 1971; Shearman et al., 1972; Walker, 1976). Immunoglobulin A also possesses bactericidal activity against gram-negative organisms, but only in the presence of lysozyme (Adinolfi et al., 1972), and can act as an agglutinating antibody (McClelland et al., 1972; Parkin et al., 1973). It does not activate the complement cascade nor act as an opsonin (Shearman et al., 1972).

1.2.4 Immunoglobulin D (IgD)

The IgD molecule consists of two γ heavy chains and either two κ or two λ L chains. Immunoglobulin D is present as a monomer with a molecule weight of 180000 daltons, and a sedimentation coefficient of 7S (Ballabriga et al., 1974/75). Immunoglobulin D is present in serum in very low and variable amounts, probably because it is not secreted by plasma cells and is uniquely susceptible to proteolytic degradation (Tizard, 1992a).

Immunoglobulin D is present on the surface of B lymphocytes and is thought to be primarily a B cell antigen receptor involved in the maturation of these cells. Immunoglobulin D has specificity against antigens but has not been demonstrated to serve a protective function (Janeway & Travers, 1994).

1.2.5 Immunoglobulin E (IgE)

The IgE molecule consists of two ε H chains and two (λ or κ) L chains with a molecular weight of approximately 200000 daltons, and a sedimentation coefficient of 8S (Tizard, 1992a). Immunoglobulin E is known as reaginic antibody. The H chain of IgE contains an extra domain, by which it attaches with unusually high affinity to specific receptors on mast cells and basophils, where the IgE molecule may remain bound to these receptors for weeks or months (Janeway & Travers, 1994). When antigen reappears, it combines with and cross links the IgE molecules on the surface of the mast cells, leading to the discharge of the contents of the mast cell granules which results in inflammatory reactions and ensuing symptoms of anaphylaxis. Immunoglobulin E is also largely responsible for immunity against invading parasites (Benjamini & Leskowitz, 1991).

1.2.6 Immunoglobulin Y (IgY)

Chicken IgG is termed IgY which is similar to mammalian IgG but somewhat larger (Tizard, 1992a). Immunoglobulin Y has the same general structure as an IgG, with two heavy chains ("nu" chains) and two light chains. The molecular weight of the whole IgY is about 180kDa, but it often runs as a smear on SDS-ploy acrylamide gels due to the presence of about 3% carbohydrate (Tizard, 1992a). The "nu" chains of IgY are composed of four constant domains and one variable domain, which contains the antigen-binding site. The "nu" chain of IgY lacks Fc domains, which means that IgY's do not fix complement nor bind protein A or protein G. Chickens also produce an alternatively spliced form of the heavy chain in which the Cnu3 and Cnu4 domains are deleted (http://www.aveslab.com/faqs/html).

Antibodies are generally present in tears, respiratory tract mucus, saliva, intestinal contents, urine, milk and blood serum. Antibodies in the blood serum are in high concentrations and are easily obtainable in large quantities.

1.3 OCCURRENCE OF IMMUNOGLOBULINS IN MILK AND OTHER SECRETIONS

Milk being the first substance ingested by a neonate, in addition to providing nourishment has an important function of protection against infection (Lönnerdal, 1985). Several substances in milk known as "immune factors" provide resistance to diseases, particularly to diseases related to the gastrointestinal tract. The immune factors consist of highly specialized antibodies, the complement system, certain enzymes, two or more iron-binding proteins, various cellular components, and a factor that encourages growth of a bifidobacterium (Lönnerdal, 1985). The most functional factors include IgA, IgG, IgM, IgE IgD, complement, lysozyme, lactoferrin, transferin, inferrins, leukocytes, lactoperoxidase, and the bifidus factor. Only Igs, complement, and certain cellular systems act with specificity. The rest are non-specific defenders. All classes of the Igs are present in milk (Larsen, 1992). The Ig concentrations in the milk of different species are presented in Table 1.2 (Larsen, 1992). Milk antibodies are preferred more than serum antibodies as they are resistant when exposed to pepsin at pH 4 (Parkin et al., 1973).

Table 1.2
Concentration of immunoglobulins$^{\Phi}$ in the milk from different species

Species	Immunoglobulin concentration (mg/ml)		
	IgG♣	IgA	IgM
Human	0.04	1.00	0.10
Rat	1.53	0.59	ND♥
Dog	0.24	2.63	0.22
Pig	3.00	7.70	0.30
Horse	0.39	0.48	0.03
Cow	0.72	0.13	0.04

$^{\Phi}$ Reference Larsen (1992)
♣ Total amount of IgG (all IgG subclasses total); ♥ Not consistently detected

1.3.1 Human

Concentrations of the major Ig isotypes in human milk are quite different from those found in the blood and intestinal fluid. The major proportion of human milk antibodies belongs to the secretory IgA class (sIgA), which is different from serum IgA, and comprises 95% of milk Igs (Shearman et al., 1972). IgA in milk is usually synthesized locally in the mammary glands. It is quite resistant to proteolytic enzymes as well as pH changes, which is probably related to the secretory component covering the degradation sensitive Fc portion of the IgA monomers (Kenny et al., 1967; Brown et al., 1970). Secretory IgA antibodies from milk are thus specially suited to function in the hostile environment of the gastrointestinal tract (Hizono et al., 1994). Breast fed children may be protected against infections due to the presence of antibodies in the human milk (Freed & Green, 1977; Ellestad et al., 1979; Lepage et al., 1981; Lönnerdal, 1985; Reddy et al., 1988).

The concentration of IgM is much lower in human milk than in the serum and the ratio of milk antibodies/serum antibodies for the IgM class is less than one. However, some IgM is transferred from the serum to the milk and under certain circumstances IgM may also be produced locally in the mammary glands (Ruiz, 1994).

Immunoglobulin G, the principal Ig in human serum, is also present in modest amounts in human milk (Ruiz, 1994). Each IgG subclass has been detected, but the relative proportion of IgG_4 is higher in human milk than serum (Kim et al., 1992; Ruiz, 1994).

The quantity of IgD is lower in milk than in serum, but the decrease is proportionally less than is reported for IgG and IgM. Immunoglobulin E is absent in human milk (Kelen, 1990).

1.3.2 Bovine

The bovine Ig closely resembles that of the Ig of other species with respect to both physiochemical properties and nomenclature. The Ig classes include IgG, IgA, IgM, IgD and IgE (Butler, 1969).

The IgGs present in bovine milk, include the two subclasses, IgG_1 and IgG_2 (Butler, 1969). Immunoglobulin G_1 is actively and selectively transported by the mammary glands from the blood to the milk, during lactation (Larson, 1958; Butler, 1969; Butler, 1971; Pierce & Feinstein, 1972). Very high levels of IgG_1 are present in colostral and precolostral lacteal secretions (Butler, 1971). Several observations indicate that a large fraction of the bovine milk IgG_1, like human sIgA, is able to resist complete proteolysis in the digestive tract (Brown et al., 1970; de Rham & Isliker 1977). Immunoglobulin G_2 appears more homogeneous than IgG_1 and occurs in much smaller amounts in serum, colostrum and milk. Immunoglobulin G_1 is prominent in disease protection and with its long serum half-life (approximately 18 days), it is the only passively acquired Ig persisting in substantial amounts throughout the period of high susceptibility to neonatal enteric diseases (Butler, 1969; Butler, 1971).

A change in the ratio of IgA to IgG_1 in lacteal secretions before and after calving, combined with the level of IgA in serum, is consistent with the view that IgA is locally synthesized in the udder (Butler, 1969; Butler, 1971; Butler et al., 1972). Bovine IgA occurs as sIgA in milk and colostrum. The most typical form for bovine IgA is an 11s protein containing a secretory component (Mach et al., 1969; Butler et al., 1972; Duncan et al., 1972; Hizono et al., 1994), although both physiochemical and antigenic heterogeneity can be demonstrated.

The nature and distribution of bovine IgM is similar to other species (Butler, 1969). Immunoglobulin M in the mammary secretions comes both from plasma cells adjacent to the secretory epithelium and blood serum (Frenyo et al., 1987).

1.3.3 Composition of human milk versus bovine milk immunoglobulins

The Ig level is highest in colostrum or early milk, decreasing rapidly as lactation becomes established in both species (Blanc, 1981). Qualitatively, bovine and human milk contain the same Ig classes (IgG, IgA, IgD and IgM), but there are large quantitative differences (Blanc, 1981). In human milk, sIgA is predominant whereas IgG_1 is abundant in bovine milk (Hilpert and Link-Amster, 1983). However, both appear to be relatively resistant to *in vivo* digestion (Brock et al., 1977; Blum et al., 1981) and therefore may perform similar functions in protecting the gastrointestinal tract from pathogenic organisms.

1.4 IMMUNE RESPONSE TO INFECTION

An animal must protect itself against disease and death caused by invading micro-organisms such as bacteria, fungi, viruses and parasites, as well as the development of abnormal cells within the body, such as cancer cells or virus-infected cells. (Tizard, 1992a). In infectious diseases, the micro-organisms colonize and utilize a host for their own metabolic benefit and reproduction. If an animal is to survive and function effectively, it must be able to defend itself against invasion by these organisms. Failure to do so will result in death from overwhelming infection.

The immune response occurs in of two phases: one is recognition, and the other is, resistance to the foreign molecule (Barrett, 1983). In the recognition phase, the antigen is recognized as foreign by antigen-presenting cells (including B cells) and T lymphocytes (Janeway & Travers, 1994). The resistance phase to infection is based on several defence lines erected at different levels of specificity. Innate immunity refers to the ability of an individual to resist infection through normal body functions. The failure of this system usually results in activation of the acquired or adaptive immune system which produce a specific reaction to individual infectious agents (Benjamini & Leskowitz, 1991). Specific immune responses can be divided into humoral and cellular

responses (Bighey, 1975). Humoral immunity is mediated by serum antibodies which are proteins secreted by B cells, that react with a given antigen to eliminate it (Bellanti, 1971). The antigen specific arm of cell mediated immunity consists of T lymphocytes that bear many identical non secreted receptors, that enable them to interact with the antigens they encounter. Cellular immune responses are dependent on an intact thymus, so the lymphocytes responsible are known as thymus-dependent (T) cells. Similarly, antibody-producing lymphocytes, which are dependent on the bursa-equivalent are known as B cells. In response to antigen stimulation, B cells will mature to antibody-secreting plasma cells (Bighey, 1975). Cellular and humoral types of the immune responses are most efficient when adequately supported by the other two important adjuncts, the complement system and the polymorphonuclear-monocyte system (Carpenter, 1975). The specific immune response is quicker and larger when a particular antigen is encountered for a second time (Dias de Silva & Ivanmota, 1981).

Specific immunity can be shown by several activities:

- Coating or aggregating the bacteria and hence enhancing phagocytosis and/or the removal of pathogens by intestinal peristalsis (Wernet et al., 1971; Bricker et al., 1991).
- Blocking adherence of pathogen micro-organisms to mucosal cell surfaces and hence preventing colonization and/or the spread of infection (Jones & Rutter, 1972; Facon et al., 1995; Pollack et al., 1995).
- Involvement with antibodies-complement-lysozyme mediated cell lysis, and hence inhibiting or killing pathogenic micro-organisms (Porter, 1979).
- Neutralizing toxins and hence limiting the pathological consequences of their presence (Fubara & Freter, 1973; Brüssow et al., 1987; Nomoto et al., 1992; Matsumura, 1995; Kelly et al., 1996; Lissner et al., 1996).

When the immunity is hyperactive certain undesirable features like allergies or hypersensitivity may be seen (Falth-Magnusson et al., 1986; Poulsen et al., 1990; Farges et al., 1995). Conversely, when the elements are hypoactive, there may be an increased

susceptibility to repeated infections by the host (Joyner et al., 1987; Ferguson & Watret, 1988; Slacek et al., 1996). Natural resistance and adaptive immunity are immediately connected and operate together: deficiencies on either part will result in a marked increase in frequency and severity of infections.

1.5 CONTROL OF INFECTION

Infectious disease prevention depends on controlling or eliminating the source of infection, breaking the chain of transmission or increasing the resistance of the individual. The major factors in the virtual elimination of certain infectious diseases are, the supply of clean water and an increased resistance to infection resulting from better nutrition and improved personal hygiene (Barrett, 1983). When infection sets in antibiotics is normally used for treatment.

Antibiotics have side effects that may occasionally be serious and would be encountered more frequently if chemoprophylaxis were to be used routinely. Prolonged antibiotic use is likely to lead to bacterial overgrowth, drug resistant strains and altered normal intestinal flora, perhaps leading to an enhanced susceptibility to other pathogens such as *Shigella* and *Salmonella* species (Bartlett et al., 1978; Chang et al., 1978; Murray et al., 1982; DuPont et al., 1986; Murray, 1986; Sack, 1986). Prevention of infection that can be achieved by immunization is the most cost effective activity in health care (Chapel & Haeney, 1994).

1.6 IMMUNIZATION

Immunity is quantitative rather than qualitative (Bighey, 1975). An individual may posses sufficient immunity to protect against ordinary contact but it is not enough to overcome massive exposure (Dias De Silva & Ivanmota, 1981). In recent years, however, immunization has been one of the most effective measures in controlling infectious diseases. There are two methods of immunization namely active and passive, both of which can be achieved naturally or artificially. (Carpenter, 1975).

Natural active immunity is acquired when exposure to an immunogenic stimulus triggers an immune response in the host. The best type of natural active immunization

follows natural infection (unintended), which may be clinical or subclinical: with many diseases, this gives lifelong protection at little or no cost to the individual or to the community. Artificial active immunization involves the deliberate administration of an antigen in the form of vaccine (Reeves & Todd, 1991).

The natural type of passive immunity normally occurs when Igs are passed from mother to child, before birth through the placenta or after birth via colostrum or milk or both (Roger, 1970; Packard, 1982). In all species, passive protection is especially important during the early days of life, when the neonate has a reduced capacity to mount an active response and needs to become immunologically more mature than in foetal life. Passive protection is two phased, placental transfer and uptake of colostrum/early milk protects against systemic infection, and suckling of mature milk for extended periods during neonatal growth and development assures local gastrointestinal protection against enteric pathogens (Kabat, 1976; Chidlow & Porter, 1979).

Different species exhibit three distinct patterns regarding transmission of antibodies before and/or after birth. In the first group, consisting of cattle, goats, sheep, pigs and horses, the young are born with no significant humoral immunity, characterized by a virtually complete absence of Igs in the blood plasma. These offspring receive maternal antibodies via the ingestion of colostral milk, which is rich in Igs (Ballabriga et al., 1974/75). In the second group, that includes rat, mouse, hedgehog, dog and cat, passive immunity is transferred from mother to offspring both before birth across the foetal membranes and after birth by resorption of milk antibodies through the intestinal wall (Ballabriga et al., 1974/75; Hemmings & Hemmings, 1979; Williams & Ibbotson, 1979). In the third group, that includes man, monkey, rabbit and guinea pig, the ingestion of colostral milk by the offspring does not significantly contribute to humoral passive immunity. In these species, maternal serum IgG is transmitted to the foetus across the foetal membrane before birth (Ballabriga et al., 1974/75; Contractor, 1979; Ress & Wallace, 1979; Schlamowitz, 1979; Thornberg & Faber, 1979), and it is now thought that the foetus itself may have a limited ability to respond to foreign components.

The passage of maternal antibodies across the placenta is a highly selective process that is probably dependent on the receptors provided by the Fc portions of the antibody molecule, since Fc fragments pass through the placenta, whereas IgG antibodies devoid of the Fc region do not (Schlamowitz, 1979; Wild, 1979; Van de Winkel & Anderson 1991; Raghavan et al., 1995).

Passive artificial immunity is generally achieved through injection of hyperimmune serum, particularly of antitoxin sera, that is either produced in animals or obtained from hyperimmunized animals to treat tetanus, diphtheria, rabies, hepatitis A and B, varicella and zoster viruses (Benjamini & Leskowitz, 1991).

Active immunization is known to persist for relatively long periods, usually years, without restimulation through booster immunization. In passive immunization, the injected antibodies are removed from the circulation without internal replacement, so the immunity can be directly correlated with the amount of antibodies injected and their half-life (Reeves & Todd, 1991). The disadvantage of passive immunization is that the administered antibody concentration decreases fairly rapidly as the protein catabolises. This decreases the protection and the receipient becomes susceptible to infection once again. However, in passive immunization, protection is provided immediately on completion of administration (Barrett, 1983). Active immunization is of high efficiency but it requires 5-14 days to develop protective quantities of antibodies in the serum, depending on whether it is a primary or secondary response (Janeway & Travers, 1994). Active immunization is usually restricted to prophylactic applications, by which the person receives the immunization in advance of the exposure to the infective agent. Passive immunization can also be used prophylacticly, that is, prior to or immediately after exposure, in individuals who have not been actively immunized. In addition, passive immunization can be used therapeutically but active immunization cannot. Passive immunity can also be used to enhance the immune system in people. For example,

When people from developed countries travel to under-developed countries, traveller's diarrhoea can be prevented by the oral administration of Igs orally (Tacket et al., 1988).

In conclusion, there is evidence from the above comparisons that passive immunization is suited than active immunization for some situations e.g. the prevention/ treatment of diarrhoea.

1.7 ROUTES OF ADMINISTERING IMMUNOGLOBULINS FOR PASSIVE IMMUNIZATION

The aim of prophylaxis is to ensure that sufficient amount of antibodies is administered so that an individual does not suffer from more frequent infections. Immunoglobulin can be administered by subcutaneous injections, by intramuscular (IM), by intravenous infusion (IV), and orally. The site into which an antibody is introduced into an organism influences the immune response (Bighey, 1975).

1.7.1 Subcutaneous administration

Rapid subcutaneous infusions of gammaglobulin preparations is presently used in a variety of immuno-deficiency diseases (Gardulf et al., 1993), because serum levels of IgG can be restored to within the normal range by resorption of IgG from the subcutaneous tissue (Berger et al., 1980; Bayston et al., 1985; Lehay, 1986). Levels of IgM and IgA remain unaltered by this treatment. Subcutaneous administration results in a very low incidence of adverse reactions (Hammarström et al., 1994), is less expensive than other options, and can be administered at home. Furthermore, this method is preferred more by both adults and children (Gardulf et al., 1991).

1.7.2 Intramuscular administration

Intramuscular administration of gammaglobulins has been used prophylacticly (Smith et al., 1972; Hammarström & Smith, 1990; Gardulf et al., 1993; Husu et al., 1993). Concentrated Igs can be safely given by IM injections, thereby minimizing the absorption of phlogistic molecules into the circulation (Carpenter, 1975). They may also be administered at home. The administration of higher doses of Igs has shown to be clinically more effective than low doses. However, in patients with antibody deficiency who require regular long term therapy with Igs, the volumes administered

tend to be large to be able to restore the normal level, but often, less than the optimal dosage is given as it is very painful. Patients often refuse further maintenance of therapy due to painful sites for several days and this leads to difficulty in maintaining the normal serum level of Igs (Smith et al., 1972; Gardulf et al., 1993). Administered Igs are partially (40-60%) destroyed *in situ* by proteolytic enzymes (Thompson, 1981). There is a significant incidence of adverse systemic reactions due to large volumes administered (Redmond et al., 1996). As the above mentioned problems are encountered in IM, and as the Igs absorption is better in IV than in IM (Steinbruchel et al., 1989) it leads to the conclusion that IV is better than IM.

1.7.3 Intravenous administration

Intravenous administration of Ig is used to treat primary immuno-deficiencies, (Imbach et al., 1981; Levin et al., 1981; Stephan & Dichtelmuller, 1983; Baumgartner & Glauser, 1987; Kurlander, 1987; Salama et al., 1991; Hassett et al., 1995), bone marrow transplant recipients (Spath et al., 1989), to maintain IgG levels in low birth weight infants (Chirico et al., 1990; Wolf & Eibl, 1991), guard against infections in human (Cuthbertson et al., 1987; Yap, 1987; Hammarström & Smith, 1990), as a replacement therapy in burn patients (Khan et al., 1984), and in the treatment of haemologic diseases (Bussel, 1984). Intravenous therapy requires careful medical supervision. This method is accompanied by a high incidence of some adverse reactions as, flushing of the face, back pains, dyspnoea, nausea, vomiting, serum sickness, circulatory collapse and hypersensitivity reactions if the preparations used are not immunologically compatible with the patient (Sorensen & Polmer, 1984; Garbett et al., 1989; Frey, 1991; Schifferli et al., 1991). The antibodies in the serum will not prevent gastrointestinal tract infection (Snodgrass & Well, 1976). The failure of colostrum administered by IV to prevent diarrhoea in lambs may be due to the inability of the whey to reach the lumen of the gastrointestinal tract in effective quantities.

1.7.4 Oral administration

Oral administration of bovine and human Igs had been used successfully to treat diarrhoea (Hilpert et al., 1977; Pahud et al., 1987; Hilpert et al., 1988), dental diseases (Michalek et al., 1987; Filler et al., 1991), multiple sclerosis (Tenser et al., 1993), polio

virus (Gonzaga et al., 1963), marrow transplant recipients (Copelan et al., 1988), and immuno-deficiency diseases (Tzipori et al., 1986; Joyner et al., 1987; Melamed et al., 1991; Shield et al., 1993; Greenberg & Cello, 1996).

Although the gastrointestinal tract is an efficient barrier to macromolecular absorption, there is increasing experimental evidence that, these molecules can penetrate the intestinal epithelial cell surface in relatively minute quantities (McClead et al., 1988). It has been found that there is absorption of whole protein from the intestinal lumen into the circulation in the neonatal animal (Brambell, 1970). Studies carried out in the adult rat had shown that a significant amount of whole protein absorbed from the gut reaches various tissues within the body (Kulangara, 1979). This may occur in other species including man (Kulangara, 1979). The intact IgG may be absorbed by the proximal region of the small intestine and the partially degradation products by the ileal and jejunal regions (Agarwal & Moore, 1979; Bridge & Woodley, 1979; Jones, 1979; Morris & Morris, 1979; Seifert et al., 1979; Williams & Ibbotson, 1979). These absorbed high molecular weight protein is able to perform certain immunological (Thomas & Parrot, 1974) and biological functions for example reduce antibody production (Seifert et al., 1974).

Oral therapy is often given after the capacity for macromolecular absorption in the gastrointestinal tract is almost lost and therefore orally administered Igs only effective locally in the gut lumen. Orally administered Igs may also stimulate the host's own immune system (Freter & Gangarosa, 1963; Porter et al., 1974), for example by enhancing antigen presenting activities of the gut associated immune cells but in general, oral administered Igs will be most effective *in situ* against gastrointestinal tract infection. Oral immunization protects fluid loss, diarrhoea and intestinal distension whereas IV protects against diarrhoea and not the fluid loss. Furthermore 15-48 hrs after IV treatment the small intestine, caecum and sometimes the colon can be distended with fluid (Kohler, 1967; Shearman et al, 1972). Oral therapy does not involve any side effects like IV or IM therapy.

1.8 USE OF IMMUNOGLOBULINS FROM DIFFERENT SPECIES TO PROVIDE PASSIVE IMMUNIZATION

Immunoglobulins from various species have been used for prophylaxis and therapy against infection of bacteria, virus, and protozoa for more than one hundred years. Antibodies are thought to be effective by binding and neutralization of microbial antigens (Woode et al., 1975; Zinkernagel & Colombini, 1975; Acres & Babjuk, 1978; Yolken et al., 1985; Kelly et al., 1996), blocking the binding of virus and bacteria to target cells (Demierre et al., 1975; Pahud et al., 1987) and by acting as opsonins thereby initiating uptake and degradation by phagocytes and in complement mediated bactericidal mechanisms (Rivier & Sobotka, 1978; Hilpert et al., 1977; Van Furth, et al., 1984; Porter et al., 1987).

1.8.1 Use of human immunoglobulins

Experimental approaches to provision of passive prophylaxis and therapy have used human milk (Eibl et al., 1988), intestinal fluids (Girard & de Kalbermatten, 1970) and serum (Leung et al., 1991; Tjellström et al., 1993). Several studies have reported the efficiency of human milk or human serum Igs in low birth weight children and in managing the immuno-compromised child with chronic rotavirus infection (Eibl et al., 1988; Rubaltelli et al., 1991), viral infections (Levin et al., 1981; Prince, 1981), cryptosporidial infection (Borowitz & Saulsbury, 1991) or bacterial infections (Michalek et al., 1987). Using whole plasma from volunteers immunized with a mutant *Escherichia coli* (*E.coli*)strain, showed a significant reduction in the mortality rate of patients with gram negative bacteria infections (Ziegler et al., 1982; Baumgartner & Glauser, 1987).

The titer against most pathogens is low in human gammaglobulin preparations, however, the vaccination of volunteers for the production of hyperimmune preparations is not feasible. In addition, the costs for these preparations are high and immunization of the human is not a practical proposition for some diseases like cancer and Acquired immunodeficiency syndrome (AIDS). There is also an increasing risk of contamination

of human blood products with diseases such as AIDS, hepatitis, and Creutzfeldt Jakob Disease, so alternative sources to obtain antibodies must be sought.

1.8.2 Use of non-human origin immunoglobulins

Antibodies in chickens (IgY) are normally transferred from the serum of the hen to the egg yolk and where they may reach higher concentrations than in the serum. This transfer includes antibodies which have arisen as a consequence of deliberate immunization (Altschuh et al., 1984). The amount of antibodies present in a single egg (0.1-0.3g) may be more than sufficient as a daily prophylactic dose against gastrointestinal tract infections provided that the hen has been immunized against the offending micro-organism (Altschuh et al., 1984). Chicken antibodies are most likely less stable with regard to proteolysis *in vitro* than bovine antibodies, but *in vivo* the stability of chicken antibodies is still not known. Immunoglobulin Y prophylactic or therapeutic effect in human has not been investigated to date either but there is reason to believe based on animal studies (Otake et al., 1991; Hammarström et al., 1994; Kuroki et al., 1994; Bogstedt et al., 1996; Ebina, 1996), that chicken antibodies may be useful in human therapy.

Bovine antibodies are present in high concentrations in colostrum from which they can be harvested. In addition, Igs from immunized cows have been shown to effectively protect against challenges with live bacteria such as *E.coli* (Tacket et al., 1988) and *Shigella flexneri* (Tacket et al., 1992) and to prevent or mitigate natural infection with rotavirus (Ebina et al., 1985; Davidson et al., 1989) in man. Bovine milk Ig is used in many experiments because it is resistant to proteolytic enzymes compared to human milk Igs (Martinsson, 1970). Tables 1.3, 1.4 and 1.5 illustrate the range of studies that have been done using orally administered bovine milk Igs.

Bovine Ig products offer several advantages over traditional therapeutic products because they:

- are orally delivered,
- are relatively biologically stable in the gastrointestinal tract,
- are not significantly absorbed or translocated into the systemic circulation,

- can target bacteria as well as their toxins and can prevent and treat viral and parasitic diseases of the gastrointestinal tract,
- are pathogen-specific, unlike conventional broad spectrum antibodies which tend to disrupt the natural, beneficial gastrointestinal flora and
- are polyclonal, enabling them to bind to multiple sites on the pathogen or associated toxin.

For oral Igs to be effective they must be able to resist, at least to some extent, digestion/denaturation in the gastrointestinal tract and hence be biologically active when and where they are needed to inactivate or reduce the necessary virulence of the antigens to cause disease.

1.9 DIGESTION OF IMMUNOGLOBULINS IN THE GASTROINTESTINAL TRACT

Absorption of the Igs in the gut may provide temporary passive immunity. The absorption of whole proteins by a newborn occurs only during the first few days of life (Baintner, 1979; Ekström & Weström, 1992; Hunneyball et al., 1979; Smith et al., 1979). Colostrum ingested is non-selectively absorbed from the gastrointestinal lumen by heightened pinocytic activity of the "absorptive cells" of the small intestine for about 24 – 48 hrs after birth by the piglets and neonates. Absorption of globulins, may be favoured because of the low levels of proteolytic enzymes in the stomach and gut of the newborn (Weiser, 1971). In addition, the colostrum and milk of certain mammalian species contain a trypsin inhibitor of polypeptide nature, which has the function of protecting the colostral antibodies from digestion (Champerlain & Perry, 1965; Baintner, 1973; Baintner, 1979, Weström et al., 1979) and thereby ensuring that they are absorbed in a biologically active form. The relative concentration of this inhibitor in the colostrum of sows, cows, and women was found to be 67:10:1 (Baintner, 1973) indicating the respective need of each species to deliver passive immunity via the colostrum. The trypsin inhibitor disappears from the intestine when trypsin appears (Hill 1956; Deustch & Smith 1957). In pigs, the absorption of intact dietary proteins is favoured by a low proteolytic activity in the gastrointestinal tract of the piglet, together with the colostrum, which also contains trypsin inhibitors (Chamberlain & Perry, 1965).

Colostral trypsin inhibitor totally suppresses tryptic activity in the luminal contents for several days although chymotryptic activity is present from birth in the pig (Baintner, 1973; Brock et al., 1977).

About a day or two after birth, the neonate's ability to absorb colostral Ig is dramatically reduced to the point at which further ingestion of colostrum does not enhance serum Ig concentration any further (Lecce et al., 1991). However, colostrum ingested after the Ig absorption has stopped still has a significant effect in neutralizing enteric pathogens. The IgG is split by the protease pepsin and trypsin into $F(ab')_2$ fragments (Ross et al., 1995). Intact IgG and fragments of $F(ab')_2$, both have neutralizing characteristics (Hilpert et al 1987; Ross et al., 1995). Kenny et al. (1967) found that high temperatures could inactivate milk and colostrum Igs during processing. Immunoglobulins must be purified at low temperatures to be used as passive immunizers.

Human colostrum IgA is fairly resistant to both acid and proteolytic enzymes (Kenny et al., 1967; Brown et al., 1970; Parkin et al., 1973; Sato et al., 1987; Kelly et al 1996) and can be found in significant quantities in faeces (Haneberg, 1974; Ogra et al., 1977; Davidson & Lönnerdal, 1985). Losonsky et al. (1985) showed that human serum Ig survived passage through the gut in immuno-compromised children in an immunologically active form.

Like human IgA, bovine IgG_1 resistance against complete proteolytic digestion has been shown *in vitro* (Hilpert et al., 1987; Sato et al., 1987; Kelly et al 1996). McClead et al. (1988) demonstrated partial recovery of functionally active bovine Ig preparations against *Cholera*, following passage through the gut of human. Using an *in vitro* model of gut digestion, however, Petschow & Talbott (1994) demonstrated a significant reduction in rotavirus neutralizing titer in a bovine colostrum Ig concentrate by gastric acid, pepsin and selected pancreatic enzymes. Depending on the initial pH, pepsin digestion of Ig antitoxin activity results in a more or less pronounced loss of antitoxin activity in comparison with initial antitoxin activity. Brock et al. (1977) showed that the biological activity of bovine antibody is reduced by exposure to acid and protease and suggested that neutralization of both gastric acid and pancreatic trypsin may enhance the

effectiveness of bovine Ig (Petschow & Talbott, 1994). Exposure of bovine Ig preparations to pepsin at pH 2 resulted in a significant reduction in virus neutralizing activity with about a 45% increase in trinitrobenzenesulfonic acid (TNBS) reactivity, the latter indicating an increase in free amino groups after degradation of bovine protein (Petschow & Talbott, 1994). Incubation at pH 2 in the absence of pepsin caused a similar reduction in virus neutralizing titer with no change in TNBS reactivity (Petschow & Talbott, 1994) suggesting that gastric acid alone is capable of reducing the functional activity of bovine milk antibody by altering conformation without cleavage of peptide bonds. The greatest reductions in neutralizing activity were observed when bovine Igs were subjected to combined gastric phase digestion at low pH and duodenal phase digestion, resulting in a 50 fold or greater reduction in virus neutralizing titer and a four-fold increase in TNBS score (Petschow & Talbott, 1994). Trypsin and carboxypeptidase contributed to significant bovine Ig proteolysis (Petschow & Talbott, 1994). Trypsin caused a reduction in virus neutralizing activity (Petschow & Talbott, 1994). Hilpert et al. (1987) also found a significant reduction in virus neutralizing activity after exposure of hyperimmune bovine Ig samples to low pH in the presence and absence of pepsin. In contrast, McClead & Gregory (1984) observed complete recovery of anti-*Cholera* toxin antibody activity after exposing milk antibodies to pepsin at pH 2 or 4. Several investigators have also shown reductions in the functional activity of bovine milk antibodies after exposure of hyperimmune bovine Igs to trypsin at an enzyme/substrate ratio of about 1:50 (de Rham & Isliker, 1977; Hilpert et al., 1987). Hilpert et al. (1977) found that incubation of bovine Ig preparations with pepsin led to reductions in tetanus antitoxin effect.

Ross et al. (1995) found that there is a low digestibility of the nitrogen fraction of Ig in healthy adult humans with a normally functioning digestive system. The prececal absorption of protein nitrogen from orally administered Ig of bovine origin was 79% which is below the known prececal absorption values of other dietary proteins of animal origin, which range from 94 to 97% in human adults (Ross et al., 1995). The ileal digestibility of milk proteins is usually between 90 to 93% in humans (Mahé et al., 1994). *In vivo* studies found Ig activity in the ileal part of the intestine indicationg that the Igs were still active in this part of the intestine. Nearly 70% of the exogenous nitrogen recovered in the ileum originated from IgG of bovine origin (Ross et al., 1995).

From the ingested amount of IgG, 19% was still immunologically active in the ileum, whereas 59% of the IgG in the jejunum was active (Ross et al., 1995). Brock, et al. (1977); Blum et al. (1981); Losonsky et al. (1985); Hilpert & Gerber (1994) found in their *in vivo* studies that Ig activity could be detected even in the faeces of infants. In the faeces of infants, 10% of the orally given bovine Igs could be detected (Hilpert et al., 1987). This is roughly half of what Ross et al., (1995) found in the ileum, which indicates that a substantial amount of Ig may be degraded by micro-organisms in the colon. Ross et al. (1995) found 19% of the ingested IgM in the ileum, but no IgA was detected.

As has been discussed above, bovine IgG_1 has been shown to be partially resistant to digestion in the gut and hence can be used for passive immunization.

1.10 ORAL ADMINISTRATION OF IMMUNOGLOBULIN DERIVED FROM BOVINE COLOSTRUM AND MILK USED AS PASSIVE IMMUNITY THERAPY OR PROPHYLAXIS

Peterson & Campbell (1955) first suggested that orally administered bovine Igs could provide passive immune protection to humans. The most important protective factor for many gastrointestinal infections is the presence of specific antibody in the lumen of the small intestine. In ruminants, the predominant milk antibody IgG_1 is mainly serum-derived (Pierce & Feinstein, 1965), has specificity for enteric pathogens (Williams, 1992) and is able to passively protect against infection by various enteric pathogens in animals and humans (Mietens et al., 1979; Tacket et al., 1988; Davidson et al., 1989; Tacket et al., 1992). Fernandez et al. (1978) successfully used lyophilized bovine colostrum to treat infants with diarrhoea.

Much research has been done recently using bovine milk derived Igs as an oral therapy against gastrointestinal tract infections caused by micro-organisms such as *E.coli* (see Table 1.3), Rotavirus (see Table 1.4), Vibrio cholera, *Clostridium difficile*, Shigella flexneri, *Helicobacter pylori*, Cryptosporidosis, *Candida,* and *Salmonella* (see Table 1.5). In most cases, the Igs were produced from the colostrum or milk of cows which had been immunized against specific pathogens, and were tested against the specific

organism in human and animal studies. Efficacy in prophylaxis and therapy (See Table 1.3, 1.4 and 1.5) varied considerably between the different studies, which probably reflects different antibody specificities (titer measurement), different Ig preparations (amount of Igs in the product), dose rates (amount given and number of times), mode of administration, time of inoculation (before or after feeding Ig and time intervals between feeding and inoculation), the type of model animal (age, health conditions, feeding method, different species and the numbers), the clinical outcome, number of days experiment conducted, and the use of any other additional products (antibiotics or any other medications or probiotics). Wilson, (1972); Logan et al. (1974); Hilpert et al. (1977); Mietens et al. (1979); Cordle et al. (1991); Ebina, (1996) and others found that Igs were effective in reducing the frequency of diarrhoea, lessening its symptoms or decreasing the duration of shedding the infecting microorganisms. Ebina et al. (1985); Hilpert et al. (1987); Brunser et al. (1992); Syväoja et al. (1994), however, found that Igs were not effective against infections.

It would appear that the Igs employed in immuno-therapy are not always fully effective. The following factors seem to be of importance in determining efficacy; the time of Ig administration, differences in pathogenesis, and the content of the specific antibodies. For example, IgM seems to be more effective than IgG in agglutinating bacteria and in activating the complement system indicating greater bactericidal, bacteriolytic, and opsonic properties (Martinsson, 1970). On the other hand, IgG seems to be more effective than IgM in neutralization and precipitation reactions (Martinsson, 1970). Many experiments have investigated the prophylactic or therapeutic benefits associated with multiple doses of Ig or continuous feeding of Ig (Wilson, 1972; Logan et al., 1974; Hilpert et al., 1977; Mietens et al., 1979; Cordle et al., 1991; Turner & Kelsey, 1993;Sarker et al., 1996) and a few studies have investigated the efficacy of a single dose or bolus feeding of Ig (Ebina et al., 1985; Ebina et al., 1992; Ebina, 1996). The disadvantage of continuous feeding is that people may at times tend to forget to take their medication and thus reduce the effectiveness of the treatment. Hence it would be more convenient to administer Ig as a prophylactic therapy once a day.

It should be noted that most preparations did not contain purified Igs and in fact in some studies whey products were shown to be more effective than purified Igs alone (Logan et al., 1974). Other factors too may play a role in providing passive immunity associated with anti-infective and toxin neutralizing agents in colostrum and whey products, such as the antistaphylococcal factor, complement, cellular components, lactoferrin, lactoperoxdiase, glycoproteins, glycolipids, lysosome, and oligosaccharides (Ballabrica et al., 1974/75; Takahashi et al., 1992).

1.11 THE NEED FOR A SUITABLE ANIMAL MODEL

The search for new or improved approaches to treat human diseases is never ending. Appropriate control studies of pathogenesis, therapy, and prophylaxis of serious infections in humans are usually not feasible, because, untreated infected groups should not be allowed to mix with healthy individuals till experimental procedure is over, furthermore hypothesis about disease processes cannot be tested directly on human. A model that is beneficial in understanding infectious disease processes should have the following properties. It should be:

- simpler than the natural infection and therefore be easier to manipulate and to reproduce
- simulate the features of the natural-parasite system
- demonstrate the features of infection at both the cellular and molecular level and
- also have characteristics resembling those of humans in physiology, anatomy, disease susceptibility, and nutrient requirements (Yang & Mickelesen, 1974).

