FACTORS CAUSING FEED INTAKE DEPRESSION IN LAMBS INFECTED BY GASTROINTESTINAL PARASITES

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To Mum (in memory) and Dad

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A reduction in voluntary feed intake is a major factor in the lost productivity of grazing lambs infected by gastrointestinal parasites yet the mechanisms involved are poorly understood. Potential pathways involved in parasite-induced feed intake depression were investigated in lambs with minimal previous exposure to parasites and artificially infected by the small intestinal parasite *Trichostrongylus colubriformis*. Six *in vivo* experiments were conducted on lambs housed in individual pens or metabolism crates with similar feeding and experimental procedures.

In Experiment 1 (Chapter 4) the effect of *T. colubriformis* infection on short term feed intake in lambs and of some pharmacological agents on feed intake depression were investigated. Prior to and for the duration of infection, lambs were fed once per day and feed intake recorded at regular intervals over the day (8 h). Following the onset of feed intake depression in the infected group (9 weeks after commencing dosing), all animals were treated with an analgesic (codeine phosphate *per os*), an anti-inflammatory agent (indomethacin *per os*), a CCK antagonist (L364-718 by subcutaneous injection) or saline (control) in a replicated Latin square design (n = 8). Although the pattern of feed consumption was similar in infected and non-infected lambs, average daily intake was reduced 32 % and short term intake (recorded at 10 minute intervals for the first hour of feeding, 15 minute intervals for the second hour and hourly for the next 6 hours of feeding) reduced 40 % by infection. This identified the key component by which intake was depressed and enabled the use of a short term intake model and short duration of action compounds to identify the pathways involved in intake depression in this sequence of experiments. None of the pharmacological treatments increased intake in the infected group. These results suggest a reduction in the rate of consumption due to reduced hunger signals, rather than change of meal eating patterns, is the major cause of feed intake depression.

Specific conclusions about the pathways investigated using the pharmacological agents could not be obtained.

Experiment 2 (Chapter 5) was designed to investigate the roles of pain and osmolality on feed intake depression. Digesta samples collected prior to and during parasite infection and before and after feeding had similar osmolalities (240-260 mosmol/1) which indicated that feeding or infection had no effect on osmolality of digesta. Following the onset of feed intake depression in infected animals, all animals were treated in a Latin square design (n = 4) with no treatment, saline, local anaesthetic (xylocaine) or analgesic (codeine phosphate) solution 15 minutes before feeding, by slow injection into the duodenum. There was no effect of these treatments on food intake. In the second part of the experiment, hyperosmotic solutions (mannitol and NaCl) markedly depressed short term intake in non-infected animals, suggesting a role for osmoreceptors in intake regulation. However these effects were not blocked by local anaesthetic so the depressed intake may have resulted from generalised malaise rather than from specific osmoreceptor effects.

In Experiment 3 (Chapter 6) the role of peripheral CCK on intake depression was examined by a dose-response study utilising the CCK antagonist, loxiglumide. Intravenous injection of 5, 10 or 20 mg/kg LW of loxiglumide to infected lambs 10-15 minutes before feeding (n = 6) had no effect on feed intake at any of the dose levels. In experiment 4 (Chapter 7) loxiglumide was infused intravenously for 10 minutes (30 mg/kg/h) before feeding and for the first 2 h (10 mg/kg/h) after feed was offered to minimise any effect of the rate of clearance of loxiglumide on the lack of feed intake response. As well, the rate of marker disappearance from the abomasum was recorded in both infected and non-infected animals. Continuous infusion of loxiglumide did not attenuate parasite induced intake depression nor did it have any effect on abomasal emptying. Abomasal volume was reduced by infection (66.3 vs 162 ml) as was the fractional outflow rate (2.2 vs 2.8 ml/min) but these differences were accounted for by the lower level of feed intake in the infected animals.