Various laboratory animals and farm animals have been used as animal models in studies of Ig prophylaxis or therapy. Calves (Logan et al., 1974), lambs (Snodgrass & Wells 1976), mice (Hilpert et al., 1977; Pahud et al., 1987; Hilpert et al., 1988; Vasser et al., 1983; Vasser et al., 1987), piglets (Wilson 1972), monkeys (Behrens & Mauler, 1980), rabbits (McClead & Gregary, 1984) and hamsters (Lyerly et al., 1991) have all been used for therapy and prophylaxis studies. Non-human primates are also useful (Waddell & Desai, 1981). Behrens & Mauler (1980) have conducted experiments with monkeys. The disadvantages are, however, that primates are expensive to import, breed, and are often trapped wild with unknown origin and health history, and they are often expensive and there may be lack of facilities to handle and maintain them. These

disadvantages limit the primate's use. The rat is omnivorous and can be fed the same through its lifetime and is commonly used in experiments (Yang & Mickelesen, 1974). The advantages of using rats are that they are easily handled and cared for without much problem, thus need minimal maintenance and are often docile and will thrive well in limited space (Waddell & Desai, 1981). The rat's disadvantages are that they are susceptible to respiratory and middle ear diseases for which antibiotic treatment is often necessary. The nutritional and physiological effects observed may also be due to secondary reactions initiated by gastrointestinal microbial activity due to the practice of coprophagy of rats (Waddell & Desai, 1981). These problems limit the use of rats use in immune studies. Mice are also seldom used because they are more susceptible to infection (Waddell & Desai, 1981), and therefore antibiotic treatment is necessary. Guinea pigs are extremely sensitive to penicillin, and they are also susceptible to many common bacterial infections (Waddell & Desai, 1981). The antibiotic treatment may interfere with infection duration and the shedding of micro-organisms in the experiment.

The pig is similar to humans in several aspects in relation to its nutrition. Like the human, the pig is an intermittent feeder and will consume a variety of foods similar to those eaten by man. The two species have a genetically similar gastrointestinal anatomy, physiology and metabolism. Piglets' absorption of Ig at an early age is similar to that of humans and both have trypsin inhibitors (Baintner, 1973). Hence the pig is commonly used as a model for human studies in nutrition (Moughan et al., 1992; Moughan et al., 1994; Rowan et al., 1994; Darragh & Moughan, 1995) and immune function (Schollum et al., 1997).

1.12 THE INFERENCES OF THE LITERATURE REVIEW

Living things are constantly threatened by pathogenic micro-organisms and at times the immune system may not be able to overcome massive infection. Induced immunization is method of protection against infection. Passive immunization has an immediate effect and enhances the immune system whereas in active immunization the response is delayed and takes time to produce antibodies. Immunoglobulin from different species is used for passive immunization, but bovine milk derived Igs are preferable as they are more resistant to digestion than other Igs. Although various methods of administration are available, oral therapy is the best way to protect against enteric micro-organisms.

This study was designed to determine the effect of bolus Ig feeding versus continuous Ig feeding against diarrhoea caused by *E.coli* K88. The Ig should be able to survive in the gut for 24 hours after administration as a bolus feeding to protect against infection. The first experiment was planned to find out the rate at which digesta moved and the quantity of digesta that could be collected from the gastrointestinal tract of the piglets over a period of 24 hours. The aim of the second experiment was to determine the digestibility of IgG in the gut of the piglet. The third experiment was to determine the effect of bolus immunoglobulin feeding versus continuous immunoglobulin feeding.

Table 1.3

Research into the use of bovine immunoglobulins for passive immunity against *Escherichia coli* administered orally as therapy or prophylaxis .

Treatment	No of individuals	Dose	Duration of treatment	Clinical end point	Clinical out come	Comments	Reference
Animal models							
Hyperimmune colostrum against A1 hyperimmune colostrum against P307 non-immune colostrum	15	100 ml whey, multiple doses		Survival	A1 longer than P307 in survival time	6 days old Gnotobiotic piglets	Wilson, 1972.
Nonimmune Colostral whey, IgM, IgA, IgG, and Placebo.	18	Whey, or 3 pints of milk substitute plus IgA, 250mg IgA, 5gIgG, 7g IgG, 4g IgM, multiple doses	7 - 8 days	Survival, diarrhoea	Colostral whey effective in preventing diarrhoea. Others did not prevent diarrhoea but reduced severity and mortality	New born calves	Logan et al., 1974.
Hyperimmune colostral whey proteins(CWP), Nonimmune CWP, Placebo	140	10mg, 25 mg and 100 mg of CWP containing 35 - 45 % Igs, multiple doses		Survival	100 μg effective and 25 μg partially effective for hyperimmune CWP as prophylaxis	Mouse protection test	Hilpert et al., 1977; Pahud et al., 1987; Hilpert et al., 1988
Hyperimmune milk immunoglobulin concentrate (MIC), nonimmune MIC		6.0, 2.4, 1.2 and 0.24 g IgG_1 per day, multiple doses	15 days	Diarrhoea	Hyperimmune MIC prevented diarrhoea at or above 2.4g IgG_1/day some protection at 1.2 and 0.24 g IgG_1/day	Gnotobiotic piglets	Cordle et al., 1991
Human studies							
Hyperimmune milk immunoglobulin concentrate	60	1g/Kg body weight, multiple doses	10 days	Diarrhoea	Effective against targeted strains43/51, nontargeted strains 1/9 as therapy	Hospitalized children (10 days to 18months)	Mietens et al., 1979.

Table 1.3 cont...

Table 1.3 continued

Treatment	No of individuals	Dose	Duration of treatment	Clinical end point	Clinical out come	Comments	Reference
Hyperimmune bovine Ig concentrate, versus control	232	1g/day multiple doses	6 months	Diarrhoea clinical symptom	No prophylactic effect	Children	Brunser et al., 1992.
Hyperimmune colostral Ig concentrate versus non-specific Ig concentrate control	20	1g/dose, multiple doses	7 days	Diarrhoea	Effective. Prevented 10/10 in treatment & 1/10 in control as therapy	Volunteers, challenged with *E.coli*	Tacket et al., 1988.
Trial I Hyperimmune colostral whey proteins	109	1g/kg body weight/day	10 days	Diarrhoea	Effective as therapy	Therapeutic efficiency in infants	Ballabriga et al., 1976.
Trial II Hyperimmune colostral whey protein	61	1g/kg body weight/day	10 days	Diarrhoea	Effective as therapy	Therapeutic efficiency in infants	Ballabriga et al., 1976.

Table 1.4
Research into the use of bovine immunoglobulins for passive immunity against Rotavirus administered orally as therapy or prophylaxis.

Treatment	No of individuals	Dose	Duration of treatment	Clinical end point	Clinical out come	Comments	Reference
Animal model							
Hyperimmune colostrum IgG concentrate, nonimmune colostrum IgG concentrate, pooled colostrum and negative control	135	Non immune Ig 0.9, 1.8, 3.6 IgG/ml diet immune 0.09, 0.19,0.37,0.75 IgG/ml, pooled colostrum 1.5mg IgG/ml, multiple doses	2 - 6 days	Diarrhoea	Immune effective at 0.19 mg/ml, & non-immunized at 1.8 mg/ml as therapy	4-6 days old piglets	Lecce et al., 1991
Colostrum versus control	6	40% of the diet, multiple doses	30 days	Diarrhoea, survival at 30 days	Effective as therapy	Gnotobiotic piglets	Bridger & Brown, 1981
Hyperimmune colostrum versus nonimmune colostrum	28	1% supplement, multiple doses	28 days	Diarrhoea	High titers are effective for protection, lower titers delayed onset and shortened duration.	New born calves	Saif et al., 1983
Hyperimmune bovine colostrum versus nonimmune bovine colostrum	26		30 days	Virus excretion, diarrhoea	Hyperimmune effective as therapy	New born monkeys	Behrens & Mauler, 1980
Nonimmune colostrum, serum or placebo	8	40 ml, multiple doseS	11 days	Diarrhoea, Viral presence	Prophylactic effect	Lambs	Snodgrass & Wells, 1976
Hyperimmune colostrum and milk concentrates, nonimmune colostrum & milk concentrates, placebo		12 - 30 ml multiple doses	3-5 weeks	Diarrhoea virus shedding	Dose dependent protection and/or reduction in diarrhoea. 100% effective at 1.72 g IgG/day.	Gnotobiotic piglets	Schaller et al., 1992
Hyperimmune colostrum versus normal		50µl	10 days	Diarrhoea, virus shedding	Hyperimmune prophylactic effect	5 days old mice	Ebina et al., 1992; Ebina, 1996

Table 1.4 cont...

Table 1.4 continued

Treatment	No of individuals	Dose	Duration of treatment	Clinical end point	Clinical out come	Comments	Reference
Human studies							
Trial I 1:330 titer anti rotavirus milk immunoglobulin concentrate (MIC), versus milk control	45	2g MIC/Kg body weight/day, multiple doses	5 days	Diarrhoea, virus shedding	No effect	Pediatric trial,	Hilpert et al., 1987
Trial II 1:1100 titer anti *rotavirus* virus MIC versus milk control	46	2g/Kg body weight/day, multiple doses	5 days	Diarrhoea virus shedding	No effect	Pediatric trial	Hilpert et al., 1987
Trial III 1:6000 titer anti rotavirus virus MIC versus milk control	73	2g MIC/Kg body weight, multiple doses	5 days	Diarrhoea virus shedding	Effective, decreased shedding	Pediatric trial	Hilpert et al., 1987
Concentrated bovine immunoglobulin plus *B.adolescertis*	1	20mg/kg body weight multiple doses	3 days	Diarrhoea	Effective, symptoms disappear.	Child	Paul, 1996
Anti-rotavirus colostrum immunoglobulin concentrate versus infant formula	120	50 ml/day, single or multiple doses	10 days	Diarrhoea	Prophylactic	Children	Davidson et al., 1989

Table 1.4 cont.

Table 1.4 continued

Treatment	No of individual	Dose	Duration of treatment	Clinical end point	Clinical out come	Comments	Reference
Trial I Anti-rotavirus colostrum versus milk control	13	20 ml /Kg body weight single dose	45 days	Diarrhoea	Effective 5/6 in treatment & 1/7 in control	Pediatric, prophylactic trial,	Ebina et al., 1985
Trial II Anti-rotavirus colostrum versus milk control	44	20 - 50 ml	3 days	Diarrhoea	No effect	Pediatric, therapeutic trial	Ebina et al., 1985
Hyperimmune colostrum versus nonimmune colostrum	75	100 ml, multiple doses	3 days	Diarrhoea duration	Hyperimmune effective in reducing duration and severity	Children	Mitra et al., 1995
Hyperimmune colostrum versus normal	20	20ml/day, single dose	20 days	Diarrhoea	Effective-prophylactic not therapeutic	20 infants	Ebina et al., 1992; Ebina 1996
Hyperimmune colostrum, Milk	15	2g/kg body weight multiple doses		Diarrhoea.	Prophylaxis	Children	Ebina et al., 1987
Hyperimmune colostrum versus placebo	34	10g/day, multiple doses	4 days	Diarrhoea presence of pathogens, stool rate	Effective, decreasing duration of infection and severity	children	Sarker et al., 1996
Hyperimmune colostrum concentrate versus placebo	64	200 µg/ml	16 - 202 days	Diarrhoea	Effective decreasing duration of infection and severity	Infants	Turner & Kelsey, 1993

Table 1.5
Research into the use of bovine immunoglobulins for passive immunity against other miscellaneous organisms administered orally as therapy or prophylaxis.

Treatment	No of Individual	Dose	Duration of Treatment	Clinical end point	Clinical out come	comments	Reference
CANDIDA							
Ig concentrate and *B.adolescentis*	1	5mg/kg body weight multiple dose		Symptoms	Therapeutic	Adult human	Paul, 1996
SALMONELLA							
Ig concentrate and *L.acidophilus*	1	10mg/kg body wt, multiple doses		Symptoms	Therapeutic	Adult human	Paul, 1996
SHIGELLA FLEXNERI							
Hyper immune whey protein concentrate				Dysentery	Effective as therapy	Mice	Vasser et al., 1987
Hyper immune milk				death	Effective as therapy	Mice	Vasser et al., 1983
SHIGELLA FLEXNERI							
Trial I Anti Shigella flexneri immunoglobulin concentrate versus nonimmune immunoglobulin concentrate	29	10g Ig, multiple doses	7 days	Diarrhoea, fever, bacterial shedding	Some effect, reduces illness, fever and diarrhoea. Decreased shedding	Adult volunteers	Tacket et al., 1992
Trial II Anti Shigella flexneri immunoglobulin concentrate versus nonimmune	21	10 g Ig, multiple doses	7 days	Diarrhoea, fever, bacterial shedding	Prophylaxis 100% effective in treatment group	Adult volunteers	Tacket et al., 1992

Table 1.5cont..

Table 1.5 continued

Treatment	No of Individuals	Dose	Duration of Treatment	Clinical end point	Clinical out come	comments	Reference
CHOLERA							
Hyperimmune immunoglobulin concentrate versus non specific immunoglobulin concentrate versus sham control (5% glucose water)	30	9ml/24 h multiple doses	24 hours	Motality, fluid accumulat-ion index	Protective reduced mortality rate in hyperimmune	Rabbit	McClead & Gregary, 1984
Trial I Anti *Cholera* toxin colostral immunoglobulin, water placebo	45	2g , multiple dose	16 hours	immuno-globulin recovery, diarrhoea, stool volume	Not effective against active infection	Adults	McClead et al., 1988
Trial II anti *Cholera* toxin colostral immunoglobulin versus nonimmune placebo	20	2g , multiple dose	16 hours	Immuno-globulin recovery, diarrhoea, stool volume	Not effective against active infection	Adult	McClead et al., 1988
CLOSTRIDIUM DIFFICILE							
Hyperimmune bovine immunoglobulin concentrate versus nonimmune placebo	47	Amount varies, multiple dose	1 day	Survival	Hyper immune protects against toxins, not against infection	Hamster	Lyerly et al. 1991

Table 1.5 cont..

Table 1.5 continued

Treatment	No of Individuals	Dose	Duration of Treatment	Clinical end point	Clinical out come	comments	Reference
CRYPTOS -PORDIOSIS							
Non-specific bovine immunoglobulin concentrate	3	50-250 g IgG/day, multiple doses	5-7 days	Diarrhoea, oocyst in stool	not effective	Patients	Saxon & Weinstein, 1987
Anti C.parvum, non-specific bovine immunoglobulin concentrate	5	14.4G IgG/day, continuous nasogastric infusion	10 days	Diarrhoea, oocysts in stool, number of stools/day, stool volume	Effective in 2/3 patients in treatment group	Patients	Nord et al., 1990
Anti-C.parvum bovine immunoglobulin concentrate	1	1g IgG/h, Two nasoduodenal infusion periods	24-60 hrs	Diarrhoea, oocysts	Effective	Patient	Ungar et al., 1990
CRYPTOS -PORDIOSIS							
Anti-C.parvum bovine immunoglobulin concentrate	3	10-25g IgG/day nasoduodenal or nasogastric infusion periods	10-21 days	Diarrhoea	Effective	Patients	Tzipori et al., 1987
Non-specific bovine immunoglobulin concentrate	37	6 g IgG/day, orally	10days	Stool frequency	Decreased frequency in 29/40 periods	Patients	Rump et al., 1992
Ig concentrate and L.acidophilius	1	10mg/kg body weight multiple doses		Symptom	Therapeutic	Adult	Paul, 1996
PYLORI							
Hyper immune colostrum	9	20g of colostrum powder/day	28 days	Gastritis	Effective -ulcer severity reduced or healed, reduction of colonization	High titer > 25,000 Patients with gastritis	Tarpila et al., 1994

Table 1.5 cont..

Table 1.5 continued

Treatment	No of Individuals	Dose	Duration of Treatment	Clinical end point	Clinical out come	comments	Reference
PYLORI							
Anti-H.pylori bovine immunoglobulin concentrate	13	0.6g IgG/day	14 days	H.pylori infection, symptoms	Effective in eradicating infection and symptoms in 8/13 patients,	H.pylori infection with ulcers and/ or gastritis	Ando & Nakumura, 1991
Anti H.pylori bovine immunoglobulin concentrate	12	0.6g IgG/day	14 days	H.pylori infection, symptoms	Effective in eradicating organisms and symptoms in 12/12 patients for up to 3 months	H.pylori infection with ulcers and/ or gastritis	Ando & Nakumura, 1991
Anti H.pylori immunoglobulin concentrate	12	0.6g IgG/day	3 weeks	H.pylori infection, symptoms	Effective in eradicating organisms and symptoms 12/12 patients up to 3 months	Human H.pylori infection with ulcers and/ or gastritis.	Ando & Nakumura, 1991
Anti H.pylori bovine immunoglobulin concentrate versus placebo	18	0.8g IgG/day	4 weeks	H.pylori infection, symptoms	Effective in eradicating organisms and symptoms in 9/9 treated patients; 1/9 placebo patients	Human H.pylori infection with gastritis	Ando & Nakumura, 1991
Anti H.pylori bovine immunoglobulin concentrate and amoxicillin versus cimetidine	21	1g IgG/day and 600mg/day amoxicillin 200mg/day cimetidine orally	4 weeks	H.pylori infection, symptoms	Effective in eradicating infection in 11/11 treatment group; partially effective 4/10 control group	H.pylori infection and duodenal ulcers	Ando & Nakumura, 1991
Anti H.pylori bovine immunoglobulin concentrate versus placebo	5	0.6g IgG/day	114 days	H.pylori infection, symptoms	Effective in eradicating infection in 5/5 patients up to 12 months	H.pylori infection and duodenal ulcers	Ando & Nakumura, 1991

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CHAPTER 2

TRANSIT OF DIGESTA THROUGH THE DIFFERENT PARTS OF THE GASTROITESTINAL TRACT OF PIGLETS OVER A 24 HOURS PERIOD

2.1 ABSTRACT

The efficiency of protein digestion and absorption is, in part, dependent on the rate at which digesta moves throughout the gastrointestinal tract. Digesta transit rate and the quantity of digesta that could be collected were determined in this study. Ten six-week-old piglets were used to assess the movement of digesta along the gastrointestinal tract and to determine the quantity of digesta that could be recovered from the gastrointestinal tract at particular time intervals after feeding. The piglets were given a single experimental diet equivalent to 10% of their metabolic body weight $kg^{0.75}$ in quantity. The diet was formulated from skim milk powder and cereal grains. Water soluble carmine red dye (0.12%w/w) and blue coloured glass beads were used to mark the liquid and solid phases of the diet respectively. Individual plastic troughs were used to feed each piglet. The piglets were randomly allocated to five groups with two piglets per group. One piglet from each of the five groups was selected, fed with the experimental diet and slaughtered at either 1, 5, 9, 16 and 24 hrs and the digesta collected separately at each slaughter from different parts of the gastrointestinal tract and monitored for the presence of dye, glass beads and the quantity. It was observed that the dye had travelled rapidly to the end of the small intestine at 1 hr, the caecum at 5 hrs, the beginning of the colon at 9 hrs, two third of the colon at 16 hrs and the end of the colon at 24 hrs after the feed following the experimental diet. Most of the beads were found in the stomach between 1 and 5 hrs, spread throughout the small intestine at 9 hrs, and in the caecum at 16 hrs and colon at 24 hrs post-prandially. The data collected confirmed that an adequate quantity of digesta would be present in different parts of the gastrointestinal tract of the piglets over a 24 hrs period. This information assisted in the design of the immunoglobulin digestibility trial (chapter 3) to measure the immunoglobulin concentration in the digesta.

2.2 INTRODUCTION

The rates of gastric emptying and intestinal transit are affected by the composition of the diet including the acid content (Holmes et al., 1974), viscosity (Blackburn & Johnson, 1981; Rainbird, 1986; Cherbut et al., 1990; Potkins et al., 1991a), liquid content (Rainbird & Low, 1986), fibre content (Bardon & Fioramonti, 1983; Low et al., 1985; Jorgensen et al., 1996), protein and fat content (Mangel & Koegel, 1984; Asche et al., 1989; Hiroshi et al., 1991) and the osmolarity and particle size of the diet (Clemens & Stevens, 1980; Hiroshi et al., 1991; Potkins et al., 1991b;). Stress in the animal (Enck et al., 1989), the temperature of the environment (Low et al., 1985; Jorgensen et al., 1996), the volume and physical properties of lumen contents, and a feed-back control from the duodenum to the stomach via receptors (Ruckebusch & Bueno, 1976) can also affect the rate of gut transit.

This study aimed to determine the transit time of digesta and the amounts of digesta that could be collected from the gastrointestinal tract of the piglet as a preliminary trial to assist in the design for an investigation of immunoglobulin digestibility (chapter 3). It is important to confirm that sufficient quantity of digesta would be present throughout the piglet's gastrointestinal tract over a 24 hrs period to enable immunoglobulin levels in the digesta to be assayed.

In this experiment a colour marker and beads were used to measure the gut transit times. The carmine red dye is water-soluble and was therefore, expected to travel with the liquid phase of the diet, whereas the beads were expected to travel at a rate similar to the solid portion of the diet. Implicit in the use of the beads was the assumption that as they were small in size they would not have significant effect on transit time.

2.3 MATERIALS AND METHODS

All aspects of this study were approved by the Massey University Animal Ethics Committee (Ethics protocol 97/113).

2.3.1 Animals

Ten six-week-old entire male Large White x Duroc piglets (weight range 8.7 - 10.0 kg; 8 ± SD, 9.2 ± 3.09 kg) were selected from a group of piglets originating from three different litters of pigs at a commercial piggery (Wairaka Farms Ltd, Foxton).

2.3.2 Housing

The piglets were maintained in a temperature-controlled room at 25 ± 1^0C under alternating 14hr light: 10hr dark periods. The animals were housed individually in plastic metabolic cages and provided with water *ad libitum* throughout the trial period.

2.3.3 Diets and Feeding regime

The piglets were fed a standard weaner diet (the composition of ingredients is presented in Table 2.1; the nutrient content is presented in Table 2.2) which was formulated to meet the nutrient requirements of growing pigs (A.R.C. 1981). The diets were mixed by machine (small 100kg capacity, horizontal pedal mash mixer, Baxter, UK) at the feed processing unit at Massey University (Palmerston North, New Zealand). The piglets were fed twice a day at 0800 hrs and 1800 hrs, with their total daily allowances being divided equally between the two meals. The daily allowances* were set according to each piglet's metabolic body weight (10% of metabolic body weight $(kg)^{0.75}$ per day). At the beginning of the trial, the piglets were allowed to adapt to their diet by allowing them free access to the food for more than 90 minutes. This feed time was reduced to 60 minutes by the third day. To reduce feed wastage, each meal was served to the piglets in individual plastic troughs and the dry food mixed with an equal amount of tap water just before feeding. The amount of food remaining in the troughs after specified times was measured to nearest 0.1 g each day. The troughs were washed well. The piglets were weighed every three days between 1500 and 1600 hrs. The piglets' intakes were adjusted after each weighing accordingly.

The piglets were fed individually on a single meal of an experimental diet and each slaughtered according to the specified time intervals to measure the transit time and the quantity of digesta in the digestive tract. The two markers, blue colour glass beads (diameter ± 2mm, Mill and Hill, Nelson, New Zealand) and carmine red dye (Catalog number C 1022, Sigma - Aldrich Pty, Auckland, New Zealand) were incorporated into the standard weaner diet to prepare the experimental diet. The concentration of carmine red, which was added at the stage of diet formulating, was 0.12 g per 100 g diet on an air dry matter basis as had been used by Enck et al. (1989) and mixed with the weaner

* Daily allowance=Metabolic body weight (MBW) * 0.75 * 0.1 for e.g. if MBW is 10kg; Daily allowance=10 * 0.75 * 0.1= 0.75 kg

Table 2.1
The ingredient composition (%) of the weaner diet fed to six-week-old piglets in a gut transit time trial

Ingredient	% in diet (Dry matter basis)
Skim milk powder	30.0%
Wheat	20.0%
Barley	22.5%
Maize	25.0%
Vitamin/mineral premix ♠	0.5%
DICP♣ ♠♠	2.0%

♠ Presented in Appendix 2.1
♣ Di Calcium phosphate
♠♠ Presented in Appendix 2.2

Table 2.2
The calculated nutrient content♦ of a weaner diet fed to six-week-old piglets in a gut transit time trial

Nutrient	per 100g
Energy	1249.58 kJ
Fibre	2.03 g
Crude protein	15.96 g
Calcium	0.78 g
Phosphorus	0.78 g
Sodium	0.14 g
Potassium	0.87 g
Chloride	0.27 g
Fat	1.73 g
Linoleic acid	0.77 g
Serine and glycine	1.52 g
Arginine	0.75 g
Histidine	0.54 g
Isoleucine	0.79 g
Leucine	1.74 g
Lysine	1.06 g
Methionine	0.39 g
Cystine	0.69 g
Tyrosine and phenylalanine	1.71 g
Threonine	0.73 g
Tryptophan	0.21 g
Valine	1.05 g

♦ Calculated based on Nutritive value of local feed stuff for pigs and poultry, Monogastric Research Centre, Massey University, 1981.

diet. Two hundred blue glass beads were added to each experimental diet and mixed thoroughly just before feeding the meal to the piglets.

2.3.4 Experimental procedure

The piglets were allowed to adapt to the weaner diet for seven days. Following this seven day adaptation period, the piglets were randomly allocated to five groups, with two piglets per group. One animal was selected from each group and fed with a single meal of the experimental diet and each one of them was slaughtered at either 1, 5, 9, 16, and 24 hrs after feeding. The piglets slaughtered at 1, 5 and 9 hrs were fed at 0800 hrs on the same day of sample collection, whereas piglets slaughtered at 16 and 24 hrs were fed the previous day at 1900hrs and 1000 hrs, respectively. Digesta were collected from different parts of the gastrointestinal tract of each piglet at slaughter. Faeces were collected from the piglets which were slaughtered at 9, 16 and 24 hrs after feeding. The above procedure was repeated with the remaining piglets.

2.3.5 Collection of digesta and faeces

The piglets were anaesthetized with halothane gas (Fluothane, Imperial Chemical Industries Ltd, Wellington, New Zealand), which was inhaled through a mask, and then euthanased using a 15 ml intra-cardiac injection of sodium pentobarbitone (Chemstock Animal Health Ltd, Christchurch, New Zealand). Immediately after death, each piglet was placed on a slaughter tray, in dorsal recumbancy, and the abdominal cavity opened through the midline. Forceps were used to separate the gastrointestinal tract into four sections: stomach (from cardia to pylorus), small intestine (from pylorus to the beginning of the caecum), caecum, and colon (from the ileo-caecal junction to the rectum) and excised. To prevent the movement of digesta within the small intestine, sections were ligated in numerous places with strings. The gastrointestinal tract was then washed thoroughly in water and dried well using paper towels. The small intestine was further divided into three equal segments: proximal (SI1), medial (SI2), and terminal (SI3). Digesta from the stomach, SI1, SI2, SI3, caecum and colon were collected by squeezing the digesta from each section into separate bags. All parts of the gastrointestinal tract were split open and washed in water. The wash water was strained and checked for beads, which were counted if found and recorded to add with the final bead count for the particular section of gut.

For collection of faeces, an ostomy bag (size 45mm, Active Life Plus with pre-cut stoma opening, Bristol Myers Squibb, Pharmaceuticals Pty Ltd, Auckland, New Zealand) was attached to the anal and tail region of each piglet. Each bag had a self-adhesive flange, and the attachment was further secured using adhesive tape (Elastoplast, Smith and Nephew Ltd, Auckland, New Zealand) around the edges of the flange. Faeces were collected from the ostomy bags after slaughter. All samples (digesta and faeces) were stored in the fridge at 4° C awaiting subsequent analysis.

2.3.6 Analysis of digesta and faeces

Digesta samples obtained from each segment of the gut, and the faecal samples collected during the experiment, were analyzed for the number of beads present, a visual colour observation, colour measurements and the quantity.

2.3.6.1 Dye marker

2.3.6.1.1 Visual observation

While collecting the samples from the gut, the movement of the red dye with the digesta along the gastrointestinal tract and in the faeces was observed by the same person throughout the experiment to reduce errors.

2.3.6.1.2 Colour measurement by colourimeter

A Hunter Lab colour quest 45/0 Lav with standard accessories and universal software (Hunter Associates Laboratory Inc, Virginia, USA) was used to quantitatively measure the colour of the digesta and faecal samples. This colourimeter has been designed to measure both the colour and lustre of solid materials. Though normally it is been used to assess the colour and appearance of different wools (R. Sherlock, pers. Comm.), in the current trial it was used to measure changes in the colour of the digesta due to the presence of the carmine red dye. For each sample, results were expressed as *L* (primarily a measure of colour intensity, i.e. light or dark); *a* (a measurement of red or green colour depth); and *b* (a measure of yellow or blue colour depth). The numeric values of each reading indicated the magnitude of the colour, whereas + or - indicated the direction of the colour change. For example, +*L* was lighter than -*L* which was darker, +*a* was more red in colour than -*a* which was greener, and +*b* was more yellow than -*b* which was more blue. Each individual sample was well mixed and subsampled into a petrie dish which was sealed around the edges with parafilm to prevent leaking.

The petric dish was placed on the stage of the colourimeter and the *L*, *a*, *b* measured. Carmine red absorbance at 530nm-wave length was also measured. Each sample was measured twice and the average calculated.

2.3.6.2 Beads

The beads in the digesta of each gut section and of the faecal sample were recovered and counted separately by repeated washings of the digesta and the faeces in water using a sieve which retained the beads. The total number of beads recovered from each location in the gut was determined by adding together the number of beads found during sample collection and in the subsequent washings. The number of beads recovered at each location was expressed as a percentage of the total number of beads found in the entire gastrointestinal tract of each piglet.

2.3.7 Data analysis

The main objective of this pilot study was to determine if sufficient quantities of digesta would be present and the distance the digesta had moved in the piglets' gastrointestinal tracts to allow for the study described in chapter 3. The experiment was planned to determine the transit time by observing the distance the dye moved in the gut and the number of beads recovered from the different regions of the gut and no statistical analysis was done.

2.4 RESULTS

The piglets in the present study readily consumed their daily allocation of diet with minimal spillage of food. The piglets' daily feed intakes are given in Appendix 2.3. The piglets remained in good health and gained weight throughout the trial period. At the end of the trial, the piglets' mean live weight ± SD, was 10.2 ± 3.49 kg (range of 8.6 - 12.0 kg) which represented an average daily growth rate of 100g/day. Changes in the colour of digesta throughout each piglet's gastrointestinal tract, as determined by visual observation, are represented in Table 2.3 and in full description in Appendix 2.4.

The dye marking the liquid phase of the diet moved to the SI2 and SI3 sections within one hour of feeding. Colour was also observed in the solid phase which remained in the stomach at one hour. Dye appeared at the caecum by 5 hrs, and at the beginning of the colon by 9 hrs and in two thirds of the colon by 16 hrs after the meal. After 24 hrs dye

was observed in the faeces of one piglet, but in the other piglets sampled after 24 hrs, dye was present only at the end of the colon.

One hour after the feed there was an obvious difference between the liquid and solid phases of the digesta in the intestine, but after 5 hrs there was no noticeable liquid phase. The digesta in the caecum and colon, in particular, were comparatively dry compared to those from other locations. Very little digesta was collected from the stomach of one piglet after 24 hrs and the other piglet sampled at 24hrs post-prandially had only a few food particles in the gastric juice.

Estimates of *L*, *a*, *b* and the carmine red colour intensity at 530 nm wave length were measured for each digesta and faecal sample. Unfortunately, many factors including thickness of the samples, small food particles in the liquid and the liquid content affected the measurements of colour in the samples. Consequently the results were unable to be used to assess the transit time. The results are, however, recorded in Appendix 2.5.

As beads were small in size, there appeared to be no accumulation of beads in the gastrointestinal tract but these had moved throughout the gastrointestinal tract in accordance with time. One hour after feeding, a few beads were found in the distal part of the small intestine in one piglet which may have gone with the liquid phase, and in the other piglet, beads were found only in the stomach. After 5 hrs of feeding the experimental diet, beads were found in all parts of the gastrointestinal tract. The percentage of beads recovered from the various segments of the gastrointestinal tract at different time intervals were calculated and are given in Appendix 2.5. These data are also presented in Figures 2.1 to 2.5 to show the recovery of beads throughout the gastrointestinal tract of the piglets at 1, 5, 9, 16 and 24 hrs respectively after a single experimental diet. This indicated that there was digesta movement in the gastrointestinal tract of piglets over time. The mean percentage of beads recovered throughout the gastrointestinal tract are presented in Figure 2.6. This indicated that there was a reduction in the quantity of digesta in the stomach and an increase in the caecum, colon and faeces while a small quantity was always present in the small intestine at the different times.

Table 2.3
Visual colour observation in digesta and faeces collected from piglets at different times and at different locations in the gastrointestinal tract after a single experimental diet containing 0.12% carmine red dye marker per 100 g of diet fed

Pig #	Time (hrs)	Intestinal section						FAECES
		STM	SI1	SI2	SI3	CCM	COL	
3	1	+	+	+	+	-	-	NS
8	1	+	+	+	+	-	-	NS
4	5	+	+	+	+	+	-	NS
9	5	+	+	+	+	+	-	NS
5	9	+	+	+	+	+	±	-
10	9	+	+	+	+	+	±	-
2	16	+	+	+	+	+	+	-
7	16	+	+	+	+	+	+	-
1	24	+	+	+	+	+	+	-
6	24	+	+	-	+	+	+	+

Pig identification number
Time (hrs) of sampling after feeding an experimental diet
STM = Stomach
SI1 = Small intestine 1 (first third of the small intestine)
SI2 = Small intestine 2 (second third of the small intestine)
SI3 = Small intestine 3 (final third of the small intestine)
CCM = Caecum
COL = Colon
+ = Red colour
- = No red colour
NS = No samples
± = Red present in the beginning and not the end of section

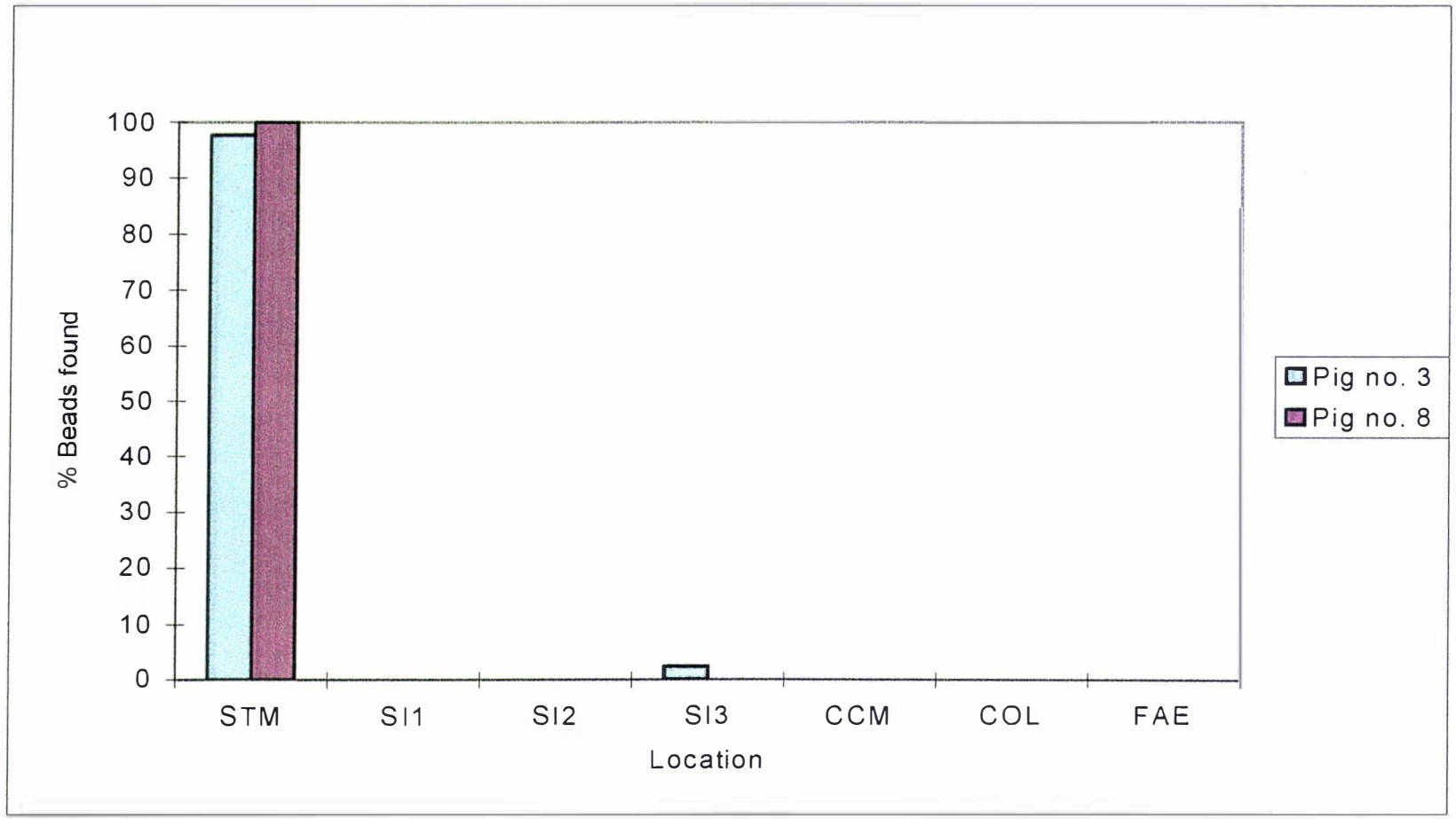

Figure 2.1 The number of beads recovered in different sections of a piglet's gastrointestinal tract expressed as a percentage of the total amount of beads recovered from the entire gastrointestinal tract 1 hr after feeding (the sites of the gastrointestinal tract from which the beads were recovered are as follows: STM = Stomach; SI1 = First third of the small intestine 1; SI2 = Second third of the small intestine 2; SI3 = Final third of the small intestine 3; CCM = Caecum; COL = Colon; FAE = Faeces).

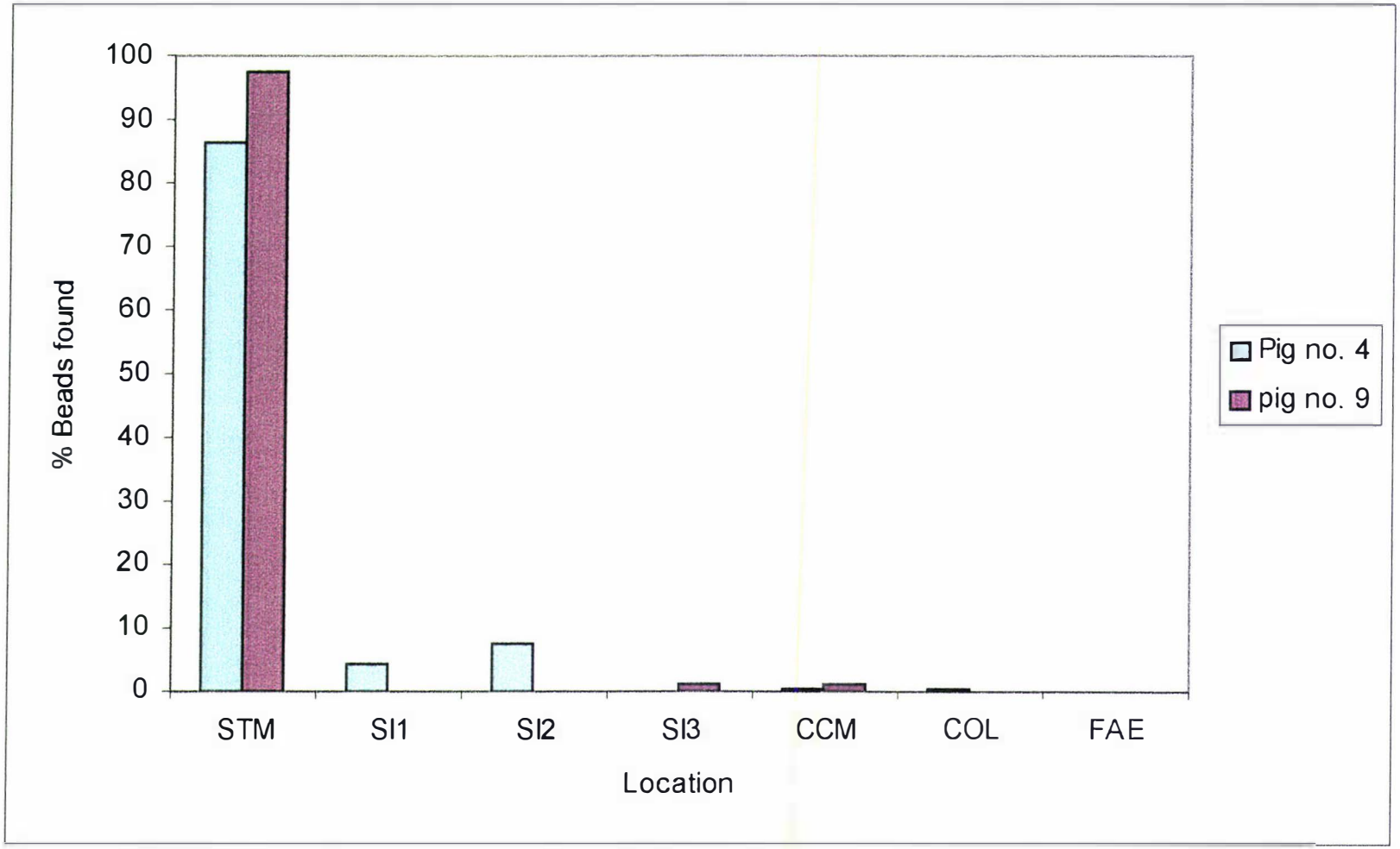

Figure 2.2 The number of beads recovered in different sections of a piglet's gastrointestinal tract expressed as a percentage of the total amount of beads recovered from the entire gastrointestinal tract 5 hrs after feeding (the sites of the gastrointestinal tract from which the beads were recovered are as follows: STM = Stomach; SI1 = First third of the small intestine 1; SI2 = Second third of the small intestine 2; SI3 = Final third of the small intestine 3; CCM = Caecum; COL = Colon; FAE = Faeces).