In Experiment 5 (Chapter 8) brotizolam, a benzodiazepine appetite stimulant, thought to act on the hypothalamus, was administered in a dose-response study to infected and non-infected animals (n = 4) immediately prior to feeding or following termination of the first meal (45 minutes after feeding) and the feed intake response recorded. Brotizolam elevated both the short term (0-0.75 h), daily (22 h) intake and all time intervals in the first 5 h after feeding in infected and non-infected animals when administered after the first meal but when

administered prior to feeding elevated intake only over the first 6 h of feeding. In both cases the magnitude of the response was greater in infected animals than in non-infected animals. Brotizolam appeared to increase the rate of eating without having a major impact on meal eating patterns when administered before feeding. Where administration was after the first meal, the effect was due to an "extra" meal being consumed. These findings showed that infected animals can respond to central stimulators of intake although the mechanism of the response is not known.

Opioids were implicated in intake depression as the rate of intake rather than meal patterns appeared to be the major parameter depressed under parasitism. This was examined in experiment 6 (Chapter 9) where animals (n = 6) were fasted for 26 h or not fasted, then treated with saline (control), brotizolam (intake stimulant) or naloxone (opioid antagonist) immediately prior to feeding. Fasting stimulated feed intake in the short term (100 % increase in 75 min) and over the day (12 % increase) in both infected and non-infected animals. Following fasting, infected animals ate a similar amount of feed to the non-infected, fasted animals and more than the non-infected, non-fasted animals. The signals resulting from a one day fast were sufficient in the short term to override parasite induced mechanisms causing feed intake depression. Naloxone suppressed the intake stimulatory effects of a 26 h fast in both infected and non-infected animals, which supports a role for endogenous opioids as hunger signals. Where animals were not fasted, naloxone reduced intake only in the non-infected animals which suggested endogenous opioid levels may be lower in infected animals than in non-infected animals.

In the final experiment (Experiment 7, Chapter 10) the role of central hunger and satiety mechanism were investigated. Infected and non-infected animals (n = 6) were treated with naloxone or saline by intravenous injection, or saline and met-enkephalinamide (an opioid analogue) by intracerebral infusion, or naloxone and the opioid analogue simultaneously to investigate the role of central opioids in feed intake depression. To determine the role of CCK induced satiety signals on feed intake at a central level, loxiglumide and CCK were infused separately and in combination for 30 minutes prior to feeding and for the first 60 minutes of feed on offer, into a lateral cerebral ventricle of the brain of infected and control animals (n = 6).

The opioid analogue tended to increase intake in infected animals but the effect was not significant probably because the dose used was too low to elicit a response in sheep.

Naloxone depressed intake only in the infected animals, which conflicted with the results of Experiment 4. As a consequence these results were inconclusive because of the single low dose of opioid analogue used and the conflicting naloxone responses.

CCK alone depressed intake by 39-52 % only in infected animals and this effect of the 90 minute infusion was evident over the 8 h short term recording period. Loxiglumide attenuated the feed intake depressive effects of CCK in the infected animals to the extent that intake was elevated above control levels. Loxiglumide alone was an intake stimulant in both infected and non-infected animals. Intake was increased over the entire 8 h but mostly in the second hour when intake was increased by 188 % in infected animals and by 16 % in the non-infected animals and resulted in almost continuous eating. These results showed loxiglumide will temporarily block the effect of parasite infection on feed intake in sheep when administered centrally and the fact that it blocked the effects of exogenous CCK on intake indicated that the effect is mediated via CCK receptors.

In conclusion GIT parasite infection reduced both short term and daily feed intake apparently by a change in rate of intake rather than any alteration in meal patterns. It was further suggested that any one of a number of potential peripheral pathways, including changes to osmolality, gut emptying, pain and inflammation of the gut, alone is not involved in anorexia in sofar as the compounds used could block these factors and the results support the idea that intake depression is mediated via a central mechanism. Intake in infected animals responded to a much greater extent when fasting, i.c.v. loxiglumide or brotizolam were employed. Feed intake thus appears to be regulated through the same mechanisms in infected and non-infected animals. The results from compounds affecting the central mechanism suggest central CCK receptors are important in parasite induced anorexia, possibly by changing the onset of satiety or by interacting with endogenous opioids to reduce the rate of feed intake. Secondly reduced endogenous opioids may be causing the reduction in the rate of feed consumption alone or as a result of other interactions. It was concluded that intake in parasitised animals could be increased to that of control animals by employing procedures and compounds thought to act on the hypothalamus.

Keywords feed intake; gastrointestinal parasites; *T. colubriformis*; CCK antagonists; L364-718; loxiglumide; fasting; opioids; naloxone; pain; osmolality; inflammation.