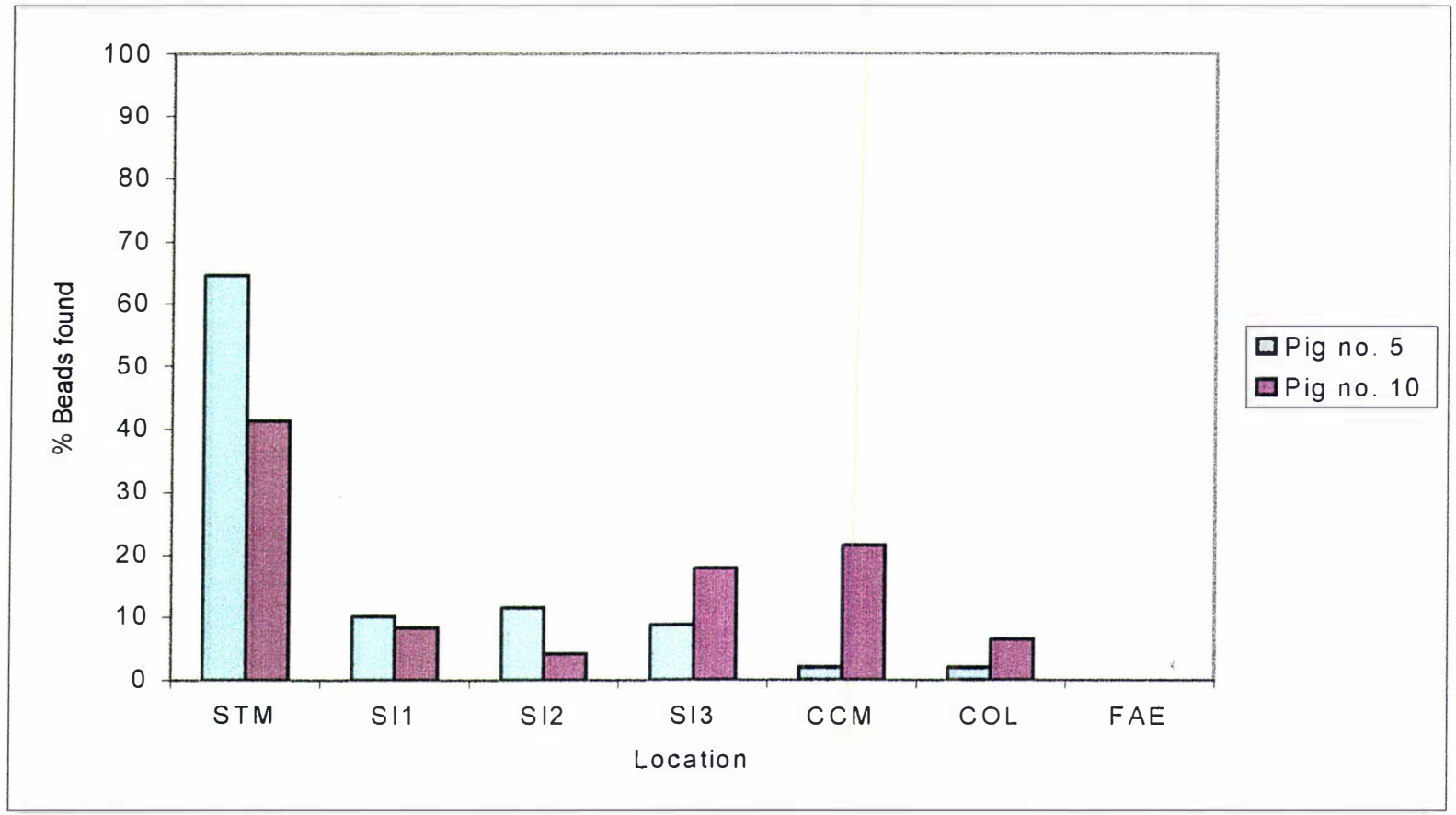

Figure 2.3 The number of beads recovered in different sections of a piglet's gastrointestinal tract expressed as a percentage of the total amount of beads recovered from the entire gastrointestinal tract 9 hrs after feeding (the sites of the gastrointestinal tract from which the beads were recovered are as follows: STM = Stomach; SI1 = First third of the small intestine 1; SI2 = Second third of the small intestine 2; SI3 = Final third of the small intestine 3; CCM = Caecum; COL = Colon; FAE = Faeces).

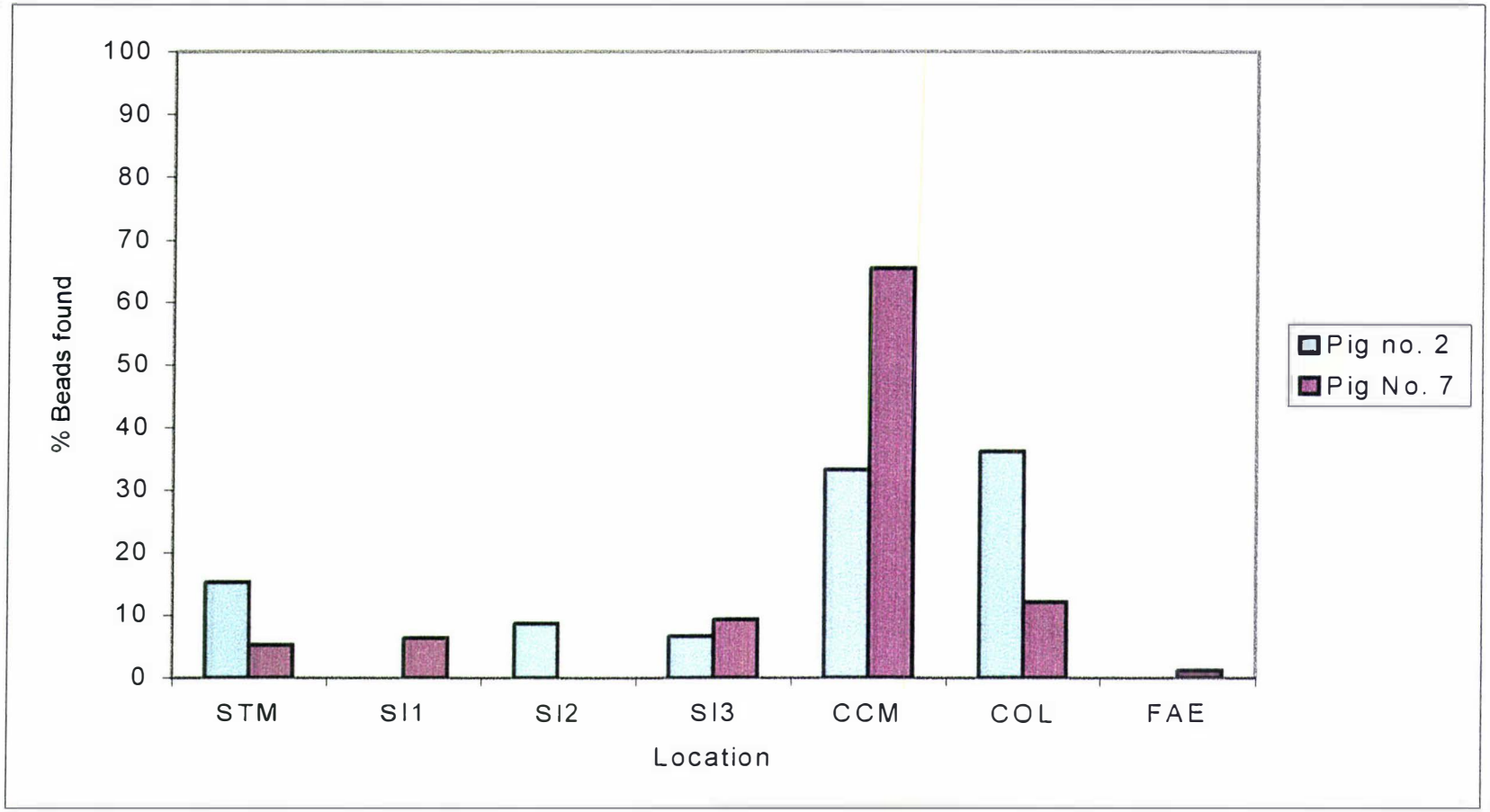

Figure 2.4 The number of beads recovered in different sections of a piglet's gastrointestinal tract expressed as a percentage of the total amount of beads recovered from the entire gastrointestinal tract 16 hrs after feeding (the sites of the gastrointestinal tract from which the beads were recovered are as follows: STM = Stomach; SI1 = First third of the small intestine 1; SI2 = Second third of the small intestine 2; SI3 = Final third of the small intestine 3; CCM = Caecum; COL = Colon; FAE = Faeces).

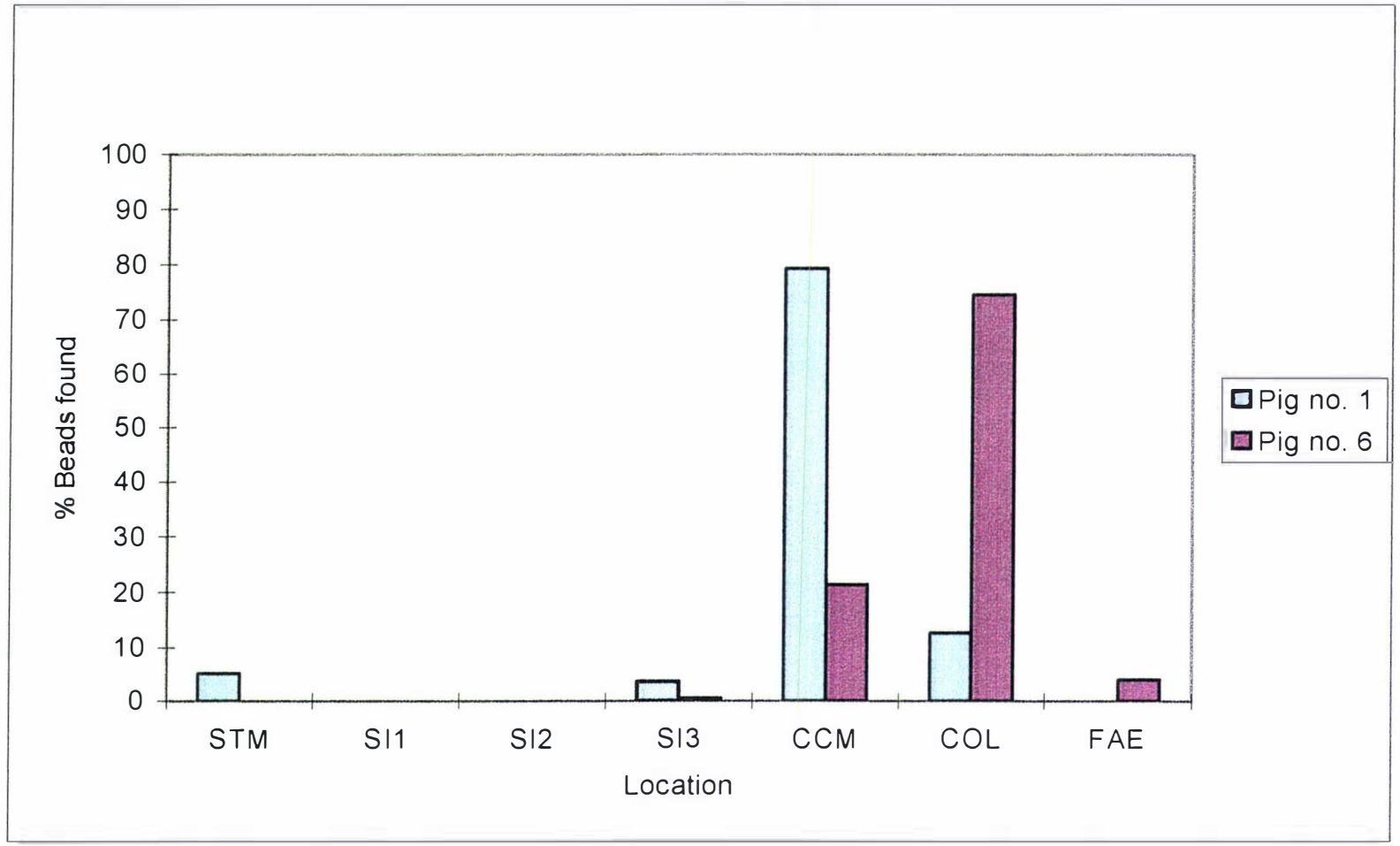

Figure 2.5 The number of beads recovered in different sections of a piglet's gastrointestinal tract expressed as a percentage of the total amount of beads recovered from the entire gastrointestinal tract 24 hrs after feeding (the sites of the gastrointestinal tract from which the beads were recovered are as follows: STM = Stomach; SI1 = First third of the small intestine 1; SI2 = Second third of the small intestine 2; SI3 = Final third of the small intestine 3; CCM = Caecum; COL = Colon; FAE = Faeces).

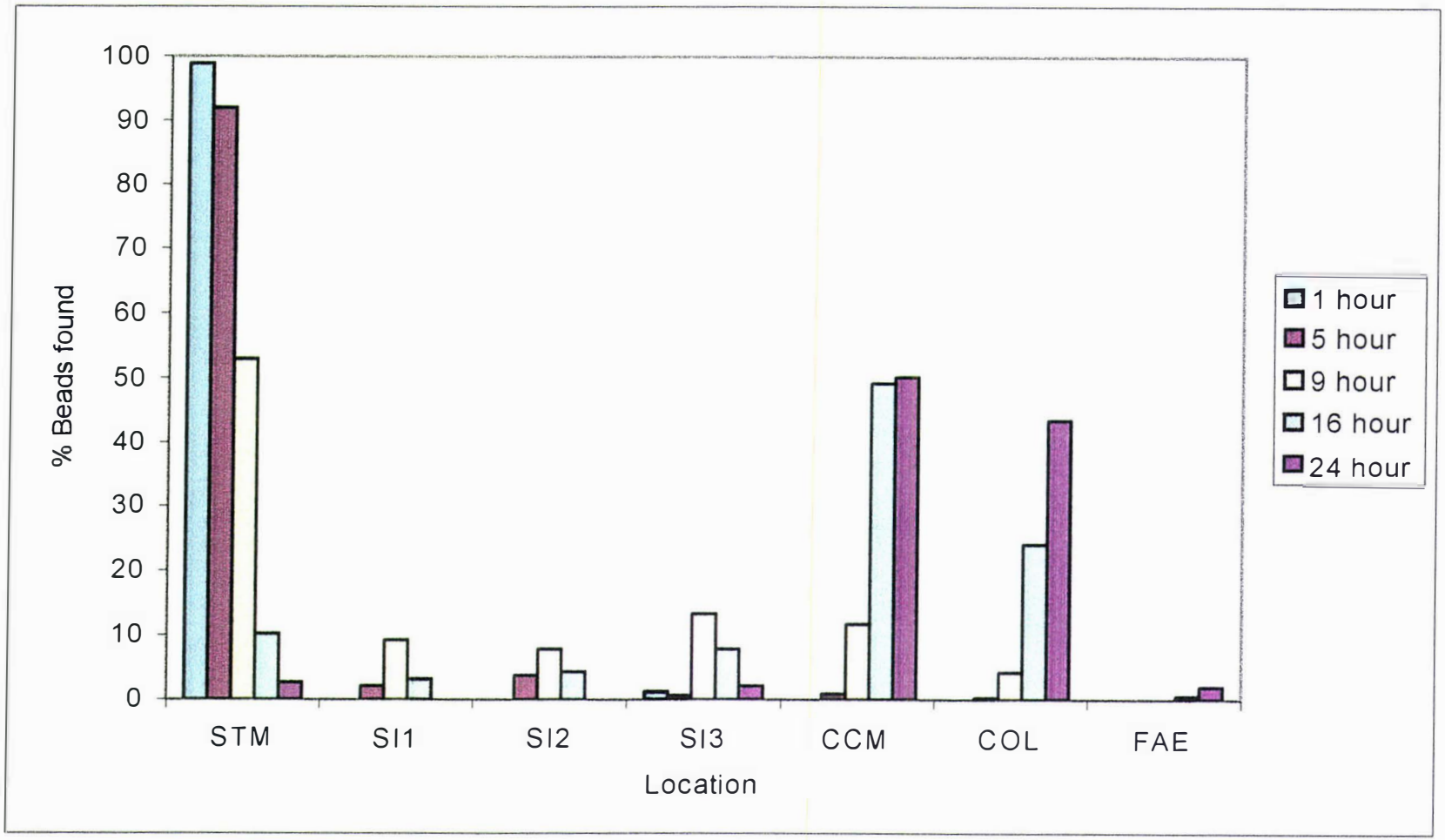

Figure 2.6 The average number of beads recovered in the different sections of the piglet's gastrointestinal tract expressed as a percentage of the total amount of beads recovered from the entire gastrointestinal tract at different times after feeding (the sites of the gastrointestinal tract from which the beads were recovered are as follows: STM = Stomach; SI1 = First third of the small intestine 1; SI2 = Second third of the small intestine 2; SI3 = Final third of the small intestine 3; CCM = Caecum; COL = Colon; FAE = Faeces).

2.5 DISCUSSION

This experiment was conducted to determine the rate of gut transit and the quantity of digesta that could be collected from various locations of the gastrointestinal tract of piglets at different time intervals, following the feeding of a single experimental diet.

Particulate and dye markers have been used extensively in studies of digesta passage and nutrient absorption in man and animals (Seerely et al., 1962; Mangel & Koegel, 1984; Forster et al., 1990; Plourdo et al., 1994; Yegen et al., 1996). In this study, it was postulated that the rate of bead and dye passage would give an indication of the rate of food passage through the gastrointestinal tract.

Movement of digesta within the gastrointestinal tract may be regulated via structural and physiological characteristics of the digestive tract (Hogan & Weston, 1969). Digesta movement is further influenced by physical, as well as nutritional characteristics of the diet (Hogan & Weston, 1969; Hintz et al., 1971). Retention of digesta generally occurs in the stomach and/or hindgut, while in the small intestine it passes rapidly. In this trial, only a small quantity of digesta was found in the small intestine indicating that the digesta passes rapidly in the small intestine but is retained longer in the caecum and colon where greater amounts of digesta were present even after 24 hrs. There was a clear distinction between the liquid and solid phases in the stomach and small intestine, and the liquid phase appeared to move more rapidly through the gut compared to the solid phase when observed after an hour. Similar observations have been made by Dozois et al. (1971) in pigs using a dye-marked meal. Prolonged retention of the solid phase can aid the digestion process by providing adequate time for host and microbial enzymatic degradation of the ingested materials (Van Barnaveld et al., 1994) and for organic acid production (Argenzio & Southworth, 1974; Clemens et al., 1975). Longer retention also enhances the absorption of nutrients (Hecker & Grovum, 1975).

The major role of the stomach is to act as a reservoir for food. The stomach has two functionally distinct areas: the proximal stomach (body and fundus) which is a reservoir and the distal stomach (antrum) which is a pump (Sleisenger & Fordtran, 1973). The stomach provides the intestine with a relatively continuous supply of nutrients for digestion over several hours. In the pig, the stomach does not usually empty completely

(Ruckebusch & Bueno, 1976). In the present study too it was found that digesta was recovered from the stomach even after 24 hrs of feeding. Cuber et al. (1980) have also found that very little amount of digesta was present in the stomach 24 hrs after feeding. Immediately after feeding, the emptying of the stomach contents is likely to be rapid due to the large volume and the resulting high intragastric pressure. However, after the initial rapid phase, there is usually a decrease in the rate of stomach emptying as the feedback from the expanding small intestine takes effect (Laplace, 1981). This results in the rate of emptying being dependent on the rate of passage along the small intestine and also on the rate of digestion of the diet. The function of the ileo-caecocolic junction is related to the retention time in the small intestine (Laplace, 1981). Small particles do not accumulate in the gastrointestinal tract whereas large particles may be retained in the stomach for a long time (Potkins et al., 1991b). In this trial, the transit of beads through the small intestine was rapid with no apparent retention of beads at any point due to their very small size

The large intestine has a major influence on the rate of passage. Most of the total time of feed residue retention is in the large intestine and the digesta movement is slow in the large intestine. The retention time of food in the caecum represents approximately 80% of total retention time (Keys & DeBarthe, 1974). In this trial too it was observed that most of the food was retained in the large intestine after 9 hrs. Food retention in the large intestine, however, is of little significance in relation to nutritional value and passive immunity protection against gastrointestinal infectious diseases. Microbial degradation takes place in the large intestine. The stomach and small intestine are more essential with regards to gut mechanics and related control factors and regulatory mechanisms especially digestion, absorption processes and passive immunity protection against gastrointestinal infectious diseases.

In this study, individual variations were observed in the gut of piglets in relation to the position of the beads, movement of dye, and the amount of digesta collected. For example, when the two piglets were slaughtered after 24 hrs of feeding, dye was observed in the faeces of one piglet and the dye travelled to the end of the colon in the other piglet and quantity of digesta recovered from the stomach varied too. When the two piglets were killed after 1 hr of feeding, beads were observed in the SI3 of one piglet and none in the other.

The colourimetric method of measuring colour intensity in the digesta and faeces was not successful. This may have been due to the *L*, *a*, and *b* measurements being affected by the thickness of the sample in the petrie dish, the presence of small food particles in the liquid, and the density of the sample evaluated. Although the samples were well mixed to achieve homogeneity, digesta from the small intestine especially had more fluid, and contained distinct small food particles in comparison to samples from other locations. These factors would have affected the light transmission/reflection and thus the colour measurements. It was concluded that this type of measurement is only appropriate for samples with a more consistent composition and consistency.

Beads and dye were used as markers in the experimental diet. The piglets were not starved, as it was assumed that the previous feeding would not have any effect on the digesta movement of the experimental diet.

The present study confirms the rate of digesta movement and that sufficient quantity of digesta could be recovered from each location of the gastrointestinal tract of the piglets at different times. These data were used in the planning of the digestibility trial described in chapter 3.

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2.7 APPENDIX 2

Appendix 2.1
Nutrient content of Vitamin and Mineral* mix added to a standard weaner diet for piglets in all trials

Nutrient	per 5kg inclusion rate
Vitamins	
A	18.0MIU
D	2.5 MIU
E	50000.0 IU
K	2.5 g
B_1	1.5 g
B_2	4.0 g
B_6	5.0 g
B_{12}	30.0 mg
C	20.0 g
Folic acid	500.0 mg
D-Calpan	12.0 g
Biotin	75.0 mg
Niacin	22.0 g
Choline	150.0 g
Minerals	
Selenium	300.0 mg
Cobalt	500.0 mg
Iodine	1.0 g
Copper	125.0 g
Iron	100.0 g
manganese	45.0 g
Zinc	120.0 g

* Nutritech, Auckland

Appendix 2.2

Nutrients content of DICP (Di calcium phosphate)[♠] added to standard weaner for piglets in all trials

Nutrient	Percentage
Phosphorous	19.0
Phosphorous oxide	43.5
Calcium	21.0

♠ Nutritech, Auckland

Appendix 2.3

Total daily feed intakes (g/day) by six week-old piglets fed over seven days during a gut transit time trial

PIG #[♦]	19/06/97	20/06/97	21/06/97	22/06/97	23/06/97	24/06/97	25/06/97
1	176	980	1063	1122	1446	1265	1213
2	85	701	925	46	1127	1199	1139
3	103	1305	883	1244	1175	1300	1385
4	110	1025	1084	1141	1418	1496	700
5	110	582	555	930	1087	1147	1143
6	25	166	205	66	230	643	679
7	72	857	870	1114	1278	1185	1203
8	54	831	1121	1171	1223	1375	1428
9	82	681	1088	1095	1369	1155	1313
10	28	118	81	47	814	1161	1146

♦ Pig identification number

Appendix 2.4
Visual Colour observation of digesta collected throughout the gastrointestinal tracts of piglets at different times after feeding a experimental diet containing 0.12g carmine red marker per 100g of diet fed.

Pig #♣	Time (hrs)♦	STM♥	SI1▲	SI2•	SI3Ψ	CCMϕ	COLφ	FAECES
3	1	Like diet	Watery with few red lumps	Watery with few red lumps	Blackish solid little water red	Black solid	Black solid	No sample
8	1	Two colours red and white; red more like diet	Watery with few reddish food particles	More blackish less red fluid	Blackish solid with little water	Dark black solid.	Black solid.	No sample
4	5	Red likes diet.	Watery red, more like diet colour	Blackish red solid little water	Dark red solid less water	Dark red solid slightly waters	Beginning of colon red and end of colon black solid slight red	No sample
9	5	Like diet	Red lumps with little water	Red watery with little solid	Dark red, fine particles	More black less red solid with little water	Black solid	No sample
5	9	Three different colours dark red, pink and white	Red lumps with more watery	More blackish less red solid with little water	More blackish less red little watery solid	Slightly watery, red	Slightly red but black solid	Black solid
10	9	Like diet	Watery with few red lumps	Dark red with little water	Dark red with fine particles	Dark red with fine particles	First one third of colon red blackish red	Black solid
7	16	Like diet but slightly less red	Red and watery	Watery with red lumps	Reddish solid with little water	Dark red blackish solid with little water	Two third of colon red and last one third of colon reddish black solid	Black solid
2	16	More yellow fluid with few red particles	Yellow liquid with few red lumps	Slightly brownish red	Solid, blackish red	Dark red more solid less liquid	Start two third of colon red and end one third of black	Black solid

Appendix 2.4 cont...

Appendix 2.4 continued

Pig #	Time (hrs)	STM	SI1	SI2	SI3	CCM	COL	FAECES[0]
1	24	Slight red more yellow, watery	More watery with little food	Watery yellows with red few food fine particles	Dark blackish red, more solid little watery	Dark red sold	Blackish dark red solid, little watery	Black solid
6	24	Yellowish fluid	Yellowish fluid with very few food particles of food	Yellowish fluid	Yellow fluid with few food particles	Red solid	Slightly red solid	Slightly red solid

♣ Pig number
• Time (Hrs) of sampling after feeding experimental diet
▾ STM = Stomach
▲ SI1 = Small intestine 1 (first third of the small intestine)
▪ SI2 = Small intestine 2 (second third of small intestine)
Ψ SI3 = Small intestine 3 (final third of small intestine)
♦ CCM = Caecum
♀ COL = Colon

Appendix 2.5
Colour measurements, *L*, *a*, *b* and 530nm wave length by colourimeter, recovered percent of beads from different parts of the gastrointestinal tract of piglets 1, 5, 9, 16, and 24 hrs after feeding a experimental diet

Time (hrs)[§]	Pig #[Φ]	Location[¥]	Colourimeter measurement			Recovered % beads[φ]	530 nm[¥¥]
			L[ω]	*a*[ω]	*b*[ω]		
1	3	Diet[£]	34.65	23.17	3.05		6.86
		STM[¥]	46.82	21.96	10.14	97.79	12.7
		SI1[£]	29.58	2.56	3.18	0.00	7.09
		SI2[♠]	0.01	0.08	0.02	0.00	0.00
		SI3[♠]	1.40	9.61	2.41	2.54	0.00
		CCM[♥]	24.99	4.44	14.40	0.00	5.77
		COL[♠]	9.25	-13 6	-4.08	0.00	1.96
		Faeces		No samples			
1	8	Diet	40.12	24.41	6.30		8.82
		STM	50.30	13.76	15.63	100.00	16.59
		SI1	43.58	14.14	6.30		12.20
		SI2	0.25	0.17	0.04		0.00
		SI3	4.69	5.31	7.12		0.89
		CCM	9.05	-2.11	1.51		1.87
		COL	5.75	-2.75	6.41		0.92
		Faeces		No samples			
5	4	Diet	37.64	24.27	5.39		8.03
		STM	38.37	22.98	10.31	86.39	8.08
		SI1	15.96	22.71	10.35	4.24	11.81
		SI2	6.38	21.99	0.51	7.42	0.00
		SI3	2.69	17.16	1.23	0.00	0.00
		CCM	20.47	24.92	12.33	0.53	2.91
		COL	8.76	5.14	4.59	0.53	1.33
		Faeces		No samples			
5	9	Diet	43.27	21.29	4.61		11.29
		STM	46.28	25.42	11.00	97.6	11.75
		SI1	19.44	26.02	6.69	0.00	2.17

Appendix 2.5 cont...

Appendix 2.5 continued

Hours	Pig #	Location	Colourimeter measurement			Recovered % beads$^{\varphi}$	530 nm
			L	*a*	*b*		
		SI2	9.26	21.26	3.45	0.00	0.49
		SI3	1.57	10.43	2.30	1.22	0.00
		CCM	13.44	16.63	5.53	1.22	2.08
		COL	4.25	0.45	3.02	0.00	0.49
		Faeces		No samples			
9	5	Diet	35.26	25.35	5.12		6.70
		STM	45.04	21.94	14.93	64.60	11.44
		SI	14.27	15.71	6.54	10.20	2.17
		SI2	9.51	15.59	2.51	11.56	0.97
		SI3	1.78	11.59	2.21	8.84	0.00
		CCM	16.62	21.60	6.21	2.04	2.18
		COL	11.78	3.16	-10.15	2.04	0.72
		Faeces	12.08	2.48	1.77	0.00	2.81
9	10	Diet	41.35	21.97	4.52		9.87
		STM	44.36	27.02	11.07	41.40	9.91
		SI1	23.16	22.25	16.16	8.40	3.47
		SI2	10.32	24.12	9.39	4.20	0.48
		SI3	5.37	25.13	1.96	18.00	0.00
		CCM	5.31	25.03	3.34	21.60	0.18
		COL	16.79	12.24	9.92	6.60	0.62
		Faeces	13.36	1.79	1.17	0.00	3.09
16	2	Diet	3.62	26.11	7.96		6.82
		STM	45.01	22.21	18.70	15.30	11.22
		SI1	56.57	0.80	6.07	0.00	25.17
		SI2	6.86	22.64	10.84	8.67	0.26
		SI3	10.71	12.87	13.97	6.63	0.72
		CCM	14.12	6.29	3.95	33.15	0.80
		COL	7.09	14.03	3.97	36.25	0.63
		Faeces	18.18	1.65	8.61	0.00	4.02
16	7	Diet	37.28	27.22	7.84		7.06
		STM	40.62	19.93	19.45	5.22	9.21
		SI1	17.27	24.81	22.96	6.38	2.19
		SI2	15.22	27.27	25.83	0.00	1.19
		SI3	6.58	30.64	11.34	9.28	0.00

Appendix 2.5 cont...

Appendix 2.5 continued

Hours	Pig #	Location	Colourimeter measurement			Recovered % beads	530 nm
			L	*a*	*b*		
		CCM	13.00	24.30	5.37	65.54	0.81
		COL	7.71	15.10	2.53	12.18	0.56
		Faeces	8.37	-3.04	3.74	1.16	1.72
24	1	Diet	31.14	33.68	4.30		4.18
		STM	41.16	19.44	40.73	5.18	10.10
		SI1	47.07	0.09	36.27	0.00	11.57
		SI2	18.39	21.02	31.41	0.00	2.41
		SI3	3.80	21.72	6.55	3.70	0.00
		CCM	5.31	25.83	3.34	79.18	0.00
		COL	16.79	12.24	9.92	12.58	3.10
		Faeces	21.71	6.44	9.26	0.00	4.50
24	6	Diet	34.88	24.10	3.67		6.68
		STM	57.25	-4.08	53.41	0.00	28.84
		SI1	51.73	-3.07	31.31	0.00	21.74
		SI2	24.14	13.83	41.09	0.00	4.33
		SI3	16.39	16.92	28.08	0.51	2.13
		CCM	15.85	24.33	12.19	21.42	1.62
		COL	37.86	20.44	0.92	74.46	2.65
		Faeces	16.58	13.20	2.80	4.08	2.80

[s] Time (hrs) sampling after feeding experimental diet
[Φ] Pig identification number
[3] Location in the gastrointestinal tract of piglets
[0] Colourimeter L measurement, measured by Hunter Lab colourimeter
[a] Colourimeter a measurement, measured by Hunter Lab colourimeter
[b] Colourimeter b measurement, measured by Hunter Lab colourimeter
[p] Recovered percentage of beads in the gastrointestinal tract of piglets after feeding
[••] Carmine red measurement at 530 nm wave length
[1] Diet given to piglets before killing
[ψ] STM = Stomach
[ε] SI1 = Small intestine 1 (first third of the small intestine)
[▲] SI2 = Small intestine 2 (second third of small intestine)
[•] SI3 = Small intestine 3 (final third of small intestine)
[▼] CCM = Caecum
[▲] COL = Colon

CHAPTER 3

DIGESTION OF IMMUNOGLOBULIN G DERIVED FROM BOVINE MILK FED AS A SINGLE BOLUS TO WEANER PIGLETS

3.1 ABSTRACT

It has been suggested that bovine milk immunoglobulin concentrates may be used to induce passive immunity against various enteric pathogens. Orally ingested Igs must resist denaturation and proteolysis by intestinal secretions to be effective against pathogens that reside in the gastrointestinal tract. This experiment was conducted to measure the digestibility of bovine milk derived immunoglobulin G in the gut at different times after oral administration. Twenty-five, four to five-weeks-old piglets were fed at 10% of their metabolic body weight $kg^{0.75}$ per day on a weaner diet. The feed consisted of a diet formulated from skim milk powder and cereal grains. The experimental diet was formulated with 30% (weight/weight) bovine whey globulin concentrate and cereal grains. Individual plastic troughs were used to feed piglets. Twenty-five piglets were randomly divided into five groups. Samples of digesta from different parts of the gastrointestinal tract, blood from the hepatic portal vein and faeces were collected from each piglet slaughtered at either 1, 5, 9, 16 and 24 hrs after feeding the experimental diet. Data collected from the experiment showed that a certain percentage of orally administered IgG can resist digestion for 24 hrs after ingestion. No bovine immunoglobulin G was detected in the hepatic portal vein blood of these piglets. This indicates that Igs ingested in the diet do not pass through the intestinal wall into the blood stream. It is unlikely therefore that they will induce an allergic reaction.

3.2 INTRODUCTION

Immunoglobulins (Igs) are found in high concentrations in colostrum and milk. They exhibit antibacterial (Morris & Edwards, 1950; Carrol & Jian, 1969) and antiviral (Snodgrass et al., 1980; Brüssow et al., 1993; Tache et al., 1995) effects for a wide range of micro-organisms and are thought to protect the new-born against local enteric infections. Colostrum and milk are the first specific foods for mammalian neonates, and they are therefore crucial in providing temporary passive immunity to the new-born (Logan et al., 1974).

Diarrhoea is a major cause of mortality in all ages especially infants and is hence a grave health problem in developing countries (Black et al., 1980; Stoll et al., 1982; Hone & Hackett, 1989). Travellers to developing countries are liable to be at risk (Gorbach & Edelman, 1986). Safe and effective vaccines for many common enteric pathogens are not currently available. In addition, the widespread use of prophylactic antibacterial agents is not encouraged because of the possible side effects (Murray et al., 1982; Murray, 1986).

Investigations suggest that oral administration of milk immunoglobulin (Ig) concentrates of bovine origin could be a safe and effective alternative to prevent or treat infantile gastro-enteritis (Brüssow et al., 1981; Hilpert et al., 1987; Mietens et al., 1979) and traveller's diarrhoea (Tacket et al., 1988). This approach is further supported by results from epidemiologic studies that suggest certain disease specific antibodies in human milk provide protection against diarrhoea in breast-fed infants (Kenny et al., 1967; Brambel, 1970; Ringenbergs et al., 1988).

Resistance to degradation and digestibility of Igs are important factors for consideration. Orally administered Ig must resist digestion in the stomach and the small intestine by resisting degradation by gastric acid, pepsin, pancreatic and small intestinal enzymes in order to be therapeutically active against diarrhoea. It can then reach the site of enteric infection intact, having retained its functional activity.

Previous work has shown that bovine colostrum contains antibodies to enteropathogenic *Escherichia coli* and that in the presence of complement these antibodies can exert a bactericidal effect (Brown et al., 1970; Reiter & Brock, 1975; Brock et al., 1977). The complement system is liable to proteolysis, however, making it unlikely that the antibody complement system could function in the gastrointestinal tract.

Several studies have led, therefore, to the conclusion that bovine milk Igs demonstrate resistance to proteolytic degradation (de Rham & Isliker 1977; McClead & Gregory, 1984; Petschow & Talbott, 1994).

So far, there is no experimental evidence to show that Ig can persist in the gut for 24 hrs after ingestion. This experiment was conducted to measure the undigested immunoglobulin G (IgG) remaining in the gastrointestinal tract 24 hrs after degradation by gastric acid and intestinal enzymes, following a single meal of an experimental diet containing 30% (w/w) whey globulin concentrate (WGC).

3.3 MATERIALS AND METHODS

All aspects of this study were approved by the Massey University Animal Ethics Committee (Protocol Number 97/113).

3.3.1 Animals

Twenty five Large White x Duroc, four to five-week-old male piglets (average body weight (8 ± SD) 9.4 ± 1.89 kg; range 8.0-12.0 kg) were selected from a group of piglets at a commercial piggery (Wairaka Farms Ltd., Foxton).

3.3.2 Housing

The piglets were housed individually in plastic metabolic cages in a temperature controlled room maintained at 25 ± 1^0C under alternating 14hr light, 10hr dark periods. They were allowed water *ad libitum* throughout the trial period.