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CHAPTER 1

Introduction

Gastrointestinal parasite infection is a common and ongoing source of lost production, particularly for young animals grazing pasture. In excess of 70 million dollars (NZ) is spent annually on anthelmintic treatment in New Zealand. However subclinical parasite infection remains the hidden cost to farmers in terms of unseen lost production and development of anthelmintic resistance which often remains undetected until a crisis in the flock health occurs.

Research workers have implicated lesions within the relevant organs for the depressed intake, with the focus on gut pathology rather than on the feed intake depression. This is perhaps understandable since much of the local damage occurs in locations known to house receptors which monitor wall tension and changes in digesta content. However this appears rather simplistic because intake falls progressively and is more marked in association with low quality diets. Identification of the mechanisms or pathway(s) involved in intake depression may provide suggestions for a means to block their action. If intake can be increased in parasitised animals this is likely to minimise the reduction in animal performance observed in parasite infection and hasten the development of immunity or self cure.

In this thesis potential mechanisms involved in causing feed intake depression are investigated. The effects of infection on feed intake were studied initially and led to the use of a short term feed intake model to screen a number of potential pathways in parasite induced anorexia.

CHAPTER 2

Review of the Literature

2.1 Introduction

Effects on productivity

Gastrointestinal nematodes are a major cause of impaired productivity in grazing ruminants, which is manifest in a variety of ways, including reduced voluntary feed intake, live-weight gain, wool growth and changes in carcass composition.

The magnitude of losses in production varies with the parasite species and the level of infection. Lower levels of infection do not measurably affect production, but with heavier infections the effects may be more dramatic and in severe cases death may result. Young animals or those on a low plane of nutrition are most severely affected by infections of the gastrointestinal tract (GIT).

The losses in production outlined above are the end result of parasite infection of the GIT. Research activity has focused on aspects of the disease process, particularly disturbances to the function and/or structure of tissues, feed intake and the efficiency of use of metabolisable energy. This review will examine the scientific literature on these effects of parasite infection on the host.

Two factors have been identified as major causes of impaired productivity. The first, anorexia or a depression in voluntary feed intake, is the focus of this study but one which remains poorly understood. This review will therefore include a major section on factors thought to control feed intake in healthy animals. The second, reduction in utilisation of metabolisable energy has been extensively studied (Bown 1986). However, the effects of parasites on nutrient utilisation by the host are reviewed here because these disturbances to gut function and nutrient availability to the tissues may be blocking or potentiating existing pathways in feed intake regulation or influencing other regulatory pathways to produce feed intake depression.

2.2 Feed intake

The most significant effect of gastrointestinal parasitism on the host is a depression in voluntary feed intake. The extent to which this reduction in feed intake or anorexia occurs is dependent on the numbers of larvae ingested and the species of helminth involved (Symons, 1985). Reduced intake *per se* is always important in infections and invariably a major contributor to lost production, accounting for 40-90 % of the observed weight differences between infected and non-infected animals.

Where possible the review of the literature will be restricted to situations in which trickle infections have been used to produce a sub-clinical infection which is more akin to the situation faced by grazing ruminants than the acute infections caused by single massive doses of parasites.

2.2.1 Effect of number of larvae ingested

Generally the greater the larval intake the greater the degree of inappetence suffered by the host. However some caution is needed in the interpretation of experimental data, because of the effect of different experimental conditions including the unknown effect of relative pathogenicity of different parasite strains and any differences in susceptibility of sheep breeds to parasite induced intake depression.

The small intestinal parasite *Trichostrongylus colubriformis* (*T. colubriformis)* depresses feed intake in a dose dependent manner. Steel, Symons and Jones, (1980) found anorexia occurred mainly during weeks 8-12 of infection, with feed intake being depressed relative to non-infected animals by 30, 40 and 60 % at larval intakes of 3 000, 9 500 and 30 000 per week respectively. In contrast 17 500 larvae/week depressed intake by only 20 % (Sykes and Coop, 1976; Coop, Sykes and Angus, 1976) to 40 % (Kimambo, M^{ac}Rae, Walker, Watt and Coop, 1988).