3.3.3 Diet and Feeding regime

The preparation of the weaner diet and feeding procedures of the piglets were carried out as described in chapter 2 section 2.3.3 except for addition of (0.4%) chromium to the weaner diet. Chromium was added as a non-digestible marker. The piglets were weighed once every three days between 1500 - 1600hrs. On the day of weighing, each piglet's daily intake was adjusted to the new body weight. Piglets were given an experimental diet prior to slaughter (the composition of ingredient is presented in Table 3.1, the nutrient content is presented in Table 3.2) containing 30% (w/w) WGC.

The bovine milk Igs were supplied as a WGC (New Zealand Dairy Board, Wellington, New Zealand) which was produced from pooled mature milk rather than colostrum. The cows that supplied the milk were exposed naturally to *Escherichia coli* pathogens and produced milk containing Igs, which are assumed to have a protective effect against *Escherichia coli.* This milk is termed 'natural harvest' immune milk. The WGC is manufactured under specific conditions (for example, low temperatures are maintained throughout processing) to preserve its immune properties. These products, when consumed, are expected to reduce the effect of pathogenic micro-organisms in the gut by reducing their numbers and neutralising the toxins in the gut, thus providing protection against diarrhoea (Schollum et al., 1997).

3.3.4 Experimental procedure

The experimental procedure was followed as described in chapter 2 section 2.3.4 with 25 piglets instead of 10 i.e. five piglets with each time group. Blood was collected from all piglets and faeces were collected from those piglets sampled at 16 hrs and 24 hrs.

3.3.5 Collection of digesta, faeces and blood

For digesta and blood collections, the procedures from anaesthesia to separation of the small intestine into three sections was carried out as described in chapter 2 section 2.3.5. Blood was collected from the hepatic portal vein immediately after opening the abdominal cavity and stored in vacutainer tubes (Biolab Scientific Ltd, Palmerston North, New Zealand). Digesta from the stomach, SI1, SI2, SI3, colon and caecum were

Table 3.1

The ingredient composition (%) of an experimental diet fed to four to five-week-old piglets as a single bolus♥ experimental diet in an IgG digestibility trial

Ingredients	% in diet (Dry matter basis)
WGC	30.0%
Wheat	20.0%
Barley	22.5%
Maize	25.0%
Vitamin/mineral premix♣	0.5%
DICP♀♣♣	2.0%

♥ Single bolus= one single meal containing 30% WGC given prior to slaughter
♣ Presented in Appendix 2.1
♀ Di calcium phosphate
♣♣ Presented in Appendix 2.2

Table 3.2

The calculated nutrient♥ content of the experimental diet fed to four-five-week-old piglets as a single bolus experimental diet in an IgG digestibility trial

Nutrients	per 100g
Energy	1144.37 kJ
Crude protein	33.19 g
Calcium	0.49 g
Phosphorus	0.48 g
Sodium	0.02 g
Potassium	0.38 g
Chloride	0.06 g
Fat	3.37 g
Linoleic acid	0.59 g
Fibre	2.05 g
Arginine	1.04 g
Histidine	0.76 g
Isoleucine	1.94 g
Leucine	3.49 g
Lysine	2.70 g
Methionine	0.67 g
Cystine	1.31 g
Tyrosine and phenylalanine	3.28 g
Threonine	2.14 g
Tryptophan	0.94 g
Valine	1.94 g

♥ Calculation based on the nutritive value of local feed stuff for pigs and poultry, Monogastric Research Center, Massey University, 1981.

separately collected and stored in individual plastic containers (Carter Holt Harvey, Palmerston North, New Zealand).

To collect faeces, the same procedure as that described in chapter 2 section 2.3.5 using ostomy bags was followed. All samples of faeces, digesta and blood collected were stored at -20° C until further analysis.

3.3.6 Sample analysis

Faeces and digesta were freeze-dried and accurately weighed to three decimal places before and after freeze-drying. After freeze-drying, the samples were ground using a 0.25mm sieve and analysed for chromium and bovine immunoglobulin G (IgG). Blood samples were analysed for bovine IgG.

3.3.6.1 Measurement of bovine immunoglobulins

The concentration of immunologically active Igs of bovine origin in the diet, digesta, faeces and blood was measured by Radial Immuno-Diffusion (RID). The RID assay used is a commercial kit supplied by "The Binding Site" (Institute of Research and Development, Birmingham, UK), which detects bovine Igs and not porcine Igs. The method is based on the antigen (bovine Igs) diffusing radially from a cylindrical well through an agarose gel containing uniformly distributed appropriate mono-specific antibody (anti-bovine Ig antibody). Antigen is added to the wells at different concentrations, and the antibody-antigen reaction results in the formation of insoluble complexes which appear as a precipitin ring around the well. Once diffusion and complex formation is complete, as instructed by the manufacturer, the diameter squared of the precipitin ring is linearly related to the antigen concentration of the sample in that well (Mancini et al., 1964; Fahey & McKelvey, 1965; Mancini et al., 1965). The equations used to calculate Ig concentrations are given in Appendix 3.1.

The RID method: There are two kinds of RID plates, low level which measures the Ig concentration range between 50 – 500 mg/L and normal level ranging from 250 – 2500mg/L. Each RID plate kit was stored at 4°C and brought to room temperature before use. The plates were removed from their foil pouches, and kept upside down to

prevent condensation of water droplets falling onto the agarose gels, until the samples were added.

Calibrator preparation: For the low level plates, lyophilised calibrators of 50mg IgG/L, 250mg IgG/L, and 500mg IgG/L were reconstituted by adding 415µl of distilled water and the weight recorded to four decimal places. For normal level plates, lyophilised calibrators of 250mg IgG/L, 1500mg IgG/L and 2500mg IgG/L for IgG; 25mg immunoglobulin A (IgA)/L, 50mg IgA/L and 250mg IgA/L for IgA; and 100mg immunoglobulin M (IgM)/L, 600mg IgM/L and 1000mg IgM/L for IgM were reconstituted by adding 390µl, 445µl and 490µl of distilled water respectively and the weight recorded to four decimal places.

Sample preparation: Freeze-dried faecal and digesta samples, awaiting measurement for IgG content, were put into a beaker and the weight recorded accurately to four decimal places. An appropriate quantity of phosphate buffered saline (PBS) diluent was added to reconstitute to different concentrations (w/w) and the total weight recorded. These prepared samples were covered with parafilm and allowed to rehydrate with continuous stirring using a magnetic multi-stirrer for 45 min and then transferred to a 1.5ml microcentrifuge tube (Bio Lab Scientific Ltd, Palmerston North, New Zealand) and centrifuged at 10000 RPM for 5 minutes. After centrifugation, the supernatant was pipetted into another 1.5ml tube (Bio Lab Scientific Ltd, Palmerston North, New Zealand) and stored at room temperature until loading onto RID plates.

Loading RID plates: Calibrators and sample supernatants were loaded into wells of the RID plates using the following method. Each sample was gently mixed, and 5µl loaded into each of two wells. Samples were allowed to completely diffuse into the agarose gel before incubation. To minimise evaporation, the plates were resealed in their foil pouches and incubated at 22 ± 1°C for 96 hours before reading. Ring diameters were measured to the nearest 0.1mm using an electronic RID plate reader ("The Binding Site", Birmingham, UK) and the measurements recorded.

Measurement of bovine Igs in the ingredients and diets fed to the piglets in the trial: The quantity of IgG present in the skim milk powder (Kiwi Co-operative Dairies, Hawera, New Zealand) used in the weaner diet was checked by the RID assay, using low level plates. Two different concentrations were prepared: approximately 10% (w/w with PBS) and approximately 37% (w/w with PBS).

The quantity of bovine IgG, IgM, and IgA in the whey globulin concentrate used in the experimental diet was measured by the RID method using normal level plates. A range of concentrations of WGC from approximately 0.5% to 10% (w/w with PBS) were assayed and the sample concentrations calculated (Appendix 3.2).

The quantity of bovine IgG present in the weaner and experimental diets was checked by RID assay, using normal level plates. The weaner diet was measured at approximately 50% (w/w with PBS) concentration and the experimental diet at approximately 10% (w/w with PBS). The calculation of sample concentration of these diets is recorded in Appendix 3.12.

Measurement of bovine IgG in the digesta: The amounts of IgG in the digesta prepared as described above were assayed by the RID method at a sample concentration of 33.33% (w/w with PBS). If the ring diameters of the test samples fell outside the range of the standard calibrator ring diameter values, the samples were re-assayed. Those that fell below the standard values were tested at 50% concentration (w/w with PBS). The samples were covered with parafilm and allowed to rehydrate, as previously described. They were then transferred to a 1.5 ml microcentrifuge tube and centrifuged at 10000 RPM for 45 minutes to obtain the supernatants. For some samples, where a supernatant was not obtained by this method, a 10% concentration (w/w with PBS) supernatant was prepared. After 45 minutes continuous stirring using a magnetic stir bar, the rehydrated samples were transferred to 15 ml centrifuge tubes (Bio Lab Scientific Ltd, Palmerston North, New Zealand) and centrifuged at 4000 for 5 minutes at 4°C. The supernatants from this procedure were pipetted into small plastic containers (Carter Holt Harvey, Palmerston North, New-Zealand) and freeze-dried for three days. The samples were weighed accurately to four decimal places before and after freeze-drying. The samples were reconstituted with 1000µl, 1400µl and 2800µl of PBS

according to the freeze-dried amount, and a final concentration to assay was determined from previous experience using the reconstituted samples. Calibrator preparation and loading RID plates procedure described earlier was followed. If the samples were above the RID standard calibrator ring diameters they were further diluted and re-assayed.

Measurement of bovine IgG in the faeces: A 10% concentration (w/w with PBS) supernatant was prepared according to the methods described above. The supernatant was pipetted into small plastic containers (Carter Holt Harvey, Palmerston North, New Zealand) and freeze-dried for three days. The sample was weighed accurate to four decimal places before and after the freeze-drying. The samples were reconstituted with 1000µl PBS and then calibrator preparation and the procedure for loading RID plates described above was followed.

Measurement of bovine IgG in the serum: The blood was stored in the vacutainer tube and left over night (approximately 15-20 hrs depending on sample collection time) at room temperature. The following morning, the serum was pipetted into 1.5 ml microcentrifuge tubes and stored at –20°C until further analysis. Before loading onto the RID plate, the samples were brought to room temperature. Each of the samples was loaded neat (not diluted) twice into the well to make up a test volume of 10 ml i.e. the first 5 µl aliquot was allowed to diffuse into the agarose gel completely, before the second 5 µl aliquot was added. After the second application, the method for loading RID plates was followed.

3.3.6.2 Measurement of Chromium

Digesta, faecal and diet samples were analysed in duplicate for chromium content using the Costagan and Ellis (1987) method, at the Nutritional Laboratory, Institute of Food, Nutrition and Human Health, Massey University, Palmerston North.

3.3.7 Data analysis

To calculate the concentration of Igs in the WGC, standard curves were generated (Appendix 3.4, 3.7, 3.10) by linear regression using the true calibrator concentration values and the squared diameter of their precipitin rings from the RID plates (Appendix 3.3, 3.6, 3.9). The quantities of IgG, IgA and IgM in the WGC were calculated using

the regression coefficients from the standard curves, the diameters squared of the samples as measured by the RID assay and the original concentration of the samples (Appendix 3.5, 3.8, 3.11).

Similarly, the amount of bovine IgG in the weaner diet and experimental diet was calculated by generating a linear regression curve (Appendix 3.14) using the true calibrator concentration values and the diameter squared of their precipitin rings from the RID plates (Appendix 3.13). The amount of IgG in the sample was calculated using this regression coefficient and the squared diameter of the samples as measured by the RID assay (Appendix 3.15).

The amount of bovine IgG in digesta and faeces was calculated from a standard curve (appendix3.17) generated by linear regression using the true calibrator concentration values and the diameter squared of their precipitin rings from the RID plates (Appendix 3.16). The amount of IgG in the samples (Key 9 in Appendix 3.19) was calculated using this regression coefficient and the squared diameter of the samples as measured by the RID assay (Appendix 3.18).

A split plot model (Appendix 3.20) was used to estimate the effects of sampling time (hours), piglets within time, location in the digestive tract, and time by location interaction on chromium content, IgG content, IgG/unit chromium ratio, and the percentage of undigested IgG. In addition, Pearson correlation coefficients were used to estimate the correlation between feed intake, chromium content, sample IgG content and percentage of undigested IgG (SAS manual, 1985).

3.4 RESULTS

The piglets used in the present study remained in good health throughout the trial period and gained weight. At the end of trial, the piglets were five or six weeks old and had a live weight average (8± SD) of 10.5 ± 2.14 kg (weight range 8.0-13.5 kg). Feed intake was measured each day and recorded (Appendix 3.21). Digesta from each intestinal location was collected from all the piglets except at SI2 at 16hrs in one piglet (pig no 18) and at SI2 at 24 hrs in another piglet (pig no. 17). In addition, the SI2 content at 24 hrs in one piglet (pig no. 2) was too scanty after freeze-drying to allow further analysis.

An anomalous result was obtained for chromium in another 24hr piglet (pig no. 12). Consequently, the data from this piglet were also excluded from the numerical analysis.

Duplicate samples were analysed for chromium and IgG and an overall mean coefficients of variation (between the duplicate samples at each assay) were 1.79 for chromium and 3.02 for IgG.

The concentrations of IgG, IgA and IgM in the WGC were 6.19%, 0.82% and 0.65%, respectively (Appendix 3.5, 3.8, 3.11). There was no visible precipitin ring around the wells in the RID plate for skim milk powder, indicating that no detectable IgG was present in the skim milk powder. The weaner diet too had no detectable IgG, as there was no visible precipitin ring around the wells in the RID assay. The experimental diet had 2% IgG (Appendix 3.15).

The percent of the undigested IgG that remained in each digesta sample at 1, 5, 9, 16, and 24 hrs after consumption of the bolus meal was calculated and is given in Appendix 3.19. A summary of the data is represented by Figures 3.1 to 3.5. This indicated that the largest amount of undigested IgG was found in the stomach with a rapid decrease in the small intestine and absent in the caecum and colon except in a few piglets. The mean percentage of IgG that remain undigested in each gastrointestinal location at 1, 5, 9, 16, and 24 h after consumption of the bolus meal is given in Figure 3.6. This indicated that the quantity of undigested IgG decreased over time in the stomach and increased in the small intestine.

Statistical analysis of these results showed a significant effect of hours after feeding, location within the gastrointestinal tract, and a location by hours interaction on the amount of IgG in the freeze-dried digesta samples, the amount of chromium (Cr) in the freeze-dried digesta samples, the IgG:Cr ratio, and the percentage of undigested IgG (Table 3.3).

The IgG:Cr ratio had a highly significant ($p<0.001$) correlation with the amount of undigested IgG in the digesta. There was no correlation ($r=0.2$) found between the feed intake and the amount of undigested IgG in the digesta.

Table 3.3

Statistical significance of effect of hours after feeding, location, and the location by hours interaction on the amount of IgG in the freeze-dried digesta sample, the amount of Chromium (Cr) in the freeze-dried digesta sample, the IgG:Cr ratio, and percentage of undigested IgG.

	Hours[φ]	Location[θ]	Location x hours
IgG FD[♣]	***	***	***
Cr FD[♦]	***	***	***
IgG:Cr[♥]	*	***	***
% of undigested IgG[♠]	*	***	***

*** $p < 0.001$ * $p < 0.05$

[φ] Hours sampling after feeding the experimental diet

[θ] Location in the piglets' gastrointestinal tract

[♣] IgG FD = The amount of IgG in the freeze-dried sample

[♦] Cr FD =-The amount of chromium in the freeze-dried sample

[♥] IgG:Cr = IgG:Chromium ratio in the freeze-dried sample

[♠] % of IgG in the freeze-dried sample

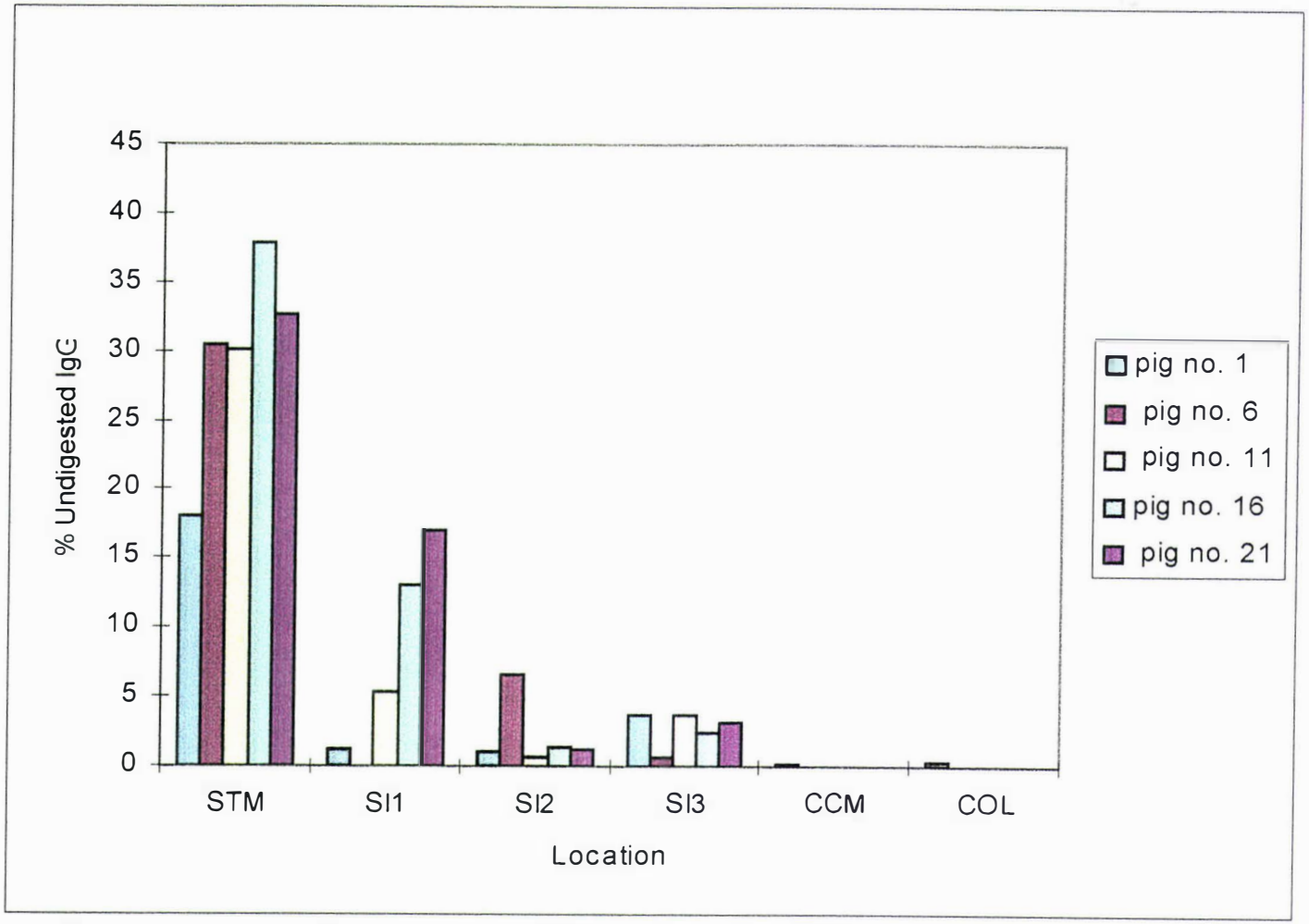

Figure 3.1 The percent of undigested bovine IgG found in different sections of the piglets' gastrointestinal tract expressed relative to the amount consumed as a single bolus experimental diet containing 2% bovine IgG, 1 hour after feeding (the sites of the gastrointestinal tract from which the undigested IgG was recovered correspond to the locations: STM = Stomach; SI1 = First third of the small intestine 1; SI2 = Second third of the small intestine 2; SI3 = Final third of small intestine 3; CCM = Caecum; COL = Colon).

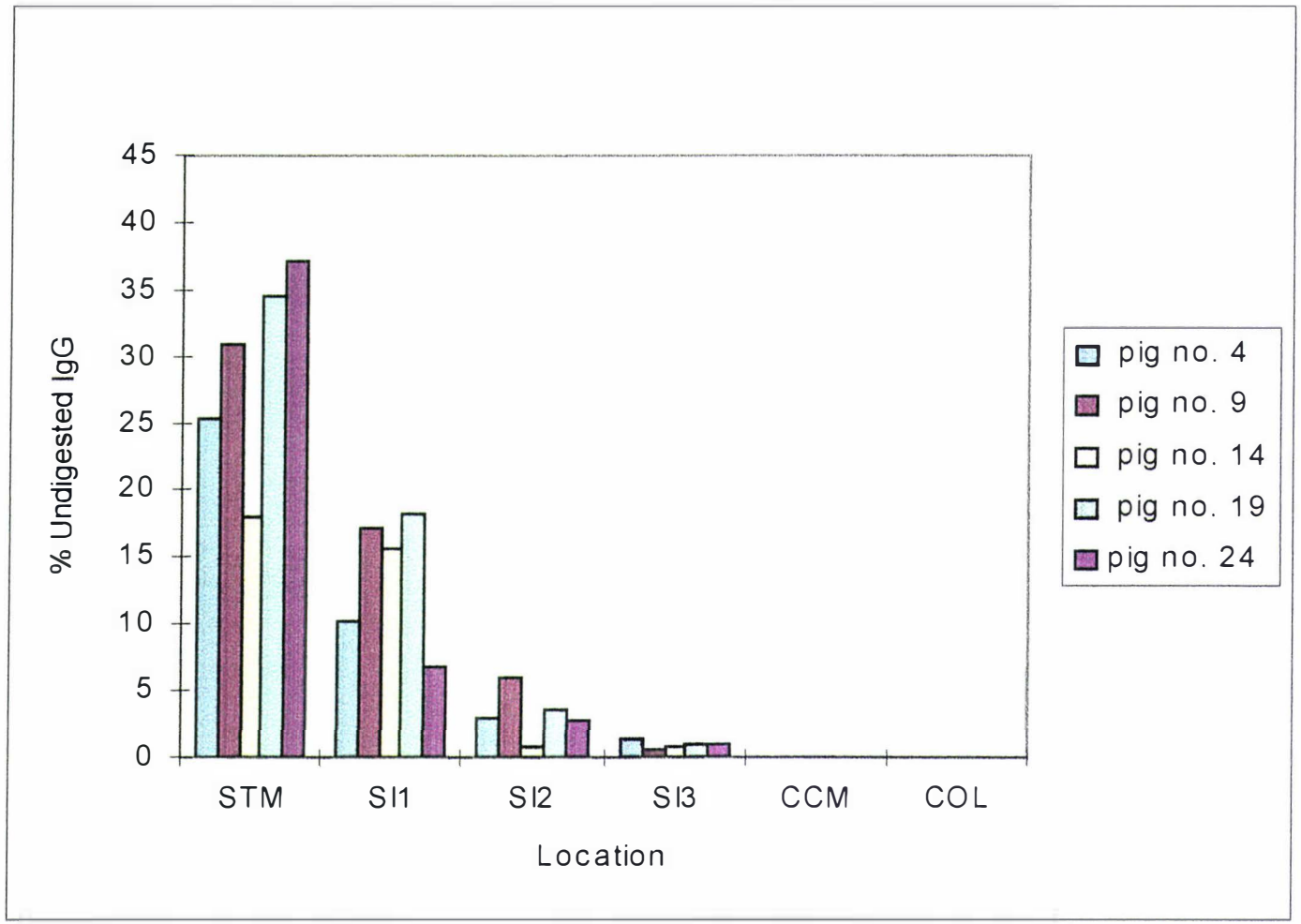

Figure 3.2 The percent of undigested bovine IgG found in different sections of the piglets' gastrointestinal tract expressed relative to the amount consumed as a single bolus experimental diet containing 2% bovine IgG, 5 hour after feeding (the sites of the gastrointestinal tract from which the undigested IgG was recovered correspond to the locations: STM = Stomach; SI1 = First third of the small intestine 1; SI2 = Second third of the small intestine 2; SI3 = last third of small intestine 3; CCM = Caecum; COL = Colon).

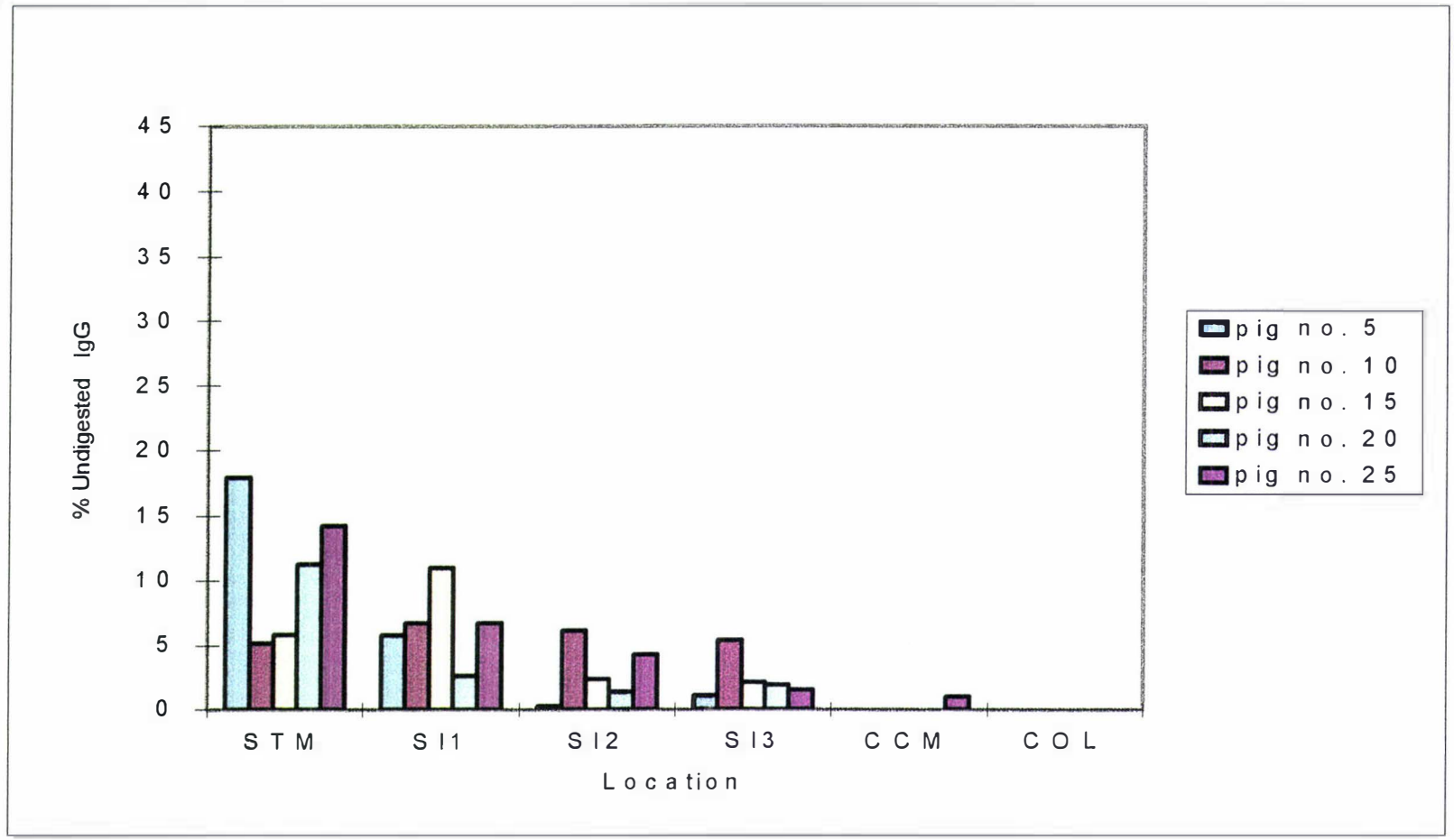

Figure 3.3 The percent of undigested bovine IgG found in different sections of the piglets' gastrointestinal tract expressed relative to the amount consumed as a single bolus experimental diet containing 2% bovine IgG, 9 hour after feeding (the sites of the gastrointestinal tract from which the undigested IgG was recovered correspond to the locations: STM = Stomach; SI1 = First third of the small intestine 1; SI2 = Second third of small intestine 2; SI3 = Final third of small intestine 3; CCM = Caecum; COL = Colon).

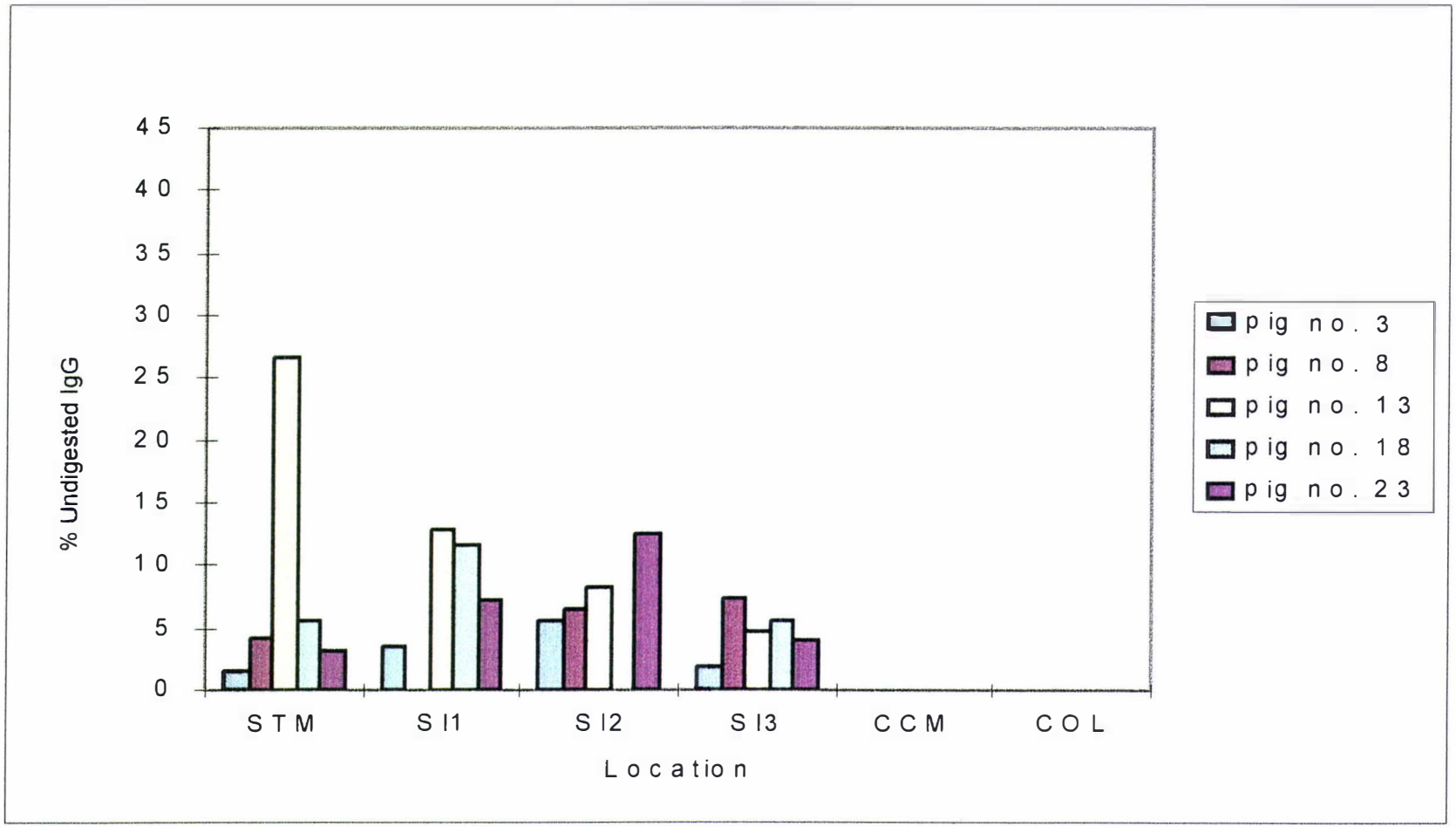

Figure 3.4 The percent of undigested bovine IgG found in different sections of the piglets' gastrointestinal tract expressed relative to the amount consumed as a single bolus experimental diet containing 2% bovine IgG, 16 hour after feeding (the sites of the gastrointestinal tract from which the undigested IgG was recovered correspond to the locations: STM = Stomach; SI1 = First third of small intestine 1; SI2 = Second third of small intestine 2; SI3 = Final third of the small intestine 3; CCM = Caecum; COL = Colon).

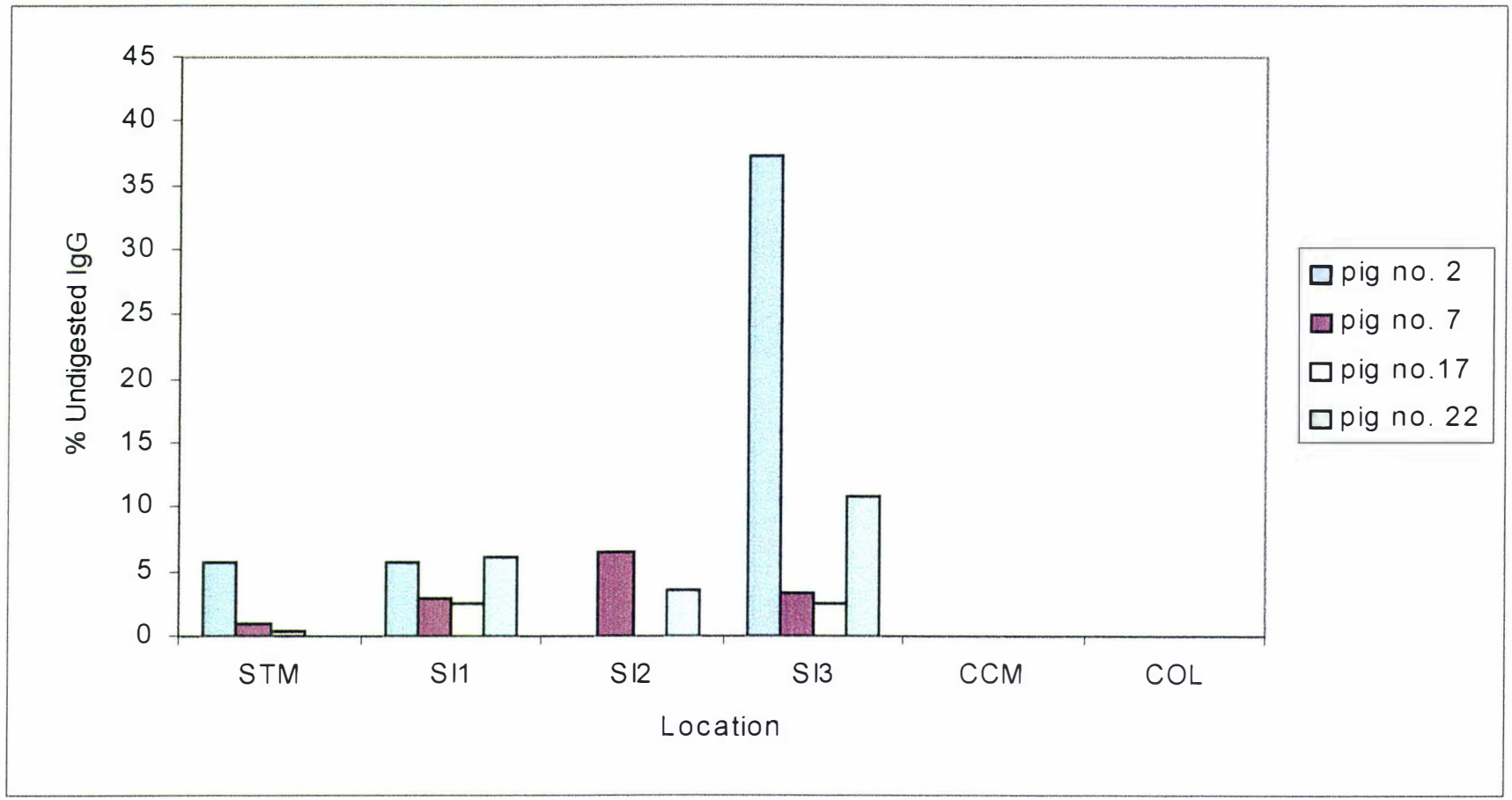

Fig 3.5 The percent of undigested bovine IgG found in different sections of the piglets' gastrointestinal tract expressed relative to the amount consumed as a single bolus experimental diet containing 2% bovine IgG, 24 hour after feeding (the sites of the gastrointestinal tract from which the undigested IgG was recovered correspond to the locations: STM = Stomach; SI1 = First third of small intestine 1; SI2 = Second third of small intestine 2; SI3 = final third of the small intestine 3; CCM = Caecum; COL = Colon).

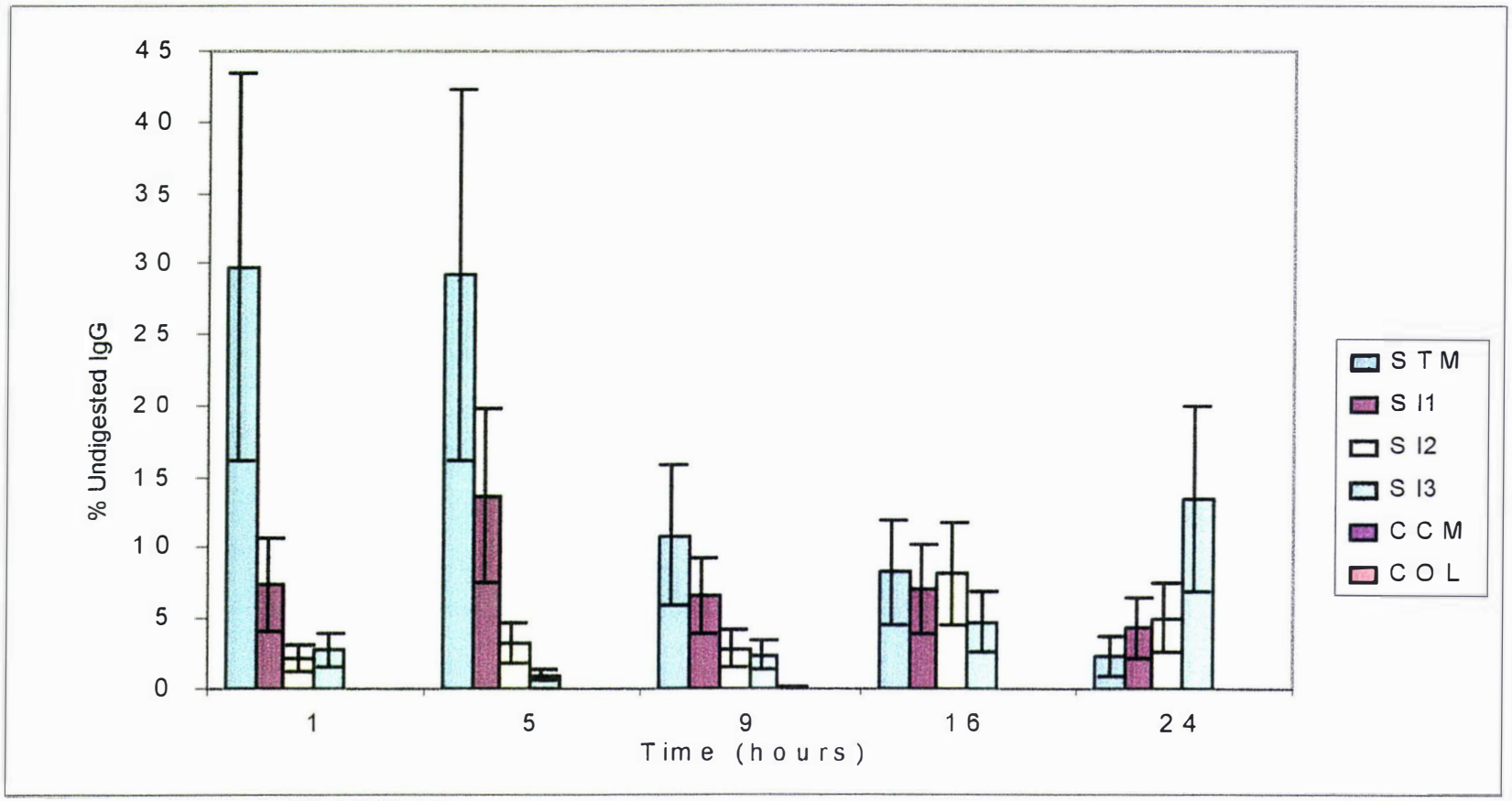

Figure 3.6 The average percent ± SD of undigested bovine IgG found in different sections of the piglets' gastrointestinal tract expressed relative to the amount consumed as a single bolus experimental diet containing 2% bovine IgG from the entire gastrointestinal tract at different times after feeding (the sites of the gastrointestinal tract from which the undigested IgG was recovered correspond to the locations: STM = Stomach; SI1 = First third of the small intestine 1; SI2 = Second third of the small intestine 2; SI3 = Final third of the small intestine 3; CCM = Caecum; COL = Colon).