The abomasal parasite *Teladorsagia circumcincta (O.circumcincta)* depressed intake by up to 20 % following a trickle infection of 4 000 larvae/day (Sykes and Coop, 1977) and 5 000 larvae/day (Coop *et al.*, 1977). In conflict with this, and highlighting the impact of differences in experimental conditions, Steel *et al.* (1980) found dose rates up to 5 300/day had no effect on feed intake and that dose rates of up to 17 000/day were required to reduce

intake by 20 %.

2.2.2 Effect of parasite species

Sheep with either trichostrongylosis or ostertagiasis suffer reduced voluntary feed intake, but as discussed in the previous section, there are species differences in the number of larvae required to induce anorexia.

Much of the work has considered only monospecific parasite infections (Sykes and Coop, 1976; Coop *et al.*, 1977; Steel *et al.*, 1980 and Symons *et al.*, 1981) whereas polyparasitic infections are more common in sheep grazing pasture. Sykes, Poppi and Elliott (1988) found *T. circumcincta* and *T. colubriformis* depressed feed intake by 8 and 10 %, respectively, while a simultaneous infection with the same number of larvae reduced feed intake by 30 %, demonstrating a multiplicative rather than additive effect of simultaneous infection on feed intake.

The multiplicative rather than additive effect of polyparasitic infections on feed intake suggests that more than one pathway may have a role in signalling feed intake depression.

2.2.3 Effect of nutritional status of host

It is generally accepted that the nutritional status of sheep can affect their susceptibility to gastrointestinal parasites (Whitlock, 1949 cited by Holmes, 1986; Gibson, 1963).

Where lambs infected by *H. contortus* (Abbott *et al.*, 1985a, b; Abbott *et al.*, 1988) were offered a diet containing either 88 or 199 g crude protein per kg dry matter, the results suggested that crude protein *per se* did not influence the establishment of a single infection. However, lambs on the low protein diet showed more severe clinical signs including weight loss, anaemia and inappetence despite apparently similar levels of blood loss. Bown, Poppi and Sykes (1986) infused protein (50 g/day) or isocaloric glucose into the abomasum, calculated to provide sufficient protein to increase nitrogen retention to that of a non-infected lamb. Feed intake depression was less in both infused groups than in the control group (22 % vs 32 %) while post-mortem worm burdens were much lower in the protein infused animals than in both the glucose infused and control groups. The development of resistance to parasite infection was impaired by a low protein status.

2.2.4 Summary

In summary, a reduction in voluntary feed intake has a major impact on the productivity of sheep. Both the number and type of parasite present affect the degree of inappetence and therefore losses to production. Doses of 3 000 - 30 000/week *T. colubriformis* or *T. circumcincta* generally reduced feed intake by 20 - 60 % in young lambs, usually during weeks 6-12 of infection. Polyparasitic infections probably result in greater inappetence than monospecific infections. Finally the nutritional status of the sheep may affect their susceptibility to parasites. A higher intake of protein reduces but does not prevent anorexia and has a beneficial effect on the immunological response of the sheep to parasitic challenge.

2.3 Gastrointestinal structure and function

Parasites reduce the efficiency of utilisation of metabolisable energy and numerous disturbances to the GIT have been identified which appear likely to contribute to this reduction in efficiency. One or a combination of these disturbances may have a role in stimulating or blocking chemical, neural, hormonal or a combination of these potential regulators of feed intake. Alternatively another pathway not usually involved in feed intake regulation may be triggered by other disturbances to the gastrointestinal tract.

2.3.1 Structural and histopathological changes of gastrointestinal tissues

Marked changes in the histological appearance of tissues are a feature of GIT parasite infection. Many of these changes and the associated tissue dysfunction can be attributed directly to penetration, migration and feeding habits of the parasite concerned. For example *T. circumcincta* and *T. axei* lesions are the result of destruction of gastric glands, coinciding with the growth of developing larvae and their emergence from the gastric mucosa. Extensive replacement of functional GIT cells by undifferentiated cell types as well as epithelial hyperplasia, crypt elongation and inflammation which together produce thickening of the mucosa and leak lesions (Dargie, 1980) are common.

2.3.2 Gastrointestinal motility

Diarrhoea, commonly associated with GIT parasite infection, is indicative of disturbances to

digestive tract motility. Diarrhoea is usually only recorded in acute infections and occurs transiently, commonly at the time of adult emergence, disappears spontaneously (Sykes and Coop, 1976; Coop *et al.*, 1977) and does not appear to have a role in or result from feed intake depression.