The percentage of chromium in the freeze-dried digesta increased as the bolus passed through the digestive tract. This is assumed to be a result of nutrient and water absorption (Key 15 in Appendix 3.19).

There were no visible precipitin rings around the wells in the RID assays of faeces and blood (Appendix 3.18). It was assumed, therefore, that no detectable bovine IgG was present in either the faeces or blood.

3.5 DISCUSSION

This experiment was designed to determine the digestibility of bovine IgG by measuring the amount of undigested IgG in the digesta of weaner piglets at various time intervals after feeding a single bolus meal containing 2% IgG.

As there is a possibility for other nutrients in the diet will be digested and absorbed, it is not appropriate to simply measure IgG per weight of digesta collected. Instead, chromium was added as a non-digestible marker for the single bolus meal. The IgG content of the diet and digesta were expressed as IgG/unit chromium (g/g). The percentage of undigested IgG, which is a measure of the resistance of IgG to the digestive process, was calculated as the ratio of IgG/unit chromium in the digesta to IgG/unit chromium in the diet.

Biological effects after oral administration of Igs can be expected only if the Igs are significantly more resistant to total digestion than normal dietary proteins. The prospect of using oral Igs from bovine milk or colostrum to provide passive immune protection from enteric diseases has been considered for a long time. A number of studies have reported on the impact of proteolytic digestion on bovine IgG_1, the predominant Ig in bovine milk. Results from several of these studies led investigators to conclude that bovine milk Igs are resistant to proteolytic degradation (Haneberg & Tender, 1973; Haneberg, 1974; Hilpert et al., 1987; Hilpert & Gerber, 1994). For example Haneberg & Tender (1973); Hilpert & Gerber (1994) detected 10-20% of an orally administered dose of hyperimmune bovine concentrate in the faeces of children using RID assays. Other studies have demonstrated at least partial recovery of the functional activity of bovine Ig preparations after *in vitro* digestion with arbitrary amounts of proteolytic

enzymes or in faecal samples from infants fed bovine Ig preparations (Deren, 1971; McClead & Gregory, 1984; Hilpert et al., 1987). The above studies indicate that undigested Ig is present in the gut. Ross et al. (1995) concluded that the ileal digestibility of the nitrogen fraction of an Ig concentrate from bovine colostrum is lower than that of other milk proteins, indicating that bovine Ig concentrates may have the potential to support the host defence system by passive immunisation of the gut. In contrast, however, exposure of a bovine Ig preparation to pepsin at pH 2 resulted in a significant reduction in virus neutralising activity (Petschow & Talbott, 1994) yet a certain amount of activity was present.

In the present study, orally-fed bovine IgG was recovered in variable amounts from different locations in the gastrointestinal tract of the piglet. The undigested IgG was solely from the experimental diet not from the weaner diet. Significant amounts of IgG were found in the stomach ($p<0.001$), the SI1 ($p<0.05$) and the SI2 ($p<0.05$). There were no significant ($p>0.05$) amounts detected in the SI3, and no IgG was found in the caecum and colon of most piglets. It may be, however, that there were small amounts of IgG in the digesta from the lower gastrointestinal tract that could not be detected because of the sensitivity of the RID method. More sensitive methods may have detected these low levels of IgG, but it is unlikely that they would be significant in providing passive immunity. There was no detectable IgG in the faeces. Other researchers who reported the presence of Ig in the faeces may have given a higher dose than that used in the present study.

There was no evidence that bovine IgG was present in the porcine blood samples. This demonstrates that orally administered IgG did not cross the intestinal wall in detectable amounts, but remained in the gut and had the potential to be protective. This result also demonstrated that there is no cross reaction between porcine and bovine IgG in the RID assay thus confirming that the IgG measured in the digesta was of bovine origin.

No correlation was observed between feed intake and percentage of undigested IgG in the gastrointestinal tract. This may indicate that the digestibility of IgG is not affected by the amount consumed. The desired health benefit will, however, depend on the amount of undigested IgG present in the gut.

The present study confirmed that a certain amount of undigested IgG could be found in the gastrointestinal tract even after 24 hours of feeding. Further research is necessary to find out the factors affecting Ig digestibility in the gastrointestinal tract.

The next experiment involved giving oral bovine IgG once a day as a large single dose and then determining whether this could prevent infection when the piglets were challenged with *Escherichia coli* K88. This will measure biological activity of Igs rather than their presence only.

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3.7 APPENDIX 3

Appendix 3.1.

Equations used to calculate immunoglobulin concentrations in the whey globulin concentrate, the diets used in Chapters III and IV, and in the faeces and digesta collected, after detection using a Radial Immunodiffusion assay ("The Binding Site", Birmingham, UK).

1) Calibrator concentration

$$\text{True calibrator concentration} = \frac{\text{Calibrator nominated concentration x amount of distilled water added}}{\text{Final weight}}$$

$$= \text{mg/L}$$

2) Sample concentration

$$\text{Sample concentration} = \frac{\text{Dry weight X}10^{6}}{\text{Final weight}}$$

$$= \text{mg/L}$$

3) Immunoglobulin (Ig) Concentration

$$\text{Ig concentration} = \frac{\text{Y}^{*}\text{ value X } 100}{\text{Sample concentration}}$$

$$= \text{g/L}$$

* From regression equation of RID calibrator standards (average ring diameter squared) and the true calibrator concentration curve.

Appendix 3.2

Calculation of sample concentrations used to measure bovine Igs concentrations in whey globulin concentrate by the radial immunodiffusion* assay

Sample	Sample number	Dry weight (g)	Final weight* (g)	Sample concentration (g/L)
WGC				
	I	0.05	10.02	4.98
	II	0.11	10.05	11.48
	III	0.24	10.01	24.51
	IV	0.49	10.09	49.38
	V	1.00	10.01	100.71
IgG calibrator	250		0.38	0.25
~390ml of distilled water added			0.38	1.50
	2500		0.39	2.49
IgM calibrator	100		0.48	0.10
~490ml of distilled water added			0.49	0.59
	1000		0.49	0.99
IgA calibrator	25		0.44	0.02
~445ml of distilled water added			0.44	0.15
	250		0.44	0.24

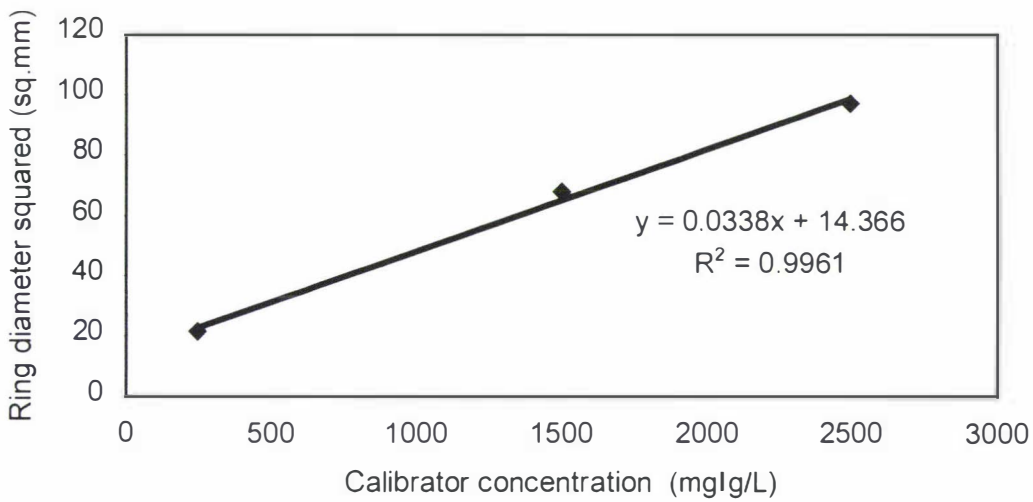
* Final weight = dry weight + PBS

Appendix 3.3

IgG calibrator readings for the radial immunodiffusion assay used to measure bovine IgG levels in whey globulin concentrate.

Calibrator concentration (mgIg/L)	Ring diameter reading 1 (mm)	Ring diameter reading 2 (mm)	Average ring diameter (mm)	Ring diameter^2 (sq.mm)$^{\varphi}$
251.70	4.53	4.78	4.66	21.67
1504.20	8.24	8.25	8.25	67.98
2494.90	9.84	9.88	9.86	97.22

Sq.mm = millimeter^2



Appendix 3.4 IgG calibrator curve$^{\clubsuit\clubsuit}$ for the radio immunodiffusion assay used to measure bovine IgG concentration in whey globulin concentrate.

$^{\clubsuit\clubsuit}$ Calibrator curve generated for true calibrator concentration and ring diameter squared

Appendix 3.5

Calculation of bovine IgG concentration in whey globulin concentrate (WGC) as measured by the radial immunodiffusion assay

Sample	Type	Ring diameter reading 1	Ring diameter reading 2	Ring diameter reading 3	Ring diameter reading 4	Average ring diameter	Ring diameter[2]	Sample concentration[♣]	IgG concentration (Y)[♦]	IgG%[♦]	Average IgG%
		(mm)	(mm)	(mm)	(mm)	(mm)	(sq.mm)	(g/L)			
WGC											
	I	5.01	4.97	4.55	-	4.84	23.43	4.98	268.04	5.38	6.19%
	II	6.45	6.83	6.17	6.64	6.52	42.25	11.48	824.97	7.18	

[♀] Sq.mm = millimeter[2]

[♦] From Appendix 3.4

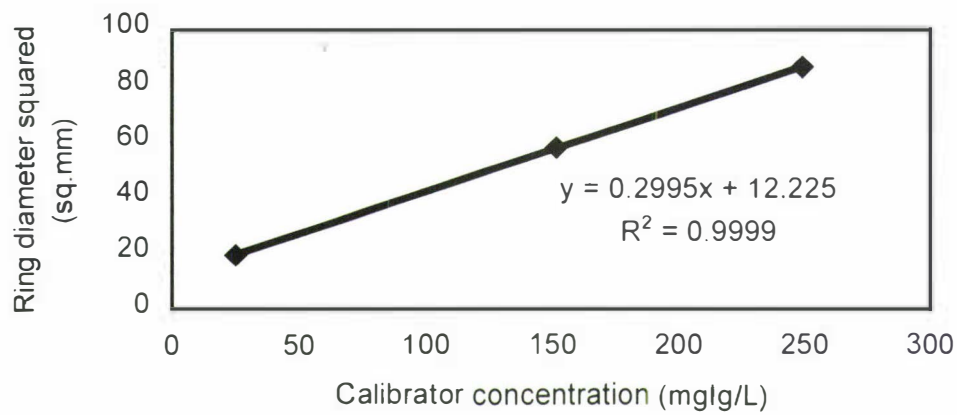
[♦] % Represents the amount of Ig in the powder

Appendix 3.6

IgA calibrator readings for the radial immunodiffusion assay used to measure bovine IgA concentration in a whey globulin concentrate.

Calibrator concentration (mgIg/L)	Ring diameter reading 1 (mm)	Ring diameter reading 2 (mm)	Average ring diameter (mm)	Ring diameter^2 (sq.mm)$^{\varphi}$
25.10	4.48	4.38	4.43	19.61
151.60	7.61	-	7.61	57.92
249.10	9.20	9.42	9.31	86.67

$^{\varphi}$ Sq.mm = millimeter^2



Appendix 3.7 IgA calibrator curve$^{\clubsuit\clubsuit}$ for the radial immunodiffusion assay used to measure bovine IgA concentration in a whey globulin concentrate

$^{\clubsuit\clubsuit}$ Calibrator curve generated for true calibrator concentration and ring diameter squared

Appendix 3.8

Calculation of bovine IgA concentration in a whey globulin concentrate as measured by the radial immunodiffusion

Sample	Type	Ring diameter reading 1	Ring diameter reading 2	Ring diameter reading 3	Average ring diameter	Ring diameter²	Sample concentration♦	IgA concentration (Y)♥	IgA %ϕ	Average IgA %
		(mm)	(mm)	(mm)	(mm)	(sq.mm)φ	(g/L)	(mgIgA/L)		
97001										
	I	5.02	5.42	5.08	5.17	26.76	4.98	48.52	0.97	0.82
	III	7.75	7.64	8.15	7.85	61.57	24.51	164.74	0.67	
	IV	9.77	10.23	Out side calibrator reading						

φ Sq.mm = millimeter²

♦ From Appendix 3.2

♥ Y= 0.2995X + 12.229; R²= 0.9999 from Appendix 3.7

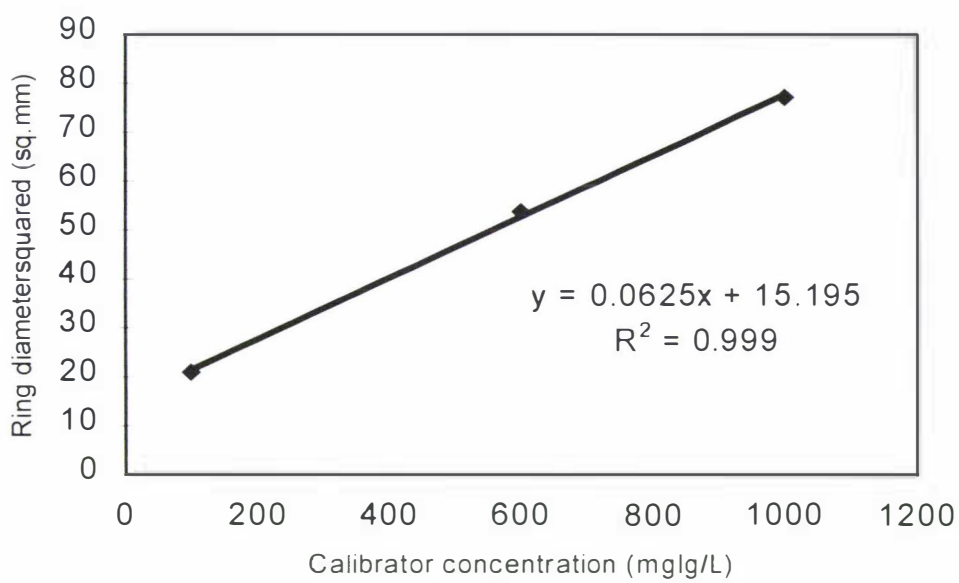
ϕ % Represents the amount of Ig in the powder

Appendix 3.9

IgM calibrator readings for the radial immunodiffusion assay used to measure bovine IgM concentration in a whey globulin concentrate

Calibrator concentration (mglg/L)	Ring diameter reading 1 (mm)	Ring diameter reading 2 (mm)	Average ring diameter (mm)	Ring diameter^2 (sq.mm)[φ]
100.20	4.63	4.54	4.58	20.97
592.00	7.22	7.44	7.33	53.72
996.30	8.65	8.91	8.78	77.08

Sq.mm = millimeter^2



Appendix 3.10 IgM calibrator curve[♣♣] for the radial immunodiffusion assay used to measure bovine IgM concentration in a whey globulin concentrate

[♣♣] Calibrator curve generated for true calibrator concentration and ring diameter squared

Appendix 3.11

Calculation of bovine IgM concentration in a whey globulin concentration (WGC) as measured by the radial immunodiffusion assay

Sample no	Type	Ring diameter reading 1	Ring diameter reading 2	Ring diameter reading 3	Ring diameter reading 4	Average ring diameter	Ring diameter^2	Sample concentration	IgM concentration (Y)$^{\blacktriangledown}$	IgM %$^{\phi}$	Average IgM %
		(mm)	(mm)	(mm)	(mm)	(mm)	(sq.mm)$^{\varphi}$	(g/L)$^{\blacktriangle}$	(mgIgM/L)		
WGC											
	IV	6.36	6.34	5.86	6.25	6.20	38.47	49.38	372.40	0.75	0.65
	V	6.88	6.71	7.19	7.38	7.04	49.56	100.71	549.84	0.55	

$^{\varphi}$ Sq.mm = millimeter^2

$^{\blacktriangle}$ From Appendix 3.2

$^{\blacktriangledown}$ $Y = 0.0628X + 15.275$; $R^2 = 0.999$ from Appendix 3.10

$^{\phi}$ % Represents the amount of Ig in the powder

Appendix 3.12

Concentration of diet samples assayed for bovine IgG concentration by the radial immunodiffusion assay

Sample	Dry weight (g)	Final weight$^{\phi}$ (g)	Sample concentration (g/L)
Weaner diet	0.75	1.56	48.16
Experimental diet	0.15	1.54	100.07

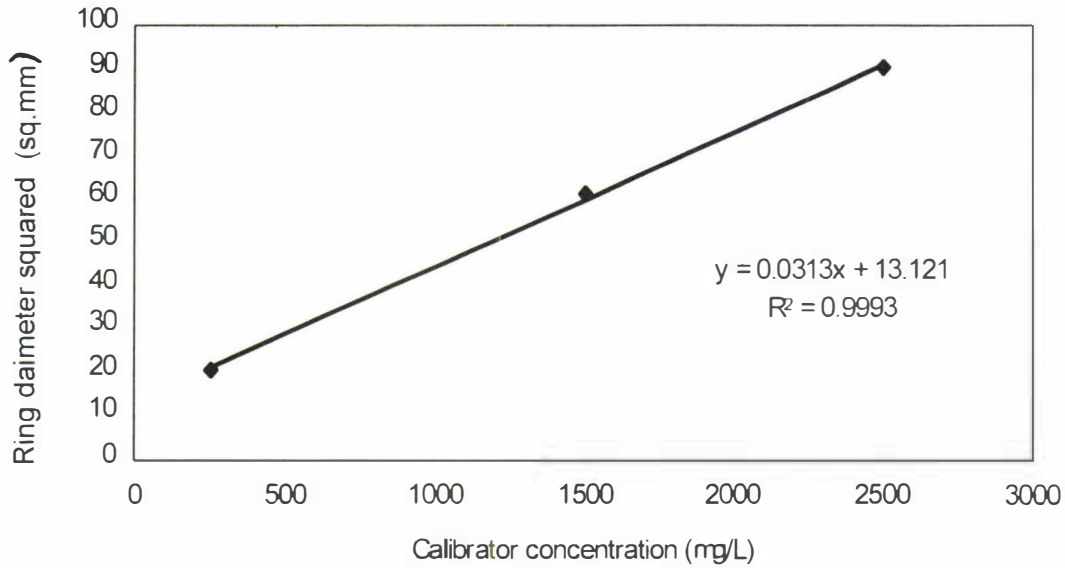
$^{\phi}$ Final weight = Dry weight +PBS

Appendix 3.13

IgG calibrator readings for the radial immunodiffusion assay used to measure bovine IgG concentration in weaner diet and experiment diet fed to four to five-week-old piglets in the IgG digestibility trial

Calibrator concentration (mg/L)	Ring diameter reading 1 (mm)	Ring diameter reading 2 (mm)	Ring diameter reading 3 (mm)	Ring diameter reading 4 (mm)	Average ring diameter (mm)
251.7	4.53	4.78	4.28	NR$^{\clubsuit}$	4.53
1504.2	8.24	8.25	7.00	NR	7.83
2494.9	9.84	9.88	9.26	9.73	9.53

$^{\clubsuit}$ No readings



Appendix 3.14 IgG calibrator curve** for the radial immunodiffusion assay used to measure bovine IgG concentrations in weaner diet and experimental diet fed to four to five-week-old piglets in the IgG digestibility trial.

** Calibrator curve generated for true calibrator concentration and ring diameter squared

Appendix 3.15

Calculation of bovine IgG concentration in the weaner and experimental diets fed to five-week-old piglets in the IgG digestibility trial as measured by the radial immunodiffusion assay

Sample	Ring Diameter reading 1	Ring diameter reading 2	Average ring diameter	Ring diameter^2	Sample Concentration	IgG concentration in sample Y♦	IgG%ϕ
	(mm)	(mm)	(mm)	(sq.mm)φ	(g/L)♣	(mgIgG/L)	
Weaner diet)	No visible ring	No visible ring	No visible ring	TDTL♠	48.16	TDTL	
Experimental diet	9.08	8.33	8.71	75.78	100.07	2001.79	2.00

φ Sq.mm = millimeter^2

♣ From Appendix 3.12

♦ y= 0.0313X = 13.12; r^2= 0.9993 from Appendix 3.14

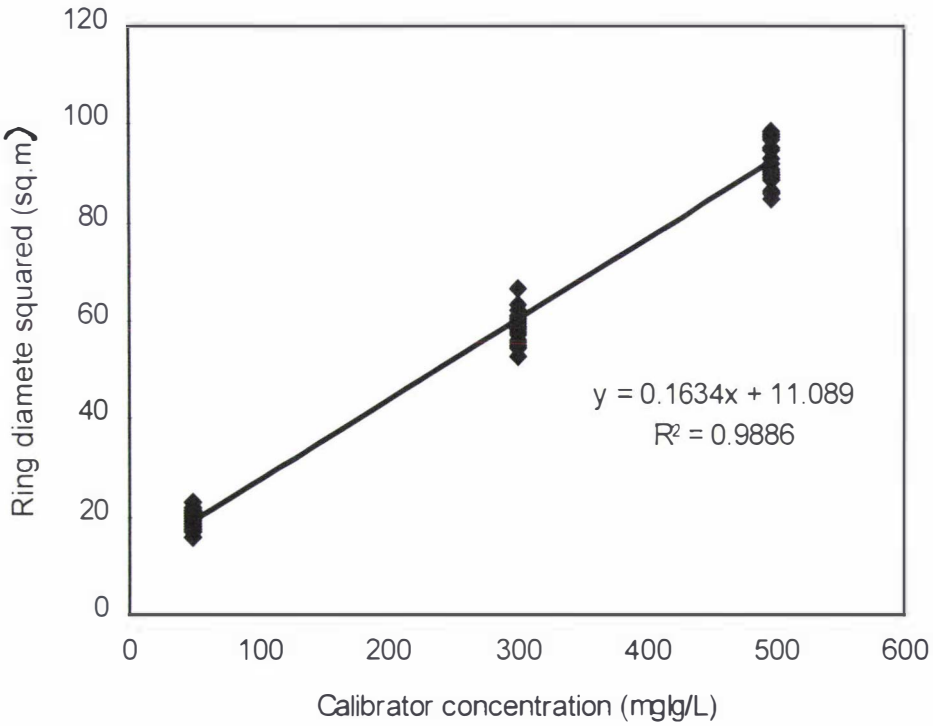
ϕ % Represents the amount of Ig in the powder

♠ TDTL = Too low to detect

Appendix 3.16 IgG calibrator readings in the radial immunodiffusion assay used to measure bovine IgG concentrations in digesta from weaner piglets in the IgG digestibility trial (in each plate two different concentrations of calibrators were used)

Calibrator concentration (mgIg/L)					
50		300		500	
Ring diameter (mm)	Ring diameter² (sq.mm)[φ]	Ring diameter (mm)	Ring diameter² (sq.mm)[φ]	Ring diameter (mm)	Ring diameter² (sq.mm)[φ]
4.67	21.80	7.68	58.98	9.64	92.926
4.72	22.27	7.64	58.36	9.73	94.67
4.57	20.88	7.41	54.90	9.47	89.68
4.39	19.27	7.87	61.93	9.43	88.92
4.23	17.89	7.70	59.29	9.86	97.21
4.00	16.00	7.69	59.13	9.90	98.01
4.68	21.90	7.97	63.52	10.44	108.99
4.29	18.40	7.78	60.52	9.76	95.25
4.48	20.07	7.59	57.60	9.42	88.73
4.43	19.62	7.55	57.00	9.26	85.74
4.63	21.43	7.46	55.65	9.50	90.25
4.66	21.71	8.15	66.42	9.76	95.25
4.51	20.34	7.39	54.612	9.40	88.36
4.81	23.13	7.76	60.217	9.52	90.63
4.30	18.49	7.66	58.67	9.30	86.49
4.54	20.61	7.80	60.84	9.85	97.02
4.44	19.71	7.90	62.41	9.94	98.80
4.26	18.14	7.26	52.70	9.60	92.16
4.40	19.36	7.49	56.10	9.93	98.64
4.38	19.18	7.37	54.31	9.72	94.47
4.21	17.72	7.41	54.90	9.94	98.80
4.48	20.07	7.65	58.52	9.91	98.20
3.94	15.57	7.89	62.25	9.46	89.49
4.14	17.13	7.75	60.06	9.86	97.21
4.71	22.18	7.87	61.93	9.60	92.16
4.67	21.80	7.63	58.21	9.53	90.82
4.23	17.89	7.71	59.44	9.86	97.21
4.61	21.25			9.75	95.06
4.32	18.66			9.22	85.00
4.13	17.05				
4.45	19.80				
4.24	17.97				

[φ] Sq.mm = millimeter²



Appendix 3.17 Standard curve** for IgG calibrators in the radial immunodiffusion assay used to measure bovine IgG concentrations in digesta from weaner piglets in the IgG the digestibility trial

** Calibrator curve generated for true calibrator concentration and ring diameter squared

Appendix 3.18

Radial immunodiffusion plates readings for digesta, faeces and blood from weaner piglets in the IgG digestibility trial.

Sample no.[♣]	Pig #[♠]	Location[♦]	Ring diameter[♥] reading 1 (mm)	Ring diameter[♥] reading 2 (mm)	Average ring diameter (mm)
97159	1	STM[♣]	8.98	9.02	9.00
97160		SI1[Ψ]	4.69	4.57	4.63
97161		SI2[♥♥]	8.70	8.84	8.77
97162		SI3[♣♣]	8.58	8.68	8.63
97163		CCM[ΨΨ]	4.45	4.20	4.33
97164		COL[♦♦]	4.61	4.59	4.60
97165	2	STM	6.38	5.30	5.84
97166		SI1	4.24	4.36	4.30
97168		SI3	6.24	6.42	6.33
97169		CCM	No visible rings		
97170		COL	No visible rings		
97171	3	STM	4.72	4.53	4.63
97172		SI1	5.27	5.16	5.22
97173		SI2	5.02	5.07	5.05
97174		SI3	5.12	5.09	5.11
97175		CCM	No visible rings		
97176		COL	No visible rings		
97177	4	STM	7.81	7.80	7.81
97178		SI1	8.88	8.42	8.65
97179		SI2	8.21	8.43	8.32
97180		SI3	8.32	8.14	8.23
97181		CCM	No visible rings		
97182		COL	No visible rings		
97183	5	STM	9.09	9.22	9.16
97184		SI1	7.01	6.7	6.86
97185		SI2	4.46	4.29	4.38
97186		SI3	6.71	6.55	6.63
97187		CCM	No visible rings		
97188		COL	No visible rings		
97189	6	STM	8.70	8.70	8.70
97190		SI1	No visible rings		
97191		SI2	9.85	9.91	9.88
97192		SI3	5.02	5.04	5.03

Appendix 3.18 cont...

Appendix 3.18 continued

Sample no.	Pig #	Location	Ring diameter reading 1 (mm)	Ring diameter reading 2 (mm)	Average ring diameter (mm)
97193		CCM	No visible rings		
97194		COL	No visible rings		
97195	7	STM	4.27	4.18	4.23
97196		SI1	4.63	4.60	4.62
97197		SI2	8.36	8.17	8.27
97198		SI3	5.13	5.35	5.24
97199		CCM	No visible rings		
97200		COL	No visible rings		
97201	8	STM	5.66	5.66	5.66
97202		SI1	No visible rings		
97203		SI2	5.75	5.58	5.67
97204		SI3	5.22	5.26	5.24
97205		CCM	No visible rings		
97206		COL	No visible rings		
97207	9	STM	8.12	8.49	8.31
97208		SI1	8.75	8.05	8.40
97209		SI2	8.70	8.44	8.57
97210		SI3	5.07	5.52	5.30
97211		CCM	No visible rings		
97212		COL	No visible rings		
97213	10	STM	6.90	6.47	6.69
97214		SI1	7.46	7.49	7.48
97215		SI2	8.03	7.85	7.94
97216		SI3	8.39	8.16	8.28
97217		CCM	No visible rings		
97218		COL	No visible rings		
97219	11	STM	8.16	8.04	8.10
97220		SI1	8.19	7.58	7.89
97221		SI2	4.78	4.76	4.77
97222		SI3	8.12	8.65	8.39
97223		CCM	No visible rings		
97224		COL	No visible rings		
97225	12	STM	4.36	4.83	4.60
97226		SI1	9.22	9.15	9.19
97227		SI2	6.61	6.55	6.58
97228		SI3	5.74	5.86	5.80
97229		CCM	No visible rings		
97230		COL	No visible rings		

Appendix 3.18 cont...

Appendix 3.18 continued

Sample no.	Pig #	Location	Ring diameter reading 1 (mm)	Ring diameter reading 2 (mm)	Average ring diameter (mm)
97231	13	STM	8.56	8.79	8.68
97232		SI1	7.43	7.34	7.39
97233		SI2	8.04	5.06	6.55
97234		SI3	4.84	4.94	4.89
97235		CCM	No visible rings		
97236		COL	No visible rings		
97237	14	STM	6.80	6.82	6.81
97238		SI1	7.65	7.44	7.55
97239		SI2	5.3	5.61	5.46
97240		SI3	7.11	7.14	7.13
97241		CCM	No visible rings		
97242		COL	No visible rings		
97243	15	STM	4.82	4.54	4.68
97244		SI1	5.85	5.98	5.92
97245		SI2	6.58	6.13	6.36
97246		SI3	6.39	6.68	6.54
97247		CCM	No visible rings		
97248		COL	No visible rings		
97249	16	STM	9.46	8.93	9.20
97250		SI1	9.27		9.27
97251		SI2	6.36	6.02	6.19
97252		SI3	7.61	7.31	7.46
97253		CCM	No visible rings		
97254		COL	No visible rings		
97255	17	STM	3.90	3.66	3.78
97256		SI1	5.38	5.55	5.47
97258		SI3	7.65	7.53	7.59
97259		CCM	No visible rings		
97260		COL	No visible rings		
97261	18	STM	6.80	6.37	6.59
97262		SI1	6.65	6.92	6.79
97264		SI3	5.59	5.49	5.54
97265		CCM	No visible rings		
97266		COL	No visible rings		
97267	19	STM	7.59	7.84	7.72
97268		SI1	8.59	8.48	8.54
97269		SI2	7.54	7.67	7.61
97270		SI3	7.54	7.12	7.33

Appendix 3.18 cont...

Appendix 3.18 continued

Sample no.	Pig #	Location	Ring diameter reading 1 (mm)	Ring diameter reading 2 (mm)	Average ring diameter (mm)
97271		CCM	No visible rings		
97272		COL	No visible rings		
97273	20	STM	6.48	6.52	6.50
97274		SI1	5.04	4.80	4.92
97275		SI2	4.89	5.03	4.96
97276		SI3	7.12	6.92	7.02
97277		CCM	No visible rings		
97278		COL	No visible rings		
97279	21	STM	8.67	8.49	8.58
97280		SI1	9.64	10.06	9.85
97281		SI2	5.53	5.18	5.36
97282		SI3	7.28	7.26	7.27
97283		CCM	No visible rings		
97284		COL	No visible rings		
97285	22	STM	No visible rings		
97286		SI1	5.47	5.55	5.51
97287		SI2	5.68	5.78	5.73
97288		SI3	9.31	9.08	9.20
97289		CCM	No visible rings		
97290		COL	No visible rings		
97291	23	STM	4.70	4.85	4.78
97292		SI1	6.71	6.28	6.50
97293		SI2	9.19	9.98	9.59
97294		SI3	8.61	8.23	8.42
97295		CCM	No visible rings		
97296		COL	No visible rings		
97297	24	STM	7.09	7.10	7.10
97298		SI1	4.94	5.15	5.05
97299		SI2	7.22	7.29	7.26
97300		SI3	6.98	7.03	7.01
97301		CCM	No visible rings		
97302		COL	No visible rings		
97328	25	STM	5.59	5.72	5.66
97329		SI1	7.14	7.16	7.15
97330		SI2	7.00	6.87	6.94
97331		SI3	7.85	7.29	7.57
97332		CCM	6.86	6.68	6.77
97333		COL	No visible rings		

Appendix 3.18 cont...

Appendix 3.18 continued

Sample no.	Pig #	Location	Ring diameter reading 1 (mm)	Ring diameter reading 2 (mm)	Average ring diameter (mm)
97583	2	Faeces	No visible rings		
97584	3	Faeces	No visible rings		
97585	7	Faeces	No visible rings		
97586	8	Faeces	No visible rings		
97587	12	Faeces	No visible rings		
97588	13	Faeces	No visible rings		
97589	17	Faeces	No visible rings		
97590	18	Faeces	No visible rings		
97591	22	Faeces	No visible rings		
97592	23	Faeces	No visible rings		
97135	1	Serum	No visible rings		
97136	2	Serum	No visible rings		
97137	3	Serum	No visible rings		
97138	4	Serum	No visible rings		
97139	5	Serum	No visible rings		
97140	6	Serum	No visible rings		
97141	7	Serum	No visible rings		
97142	8	Serum	No visible rings		
97143	9	Serum	No visible rings		
97144	10	Serum	No visible rings		
97145	11	Serum	No visible rings		
97146	12	Serum	No visible rings		
97147	13	Serum	No visible rings		
97148	14	Serum	No visible rings		
97149	15	Serum	No visible rings		
97150	16	Serum	No visible rings		
97151	17	Serum	No visible rings		
97152	18	Serum	No visible rings		
97153	19	Serum	No visible rings		
97154	20	Serum	No visible rings		
97155	21	Serum	No visible rings		
97156	22	Serum	No visible rings		
97157	23	Serum	No visible rings		
97158	24	Serum	No visible rings		
97327	25	Serum	No visible rings		

♦ ♣ Sample identification number; ♀ Pig identification number; ♦ Location in the gastrointestinal tract of piglet; ♥ Ring diameter reading in RID assay; ♣ Stomach; Ψ Small intestine 1 (First third part of small intestine); ♥♥ Small intestine 2 (second part of small intestine); ♣♣ Small intestine 3 (last third part of small intestine); ΨΨ Caecum; ♦♦ Colon

NB: No samples obtained for sample no 97167 (Pig no. 2 SI2), 97257pig no. 17 SI2) and 97263 (Pig no. 18 SI2)

The quantity of undigested bovine IgG in the digesta of weaner piglets in the IgG digestibility trial

Key to Appendix 3.19

Key

K1	Sample Number
K2	Pig number
K3	Location in the gastrointestinal tract
K4	Supernatant weight after freeze-drying (g)
K5	Amount of diluent added (ml)
K6	Supernatant plus diluent weight (g) [K6 = K4 + K5]
K7	Freeze-dried (FD) supernatant concentration g FD matter/g solution [K7 = K4/K6]
K8	Additional dilution factor if needed to bring RID readings within calibrator ring diameter range.
K9	Amount of IgG in assayed solution (mgIgG/L) Y= X-11.089)/0.1634
K10	The amount of IgG in FD supernatant corrected for additional dilution factor [K10 = K9/K8]
K11	mgIgG mg/L in original FD material [K11 = K10/K7]
K12	IgGmg/ 100g original F D material
K13	Mean % Dry Matter (DM) for Cr
K14	Corrected % Cr/DM
K15	% chromium corrected for DM of FD material [K15 = K13/K14*100]
K16	gIgG/gCr=IgG/Cr ratio [K16 = K12/K15]
K17	IgG:Cr ratio of digesta/IgG:Cr ratio of diet [K17 = K16/6.82•]
K18	% of undigested IgG in digesta [K18 = K17*100]

• 6.82 % = The amount of IgG in the diet
♣ STM = Stomach
♦ SI1 = Small intestine 1 (First part of small intestine)
♥ SI2 = Small intestine 2 (The second part of small intestine)
♠ SI3 = Small intestine 3 (rest of small intestine)
• CCM = Caecum
♀ COL = Colon
Φ TLTD = Too low to detect

NB: No samples obtained for sample no 97167 (Pig no. 2 SI2), 97257pig no. 17 SI2) and 97263 (Pig no. 18 SI2)

Appendix 3.19

Calculation of percentage of undigested bovine IgG (K18) in digesta and faeces from weaner pigs in the IgG digestibility trial

K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	K12	K13	K14	K15	K16	K17	K18
97159	1	STM♣	0.54	2.00	2.54	0.21	0.50	427.90	855.70	3984.40	0.40	96.80	0.30	0.29	1.35	0.18	18.13
97160		SI1♦	1.09	1.40	2.49	0.43	0.33	63.30	191.90	436.80	0.04	95.37	0.47	0.45	0.10	0.01	1.32
97161		SI2♥	0.83	2.00	2.83	0.29	1.00	402.80	402.80	1367.70	0.14	93.73	1.83	1.71	0.08	0.01	1.15
97162		SI3♠	0.63	2.00	2.63	0.24	0.50	387.90	775.90	3211.80	0.32	94.87	1.17	1.11	0.29	0.04	3.91
97163		CCM•	0.29	1.00	1.29	0.22	1.00	46.90	46.90	204.70	0.02	96.60	1.20	1.16	0.02	0.00	0.28
97164		COL♀	0.26	1.40	1.66	0.15	1.00	61.60	61.60	393.50	0.04	98.33	1.84	1.81	0.02	0.00	0.30
97165	2	STM	0.23	1.40	1.63	0.14	0.50	140.90	281.70	1981.80	0.20	93.99	0.49	0.46	0.43	0.06	5.83
97166		SI1	0.11	0.40	0.51	0.21	1.00	45.30	45.30	208.50	0.02	93.35	0.05	0.05	0.42	0.06	5.64
97168		SI3	0.81	2.80	3.61	0.22	1.00	177.40	177.40	790.50	0.08	91.43	0.03	0.03	2.80	0.37	37.42
97169		CCM	0.26	1.40	1.66	0.16	1.00	Too low to detect				91.53	0.73	0.67	0.00	0.00	TLTD♀
97170		COL	0.25	1.40	1.65	0.15	1.00	Too low to detect				95.60	1.70	1.63	0.00	0.00	TLTD
97171	3	STM	0.21	1.40	1.61	0.13	1.00	63.30	63.30	471.90	0.05	97.60	0.40	0.39	0.12	0.02	1.63
97172		SI1	0.43	0.80	1.23	0.35	0.50	98.90	197.80	564.90	0.06	88.94	0.24	0.22	0.26	0.04	3.52
97173		SI2	0.50	0.80	1.30	0.38	0.33	88.20	267.30	692.40	0.07	84.70	0.20	0.17	0.42	0.06	5.63
97174		SI3	0.47	1.40	1.87	0.25	0.50	91.90	183.90	725.80	0.07	93.81	0.51	0.48	0.15	0.02	2.01
97175		CCM	0.30	1.40	1.70	0.17	0.50	Too low to detect				95.29	1.39	1.33	0.00	0.00	TLTD
97176		COL	0.25	1.40	1.65	0.15	0.50	Too low to detect				94.72	2.32	2.19	0.00	0.00	TLTD

Appendix 3.19 cont...