2.3.3 Endogenous losses

Mucosal cells

Increased desquamation of mucosal epithelium cells and/or increased mucus production may explain some of the unaccounted endogenous N losses in infected sheep. Increased cell proliferation has been suggested from increased mitotic figures in the crypts with *T. vitrinus* (Coop and Angus, 1975; Coop *et al.*, 1979) and *H. contortus* (Rowe *et al.*, 1982). Proliferation of goblet cells and increased mucus production have been reported with *ostertagiasis* (Armour *et al.*, 1966; Murray *et al.*, 1970) and in some cases *T. vitrinus* (Jackson *et al.*, 1983).

Plasma proteins

Loss of protein into the GIT is a distinctive feature of GIT parasite inf dependant with *T. colubriformis* infection but greater than could be explained by a reduction in feed intake aloections and usually associated with lowered plasma albumin and elevated plasma globulin concentration. Hypoalbuminaemia was dose dependent with *T. colubriformis* infection but greater than could be explained by a reduction in feed intake alone (Coop *et al.*, 1976; 1977). Increased enteric catabolism and decreased synthesis appears responsible for the observed depletion in the plasma albumin pool (Steel *et al.*, 1980). Parasitised sheep seem to be unable to increase the synthesis of albumin in response to excessive enteric loss, especially when N intake is depressed (Dargie, 1975; 1980). The increased plasma concentration of globulin results from increased synthesis, greater than enteric catabolism, which may be associated with the development of an immunological response to infection.

2.3.4 Digestion, absorption and metabolism

Protein digestion and absorption

The amount of protein available to parasite infected lambs is reduced by anorexia, but pair feeding experiments indicate that anorexia alone does not account for all the observed changes in protein availability. The nitrogen (N) content of digesta at the ileum increased

et al., 1985b). However true digestibility and absorption in the small intestine are unchanged during infection (Poppi *et al.*, 1981; Symons and Jones, 1970). These findings led the authors to conclude that the extra protein was of endogenous origin.

Protein metabolism

The fractional synthetic rate and amount of protein synthesis per day appear to increase in infected lambs but not in pair-fed controls (Jones and Symons, 1982). Symons *et al.* (1981) concluded that anorexia, enteric protein losses and increased intestinal tissue protein metabolism resulted in a net movement of amino acid N from muscle and possibly skin to the liver and intestines, which decreases the availability for deposition in muscle and wool.

Energy digestibility and metabolism

Infection with either *T. circumcincta* or *T. colubriformis* has little effect on feed digestibility (Sykes *et al.*, 1988; Sykes and Coop, 1976; Dargie, 1980). A reduction in efficiency of use of metabolisable energy does occur with both *T. circumcincta* and *T. colubriformis* (Sykes and Coop, 1976; M^{ac}Rae *et al.*, 1979; Dargie, 1980; Sykes *et al.*, 1988).
Sykes *et al.* (1988) concluded that the predominant effect of simple infection with *T. colubriformis* was to reduce energy retention by reducing the efficiency of use of metabolisable energy, while infection with *T. circumcincta* reduced energy retention by reducing feed intake.

2.3.5 Minerals

Intestinal infection with *T. colubriformis* results in marked villous atrophy of the proximal intestine. The intestine is also the primary site of P absorption, so it appears likely the physical damage caused by the parasites might affect the mechanism of P absorption. Apparent absorption is depressed in infected animals (Reveron *et al.*, 1974; Sykes and Coop, 1976) by up to 33 % (Wilson and Field, 1983). The changes in pH of digesta associated with infection may also hinder absorption by precipitating P as insoluble Ca and Mg complexes in the intestinal lumen (Smith and M^{c}Allan, 1966; 1967).

Increased faecal Ca levels occur with both *T. circumcincta* and *T. colubriformis* infections (Wilson and Field, 1983, Poppi *et al.*, 1985b; Bown *et al.*, 1989), probably originating from extra endogenous losses. Hypocalcaemia has been reported in recent studies (Bown *et al.*, 1989), but not in earlier studies (Wilson and Field, 1983; Poppi *et al.*, 1985b).