Appendix 3.19 continued

K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	K12	K13	K14	K15	K16	K17	K18
97177	4	STM	0.26	2.00	2.26	0.11	0.50	305.40	610.90	5169.51	0.52	98.71	0.28	0.27	1.90	0.25	25.41
97178		SI1	1.01	2.00	3.01	0.33	0.50	390.10	780.10	2324.93	0.23	91.74	0.33	0.30	0.77	0.10	10.23
97179		SI2	0.90	1.40	2.30	0.39	0.50	355.80	711.50	1818.45	0.18	92.97	0.88	0.82	0.22	0.03	3.05
97180		SI3	0.74	1.40	2.14	0.34	0.50	346.70	693.30	1996.27	0.20	94.40	1.96	1.85	0.11	0.01	1.47
97181		CCM	0.29	1.40	1.69	0.17	1.00		Too low to detect			94.72	1.67	1.58	0.00	0.00	TLTD
97182		COL	0.25	1.40	1.65	0.15	1.00		Too low to detect			96.85	1.71	1.66	0.00	0.00	TLTD
97183	5	STM	0.22	1.00	1.22	0.18	0.50	445.60	891.30	4905.99	0.49	99.68	0.37	0.37	1.34	0.18	17.99
97184		SI1	0.74	1.40	2.14	0.34	0.50	220.10	440.30	1266.52	0.13	89.83	0.32	0.29	0.44	0.06	5.82
97185		SI2	0.75	1.40	2.15	0.35	1.00	49.50	49.50	141.34	0.01	92.26	0.81	0.75	0.02	0.00	0.34
97186		SI3	0.53	1.40	1.93	0.27	0.50	201.20	402.30	1461.06	0.15	95.59	1.87	1.79	0.08	0.01	1.16
97187		CCM	0.38	1.40	1.78	0.21	1.00		Too low to detect			93.71	2.07	1.94	0.00	0.00	TLTD
97188		COL	0.25	1.40	1.65	0.15	1.00		Too low to detect			98.24	2.38	2.34	0.00	0.00	TLTD
97189	6	STM	0.52	1.40	1.92	0.27	0.20	395.40	1976.80	7298.88	0.73	97.47	0.33	0.32	2.28	0.31	30.58
97190		SI1	0.90	1.40	2.30	0.39	0.20		Too low to detect			96.09	0.50	0.48	0.00	0.00	TLTD
97191		SI2	1.11	1.40	2.51	0.44	0.20	529.50	2118.10	4787.20	0.48	92.52	1.05	0.97	0.49	0.07	6.60
97192		SI3	0.61	1.40	2.01	0.30	0.50	87.00	174.00	570.01	0.06	90.55	1.08	0.98	0.06	0.01	0.82
97193		CCM	0.28	1.40	1.687	0.170	0.50		Too low to detect			92.48	1.34	1.24	0.00	0.00	TLTD
97194		COL	0.25	1.40	1.656	0.155	1.00		Too low to detect			96.40	2.17	2.09	0.00	0.00	TLTD
97195	7	STM	0.24	1.40	1.646	0.149	1.00	41.60	41.60	278.63	0.03	94.93	0.43	0.41	0.07	0.01	0.96
97196		SI1	0.21	0.80	1.012	0.209	0.66	62.80	95.10	453.97	0.05	90.66	0.23	0.21	0.22	0.03	2.95
97197		SI2	0.27	0.70	0.979	0.285	1.00	350.70	350.70	1230.65	0.12	90.81	0.28	0.25	0.49	0.07	6.64

Appendix 3.19 cont...

Appendix 3.19 continued

K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	K12	K13	K14	K15	K16	K17	K18
97198		SI3	0.55	1.40	1.95	0.28	0.50	100.26	200.41	707.61	0.07	90.73	0.31	0.28	0.25	0.03	3.33
97199		CCM	0.35	1.40	1.75	0.20	1.00		Too low to detect			92.26	0.88	0.82	0.00	0.00	TLTD
97200		COL	0.29	1.40	1.69	0.17	1.00		Too low to detect			93.98	1.85	1.74	0.00	0.00	TLTD
97201	8	STM	0.27	1.00	1.27	0.21	0.50	128.28	256.44	1185.34	0.12	94.68	0.39	0.37	0.32	0.04	4.36
97202		SI1	0.20	1.00	1.20	0.16	1.00		Too low to detect			87.71	0.25	0.22	0.00	0.00	TLTD
97203		SI2	0.61	1.40	2.01	0.30	0.33	128.95	390.67	1275.47	0.13	90.54	0.29	0.26	0.49	0.07	6.59
97204		SI3	0.49	1.40	1.89	0.26	0.25	100.22	400.70	1538.70	0.15	91.15	0.30	0.28	0.56	0.07	7.42
97205		CCM	0.27	1.40	1.67	0.16	0.50		Too low to detect			93.93	0.96	0.90	0.00	0.00	TLTD
97206		COL	0.28	1.40	1.68	0.17	0.50		Too low to detect			96.09	2.01	1.93	0.00	0.00	TLTD
97207	9	STM	0.43	1.40	1.83	0.23	0.25	354.81	1419.02	6039.22	0.60	97.95	0.27	0.26	2.32	0.31	30.95
97208		SI1	0.82	1.40	2.22	0.37	0.25	364.04	1455.85	3926.45	0.39	92.74	0.33	0.30	1.30	0.17	17.48
97209		SI2	0.91	1.40	2.31	0.39	0.50	381.67	763.28	1931.08	0.19	87.65	0.48	0.42	0.46	0.06	6.11
97210		SI3	0.65	1.40	2.05	0.31	0.50	104.10	208.13	653.63	0.07	93.67	1.45	1.35	0.05	0.01	0.64
97211		CCM	0.33	1.40	1.73	0.19	1.00		Too low to detect			94.91	1.52	1.44	0.00	0.00	TLTD
97212		COL	0.29	1.40	1.69	0.17	1.00		Too low to detect			97.64	2.15	2.10	0.00	0.00	TLTD
97213	10	STM	0.21	1.40	1.61	0.13	1.00	206.02	206.06	1547.76	0.16	98.04	0.40	0.39	0.39	0.05	5.27
97214		SI1	0.85	1.40	2.25	0.37	0.50	274.65	549.19	1448.29	0.15	91.78	0.31	0.29	0.51	0.07	6.70
97215		SI2	0.73	1.40	2.13	0.34	0.50	318.08	635.92	1840.62	0.18	93.38	0.42	0.40	0.47	0.06	6.22
97216		SI3	0.61	1.40	2.01	0.30	0.50	351.73	703.47	2299.55	0.23	95.14	0.59	0.56	0.41	0.06	5.57
97217		CCM	0.37	1.40	1.77	0.20	1.00		Too low to detect			93.88	2.15	2.02	0.00	0.00	TLTD

Appendix 3.19 cont...

Appendix 3.19 continued

K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	K12	K13	K14	K15	K16	K17	K18
97218		COL	0.27	1.40	1.67	0.16	1.00		Too low to detect			97.80	2.00	1.95	0.00	0.00	TLTD
97219	11	STM	0.43	1.40	1.83	0.23	0.17	333.7	1962.8	8323.63	0.83	99.68	0.37	0.37	2.25	0.30	30.14
97220		SI1	1.06	1.40	2.46	0.43	0.33	313.1	948.8	2202.01	0.22	96.36	0.57	0.55	0.40	0.05	5.47
97221		SI2	0.82	1.40	2.22	0.37	0.50	71.4	142.8	385.37	0.04	96.74	0.81	0.78	0.05	0.01	0.70
97222		SI3	0.48	1.40	1.88	0.25	0.50	362.9	725.9	2816.88	0.28	96.32	1.04	1.00	0.28	0.04	3.81
97223		CCM	0.25	1.40	1.65	0.15	0.50		Too low to detect			97.77	1.25	1.22	0.00	0.00	TLTD
97224		COL	0.24	1.40	1.64	0.14	1.00		Too low to detect			97.94	2.17	2.13	0.00	0.00	TLTD
97225	12	STM	0.21	0.80	1.01	0.21	1.00	61.6	61.6	289.99	0.03	97.61	0.09	0.09	0.33	0.04	4.36
97226		SI1	0.16	0.60	0.76	0.21	0.50	449.0	898.0	4163.50	0.42	95.88	0.03	0.03	15.75	2.10	210.09
97227		SI2	0.33	0.80	1.13	0.29	0.66	197.1	298.7	1016.21	0.10	90.75	0.02	0.02	6.71	0.90	89.63
97228		SI3	0.44	1.40	1.84	0.24	0.50	138.0	276.0	1150.36	0.12	90.62	0.07	0.06	1.81	0.24	24.21
97229		CCM	0.25	1.40	1.65	0.15	1.00		Too low to detect			96.59	1.09	1.05	0.00	0.00	TLTD
97230		COL	0.25	1.40	1.65	0.15	1.00		Too low to detect			97.16	2.12	2.05	0.00	0.00	TLTD
97231	13	STM	0.24	1.40	1.64	0.14	0.50	393.2	786.5	5317.58	0.53	98.33	0.27	0.27	2.00	0.27	26.74
97232		SI1	0.86	1.40	2.26	0.38	0.50	266.4	532.7	1391.09	0.14	89.70	0.16	0.14	0.97	0.13	12.97
97233		SI2	0.08	0.60	0.68	0.12	0.66	194.7	295.0	2329.52	0.23	93.87	0.40	0.38	0.62	0.08	8.39
97234		SI3	0.46	1.40	1.86	0.24	0.25	78.5	313.9	1259.14	0.13	95.04	0.38	0.36	0.35	0.05	4.72
97235		CCM	0.34	1.40	1.74	0.19	0.50		Too low to detect			94.01	1.20	1.13	0.00	0.00	TLTD
97236		COL	0.25	2.80	3.05	0.08	1.00		Too low to detect			96.48	2.25	2.17	0.00	0.00	TLTD
97237	14	STM	0.38	1.40	1.78	0.21	0.25	216.0	863.8	3972.86	0.40	99.05	0.30	0.29	1.35	0.18	18.12

Appendix 3.19 cont...

Appendix 3.19 continued

K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	K12	K13	K14	K15	K16	K17	K18
97238		SI1	0.54	1.40	1.94	0.28	0.25	281.01	1124.01	3995.45	0.40	93.26	0.36	0.34	1.18	0.16	15.81
97239		SI2	0.81	1.40	2.21	0.36	0.50	114.62	229.21	622.82	0.06	96.35	1.03	0.99	0.06	0.01	0.87
97240		SI3	0.69	1.40	2.09	0.33	0.50	243.33	486.50	1461.02	0.15	96.17	2.51	2.41	0.06	0.01	0.83
97241		CCM	0.27	1.40	1.67	0.16	1.00		Too low to detect			93.84	1.60	1.50	0.00	0.00	TLTD
97242		COL	0.24	1.40	1.64	0.14	1.00		Too low to detect			99.68	1.69	1.68	0.00	0.00	TLTD
97243	15	STM	0.19	1.40	1.59	0.12	0.50	66.28	132.48	1087.54	0.11	98.93	0.25	0.25	0.44	0.06	5.99
97244		SI1	0.48	1.40	1.88	0.25	0.50	146.67	293.28	1139.77	0.11	90.29	0.15	0.14	0.83	0.11	111 5
97245		SI2	0.84	1.40	2.24	0.37	0.50	179.73	359.48	952.09	0.10	92.92	0.57	0.53	0.18	0.02	2.41
97246		SI3	0.52	1.40	1.92	0.27	0.50	193.92	387.82	1420.00	0.14	96.20	0.94	0.90	0.16	0.02	2.17
97247		CCM	0.30	1.40	1.70	0.17	1.00		Too low to detect			95.11	2.08	1.97	0.00	0.00	TLTD
97248		COL	0.27	1.40	1.67	0.16	1.00		Too low to detect			97.15	1.96	1.90	0.00	0.00	TLTD
97249	16	STM	0.50	1.40	1.90	0.26	0.20	450.19	2250.75	8514.91	0.85	99.77	0.30	0.30	2.83	0.38	37.83
97250		SI1	0.99	1.40	2.39	0.41	0.25	441.25	1764.63	4237.64	0.42	96.34	0.45	0.43	0.98	0.13	13.12
97251		SI2	1.11	1.40	2.51	0.44	0.33	166.66	504.93	1138.98	0.11	92.29	1.21	1.12	0.10	0.01	1.47
97252		SI3	0.54	1.40	1.94	0.28	0.50	272.72	545.46	1938.98	0.19	95.63	1.09	1.04	0.19	0.03	2.55
97253		CCM	0.30	1.40	1.70	0.17	0.50		Too low to detect			94.80	1.04	0.99	0.00	0.00	TLTD
97254		COL	0.27	1.40	1.67	0.16	1.00		Too low to detect			97.52	1.73	1.68	0.00	0.00	TLTD
97255	17	STM	0.32	1.40	1.72	0.18	1.00	19.69	19.64	103.70	0.01	96.09	0.40	0.39	0.03	0.00	0.43
97256		SI1	0.10	0.40	0.50	0.21	1.00	115.33	115.34	542.11	0.05	93.61	0.29	0.27	0.20	0.03	2.62
97258		SI3	0.64	1.40	2.04	0.31	0.50	284.71	569.42	1809.12	0.18	93.25	0.99	0.92	0.20	0.03	2.67

Appendix 3.19 cont...

Appendix 3.19 continued

K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	K12	K13	K14	K15	K16	K17	K18
97259		CCM	0.31	1.40	1.71	0.18	1.00		Too low to detect			95.90	0.74	0.71	0.00	0.00	TLTD
97260		COL	0.26	1.40	1.66	0.15	1.00		Too low to detect			96.85	1.76	1.70	0.00	0.00	TLTD
97261	18	STM	0.24	1.40	1.64	0.14	1.00	197.91	197.94	1347.61	0.14	98.99	0.32	0.32	0.42	0.06	5.62
97262		SI1	0.76	1.40	2.16	0.35	0.50	214.33	428.62	1217.03	0.12	85.94	0.16	0.14	0.88	0.12	11.79
97264		SI3	0.55	1.40	1.95	0.28	0.25	120.05	479.99	1688.25	0.17	95.18	0.42	0.40	0.42	0.06	5.61
97265		CCM	0.29	1.40	1.69	0.17	0.50		Too low to detect			92.13	0.97	0.89	0.00	0.00	TLTD
97266		COL	0.27	1.40	1.67	0.16	0.50		Too low to detect			97.47	2.08	2.03	0.00	0.00	TLTD
97267	19	STM	0.35	1.40	1.75	0.20	0.25	296.97	1187.57	5844.53	0.58	99.02	0.23	0.23	2.59	0.35	34.60
97268		SI1	0.71	1.40	2.11	0.33	0.25	378.59	1513.95	4499.06	0.45	92.06	0.36	0.33	1.37	0.18	18.33
97269		SI2	0.88	1.40	2.28	0.38	0.50	286.62	573.13	1481.87	0.15	91.31	0.61	0.56	0.27	0.04	3.58
97270		SI3	0.84	1.40	2.24	0.37	0.50	261.04	521.91	1388.69	0.14	92.61	2.01	1.86	0.08	0.01	1.04
97271		CCM	0.41	1.40	1.81	0.22	1.00		Too low to detect			96.60	1.54	1.49	0.00	0.00	TLTD
97272		COL	0.28	1.40	1.68	0.17	1.00		Too low to detect			94.62	1.53	1.44	0.00	0.00	TLTD
97273	20	STM	0.29	1.40	1.69	0.17	0.50	190.76	381.40	2173.20	0.22	96.99	0.26	0.26	0.85	0.11	11.37
97274		SI1	0.42	0.80	1.22	0.34	0.50	80.38	160.68	464.91	0.05	91.03	0.26	0.23	0.20	0.03	2.75
97275		SI2	0.68	1.40	2.08	0.33	0.50	82.70	165.46	502.04	0.05	93.08	0.53	0.49	0.10	0.01	1.48
97276		SI3	0.69	1.40	2.09	0.33	0.50	233.71	467.54	1406.47	0.14	93.64	1.06	1.00	0.14	0.02	1.9
97277		CCM	0.36	1.40	1.76	0.20	1.00		Too low to detect			93.57	1.93	1.81	0.00	0.00	TLTD
97278		COL	0.26	1.40	1.66	0.15	1.00		Too low to detect			95.75	2.04	1.95	0.00	0.00	TLTD
97279	21	STM	0.49	1.40	1.89	0.26	0.20	382.73	1913.32	7357.77	0.74	97.46	0.31	0.30	2.44	0.33	32.61

Appendix 3.19 cont...

Appendix 3.19 continued

K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	K12	K13	K14	K15	K16	K17	K18
97280		SI1	0.92	1.40	2.32	0.39	0.25	525.9	2103.6	5273.8	0.53	92.1	0.45	0.41	1.28	0.17	17.1
97281		SI2	1.13	1.40	2.53	0.44	0.33	108.0	327.2	729.6	0.07	89.4	0.88	0.79	0.09	0.01	1.2
97282		SI3	0.58	1.40	1.98	0.29	0.50	255.6	511.2	1734.5	0.17	92.8	0.79	0.74	0.24	0.03	3.1
97283		CCM	0.26	1.40	1.66	0.16	0.50		Too low to detect			94.7	1.20	1.13	0.00	0.00	TLTD
97284		COL	0.25	1.40	1.65	0.15	1.00		Too low to detect			96.4	1.72	1.66	0.00	0.00	TLTD
97285	22	STM	0.20	1.40	1.60	0.12	1.00		Too low to detect			96.5	0.41	0.39	0.00	0.00	TLTD
97286		SI1	0.34	1.00	1.34	0.25	0.50	117.9	235.9	929.6	0.09	88.4	0.23	0.20	0.46	0.06	6.2
97287		SI2	0.21	1.00	1.28	0.22	0.50	133.1	266.1	1203.3	0.12	91.3	0.49	0.45	0.27	0.04	3.6
97288		SI3	0.69	2.00	2.69	0.25	0.50	450.1	900.3	3498.4	0.35	93.4	0.46	0.43	0.82	0.11	10.9
97289		CCM	0.26	1.00	1.26	0.20	0.50		Too low to detect			96.9	1.85	1.79	0.00	0.00	TLTD
97290		COL	0.26	2.00	2.26	0.11	1.00		Too low to detect			93.5	1.03	0.96	0.00	0.00	TLTD
97291	23	STM	0.24	1.00	1.24	0.19	0.50	72.0	143.9	729.1	0.07	99.3	0.30	0.30	0.24	0.03	3.2
97292		SI1	0.44	1.00	1.44	0.30	0.50	190.7	381.4	1244.3	0.12	91.2	0.25	0.23	0.54	0.07	7.2
97293		SI2	0.74	1.00	1.74	0.42	0.33	495.0	1499.9	3513.3	0.35	89.8	0.42	0.37	0.94	0.13	12.6
97294		SI3	0.70	2.00	2.70	0.26	0.50	366.0	732.0	2814.7	0.28	90.7	1.03	0.94	0.30	0.04	4.0
97295		CCM	0.39	1.40	1.79	0.21	0.50		Too low to detect			91.8	1.82	1.67	0.00	0.00	TLTD
97296		COOL	0.26	1.40	1.66	0.16	0.50		Too low to detect			94.6	2.32	2.20	0.00	0.00	TLTD
97297	24	SOTM	0.28	1.40	1.68	0.16	0.25	240.6	962.6	5775.4	0.58	94.9	0.22	0.21	2.79	0.37	37.2
97298		0SI1	0.52	1.40	1.92	0.27	0.25	88.2	352.8	1295.5	0.13	94.7	0.27	0.25	0.51	0.07	6.8
97299		SI2	0.71	1.40	2.11	0.33	0.50	254.7	509.4	1508.2	0.15	88.4	0.79	0.70	0.22	0.03	2.9

Appendix 3.19 cont...

0

Appendix 3.19 continued

K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	K12	K13	K14	K15	K16	K17	K18
97300		SI3	0.45	1.40	1.85	0.24	1.00	232.91	232.95	947.8	0.10	91.5	1.39	1.27	0.07	0.01	1.0
97301		CCM	0.29	1.40	1.69	0.17	1.00	Too low to detect				91.1	1.63	1.48	0.00	0.00	TLTD
97302		COL	0.26	1.40	1.66	0.15	1.00	Too low to detect				92.6	1.80	1.66	0.00	0.00	TLTD
97328	25	STM	0.15	1.40	1.55	0.09	0.50	128.22	256.45	2587.1	0.26	95.0	0.25	0.24	1.07	0.14	14.3
97329		SI1	0.39	1.40	1.79	0.22	1.00	245.04	245.02	1109.0	0.11	90.4	0.24	0.22	0.51	0.07	6.7
97330		SI2	0.58	1.40	1.98	0.29	0.50	226.96	453.82	1545.4	0.16	87.3	0.55	0.48	0.32	0.04	4.3
97331		SI3	0.61	1.40	2.01	0.30	0.50	282.88	565.72	1853.4	0.19	91.9	1.75	1.61	0.12	0.02	1.5
97332		CCM	0.22	1.40	1.62	0.13	1.00	212.60	212.61	1535.7	0.15	90.8	2.30	2.09	0.07	0.01	1.0
97333		COL	0.18	1.40	1.58	0.11	1.00	Too low to detect				94.6	2.46	2.33	0.00	0.00	TLTD
97583	2	Faeces	0.07	1.00	1.07	0.07	1.00	Too low to detect				95.6	2.08	1.98	0.00	0.00	TLTD
97584	3	Faeces	0.06	1.00	1.06	0.06	1.00	Too low to detect				97.1	2.15	2.09	0.00	0.00	TLTD
97585	7	Faeces	0.08	1.00	1.08	0.08	1.00	Too low to detect				95.3	1.79	1.70	0.00	0.00	TLTD
97586	8	Faeces	0.08	1.00	1.08	0.08	1.00	Too low to detect				94.8	2.18	2.07	0.00	0.00	TLTD
97587	12	Faeces	0.08	1.00	1.08	0.07	1.00	Too low to detect				94.9	2.04	1.93	0.00	0.00	TLTD
97588	13	Faeces	0.08	1.00	1.08	0.07	1.00	Too low to detect				91.4	2.33	2.13	0.00	0.00	TLTD
97589	17	Faeces	0.06	1.00	1.06	0.06	1.00	Too low to detect				96.8	2.02	1.95	0.00	0.00	TLTD
97590	18	Faeces	0.07	1.00	1.07	0.06	1.00	Too low to detect				95.3	1.93	1.84	0.00	0.00	TLTD
97591	22	Faeces	0.07	1.00	1.07	0.07	1.00	Too low to detect				93.6	1.96	1.83	0.00	0.00	TLTD

Appendix 3.20
General Linear model

% Undigested IgG = $\beta_o + \beta_i + u_{ij} + \alpha_k + (\alpha\beta)_{ik} + e_{ij}$

β_o is a constant
β_i is hours
u_{ij} is a random effect for the j^{th} pig within the i^{th} hour
α_k is location
$(\alpha\beta)_{ik}$ is interaction of location and hours
e_{ij} is residual error term

Appendix3.21

Total daily feed intake (g/day) by four to five-week-old piglets in the IgG digestibility trial

Pig #♣	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
	(g)	(g)	(g)	(g)	(g)	(g)
1	25	645	978	1058	1040	1263
2	0	340	899	1097	1029	690
3	0	350	743	1009	974	1209
4	18	115	538	702	742	830
5	8	138	675	1109	1026	782
6	15	273	740	706	584	863
7	58	452	633	1141	1131	1181
8	0	377	810	1016	1042	1084
9	118	672	955	1050	897	931
10	27	270	786	909	906	1051
11	41	486	836	794	1093	687
12	249	819	1237	1209	865	NF♥
13	NM♦	690	755	1279	1515	836
14	75	538	1003	1162	1361	794
14	98	492	638	922	1145	1019
15	26	857	1218	1310	1281	700
16	289	952	1096	1164	1097	1238
17	306	998	1123	1130	1479	767
18	216	519	658	1073	1232	1256
19	271	1011	1209	1060	1335	1318
20	269	926	1404	1301	1356	261
21	36	503	741	897	1212	1233
22	154	634	1266	1413	1556	1503
23	256	917	1063	1199	1310	1178
25	7	544	878	1016	1132	1309

♣ Pig identification number
♦ NM = Not measured
♥ NF = Not fed

CHAPTER 4

THE EFFECT OF BOVINE IMMUNOGLOBULINS FED EITHER AS A BOLUS VERSUS AS CONTINUOUS ON THE DEVELOPMENT AND SEVERITY OF DIARRHOEA CAUSED BY *ESCHERICHIA COLI* K88 AS THE ENTEROPATHOGENIC CHALLENGE MICROORGANISM USING A PIGLET MODEL

4.1 ABSTRACT

Enterotoxigenic *Escherichia coli* is a common cause of diarrhoea in infants, and immuno-compromised people, and of traveller's diarrhoea. The experiment was designed to determine, whether the administration of oral immunoglobulins derived from bovine milk of a bolus feeding (once a day) or continuous feeding (three times a day) is more effective on the susceptibility to diarrhoea, and if administrating a without immunoglobulins to the above group after nine days challenge with *Escherichia coli* would have an effect on diarrhoea. The milk immunoglobulin concentrate containing antibodies to enteropathogenic *Escherichia coli* was prepared from the pooled milk of cows naturally exposed to *Escherichia coli.* This whey globulin concentrate contained six percent bovine immunoglobulin G. For this study, the experiment was conducted on 24 piglets randomly allocated into three treatment groups comprising a "continuous immunoglobulin group", a "bolus immunoglobulin group" and a "control group". The piglets were fed with 10% of their metabolic body weight $kg^{0.75}$ per day three times in individual plastic troughs. The "continuous immunoglobulin group" was fed a diet containing immunoglobulin three times a day, the "bolus immunoglobulin group" received the same total amount of immunoglobulin as a single large dose in their diet in the morning, and the control group did not receive any immunoglobulins in their diet. All piglets were challenged on the ninth day, 30 minutes before the morning feed, with $1x10^9$ cfu *Escherichia coli K88* after eight days of their specified diets. After the challenge, the piglets were fed their respective diets for a further three days and then all were fed the diet which did not contain bovine immunoglobulins (control diet) for a further nine days. The piglets were monitored for incidence of diarrhoea, length of diarrhoea episode and severity of diarrhoea by faecal cultures for *Escherichia coli,* scoring faeces for degrees of diarrhoea, and by measuring the water content of their faeces. Faecal cultures were done prior to *Escherichia coli* challenge, during the time they were fed the specified diets, and after all were given the control diet without any immunoglobulin. There was no significant ($p>0.05$) effect in the duration of bacterial shedding but there was a difference in the severity of diarrhoea (according to faecal consistency $p<0.001$) and percentage of piglets that had abnormal faeces among the three different treatment groups ($p<0.05$). Bolus immunoglobulin feeding lessened the severity of diarrhoea and limited water loss compared to continuous immunoglobulin feeding, and the control feeding. It was concluded from the data that bolus

immunoglobulin feeding could be used for prophylactic treatment of *Escherichia coli* infection.

4.2 INTRODUCTION

The term "diarrhoea" refers to disturbances of the gastrointestinal tract comprising changes in intestinal motility and absorption, leading to an increase in the number of stools and changes in their consistency. When the presumed or definite cause of these disturbances is infectious, it is known as infectious diarrhoea (Ballabriga et al., 1974/75).

In places where hygienic conditions are poor there is a possibility that food and water can be contaminated with diarrhoea causing micro-organisms. The high rates of morbidity and mortality of infants in under-developed countries are mainly caused by intestinal infections (Wadström, 1975). The gut-associated lymphoid organs are not fully developed in the newborn child, who is particularly vulnerable to enteropathogenic micro-organisms. Travellers, immuno-compromised persons and hospital patients are also at risk from these infections.

Consequently, infectious diarrhoea itself is an important problem in paediatrics, as dehydration and electrolyte losses in premature and newborn infants often have a fatal outcome (Wadström, 1975). Approximately ten percent of children with enterohemorrhagic *Escherichia coli* (*E.coli*) diarrhoea develop other complications, of which haemolytic uremic syndrome is the most common (Tarr, 1995). Thirty percent of the above mentioned can develop severe complications such as long term renal or cerebral damage (Siegler et al., 1991). Haemolytic uremic syndrome usually develops one week after the onset of *E.coli* associated diarrhoea (Lissner et al., 1996). Unfortunately, the therapeutic regimens currently available for the treatment or for prevention of the extraintestinal sequelae of *E.coli* associated diarrhoea can have severe complications.

Adhesion is the first step in the pathogenesis of enterotoxigenic *E.coli* induced diarrhoea. Adhesion occurs to specific membrane receptors or receptor sites (Sellwood, 1979; Erickson et al., 1994) on the villus enterocytes of the small intestine (Smith & Jones, 1963; Arbuckle, 1970; Jones & Rutter, 1972). The adhesion is mediated by long thread like protein polymers present on the surface of the bacteria. These surface structures are called fimbriae or pili. Colonisation of the anterior part of small intestine by these micro-organisms is followed by their multiplication to reach large numbers and

the production of enterotoxins (Moon et al., 1966; Gyles & Barnum, 1967). Antibodies in milk of human, pig and cow can prevent bacterial adherence and neutralise the toxins (Facon et al., 1995).

Different antibiotics have been used for many years for the treatment of *E.coli* infections. Antibiotic therapy is not recommended for several reasons. Antibiotic treatment in early stages of the infection may kill or disrupt the *E.coli* cells resulting in release and possibly systemic absorption of toxin (Tarr, 1995), and sub-inhibitory concentrations of some antibiotics have been reported to increase toxin production *in vitro* (Walterspiel et al., 1992). Antibiotic treatment may also have side effects, such as gastrointestinal symptoms or hypersensitivity reactions (DuPont et al., 1986; Sack, 1986). In addition, large scale use of prophylactic antimicrobial agents can contribute to development of drug resistance in these organisms, making the drugs therapeutically ineffective (Murray et al., 1982; Murray, 1986). Thus, although it may give rapid results in many cases, antibiotic therapy cannot be considered as satisfactory in the long-term perspective.

Immuno-prophylaxis is an alternative treatment to antibiotics. Human milk contains specific antibodies against some diarrhoea causing organisms (Cunningham, 1977; Larsen & Hormer, 1978; Glass et al., 1983). However, levels of immunoglobulins (Igs) or specific antibodies in breast milk are variable over time. Many children raised on infant formulae, in which Igs have been destroyed by processing, or were never present, as in the case of soya based formula can lack in immuno-prophylaxis. The cows milk and colostrum contain specific antibodies against *E.coli* (Montenegro et al. 1990; Chapman et al., 1994). The transfer of colostrum to the newborn calf is thought to be an important mechanism in early acquired passive immunity against *E.coli* infections (Pirro et al., 1995). Interest has therefore been shown in using cows milk as a source of Igs for passive protection against *E.coli* infection in humans.

It has been reported that human and bovine colostrum immunoglobulin (Ig) preparations contain safe and effective agents for the prevention and treatment of diarrhoeal diseases caused by micro-organisms (Brüssow et al., 1987; Tacket, et al., 1988; Nord et al., 1990). Inflammation of the gastrointestinal mucosa, and diarrhoea due to *E.coli* infection associated with traveller's diarrhoea have been prevented by treatment with an

Ig concentrate from bovine milk (Tacket et al., 1988). Immunoglobulin preparation from rabbit antiserum (Rivier & Sobota, 1978) and bovine milk have been used successfully to treat infantile diarrhoea caused by enteropathogenic *E.coli* and *rotavirus* (Mietens et al., 1979; Hilpert et al., 1987).

One aspect of maximizing the benefits of oral bovine Igs for passive protection against diarrhoea is to optimise the method of administration. Bovine Igs can be administered continuously (Igs fed with every meal) or bolus (Igs fed as a single large dose once a day). This experiment was conducted to compare the effect of feeding a bolus of bovine Igs with the effect of feeding a continuous supply of Igs. Bovine Ig concentrate with specific activity against enterotoxigenic *E.coli* was added to a standard diet and tested in piglets, as a prophylactic/therapeutic agent against *E.coli* strain K88 for diarrhoea.

4.3 MATERIALS AND METHODS

All aspects of this study were approved by the Massey University animal ethics committee. (Protocol Number 97/113).

4.3.1 Animals

Twenty four Large White x Duroc, four-week-old entire male piglets (weight range 5.4 - 10.3 kg, 8± SD = 7.5 ± 1.52 kg) were selected immediately after weaning, from a group of piglets originating from five different litters at a commercial piggery (Wairaka Farms Ltd, Foxton).

4.3.2 Housing

From their arrival until Day 2, the piglets were housed as groups in pens according to their litter. On day 2, the piglets were housed individually in pens with a sloping concrete floor to facilitate urine drainage and regular washing down. They were maintained in a temperature controlled room at 25 ± 1^{0}C, with alternating 16 hr light and 8 hr dark periods. They were allowed water *ad libitum* throughout the trial.

4.3.3 Diet and Feeding regime

Diets varying in ingredients and quantity were given to three different groups of piglets (the composition of ingredients which is presented in Table 4.1, and the nutrient

content is presented in Table 4.2) which was formulated to meet the nutrient requirements of growing pigs (A.R.C. 1981). The diets were mixed by machine (small 100kg capacity, horizontal pedal mash mixer, Baxter, UK) at the feed processing unit at Massey University (Palmerston North, New zealand). The control group was fed three times a day on a commercial weaner diet which did not contain any bovine Igs. The "continuous Ig" group was fed three times a day on the "continuous Ig diet" which contained 10% bovine whey globulin concentrate (WGC). The "bolus Ig" group was fed the "bolus Ig diet" which contained 30% bovine WGC in the morning, and the control weaner diet without bovine Igs in the afternoon and evening. The "continuous Ig diet" and "bolus Ig diet" group received the same amount of Igs every day. Alatal 825 (Protein type Lactalbumin, New Zealand Dairy Board, Wellington, New Zealand) was used in the control diet to make it comparable in nutritive value to the "continuous Ig diet" and "bolus Ig diet". This ensured that there was no significant difference in the protein intake of each piglet, which may have affected their growth rates.

The piglets were fed three times a day in the morning at 0730 hrs, in the afternoon at 1230 hrs, and in the evening at 1730 hrs. The amount fed was determined based on 10% of metabolic body weight $kg^{0.75}$ per day. The daily dry feed amount was divided into three equal amounts, to be fed at each meal. Each portion was mixed with 75% (w/w) water immediately prior to feeding to reduce food wastage in individual plastic trough for each piglet. The piglets were allowed to adapt to the diet by allowing them free access to the consumption of food for more than one and half hours initially. The time allowed was slowly reduced to one hour by the third day. The troughs were removed after one hour and the unconsumed food (feed refusal) was measured to the nearest 0.1g. The piglets were weighed every three or four days between 1400 - 1500 hours, using a standard balance, which gives a reading time for 5 seconds. This helped to reduce the weighing error associated with the piglets moving or jumping in the weighing container. The piglet's feed intakes were adjusted accordingly after each weighing.

Table 4.1

The ingredient composition (%) of the control diet♣, the "continuous Ig diet♦", and the "bolus Ig diet♥" fed to weaner piglets in the trial to determine the effect of "continuous Ig feeding" versus "bolus Ig feeding" on the development of diarrhoea after challenge with *Escherichia coli* K 88

Ingredient	% in diet (Dry matter basis)		
	Control diet	Continuous diet	Bolus diet
Skim milk powder	20.0%	20.0%	-
Alatal 825	10.0%	-	-
Whey globulin concentrate	-	10.0%	30.0%
Wheat	20.0%	20.0%	20.0%
Barley	22.5%	22.5%	22.5%
Maize	25.0%	25.0%	25.0%
Vitamin/mineral premix♠	0.5%	0.5%	0.5%
DICP♆ ♠♠	2.0%	2.0%	2.0%

♣ Control diet without any bovine Igs and fed three times a day

♦ Continuous Ig diet which contain 10% Igs and fed three times a day

♥ Bolus Ig diet which contain 30% Igs and fed once in the morning

♠ Presented in Appendix 2.1

♆ Di calcium phosphate

♠♠ Presented in Appendix 2.2

Table 4.2

The calculated nutrient[♥♦] content of the control diet[♠], the "continuous Ig diet[♦]", and the "bolus Ig diet[♥]" fed to weaner piglets in the trial to determine the effect of "continuous Ig feeding" versus "bolus Ig feeding" on the development of diarrhoea after challenge with *Escherichia coli* K 88

Nutrient	per 100 g		
	Control diet	Continuous Ig diet	Bolus Ig diet
Energy	1214.50 kj	1214.50kj	1144.37 kj
Fibre	2.05 g	2.05 g	2.05 g
Crude protein	22.08 g	21.71 g	33.19 g
Calcium	0.69 g	0.69 g	0.49 g
Phosphorus	0.71 g	0.68 g	0.48 g
Sodium	0.11 g	0.11 g	0.02 g
Potasium	0.71 g	0.71 g	0.38 g
Chloride	0.20 g	0.20 g	0.06 g
Fat	1.97 g	2.20 g	3.37 g
Linoleic acid	0.71 g	0.71 g	0.59 g
Glycine and serine	2.29 g	1.79 g	1.29 g
Arginine	0.96 g	0.85 g	1.04 g
Histidine	0.66 g	0.62 g	0.76 g
Isoleucine	1.16 g	1.18 g	1.94 g
Leucine	2.78 g	2.32 g	3.49 g
Lysine	1.93 g	1.61 g	2.70 g
Methionine	0.54 g	0.49 g	0.67 g
Cystine	1.09 g	0.90 g	1.31 g
Tyrosine and phenylalanine	2.19 g	2.23 g	3.28 g
Threonine	1.21 g	1.20 g	2.14 g
Tryptophan	0.39 g	0.45 g	0.94 g
Valine	1.43 g	1.35 g	1.94 g

♥♦ Calculation based on the nutritive value of local feed stuff for poultry and pigs, Monogastric Research Center, Massey University, Palmerston North

♠ Control diet without any bovine Igs and fed three times a day; ♦ Continuous Ig diet which contain 10% Igs and fed three times a day

♥ Bolus Ig diet which contain 30% Igs and fed once in the morning

The method for measurement of bovine immunoglobulins as previously described in chapter 3, section 3.3.6.1 was used to measure the bovine immunoglobulin G (IgG) in the different diets. The amount of bovine IgG present in the three diets was measured by the radial immunodiffusion assay (RID) using normal level plates at the beginning and end of the experiment. The control diet was measured at 50% concentration (w/w with phosphate buffered saline (PBS), the "continuous Ig" diet was tested at 25% concentration (w/w with PBS) and the "bolus Ig" diet was tested at 10% concentration (w/w with PBS) and the calculations are presented in Appendix 4.1.

4.3.4 Experimental procedure

The twenty four piglets were randomly divided into three groups of eight in each group: the control group (to be fed on control diet without bovine Igs three times a day), the "continuous Ig" group ("continuous Ig diet" which contained 10% bovine whey globulin concentrate (WGC) three times a day) and the "bolus Ig" group ("bolus Ig diet" which contained 30% bovine whey globulin concentrate once a day in the morning, and the other two times on control diet). The "continuous Ig group" ranged in weight from 6.7-7.9 kg with a 8 ± SD of 8.0 ± 2.82 kg. The "bolus Ig group" ranged in weight from 6.5 - 9.4 kg with a 8 ± SD of 8.0 ± 2.82 kg. The control group in weigh ranged from 5.4-10.3 kg with a 8 ± SD of 7.9 ± 2.79 kg. After an adaptation period of eight days, on Day9, 30 minutes before the first feed each piglet was inoculated with $1x10^9$ cfu *E.coli* K88 organisms into the throats using a syringe. The strain of *E.coli K88* used was isolated from a clinical case of diarrhoea in a commercial piggery by the Veterinary Microbiology Laboratory, Massey University. The health of the piglets was observed for the following nine days using faecal observations for diarrhoea, feed intake, weight gain, bacteria shedding period (based on faecal culture), presence of rotavirus, *Salmonella*, Cry*tospordium*, *Coccidia* and *Giardia*, and percentage of liquid in the faeces. On the 17^{th} day, all piglets were fed on the control diet and their health monitored for a further three days. At the end of the trial, the piglets were treated with antibiotic Biosol M (Active ingredient- Neomycin 50mg/ml, Elano Animal Health, Auckland, New Zealand) for three days from Day 21. One piglet had diarrhoea after the antibiotic treatment, as a result all the piglets were treated with Tylan 200 (Active ingredients-200mg/mL of tylosin in 50% propylene glycol, Elano Animal Health, Auckland, New

Zealand) at a dose of 5mg/kg body weight by intramuscular injection near the neck for three days in the morning. They were then returned to a commercial farm.

4.3.5 Measurement of diarrhoea

Diarrhoea score: On the fourth day, each piglet was shaved around the anal and tail region. Wafer plate flanges (Surfit stomahesive; Bristol Myers Squibb Pharmaceuticals Pty Ltd, Auckland, New Zealand), which are elastic and designed for the attachment of ostomy bags, were attached to each pig with adhesive tape (Elastoplast, Crown Medical and Dental, Auckland, New Zealand) so that the 45mm opening in each flange was directly over the anus. An ostomy bag (a closed end pouch, Surfit plus; Bristol Myers Squibb, Pharmaceuticals Pty Ltd, Auckland, New Zealand) was attached to the wafer plate flange on each piglet. This pouch was used to collect faeces and was changed as required, i.e. once or twice a day. By using the pouch, it was possible to ensure total collection of uncontaminated faeces. The consistency of the faeces was subjectively classified as hard pellets, hard but together, soft, very soft, semisolid, or watery, every morning after the ostomy bags were changed. Hard pelleted faeces were given a score of one, hard but together faeces were given a score of two, soft faeces were given a score of three, very soft faeces were given a score of four, semisolid faeces were given a score of five, and watery faeces were given a score of six. A score of one to three was classified as normal faecal consistency and from four to six was classified as evidence of diarrhoea. Faeces were collected in the ostomy bags from Day 4^{th} to 22^{nd}.

Percentage of free liquid content in the faeces: The faeces collected daily from individual piglets were mixed well in the ostomy bags to ensure a homogenous consistency before being subsampled into 50ml centrifuge tubes (Bio Lab Scientific Ltd, Palmerston North, New Zealand). The tubes were centrifuged at 6000 r.p.m for twenty minutes at 4°C. The amounts of solid precipitate and supernatant were measured and the percentage of free liquid in the faeces was calculated as the volume of supernatant divided by the total volume of faeces multiplied by hundred. More than 10% percent free liquid in the faeces by this method was classified as "abnormal faeces" and indicative of diarrhoea. The piglets that had abnormal faeces in each treatment group before challenge, during challenge and after challenge are expressed as percentages.

4.3.6 Obtaining evidence of *E.coli K88* infection

To identify the presence of *E.coli K88*, microbiological cultures on fresh faeces samples were grown twice before the challenge (on Day 3 and 6), three times after the challenge (on Day 11, 13 and 16) and on Day 21 after all piglets had been fed the control diet for three days. In addition, cultures were done on day 29 to check if animals were free of infection after antibiotic treatment.

The faecal samples of remaining days were stored in the fridge. After few days, the samples were then measured for free liquid content and restored for few days in the fridge (4^0C) and subsequently in the freezer (-20^0C) until *E.coli K88* investigation was done for which days microbial culture had not done previously been done. Samples from the freezer were defrosted and mixed thoroughly with a spatula washed in virkon (Intermed Scientific Lab, Auckland, New Zealand) and culture for *E.coli K88* was grown.

All media were supplied by the Massey Veterinary Microbiology Laboratory, (Department of Veterinary Pathology and Public Health, Massey University, Palmerston North, New Zealand). Dry cotton swabs (Bio Lab Scientific Ltd, Palmerston North, New Zealand) were used to inoculate a sample of faeces onto blood agar plates which were incubated overnight at 37 ± 1^0C. After incubation, three individual haemolytic presumptive *E.coli* colonies were picked up from the blood agar plates mixed into tryptone water and incubated at 37 ± 1°C for 4 hrs (Quinn et al., 1994). Loopfuls of tryptone water were then used to inoculate Minka agar slopes and MacConkey plates. These were incubated at 37 ± 1^0C overnight. The next day, if the MacConkey plates showed typical *E.coli* colonies (circular, convex and smooth pink colonies with distinct edges), colonies on the Minka agar slopes were tested for the presence of *E.coli* K88 by a slide agglutination test using K88 specific antisera (Seiken- Denka Seiken Co., Ltd, Tokoyo, Japan) (Guinee, 1976; Guinee, 1977; Nagy, 1977).

4.3.7 Obtaining evidence of rotavirus infection

The presence of rotavirus was checked in faeces collected on Day 3 and Day 7, using the Murex Rotavirus Latex test (Murex Diagnostics New Zealand, Auckland, New Zealand). This assay was performed according to the manufacturer's instructions. All reagents were brought to room temperature. Suspensions (10%) of faeces in extraction

buffer were prepared in centrifuge tubes and then centrifuged at 1000g for 10 minutes. Disposable droppers were used to dispense one drop of supernatant from this centrifugation onto each circle on the card. Forty microliters of either the latex reagent, or the test latex was added to the drop of supernatant and gently mixed for two minutes. The presence of rotavirus in the faeces was detected by the agglutination of the test latex supernatant mixture (Julkenen et al., 1985; Pai et al., 1985).

4.3.8 Obtaining evidence of other diarrhoea causing micro-organisms

Faecal samples were also examined for Salmonella, Giardia, Crytospordium and Coccidia by Vetinary microbiology Laboratory, Massey University.

4.3.9 Data analysis

For the measurement of bovine IgG in the diets, the calibrator ring diameters were measured (Appendix 4.2) and a standard curve (Appendix 4.3) generated by linear regression using the true calibrator concentration values and the squared diameter of their precipitin rings in the RID plates. The concentration of bovine IgG in each diet was calculated using this standard curve (Appendix 4.4).

A general repeat statistical linear model (SAS, 1985) was used to estimate the effects that time, treatment, pig within treatment, and time by treatment had on faecal consistency and the percentage of free liquid in the faeces (Appendix 4.5). Faecal culture (bacterial shedding) and piglet weight gain were analysed for the effect of treatment (Appendix 4.5).

4.4 RESULT

The amounts of bovine IgG in the "continuous Ig diet" and the "bolus Ig diet" were 0.75% and 1.88% at the beginning of the experiment, and 0.66% and 1.86% at the end of the experiment respectively. There was no visible precipitin ring around the wells in the RID plates for the control diet, which indicates that there was no measurable bovine IgG present in the control diet (Appendix 4.4). There was no significant difference in the bovine IgG content of the diets at the beginning and end of the trial and also in the nutrient content of the diet supplied to each group.

Duplicates sampled were analysed for IgG and the overall mean coefficients of variation (between the duplicate samples at each assay) was 1.75.

One piglet in the continuous Ig treatment group had diarrhoea for a day before the challenge. One piglet in the continuous Ig treatment group had diarrhoea the day following the end of antibiotic treatment.

There were no individual observations till the afternoon of Day 2 because the piglets were not individually housed. The piglets' feed intakes were recorded three times daily from the evening of Day 2 to Day 26 (Appendix 4.6). They did not refuse daily feed except for one piglet in the bolus Ig group (piglet no 1) which rejected food for one day (midday feed on Day 12) due to vomiting.

Piglets were weighed every three or four day and their weights recorded (Appendix 4.7). There was no significant difference ($p>0.05$) in the average weight of piglets in each group fed with the different diets during the trial (Figure 4.1).

The consistency of the faeces of each individual piglet was observed daily from Day 2 to 26 of the trial and is recorded in Appendix 4.8. There was a significant difference ($P<0.001$) in the faecal consistency of the piglets of each treatment group observed both before and during challenge. The consistency of the faeces from the piglets suffering from diarrhoea was soft and porridge like, followed by a watery outpour. In few other piglets, diarrhoea was observed intermittently with an irregular pattern. *Salmonella, Cryptospordium, Coccidia* and *Giardia* were not detected in the faeces of the piglets which suffered from intermittent diarrohea. Rotavirus was not detected in these faeces of all piglets.

The percentage of free liquid content of the faeces of each piglet on a daily basis is given in Appendix 4.9. The relationship between visual faecal consistency and the percentage of free liquid content in the faeces is represented in Figure 4.2. There was only a weak correlation ($R^2=0.59$) between the faecal consistency observations and the percentage of free liquid content in the faeces. However, in the most severe form of diarrhoea (as defined by a faecal score of 6) consistently had more than 10% free liquid in the faeces and it was defined vas "abnormal faeces".

The percentage of piglets that had abnormal faeces or "diarrhoea" pre-challenge, during challenge and post-challenge is calculated and given by Figure 4.3. There was a significant difference among the three treatment groups in percentage of piglets. The control group had the highest number of piglets with abnormal faeces while the "continuous Ig diets" had lesser number and the "bolus Ig group" had the least number of piglets with abnormal faeces. There was no significant difference ($p \leq 0.05$) between the time periods for any of the treatment groups.

The results of microbiological culture of the fresh faeces of the piglet to detect the presence of *E.coli* K88 is given in Table 4.3. Before challenge (on Day 3 and 7) none of the piglets showed the presence of *E.coli* K88 in their faeces. After challenge (on Day 11, 13 and 16) the presence of bacteria in the faeces of the piglet in the three diet groups varied in number on different days. No *E.coli* K88 was detected in faeces collected from piglets after they had all been fed the control diet (Day 23), or on Day 29, following antibiotic treatment. No signs of *E.coli* growth were visible in the frozen faecal samples. Since *E.coli* growth was expected, several rescue methods, such as tryptone water, glucose - tryptone water and brain heart infusion broth were tried to enhance culture success and yet no evidence of *E.coli* growth was observed.

The duration of shedding was determined from the number of animals that had consecutive positive faecal cultures. When the animals were grouped according to whether bacterial shedding lasted less than or greater than 5 days, the pattern of shedding varied among the different diet groups, but no significant ($p>0.05$) differences were found either before or during challenge (Figure 4.4). There was no correlation found between faecal score and *E.coli* faecal culture results.

4.5 DISCUSSION

This study was conducted to compare the effect of feeding a single bolus of bovine Igs once a day with the effect of feeding a continuous supply of Igs each meal as a prophylactic against the challenge of *E.coli K88* diarrhoea.

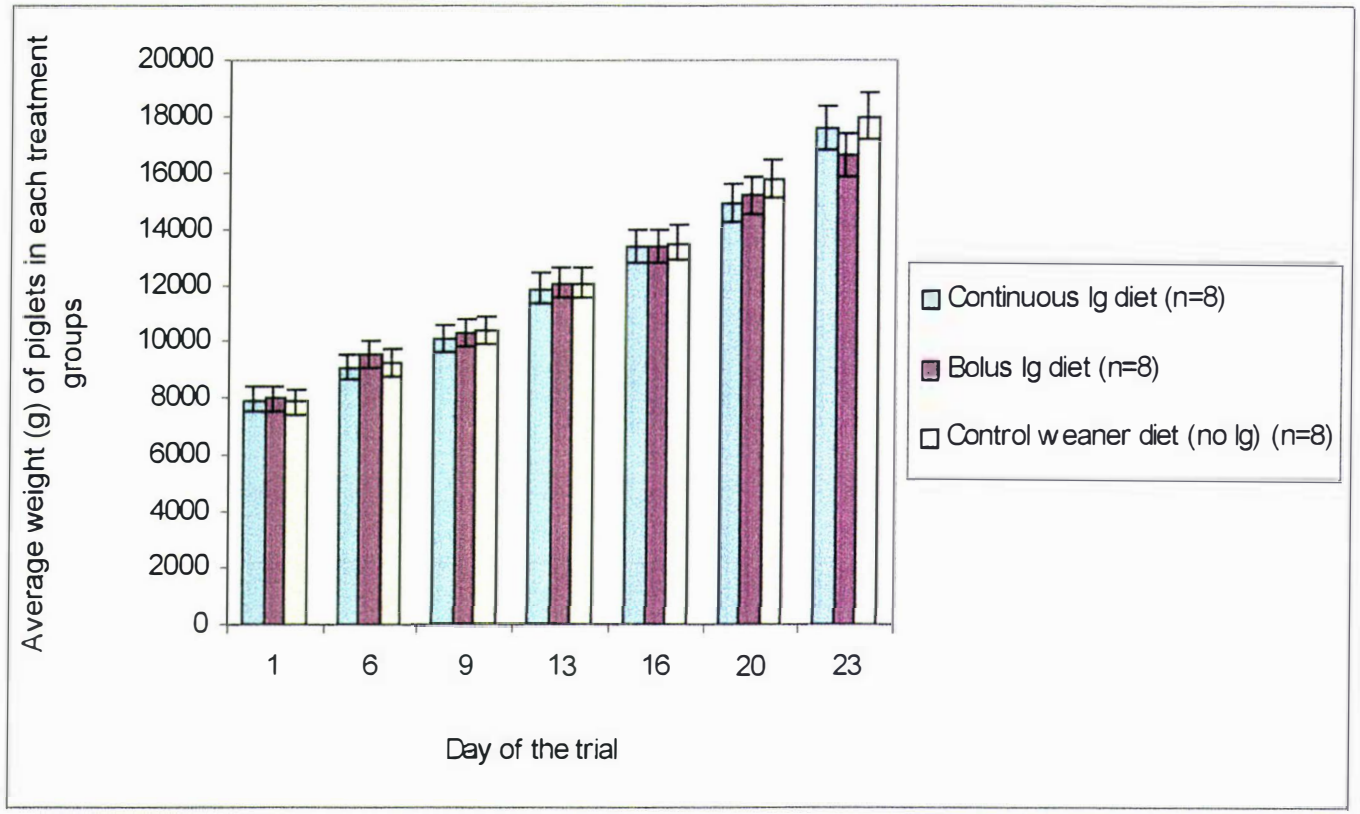


Figure 4.1 Average weight of weaner piglets fed three different diets in a trial to determine the effect of continuous immunoglobulin feeding versus bolus immunoglobulin feeding on the development of diarrhoea after challenge with *E.coli* K88.

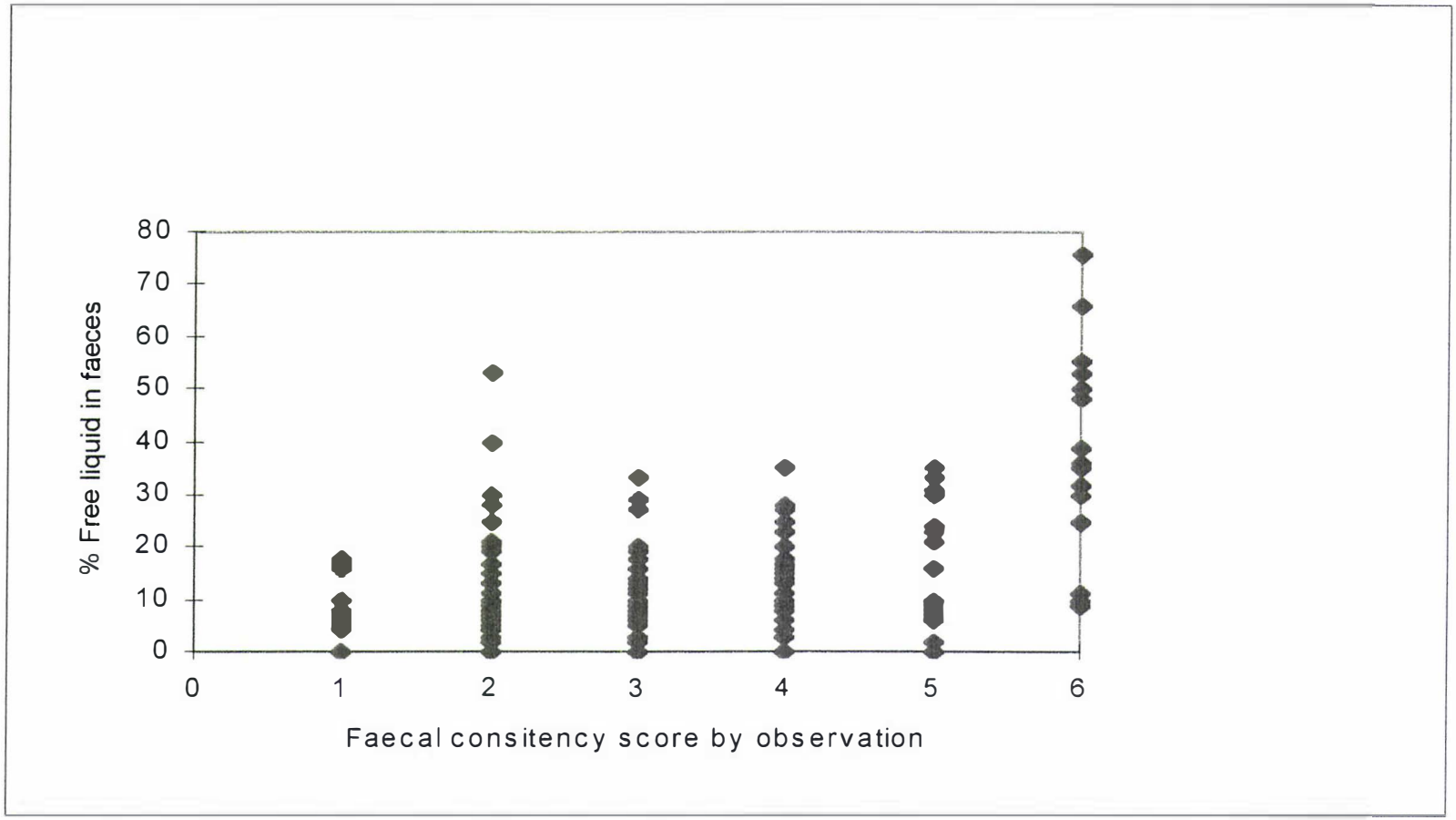


Figure 4.2 Distribution of faecal consistency versus percentage free liquid in the faeces of all piglets in the trial to determine the effect of continuous immunoglobulin feeding versus bolus immunoglobulin feeding on the development of diarrhoea after challenge with *E.coli* K88. (Faecal observation: 1 = hard pelleted; 2 = hard but together; 3 = soft; 4 = very soft; 5 = semisolid; 6 = watery).

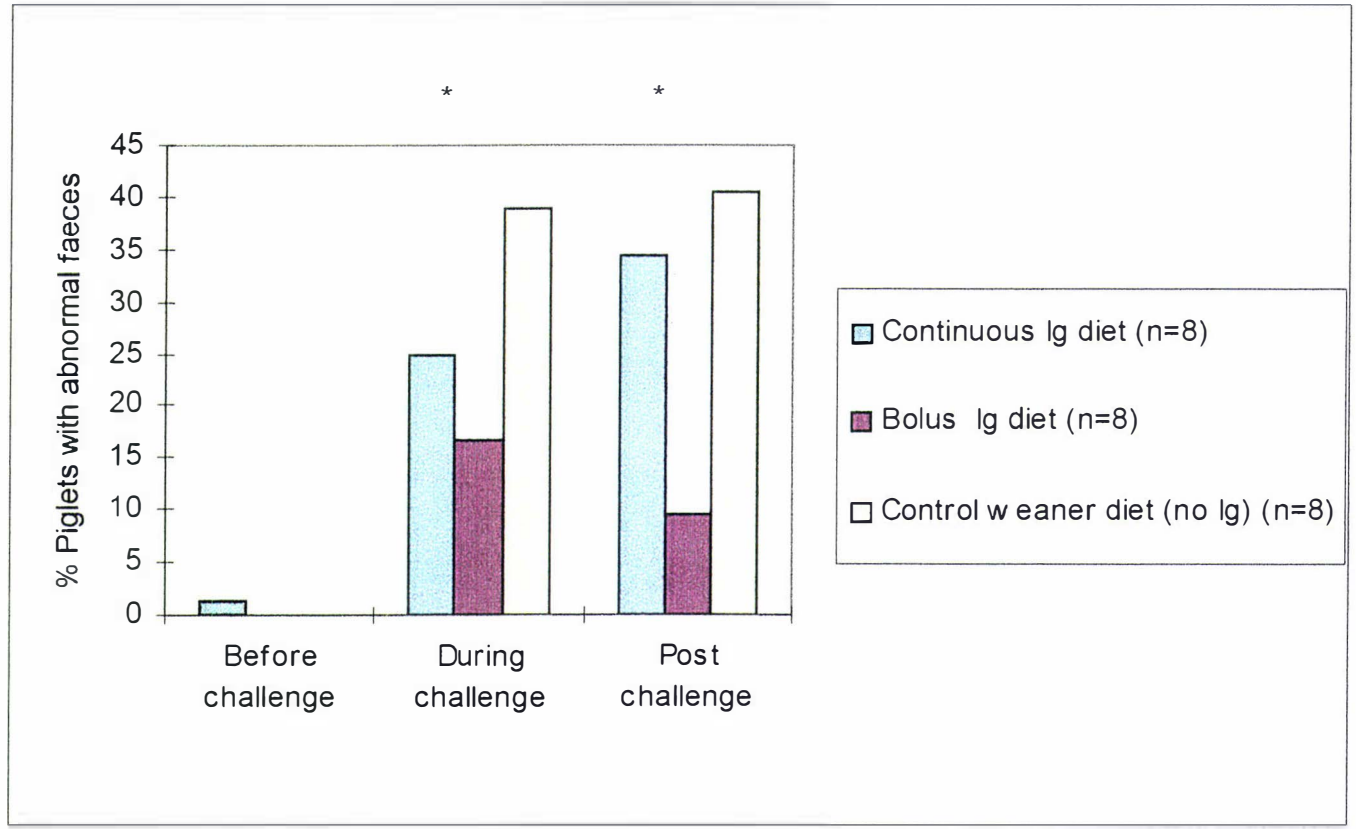


Figure 4.3 The percentage of piglets that had abnormal faeces, as defined by more than 10% of water in the faeces, in the different treatment groups before challenge, during challenge and after challenge with *E.coli* to determine the effect of continuous immunoglobulin feeding versus bolus immunoglobulin feeding on the development of diarrhoea

* $p<0.05$

Table 4.3

The presence of *E.coli K88* in the fresh faeces of piglets in the trial to determine the effect of continuous immunoglobulin feeding versus bolus immunoglobulin feeding on the development of diarrhoea after challenge with *E.coli* K88

Pig #[a]	Treatment group[b]	Before *Ecoli K88* Challenge		During challenge[c] with *E.coli* K88			On control diet	After antibiotic treatment (Biosol M)
		Day[d] 3	ay 7	Day 11	Day 13	Day 16	Day 23	Day 29
3	Continuous	N[e]	N	P[f]	P	N	N	N
6	Continuous	N	N	P	P	P	N	N
12	Continuous	N	N	P	N	N	N	M
13	Continuous	N	N	P	N	N	N	N
16	Continuous	N	N	P	N	N	N	N
19	Continuous	N	N	P	P	P	N	-[g]
22	Continuous	N	N	N	N	N	N	N
23	Continuous	N	N	N	N	N	N	N
1	Bolus	N	N	P	P	P	N	-
5	Bolus	N	N	P	P	N	N	N
9	Bolus	N	N	P	N	P	N	N
11	Bolus	N	N	P	N	P	N	N
15	Bolus	N	N	P	P	N	N	-
18	Bolus	N	N	N	N	N	N	N
21	Bolus	N	N	P	N	N	N	N
24	Bolus	N	N	P	P	N	N	N
2	Control	N	N	P	P	N	N	-
4	Control	N	N	P	P	N	P	-
7	Control	N	N	N	N	N	N	N
8	Control	N	N	P	N	N	N	P
10	Control	N	N	P	P	N	N	N
14	Control	N	N	P	P	P	N	P
17	Control	N	N	P	P	N	N	N
20	Control	N	N	P	P	N	N	N

[a] Pig identification number
[b] Treatment group whether continuous, bolus or Control feeding (no Ig)
[c] *E.coli* challenged on the ninth day
[d] Day of the trial
[e] N = *E.coli* is not present in the faeces
[f] P = *E.coli* is present in the faeces
[g] - = *E.coli* culture not done due to already killed

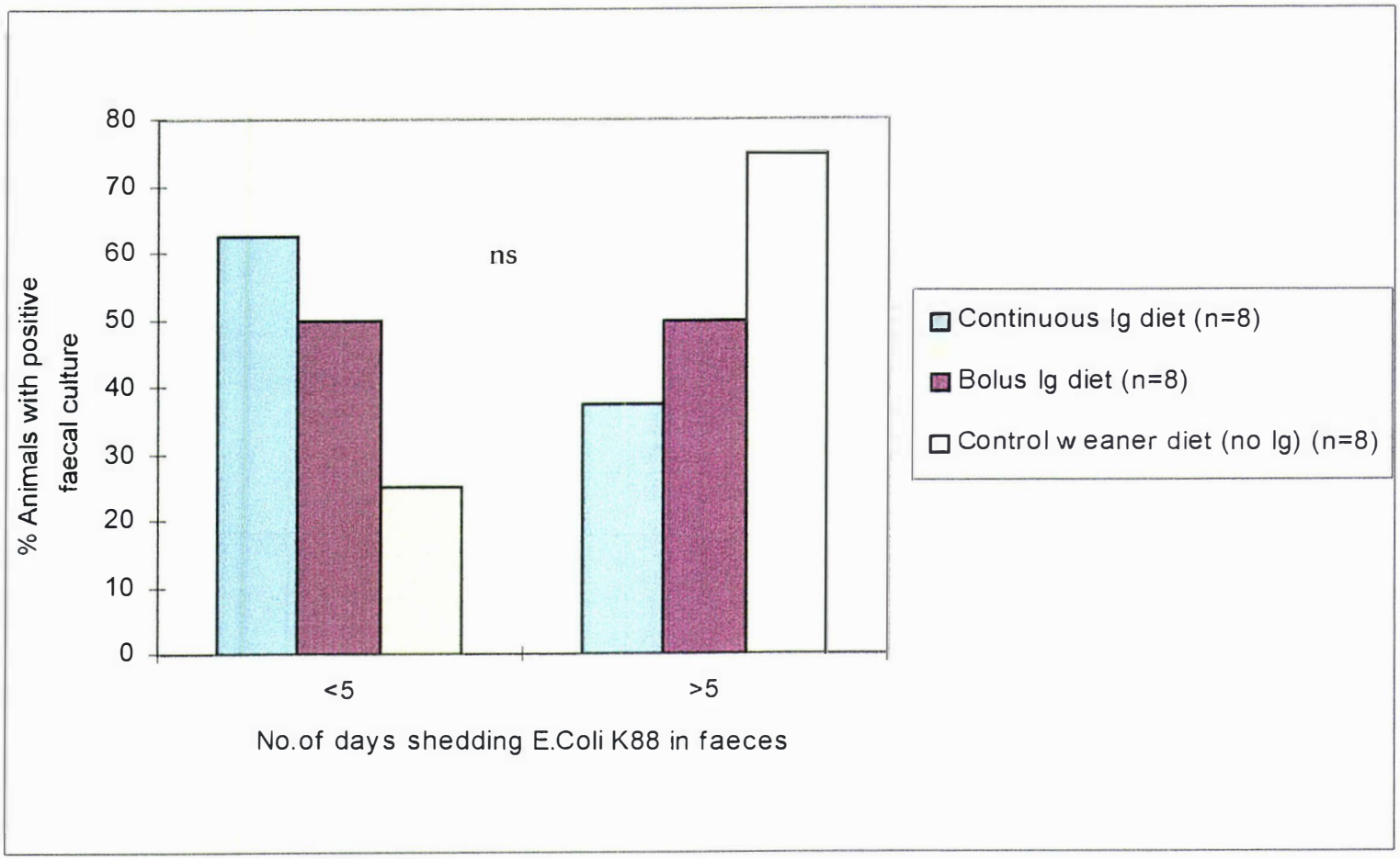


Figure 4.4 The percentage of piglets that were shedding *E.coli* K88 for less than or more than 5 days in a trial to determine the effect of continuous immunoglobulin feeding versus bolus immunoglobulin feeding on the development of diarrhoea after challenge with *E.coli* K88
ns not significant

There was no remarkable change in the levels of IgG in the bolus diet and continuous Ig diets at the beginning and at the end of the experiment. Therefore, presumably, the quantity and the protective properties of the IgG were not destroyed with time.

The method of culture used to recover *E.coli K88* from the faeces may not have been very sensitive because of the faeces placed on the blood agar plates were little in quantity and the number of colonies selected from the blood agar plates for investigation was only three in number. Appropriate colonies may have been missed during the selection due to the *E.coli* K88 being few in numbers in the colonies in the blood agar plates.

Unsuccessful culture of the frozen faecal samples led to the conclusion that the virkon on the spatula and storage in the fridge may have resulted in the destruction of *E.coli* in the faeces. Storage of samples at low temperature (4°C) reduces the number rapidly but very low temperature (-80°C) help to preserve them (Bretz & Hartsell, 1959; Straka & Stokes, 1959; Postgate & Hunter, 1963; Ray & Speck, 1973; Graham & Beveridge, 1990). Unsuccessful culture of the stored samples limited our ability to measure the duration of infection. Thus, it is recommended that in future the faeces be cultured fresh each day, and if this is not possible, faeces should be stored at -80°C immediately after collection to maximise the chance of recovering any *E.coli K88* present.

The administration of the single large bolus dose of Ig concentrate 30 minutes after the inoculation with *E.coli* K88 undoubtedly optimised the opportunity for passive protection. Although some challenge organisms may have been neutralised in the stomach by the dose of Ig concentrate given immediately after challenge, we know that some challenge organisms did pass into the bowel, because the piglets had positive faecal cultures.

The diarrhoea observed in some of the piglets throughout the trial was intermittent. As reported earlier, there was no correlation between the faecal diarrhoea score and faecal culture results. Examination of the faeces for other diarrhoea causing organisms (*Salmonella, Cryptospordia, Coccidia, Giardia* and rotavirus) was also unsuccessful. In addition, one of the piglets already had diarrhoea before the challenge, so it can reasonably be assumed that the diarrhoea was of unknown infectious origin or may have been a physiologically-based post-weaning diarrhoea. In future trials, it would be

important to control for other pathogens, espcifically by selecting only healthy animals at the start of the trial. However, this may be difficult to achieve logistically.

Enteropathogenic *E.coli* induce diarrhoea by firstly adhering to the gastrointestinal cells via specific K88 receptors and thereafter colonizing the intestinal tract. They also produce enterotoxins which induce an outpouring of fluids leading to profuse diarrhoea (Williams & Gibbons, 1972).

The rate of adhesion of *E.coli* K88 falls gradually in weaned piglets as they became older, and by the time they are four to five weeks old, the small intestine becomes markedly less receptive to the K88 type of adhesion than during the neonatal period (Wilson, 1972; Wilson and Hohmann, 1974; Erickson et al., 1994). Although the piglets in this trial were 5 weeks old, seven out of eight animals in the control group shed *E.coli* K88 in their faeces. It was assumed, therefore, that the animals were sufficiently susceptible to infection to be used as a model for *E.coli* K88 induced diarrhoea.

Enterotoxigenic *E.coli* produce a number of different toxins. Heat labile enterotoxin (LT) is a high molecular weight protein similar to cholera toxin, and is antigenically constant regardless of the serotype of *E.coli* that produces it. Stable toxin (ST) refers to low molecular weight heat stable enterotoxins, STa and STb. Both LT and ST bind to different receptors on the surface of the gut enterocytes and affect the metabolism of the enterocyte in different ways. Heat labile enterotoxin activates adenylate cyclase which stimulates the production of cyclic adenosine monophosphate resulting in an increase in the secretion of chloride, sodium, carbonate and water into the gut. It may also block the absorption of sodium by mature enterocytes. Stable toxin (Sta) activates guanylate cyclase which stimulates the production of guanosine monophosphate resulting in reduced absorption of electrolytes and water from the intestine lumen. The action of STb is not well understood but the net effect of the toxins is secretory and results in ionic inbalance, dehydration, acidosis and raised blood pressure (Fisher et al., 1994).

In the present study it is possible that the single high bolus Ig dose has been able to neutralise toxin in the small intestine. In the continuous Ig group, the Ig quantity may have not been sufficient to neutralise toxin as efficiently as Ig from the bolus diet, hence

there was more fluid loss associated with the continuous Ig group compared to the bolus Ig group. In the control group there was no IgG to neutralise toxin and therefore the loss of fluid was the greatest. Pirro et al. (1995) demonstrated the presence of antibodies in the colostrum of cows neutralised Shigela-like toxins (SLT I and SLT II) and reduced water loss.

The percentage of animals that shed *E.coli* K88 for five days after challenge was greatest in the control group, less in the bolus Ig group, and least in the continuous Ig group. Although this was not statistically significant due to very large standard deviations for the groups, additional animals within each group may have helped to produce a significant difference. Certainly the results tend to indicate that adequate Igs may limit the duration of infection with enteropathogenic *E.coli*.

When faecal consistency was measured by determining the percentage of free fluid in the faeces after centrifugation, significant differences were seen in the level of fluid secretion between the different groups of piglets. In the "bolus Ig" group of piglets, the fluid loss was less than the "continuous Ig" group, while piglets in the control group had the greatest fluid loss.

From Day 17 to 29, all the piglets were taken off their experimental dietary regimes and fed the control diet. None of the piglets showed cultural evidence of *E.coli K88* colonization except for one piglet in the control group on Day 23, and two piglets in the control group on Day 29. From these results, it can assumed that the continued presence of IgG in the diet either delivered as a bolus once a day or as part of each feed prevented recolonization of the gut and eliminated *E.coli K88* infection in the Ig fed groups. This would mean that no *E.coli* K88 was present to reinfect the piglets once IgG was no longer supplied in the diet. There may also have been an opportunity for active immunity to develop as *E.coli K88* colonization of piglets in the control group persisted intermittently (as judged by culture results) throughout the trial.

This experiment did not conclusively demonstrate a protective effect of bovine milk immunoglobulin concentrate against enteropathogenic *E.coli* K88 infection, although there was some indication that the duration of shedding may be shortened and the

severity of the disease (in term of fluid loss) was certainly lessened by the administration of a high dose of IgG as a single bolus each day.

In other studies, the effectiveness of bovine milk Igs against colonization by diarrhoea causing organisms has been demonstrated. Demierre et al. (1975) demonstrated *in vivo* that bovine anti- *E.coli* milk Ig inhibited the adhesion of pathogenic *E.coli* to the epithelial cells of rabbit intestine.

Lyophilised Ig obtained from the colostrum of cows immunized with several enterotoxigenic *E.coli* serotypes, was shown to provide complete protection against enterotoxigenic *E.coli* infection in ten adult volunteers when administered at 1g Ig per day (Tacket et al., 1988). However, Brunser et al. (1992) were unable to show that supplementing infant formula with bovine milk Ig concentrate (at 1g Ig per day) provided any protective benefit against the major enteropathogenic *E.coli* serogroups. This failure may have been associated with the high level of challenge in the field, loss of activity of Igs during formula preparation, or lack of specificity of the Igs used.

In a similar piglet study, bovine milk Ig concentrates included as 10% of the diet under continuous Ig feeding conditions, have been shown to reduce the severity of diarrhoea after oral challenge with 1×10^9 cfu *E.coli* K88 (Schollum et al., 1997).

Logan et al. (1974) demonstrated that in calves, the protection against *E.coli* afforded by each Ig classes appeared to differ in certain respects from that of the others. For example, IgA failed to modify the haemoconcentrating effect of the disease to the same extent as IgG and IgM, although it clearly had a protective effect as the calves remained bright and survived. IgG and IgM also prevented death but did not prevent diarrhoea. Thus it would appear that no class of Ig alone provided the full spectrum of protection obtained with colostrum and it seems probable that all three Ig classes may have separate prophylactic qualities which may act in synergy when present in colostrum. Thus indicating that all the classes of Igs must be present for better results.

Mietens et al. (1979) treated 60 children, age between 10 days to 18 months with hyperimmune anti-enteropathogenic *E.coli* milk Ig concentrate (1g per kg body weight) for diarrhoea due to enteropathogenic *E.coli* daily, for 10 days. The treatment was

effective in eliminating the *E.coli* infection in 43 of the 51 children infected with strains present in the vaccine, but in only one of the nine infected with strains not incorporated in the vaccine. These results suggest that the specificity of the milk Igs may be important for their efficacy.

The differences in success between our trial and others may be associated with the level of IgG used; approximately 2% IgG in diet versus a variety of other larger dose rates. In addition, the Ig classes present in mature milk rather than in colostrum may have a different impact on the disease process. To achieve effective treatment, in contrast to the prophylaxis discussed above, even higher doses of specific Igs may be needed.

In summary, these results suggest that specific milk Igs may have a role in preventing, limiting or treating *E.coli* diarrhoea. Although further work is necessary, there is some indication that a single high level bolus of IgG once a day may be an effective means of delivering passive immunity.

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4.7 APPENDIX

Appendix 4.1

Calculation♦ of sample concentrations for measurement of bovine IgG in the diets used to fed piglets in a trial to determine the effect of continuous immunoglobulin feeding versus bolus immunoglobulin feeding on the development of diarrhoea after *Escherichia coli* K88 challenge.