Feed intake in sheep of low P status is elevated significantly by P supplementation (Milton and Ternouth, 1979). The occurrence of induced P deficiency with parasite induced anorexia suggests P status of the sheep may have an additive effect with parasitism on feed intake (Poppi *et al.*, 1985b).

2.3.6 Summary

Increased endogenous losses of plasma, mucus and epithelial cells are a feature of infections of the GIT with nematodes. Much of the protein lost is reabsorbed distal to the site of infection but the cost of recycling plus that which is not reabsorbed increases the nutrient requirements of the infected animal. The resulting elevated gut tissue metabolism and large amounts of protein cycling are energetically costly (Sykes, 1982). The result is an additional nutrient demand on the host in much the same way lactation or foetal growth may be viewed (Poppi *et al.*, 1990), but this demand occurs at a time when anorexia is reducing the intake of nutrients. The role of these changes in precipitating feed intake depression are unknown.

Of the minerals studied (P, Mg, Ca) P absorption appears most affected by intestinal parasites, with the ability to absorb P being impaired. Induced P deficiency may be have a role in parasite induced feed intake depression.

Many histological changes in the appearance and dysfunction of gut tissue result from parasite penetration, migration and feeding. Enhanced permeability, mucosal thickening and loss of functional cell types are common features. These changes may directly effect feed intake due to the loss of production of neural or hormonal signals from the mucosal tissue, loss of receptors from this tissue or from stimulation of pain or distension pathways.

Parasites cause a depression in productivity and increased requirement for nutrients in the infected host. Yet another response of the host to infection is the development of anorexia so nutrient availability is falling at a time of increased demand. The fall in feed intake may result directly from one of the changes described above or indirectly via neural or hormonal pathways

2.4 Regulation of voluntary feed intake

In a comprehensive review of anorexia in parasitic infection Symons (1985) suggested that although anorexia is a symptom of many different infectious and non-infectious diseases of various organs and tissues of the body with manifold causes, even with these peripheral causes, it is probable that there is a common mechanism in the central nervous system (CNS) signalling feed intake depression. Symons (1985) highlighted the lack of understanding of likely causes and concluded "the summary of the regulation of eating and satiety in the normal animal indicates that a complete understanding of anorexia in parasitised animals is certainly complex and may be difficult to unravel".

In one of the few studies in sheep Symons and Hennessy (1981) reported elevated peripheral cholecystokinin (CCK) levels associated with the onset of feed intake depression, with a fall in CCK levels following anthelmintic treatment. More recently Fox *et al.* (1989) reported increases in plasma gastrin concentration during establishment of gastrointestinal parasite infection in calves. Both studies have shown anorexia to be temporary/reversible because the intake of both the sheep and calves was restored following anthelmintic treatment.

This section will outline the regulation of intake in the normal animal then consider possible mechanisms in the parasitised counterpart.

It appears likely that two components to feed intake regulation operate, a short term or meal regulator and a longer term (greater than 2-3 days) intake regulator. Differences probably exist between these two control mechanisms, with determinants of meal regulation (short term intake) not being determinants of longer term feed intake control (Weston and Poppi, 1987). Yet many similarities exist in the concept of energy demand and flow being involved in both short term (Forbes, 1980) and long term (Weston, 1985) regulation of feed intake.

2.4.1 Feed intake regulation (long term)

Feed intake changes to meet the tissue energy requirements of the individual, which varies with the physiological state of the animal. Long term feed intake regulation appears to respond to the energy requirements of body tissue, with rumen physical transactions, dietary, environmental and other physiological functions being constraints to the system

(Weston, 1985; Weston and Poppi, 1987).

Physiological state

As the physiological state of the animal changes through growth, maturity and successive reproductive cycles, associated changes in feed intake will usually meet the changing nutrient demands of the individual. For example, growing animals will adjust feed intake to maintain digestible energy intake and body weight gain when the energy concentration of the diet is changed (Baumgardt, 1970). Similarly, feed intake decreases when nutrients are infused into the abomasum of sheep (Weston, 1971) again such that digestible energy and live-weight gain remain unchanged.