Diet	Dry weight	Final weight♣	Sample concentration
	(g)	(g)	(g/L)
Control diet♥	0.75	1.68	446.67
Control diet♠	0.74	1.66	448.18
Continuous Ig diet♥	0.37	1.52	246.85
Continuous Ig diet♠	0.37	1.47	252.81
Bolus Ig diet♥	0.14	1.44	98.73
Bolus Ig diet♠	0.15	1.53	100.35e

♦ Equations used to calculate Ig concentration is presented in Appendix 3.1

♣ Final weight = Dry weight + PBS

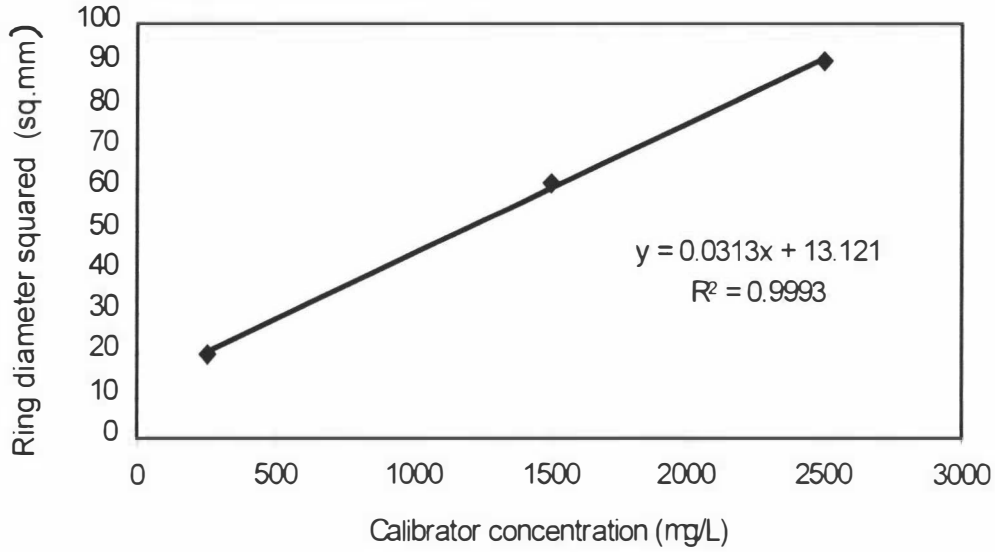
♥ Beginning of the experiment

♠ End of the experiment

Appendix 4.2

IgG calibrator readings from the radial immunodiffusion assay measuring IgG concentration in diets used to feed piglets in a trial to determine the effect of continuous immunoglobulin feeding versus bolus immunoglobulin feeding on the development of diarrhoea after *Escherichia coli* K88 challenge.

Calibrator concentration	Ring diameter reading 1	Ring diameter reading 2	Ring diameter reading 3	Average ring diameter	Average ring diameter squared
(mg/L)	(mm)	(mm)	(mm)	(mm)	(sq.mm)
251.7	4.53	4.78	4.28	4.53	20.52
1504.2	8.24	8.25	7.00	7.83	61.30
2494.9	9.84	9.88	9.26	9.66	93.32



Appendix 4.3 IgG calibrator curve♣ from the RID assay used to measure bovine IgG in the diets used to feed piglets in a trial to examine the effect of continuous immunoglobulin feeding versus bolus immunoglobulin feeding on the development of diarrhoea after *Escherichia coli* K88 challenge.

♣ Calibrator curve generated for true calibrator concentration and ring diameter squared

Appendix 4.4

Calculation of bovine IgG concentration in diets fed to piglets in the trial to determine the effect of continuous immunoglobulin feeding versus bolus immunoglobulin feeding on the development of diarrhoea after *Escherichia coli* K88 challenge.

Diet	Ring diameter reading 1	Ring diameter reading 2	Average ring diameter	Diameter squared	Sample Concentration♦	IgG concentration in sample X=(Y-13.121)/0.0313♥	IgG%♦
	(mm)	(mm)	(mm)	(sq.mm)	(g/L)	(mglg/L)	
Control diet (beginning of the experiment)			Too low to detect		446.67		
Control diet (end of the experiment)			Too low to detect		453.59		
Continuous Ig diet (beginning of the experiment)	8.49	8.46	8.48	71.82	246.85	1875.54	0.75
Continuous Ig diet (end of the experiment)	8.29	7.95	8.12	65.93	252.81	1687.32	0.66
Bolus Ig diet (beginning of the experiment)	8.35	8.53	8.44	71.23	98.73	1856.63	1.88
Bolus Ig diet (end of the experiment)	8.10	8.82	8.46	71.57	100.35	1867.43	1.86

♦ From Appendix 4.1

♥ From the regression equation; R^2=0.9993, Appendix 4.3

♦ % Represents the amount of Ig present in the powder

Appendix 4.5
General linear Model

Water % = $\beta_o + \beta_i + u_{ij} + \alpha_k + (\alpha\beta)_{ik} + e_{ij}$

β_o is a constant
β_i is the effect of the i^{th} treatment group
u_{ij} is a random effect for the j^{th} pig within i^{th} treatment group
α_k is stage
$(\alpha\beta)_{ik}$ is interaction of treatment and stage
e_{ij} is the residual error term

Average weight = $\beta_o + \beta_i + e_{ij}$
β_o is a constant
β_i is the effect of the i^{th} treatment group
e_{ij} is the residual error term

Faecal consistency = $\beta_o + \beta_i + u_{ij} + \alpha_k + (\alpha\beta)_{ik} + e_{ij}$
β_o is a constant
β_i is the effect of the i^{th} treatment group
u_{ij} is a random effect for the j^{th} pig within i^{th} treatment group
α_k is Stage
$(\alpha\beta)_{ik}$ is interaction of treatment and stage
e_{ij} is the residual error term

Bacterial shedding = $\beta_o + \beta_i + + e_{ij}$
β_o is a constant
β_i is the effect of the i^{th} treatment group
e_{ij} is the residual error term

Appendix 4.6

Total daily feed intake (g/day) by weaner piglets during the trial[v] to determine the effect of continuous immunoglobulin feeding versus bolus immunoglobulin feeding on the development of diarrhoea after *Escherichia coli* K88 challenge.

Pig #[♣]	Diet[♦]	Before challenge with *E.coli K88*							During challenge with *E.coli K88*					
		Day[♥] 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
		(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)
3	Continuous	184.0	608.3	595.2	936.5	978.5	541.1	653.9	1019.	925.4	1153.3	1164.1	1302.9	1596.5
6	Continuous	93.4	571.3	504.1	749.2	805.8	538.5	951.9	799.9	1053.6	994.5	1513.7	1320.1	1579.4
12	Continuous	10.0	553.1	512.4	599.0	775.3	559.1	678.1	1042.	1056.9	884.2	1242.9	1337.1	1418.2
13	Continuous	93.4	681.3	674.7	1035.5	1017.0	677.6	966.8	930.6	1034.7	1078.7	1153.6	1269.9	1212.4
16	Continuous	167.2	424.9	548.8	876.9	915.5	599.1	968.5	987.0	795.7	936.9	1063.0	1124.9	919.0
19	Continuous	138.1	376.6	193.4	378.6	596.3	330.1	525.7	583.2	881.3	1028.3	880.3	1007.4	1016.5
22	Continuous	81.6	610.2	503.9	829.8	967.1	518.5	826.5	735.4	688.0	731.7	877.4	1091.9	1358.6
23	Continuous	147.4	616.1	731.6	864.4	924.7	367.6	604.4	520.0	839.7	625.4	935.8	1190.9	1237.3
1	Bolus	129.1	493.3	320.7	1016.1	1071.2	353.1	618.0	915.0	757.1	596.7	300.0	866.8	1329.7
5	Bolus	33.6	374.2	181.6	501.7	640.0	294.3	642.7	464.4	580.1	602.0	841.2	1001.2	1175.1
9	Bolus	31.8	250.9	508.8	752.1	848.5	633.4	1082.4	714.5	930.6	887.0	1237.1	1358.0	1499.6
11	Bolus	87.3	487.5	411.2	904.5	1134.5	705.1	1018.9	985.8	657.2	1221.0	616.3	1291.1	1729.9
15	Bolus	122.2	294.8	361.2	395.3	905.9	695.0	1031.9	1040.	1033.7	1126.9	1387.8	1380.9	1592.4
18	Bolus	139.1	619.4	439.2	913.6	866.0	434.1	927.2	758.2	1144.8	1082.8	848.8	1012.7	1165.9
21	Bolus	63.8	457.2	543.5	845.2	805.2	326.8	838.6	765.7	902.1	918.2	1190.3	1136.4	1364.2
24	Bolus	230.1	452.2	580.6	671.3	869.1	500.2	796.4	713.4	983.9	615.6	902.4	1095.4	1193.5
2	Control	99.0	162.7	290.4	493.9	729.4	473.6	869.8	765.1	849.4	1075.6	843.9	1305.5	1369.2
4	Control	191.3	550.4	405.8	954.7	901.0	746.4	761.8	811.7	1275.2	975.0	1482.3	1217.5	1346.3
7	Control	94.1	655.4	129.8	546.4	1169.7	683.6	886.0	1188.	875.1	864.0	1112.6	1275.4	998.6
8	Control	5.7	466.6	162.4	395.4	803.0	548.8	536.8	766.1	893.3	598.4	646.2	1099.8	1085.4
10	Control	47.0	379.0	534.7	690.0	637.6	662.0	711.2	837.5	1070.5	936.2	1250.3	1100.4	1195.4
14	Control	70.4	325.5	476.4	905.2	863.5	635.2	909.8	974.3	814.7	679.9	842.3	1154.3	836.5
17	Control	46.1	415.3	246.3	494.0	597.7	548.4	858.6	723.7	761.7	754.6	947.3	1443.1	1183.7
20	Control	373.6	517.9	572.6	846.9	1014.7	876.0	980.4	935.1	1132.8	1242.7	1340.9	1332.9	1458.9

Appendix 4.6 cont...

Appendix 4.6 continued

Pig #	Diet	During challenge with *E.coli K88*						Post challenge with *E.coli K88*					
		Day 15 (g)	Day 16 (g)	Day 17 (g)	Day 18 (g)	Day 19 (g)	Day 20 (g)	Day 21 (g)	Day 22 (g)	Day 23 (g)	Day 24 (g)	Day 25 (g)	Day 26 (g)
3	Continuous	881.9	720.5	868.7	1365.5	1566.7	1595.3	1597.6	2019.3	1905.0	1959.0	2160.0	960.0
6	Continuous	1589.6	1739.1	1633.4	1488.6	1557.0	1583.0	1620.1	1996.6	1893.0	2082.0	2590.0	1489.0
12	Continuous	1355.4	1601.5	1171.1	1038.6	1653.0	1346.9	1648.1	1827.9	1689.0	1956.0	1807.0	1377.0
13	Continuous	1408.4	1236.9	1321.2	924.3	1181.5	1345.4	1434.9	1662.7	1706.0	1960.0	1925.0	999.0
16	Continuous	959.2	1226.9	1398.5	1630.6	1088.7	1340.8	1730.1	1533.9	1518.0	1578.0	1558.0	1086.0
19	Continuous	1239.6	862.4	837.9	871.7	1200.8	1641.9	1136.2	1032.1	1465.0	1798.0	1993.0	872.0
22	Continuous	1465.2	1581.6	972.7	1339.5	1270.6	1659.7	1648.9	1950.0	1965.0	2165.0	2146.0	1471.0
23	Continuous	1356.2	1606.1	1615.1	1852.7	1745.7	1538.4	1883.7	1966.7	1945.0	2204.0	2279.0	1501.0
1	Bolus	1438.5	910.6	968.3	1192.5	1564.9	1491.8	1821.7	2008.3	2024.0	1979.0	2042.0	1357.0
5	Bolus	1274.0	1366.0	1226.9	974.1	1288.4	1141.2	1742.8	1285.4	1489.0	1664.0	1950.0	926.0
9	Bolus	1490.5	1515.9	1497.1	1620.2	1538.6	1855.2	2047.1	1964.6	1760.0	2233.0	2074.0	1328.0
11	Bolus	1015.2	1335.3	1069.4	1554.6	1703.5	1841.7	2023.1	1925.7	1807.0	1889.0	2226.0	1254.0
15	Bolus	1274.9	1875.5	1172.9	1243.3	1578.6	1505.0	1696.0	1968.3	1746.0	1930.0	2006.0	1280.0
18	Bolus	1251.2	1783.6	1019.6	1122.4	1292.0	1199.4	1993.8	2002.3	1705.0	1887.0	1930.0	1254.0
21	Bolus	1486.9	1652.4	1495.8	1479.6	1629.5	1559.5	2023.4	1955.9	1786.0	2080.0	1985.0	1378.0
24	Bolus	1343.4	1133.9	1046.1	1110.5	1420.9	1354.7	1822.2	1808.6	1731.0	1787.0	1667.0	1340.0
2	Control	1240.9	1485.2	1139.5	1233.3	1644.6	1692.6	1965.6	2100.3	1496.0	2104.0	2092.0	1355.0
4	Control	1231.6	1774.1	1797.8	1652.3	1842.3	1840.1	2131.5	1653.2	2020.0	2192.0	2248.0	1482.0
7	Control	960.2	1327.8	1418.3	1356.9	1840.2	1663.9	1994.3	2073.1	2028.0	2154.0	2175.0	1310.0
8	Control	981.9	962.1	1087.9	916.1	1523.6	1404.9	1682.8	1928.0	1771.0	1794.0	1724.0	1192.0
10	Control	1539.6	1596.7	1503.2	1688.5	1787.7	1711.2	2096.2	2026.8	1831.0	2048.0	2414.0	1226.0
14	Control	710.5	822.4	1106.0	1245.5	1453.6	1307.9	1444.8	1797.3	1630.0	1919.0	1757.0	1270.0
17	Control	1386.4	1706.4	1318.6	1483.6	1570.8	1481.8	1808.1	1579.4	1776.0	2130.0	1844.0	1418.0
20	Control	1792.7	1170.0	1513.0	1320.6	1745.4	1844.3	1820.8	2327.8	1869.0	2313.0	2216.0	1596.0

Ψ Four weeks trial period

♣ Pig identification number

♦ Type of diet whether continuous Ig, bolus Ig or control (no Ig)

♥ Day of trial

Appendix 4.7

Weight of piglets throughout a trial[ψ] to determine the effect of continuous immunoglobulin feeding versus bolus immunoglobulin feeding on the development of diarrhoea after *Escherichia coli* K88 challenge.

PIG #[♠]	Diet[♦]	Day[♥] 1 (g)	Day 6 (g)	Day 9 (g)	Day 13 (g)	Day 16 (g)	Day 20 (g)	Day 23 (g)
3	Continuous	8295	9612	10688	12668	13360	15553	17800
6	Continuous	6751	8483	9626	11732	13730	15146	17800
12	Continuous	8089	9415	10374	12093	14229	15564	18200
13	Continuous	7889	9353	10083	11681	12953	14024	16600
16	Continuous	9901	10756	11936	13625	14590	15748	17800
19	Continuous	6788	7214	7857	9733	10713	12114	14000
22	Continuous	7869	9478	10445	11841	13784	15351	19000
23	Continuous	8169	8686	9941	11744	13775	15739	19800
1	Bolus	8815	9984	11279	11545	13151	15177	16600
5	Bolus	6509	7515	8014	9509	10593	12171	12200
9	Bolus	7219	11359	11768	14735	16118	18224	19800
11	Bolus	8154	10064	11281	12941	14113	15486	18400
15	Bolus	9429	9987	11105	12751	14230	16191	16800
18	Bolus	8807	10193	10783	12343	13668	14807	16000
21	Bolus	7533	8513	9070	11389	13050	15261	17400
24	Bolus	7532	8818	9638	11393	12332	14395	16000
2	Control	7645	9344	10776	12175	13590	15834	18400
4	Control	9205	10443	11629	14212	15411	17959	19800
7	Control	10316	10820	12150	14091	15483	18067	21200
8	Control	6633	6733	8647	10404	11154	13229	15600
10	Control	5462	8883	9303	11693	13287	15626	17400
14	Control	7691	8580	9497	9629	10577	12393	13800
17	Control	7072	7973	8963	10300	12047	14474	16600
20	Control	9272	11653	12511	14085	16654	18788	21400

[ψ] Four weeks trial period

[♠] Pig identification number

[♦] Type of diet, whether continuous Ig, bolus Ig or control (no Ig)

[♥] Day of trial

Appendix 4.8

Daily observation of the faeces consistency from piglets during a trial[ψ] to determine the effect of continuous immunoglobulin feeding versus bolus immunoglobulin feeding on the development of diarrhoea after *Escherichia coli* K88 challenge.

PIG #[♠]	Diet[♦]	Before challenge with E.coli K88						During challenge with E.coli K88					
		Day[♥]3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
3	Continuous	1[♠]	1	1	1	1	3[♣]	2[♣]	2	1	1	3	1
6	Continuous	1	1	1	1	1	2	2	3	3	3	2	3
12	Continuous	1	1	1	1	1	1	2	1	3	4	4	4[ψ]
13	Continuous	1	1	1	1	1	2	2	2	2	2	2	3
16	Continuous	1	1	1	1	1	1	2	2	3	2	4	3
19	Continuous	1	1	1	5[ψ]	1	1	2	2	1	2	1	1
22	Continuous	1	1	1	1	1	1	4	1	1	1	1	1
23	Continuous	1	1	1	1	1	2	2	1	1	1	1	1
1	Bolus	1	1	1	1	1	1	1	5	4	3	6[Ж]	5
5	Bolus	1	1	1	1	1	1	1	1	2	2	3	2
9	Bolus	1	1	1	1	1	1	1	1	1	1	2	2
11	Bolus	1	1	1	1	1	1	1	1	1	1	2	1
15	Bolus	1	1	1	1	1	1	2	1	2	3	2	2
18	Bolus	1	1	1	1	1	1	2	2	2	3	3	1
21	Bolus	7[♥]	1	1	1	1	2	2	2	2	2	2	5
24	Bolus	1	1	1	1	1	2	2	1	2	2	2	2
2	Control	1	1	1	1	1	1	2	2	3	2	3	2
4	Control	1	1	1	1	1	3	4	3	2	3	5	5
7	Control	1	1	1	1	1	1	2	3	2	2	2	2
8	Control	1	1	1	1	1	1	2	2	3	3	3	3
10	Control	1	1	1	1	1	1	4	6	4	4	3	3
14	Control	7	1	1	1	1	2	2	4	6	6	5	5
17	Control	1	1	1	1	1	1	1	1	2	2	2	2
20	Control	1	1	1	1	1	2	1	1	2	2	2	2

Appendix 4.8 cont...

Appendix 4.8 continued

Pig #	Diet	During challenge with E.coli K88						Post challenge with E.coli K88					
		Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26
3	Continuous	3	6	5	5	2	4	2	2	3	3	2	4
6	Continuous	4	4	6	6	5	2	2	2	2	4	4	2
12	Continuous	4	5	6	6	4	2	2	2	3	3	2	4
13	Continuous	4	3	5	6	6	3	2	2	3	2	3	3
16	Continuous	4	4	1	4	4	3	2	2	2	2	2	2
19	Continuous	2	4	6	6	2	3	2	6	2	2	3	5
22	Continuous	1	1	2	3	3	2	2	2	3	2	2	2
23	Continuous	1	1	1	2	2	2	2	2	2	2	1	2
1	Bolus	2	4	5	5	3	3	2	2	2	2	2	2
5	Bolus	1	2	2	2	2	2	2	2	2	2	2	2
9	Bolus	1	2	1	2	2	2	2	2	2	2	2	2
11	Bolus	3	1	2	1	2	1	2	2	2	2	1	2
15	Bolus	3	4	1	2	4	2	2	2	2	2	2	2
18	Bolus	1	2	1	2	3	2	2	2	2	2	2	2
21	Bolus	2	4	2	2	2	2	2	2	2	2	2	2
24	Bolus	2	2	2	1	2	2	2	2	2	2	2	2
2	Control	3	3	4	5	2	3	2	2	2	2	3	3
4	Control	3	3	5	2	3	2	5	2	5	2	4	3
7	Control	2	5	2	1	2	3	2	2	2	2	1	2
8	Control	2	2	2	2	2	3	2	2	5	2	1	2
10	Control	2	4	5	2	6	4	3	2	2	2	3	2
14	Control	2	5	2	2	2	2	2	2	2	2	2	3
17	Control	3	4	4	2	3	3	2	2	2	2	3	2
20	Control	1	2	2	2	6	2	2	2	2	2	2	2

- Four weeks trial period
- Pig identification number
- Type of diet, whether continuous Ig, bolus Ig or control (no Ig)
- Day of trial
- 1 = Hard pelleted; 2 = Hard but together; 3 = Soft, 4 = Very soft; 5= Semisolid; 6 = Watery; 7= No faeces observed

Appendix 4.9

The percentage of free liquid content in the faeces[♠♠] of piglets during the trial[Ψ] to determine the effect of continuous immunoglobulin feeding versus bolus immunoglobulin feeding on the development of diarrhoea after *Escherichia coli* K88 challenge.

Pig #[♣]	Diet[♦]	Before challenge with E.coli K88					During challenge with E.coli K88			
		Day[♥] 3	Day 4	Day 5	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
3	Continuous	0	0	0	0	0	0	0	0	6
6	Continuous	0	0	0	0	0	3	14	10	2
12	Continuous	0	0	0	0	0	3	0	33	35
13	Continuous	0	0	0	0	0	0	0	0	11
16	Continuous	0	0	0	0	0	0	0	3	0
19	Continuous	0	0	0	0	0	3	0	0	0
22	Continuous	0	0	0	0	0	0	0	6	0
23	Continuous	0	0	0	0	0	0	0	0	0
1	Bolus	0	0	0	0	0	0	9	17	13
5	Bolus	0	0	0	0	0	0	0	0	13
9	Bolus	0	0	0	0	0	0	0	0	0
11	Bolus	0	0	0	0	0	0	0	0	0
15	Bolus	0	0	0	0	0	2	0	0	3
18	Bolus	0	0	0	0	0	6	0	0	7
21	Bolus	0	0	0	0	0	0	0	0	0
24	Bolus	0	0	0	0	0	5	0	0	5
2	Control	0	0	0	0	0	0	0	9	5
4	Control	0	0	0	0	0	6	10	0	20
7	Control	0	0	0	0	0	0	0	0	3
8	Control	0	0	0	0	0	5	0	14	19
10	Control	0	0	0	0	0	20	76	14	18
14	Control	0	0	0	0	0	10	27	66	35
17	Control	0	0	0	0	0	10	0	0	11
20	Control	0	0	0	0	0	0	0	0	0

Appendix 4.9 cont..

Appendix 4.9 continued

Pig #	Diet	During challenge with *E.coli K 88*								Post challenge with E.coli K88
		Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21
3	Continuous	6	5	13	53	2	6	3	11	0
6	Continuous	4	8	11	13	11	10	31	0	0
12	Continuous	23	10	13	16	48	32	9	0	0
13	Continuous	0	8	13	29	8	50	36	0	0
16	Continuous	0	10	4	15	8	20	8	19	0
19	Continuous	0	0	0	20	9	30	0	6	53
22	Continuous	0	0	0	0	0	0	12	0	0
23	Continuous	0	0	0	0	0	0	4	0	0
1	Bolus	55	0	0	28	35	7	7	6	0
5	Bolus	3	0	7	0	3	4	3	0	0
9	Bolus	0	0	0	0	0	4	0	17	0
11	Bolus	0	5	20	0	0	0	3	5	40
15	Bolus	25	21	5	16	4	0	3	0	0
18	Bolus	8	16	0	6	8	4	8	0	0
21	Bolus	0	10	10	8	2	9	0	0	0
24	Bolus	7	4	3	0	15	18	9	0	0
2	Control	9	0	16	14	13	31	4	10	0
4	Control	21	0	0	8	24	28	27	8	31
7	Control	4	7	7	31	3	4	0	18	0
8	Control	12	16	0	5	0	0	0	3	0
10	Control	6	3	9	25	33	25	25	11	19
14	Control	23	10	15	30	0	0	7	0	0
17	Control	0	0	2	11	6	19	0	7	0
20	Control	3	3	17	0	0	39	20	30	0

** Measured as % of free liquid in the faeces after centrifugation at 6000 RPM for 20 minutes at $4^{O}C$

ψ Four weeks trial period

• Pig identification number

• Type of diet, whether continuous Ig, bolus Ig or no Ig

• Day of trial

CHAPTER 5

GENERAL SUMMARY, DISCUSSION AND RECOMMENDATIONS

Humans have many defences against microbial invasion. Non specific immune mechanisms include natural, species-specific immunity, physical and chemical barriers (e.g. skin, body secretions), phagocytosis, and inflammation (Barrett, 1976). Specific immune responses are mediated by B cells, which produce antibodies, and T cells, which control the cellular response to infection (Larson, 1995). Nutritional status, stress, age, certain congenital abnormalities, and steroids are among the human host characteristics that affect the immune system and susceptibility to infection (Rosen & Seligmann, 1993).

The important pathogenic organisms causing diarrhoea are *Escherichia coli* (*E.coli*, Mietens et al., 1979; Tacket et al., 1988), rotavirus (Hoshino et al., 1984; Green et al., 1989; Snodgrass et al., 1990), Shigella species (Levine et al., 1974; Black, 1986; Cohen et al., 1988), Campylobacter species (Skirrow, 1977; Prescott & Munroe, 1982), *Vibrio cholerae* (McClead et al., 1988), *Clostridium difficile* (Lyrely et al., 1991), and Cryptosporidium (Nord et al., 1990; Ungar et al., 1990).

Infectious diseases can be treated by antibiotics and prevented by vaccines and the use of immunoglobulins (Igs). The use of antibiotics is not very suitable for treatment as they have side effects (Bartlett et al., 1978; Chang et al., 1978; Murray et al., 1982; Sack, 1986; DuPont et al., 1986; Murray, 1986), whereas, vaccine approaches might provide an easier and possibly more cost effective means to prevent enteric diseases. Yet, safe and effective vaccines are not always available for all common enteric pathogens. The use of passive antibodies has a number of attractive features such as high potential for safety, specificity, immediate effectiveness, and efficacy. Antibodies are thought to be effective by binding and neutralising microbial antigens (Woode et al., 1975; Zinkernagel & Colombini, 1975; Acres & Babjuk, 1978; Yolken et al., 1985; Kelly et al., 1996), blocking the binding of virus and bacteria to target cells (Demierre et al., 1975; Pahud et al., 1987) and by acting as opsonins thereby initiating uptake and degradation by phagocytes and in complement mediated bactericidal mechanisms

(Rivier & Sobotka, 1978; Hilpert et al., 1977; Van Furth. et al., 1984; Porter et al., 1987).

Human breast milk, containing secretory immunoglobulin A (sIgA), had been in use since ancient times as eye "drops" and nose "drops" to combat infections. Secretory immunoglobulin A has been shown to protect against diarrhoea in infants (Cunningham, 1977; Larsen & Homer, 1978; Barnes et al., 1982; Glass et al., 1983). Enriched human immunoglobulin A (IgA) obtained from serum when given orally to prematurely born children has demonstrated a prophylactic effect against the development of necrotizing enterocolitis (a disease with a presumed infectious etiology) and a therapeutic effect in immunodeficient patients suffering from *E.coli* (Ebil et al., 1988; Firmino et al., 1991).

Administration of Igs can be done intramuscularly (IM), intravenously (IV), subcutaneously and orally. As discussed earlier in the literature review IM, IV and subcutaneous methods have side effects and obviously oral administration is preferable. Many researchers have been engaged in investigations of oral immunization against *E.coli* and other micro organisms using bovine immunoglobulins and results have indicated that oral immunization can provide an important method of controlling diseases caused by *E.coli* and other micro organisms (Mietens et al., 1979; Ebina et al., 1985; Tzipori et al., 1986; Tacket et al., 1988; Brunser et al., 1992; Tacket et al., 1992). Oral immunization provides a significant contribution to animal health and performance.

Studies have been carried out with bovine colostrum or milk Igs concentrates for passive protection against diarrhoea administered orally (Mietens et al., 1979; Ebina et al., 1985; Brüssow et al, 1987; Tacket et al., 1988; Boesman-Finkelstein et al., 1989; Davidson et al., 1989). The product was obtained from nonimmunized or hyperimmunized cows against various pathogens. In this study, whey globulin concentrate (WGC) is the source for Igs, derived from nonimmunized mature milk. The WGC contained 0.9% IgA, 6% immunoglobulin G (IgG), and 0.6% immunoglobulin M.

E.coli has been chosen for the research, as it is the main causative organism for diarrhoea in developing countries (Black et al., 1982; Guerrant et al., 1983), immuno-compromised people (Kreger et al., 1980; Tancrède & Andremont, 1985) and traveller's

diarrhoea (Tacket et al., 1988). Many researchers had administered Igs in multiple doses and found it effective as therapy or prophylaxis against *E.coli* diarrhoea. The comparison of the effect of a single large dose of bolus Ig to the same amount of Igs given in three equally divided doses was investigated as part of this study.

Immunoglobulin must be able to resist digestion in the gut by proteolytic enzymes to be effective. Several investigators suggest bovine IgG_1 is partially resistant to proteolytic enzymes and therefore may retain some specific antibody activity after passage through the gastrointestinal tract. As no one has looked at how long Igs can persist in the gut, this experiment was conducted to estimate the amount of undigested Igs in the gut 24 hr after feeding.

Piglets have been shown to be a suitable model for human gastrointestinal physiology (Darragh & Moughan, 1995).

In chapter 2, a pilot study was done to determine the rate at which digesta moved along the gut and the quantity of digesta collected in different parts of the gastrointestinal tract at particular time intervals after feeding. This information will be used in the next trial to measure the quantity of IgG present in the gut. Sufficient amount of digesta was found in various parts of the gastrointestinal tract even 24 hr after feeding and this information was helpful to plan out the IgG digestibility trial (Chapter 3).

It is recommended to conduct a study by feeding all the piglets at the same time, and determine the transit time and the quantity of digesta collected at different time intervals as specified in the experiment in chapter 2 and compare the results with it.

In Chapter 3, the experiment was conducted to measure the quantity of undigested IgG present in the various parts of gastrointestinal tract after 24 hr feeding. As there is no previous scientific evidence that IgG can resist digestion upto 24 hr after feeding, various time intervals had to be utilized. In the experiment, twenty-five piglets were divided into five equal groups and fed on an experimental diet. One piglet from each group was slaughtered according to the plan at the specified times. The largest quantity of undigested IgG was found in the stomach, with a gradual reduction in quantity throughout the small intestine, while there was no IgG found in caecum and colon

except in a few piglets. The quantity of undigested IgG (range, 8 ± SD) in the different parts of the gastrointestinal tract at various times is given in Table 5.1. The results of this experiment indicated that undigested IgG was present in stomach and small intestine even after 24 hr feeding.

In this experiment, undigested IgG was measured by radio immunodiffusion assay which is not a very sensitive method. If a more sensitive method had been used, the presence of IgG may have been detected in caecum, colon and faeces, yet the quantity would not have been sufficient to provide passive immunity.

In chapter 4, the experiment was conducted to determine the effect of a single large dose of bolus Igs feeding versus the same quantity divided into three equal doses of continuous Igs feeding against *E.coli* K88 diarrhoea. The twenty-four piglets were randomly divided into three equal groups namely control group, "continuous Ig" group and "bolus Ig" group. The control group was given an Igs free diet. The continuous Igs feeding group was given feeds containing 10% Igs three times a day. The bolus Igs feeding group was given a diet containing 30% Igs in the morning and Igs free diet at the other two feeds. The piglets were fed on their respective diets for eight days and on the day 9 inoculated with $1x10^9$ cfu *E.coli* K88 into their throats before their morning feed, and observed for nine days. Then all piglets were fed on control diet and observed for further three days. The piglets were observed for the incidence of diarrhoea, length of diarrhoea episode and severity of diarrhoea by faecal culture, scoring faeces and measurement of the free liquid content in the faeces. The highest incidence of diarrhoea was found in the control group, lesser in the continuous group and least in the bolus group. The diarrhoea incidence in each group for Day 3 – 21 is given in Figure 5.1.

The microbial culture for *E.coli* K88 could not be done as the faeces had been left in the fridge and freezer for a long duration. It is therefore recommended that microbial culture from faeces for *E.coli* K88 to be done within 24 hrs of faecal collection. If this method is not possible, faeces should be stored at –80°C. Three colonies were picked up from each agar plate at random to identify the presence of *E.coli* K88. It may be possible that the sensitive colonies may have been missed due to the small number of colonies randomly selected. It is suggested to increase the number of colonies to be

Table 5.1

The percent of undigested bovine IgG (range and $\bar{X} \pm SD$) found in different parts of a piglets gastrointestinal tract is given as relative to the amount consumed as a single bolus experimental diet containing 2% IgG at different times after feeding.

Time (hrs)[φ]	1 hr[φ]	5 hrs	9 hrs	16 hrs	24 hrs
STM[♣]	18.1-37.8	18.2-37.2	5.2-17.9	1.6-26 7	0-5.8
	$\bar{X}$±SD = 28.8±13.31	$\bar{X}$±SD = 29.2±13.01	$\bar{X}$±SD = 10.0±4.84	$\bar{X}$±SD = 8.3±3.71	$\bar{X}$±SD = 1.7 ±0.84
SI1[♦]	1.3-17.1	6.8-18.3	2.7-11.1	0-12.9	2.9-210.3
	$\bar{X}$±SD = 7.3±3.34	$\bar{X}$±SD = 13.6±6.1	$\bar{X}$±SD = 6.6±2.92	$\bar{X}$±SD = 7.1±3.28	$\bar{X}$±SD = 4.4±1.96
SI2[♥]	0.7-6.6	0.8-6.1	0.3-6.2	5.6-12.6	3.6-89.6
	$\bar{X}$±SD = 2.1±0.91	$\bar{X}$±SD = 3.2±1.43	$\bar{X}$±SD = 2.9±1.31	$\bar{X}$±SD = 8.2±3.72	$\bar{X}$±SD = 15.8±7.19
SI3[♠]	0.8-3.9	0.6-1.4	1.1-5.5	2.0-7.4	2.6-24.2
	$\bar{X}$±SD = 2.8±1.32	$\bar{X}$±SD = 0.9±0.40	$\bar{X}$±SD = 2.4±1.14	$\bar{X}$±SD = 4.8±2.14	$\bar{X}$±SD = 4.2±1.95
CCM[#]	0-0.2	0	0-1	0	0
	$\bar{X}$±SD = 0.05±0.022		$\bar{X}$±SD = 0.2±0.085		
COL[θ]	0-0.3	0	0	0	0
	$\bar{X}$±SD = 0.06±0.026				

[φ] Time (hrs) sampled after feeding experimental diet
[♣] STM = Stomach
[♦] SI1 = Small intestine 1 (first third of the small intestine)
[♥] SI2 = Small intestine 2 (second third of the small intestine)
[♠] SI3 = Small intestine 3 (last third of the small intestine)
[#] CCM = Caecum
[θ] COL = Colon

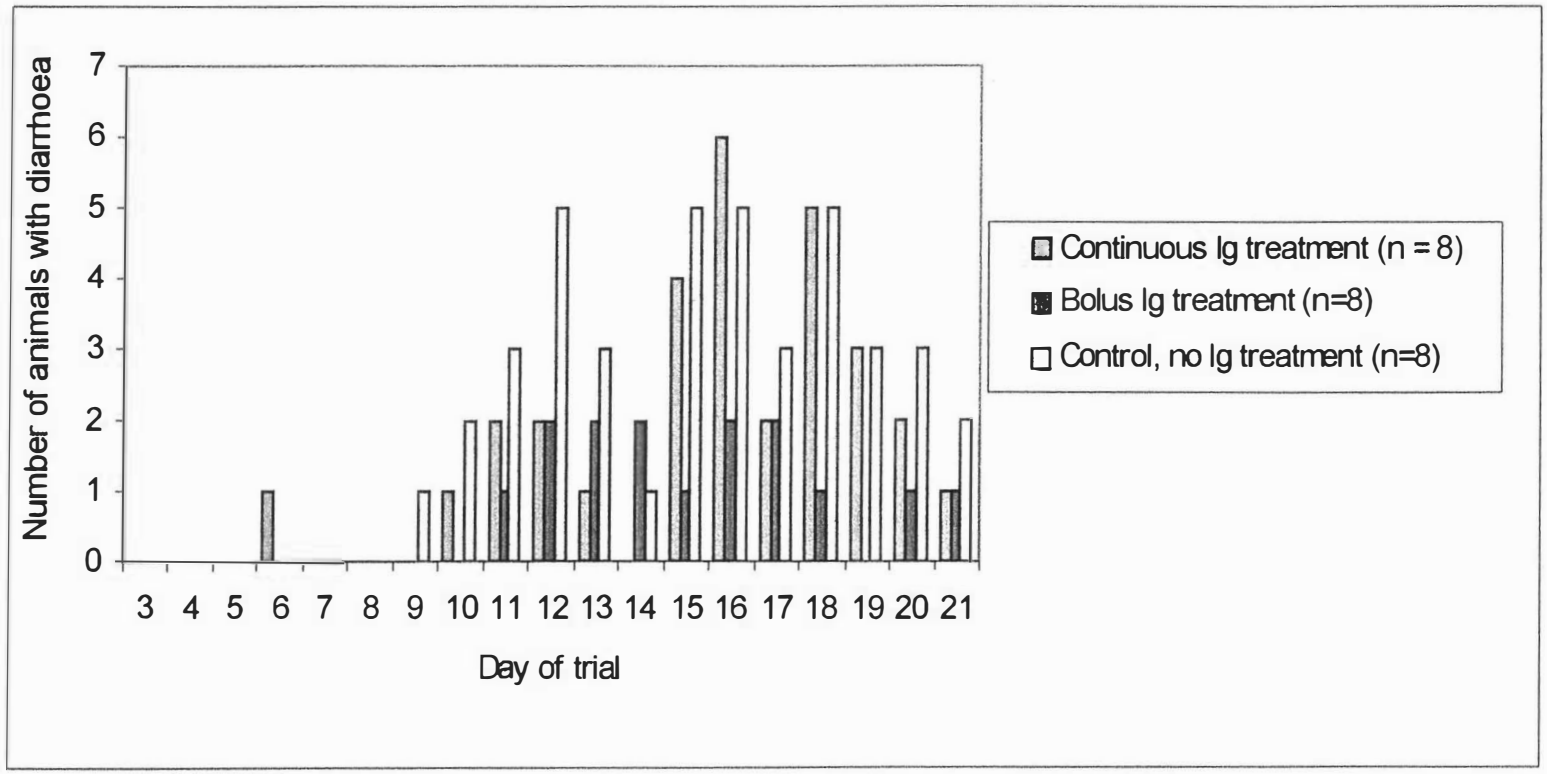


Fig 5.1 Number of piglets that had diarrhoea each day through out the trial in the continuous Ig group, bolus Ig group and control, no Ig group after challenge with *Escherichia coli K88* on Day 9 to determine the effect of continuous immunoglobulin feeding versus bolus immunoglobulin feeding on the development of diarrhoea

selected to ensure better results for the presence of *E.coli* K88. The scoring was done physically, by the feel of the faeces by the fingers. This apparently appears not to be an accurate assessment. It is recommended that another more suitable method of scoring of the faeces be used.

According to the study, "bolus Igs" had protective effect against *E.coli* K88 diarrhoea in controlling water loss but not bacterial shedding. If the dosage is increased it may possibly prevent both, water loss and shedding. It is recommended to investigate: whether Igs should be served prior or after inoculation of *E.coli* K88, and to define the best time interval, between Igs feeding and the inoculation; whether the single dose of immunoglobulin be fed with the morning or the evening meal will provide better prophylactic effect; factors that can affect the digestion of Igs; whether an increase in the dose level for an optimum effect in prevention of diarrhoea or the prevention of Igs from digestion is more cost effective.

As diarrhoea is a prevalent disease among all age groups, around the world, it is of utmost importance to investigate suitable treatment and prophylaxis. The study conducted also supports the literature review of earlier researchers and furthermore proves that bolus Ig feeding is a better prophylaxis than continuous Ig feeding. The prophylactic effect of Igs for the prevention of diarrhoea may be due to the fact that it does not undergo complete digestion in the gut. Study indicated that IgG was present in the stomach and small intestine even after 24 hr of feeding. It could be assumed that a large single (bolus) dose of Ig would have neutralized the toxins and suppressed the multiplication of *E.coli* K88. A single dose of orally administered Igs is a suitable method for prevention or treatment of diarrhoea by *E.coli* K88. The fact that individuals at risk are liable to forget to take medication at the specified times especially in the case of multiple doses, it can lead to an ineffectiveness of the medication. To overcome this problem a single large dose is recommended, as this study supports this theory.

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