During lactation in ruminants there is a considerable increase in energy demand which is positively correlated with litter size (Weston, 1982) but, at least initially, increases in feed intake may not match the increased demand (Bauman and Currie, 1980) and mobilisation of tissue reserves is required. The final trimester of pregnancy is a phase of increased energy demand to meet the demands of a rapidly growing foetus yet feed intake remains unchanged, or in some cases falls, during this time. Evidence does not support a physical limitation to intake being the sole cause (Forbes, 1986). In this case, during early lactation and possibly with the fall in intake during oestrus, associated marked endocrine changes may be responsible for the low nutrient intake (Forbes, 1986; Weston and Poppi, 1987).

Gastrointestinal tract and feed intake

Forage acquisition has a role in regulating feed intake: the time available for grazing, ease of harvesting and mass of feed available will all impact on feed intake. Weston (1985) and Weston and Davis (1986) have demonstrated a link between the energy required for comminution or grinding a forage and feed intake, so the resistance of a forage to degradation during eating may be a constraint to feed intake. There is probably variability between species of animal and between age groups in susceptibility to this type of constraint.

Factors which affect flow of digesta through the GIT may influence feed intake. An inverse relationship between reticulo-rumen load and DE or ME intake has been reported (Ulyatt, 1970; Weston, 1984) which Weston (1985) speculated may reflect a relationship between reticulo-rumen load and energy deficit of the animal (where energy deficit is the difference between tissue energy demand and the energy supplied from the diet). However this

relationship does not hold in all cases. For instance, Poppi *et al.* (1980) compared plant leaf fractions with stem fractions and found a 21-77 % increase in DE with no change in reticulo-rumen load. Particle size constraints exist, as fine particles are cleared more rapidly than coarse particles (Poppi *et al.*, 1985a) hence the decrease in load and increase in rate of emptying when forages are ground and pelleted. However there appear to be no data on upper limits to flow rates for ruminants.

Parasites may have a role in disturbing feed intake (long term) regulation by influencing the rate of digesta flow to and/or from the reticulo-rumen. Changes in digesta flow have been recorded (Gregory *et al.*, 1985b) in infected animals which were greater than could be explained by changes in feed intake alone. Slowing gut emptying would probably affect both meal and longer term regulation of intake. Parasites may change the essential nutrient balance of the individual. For instance, the fall in plasma P associated with parasite infection may depress feed intake as may the protein recycling resulting from increased losses of plasma proteins into the intestinal lumen.

2.4.2 Short term or meal regulation

Regulation of short term feed intake is concerned with nutrient intake, such that only small deviations from a constant are possible, whilst maintaining the internal milieu and preventing overload of the GIT. Meal patterning is not rigid and ruminants will vary the frequency, duration and size of meals to compensate for limited access to feed or harvesting difficulties. Early interest was in the role of metabolites of digestion as determinants of initiation and termination of meals, followed by interest in an array of hormones identified in the gut and brain of animals.

2.4.2.1 Metabolites

Volatile fatty acids

Volatile fatty acids (VFA) are collectively an essential energy substrate in ruminant metabolism, and as a result, their role in regulation of meals has received much attention.

Following large meals, increased hepatic blood flow of VFA has been noted in both sheep and cattle (de Jong, 1987) yet such changes are not evident following spontaneous meals (Adams and Forbes, 1981). Jugular or ruminal infusions of VFA depress feed intake, (Baile

and Forbes, 1974), but the unphysiological levels of VFA used, their form and the method of administration suggest the importance of this finding is now questionable (de Jong, 1986). More recently hepatic portal vein infusions of high concentrations of all 3 VFA failed to affect meal size, inter-meal interval or 4 h feed intake in goats (de Jong 1981a, b). Similarly Peters *et al.* (1983) tripled propionate concentration in portal blood without changing feed intake. In contrast Anil and Forbes (1980) and Elliot, Symonds and Pike (1985) demonstrated a reduction in meal size following portal infusions of propionate in sheep or cattle. These contradictory findings might be at least partly explained by the difference in energy status of the animals (Elliot *et al.*, 1985). Alternatively the reduced feed intake may have resulted from a disturbance to sodium receptors or osmoreceptors by the ionic strength of the VFA solutions used (de Jong, 1986) rather than changes in concentration of VFA *per se*.

In summary, spontaneous meals do not appear to be reflected by changes in blood VFA levels which could provide feedback signals and there is little evidence that experimental manipulation of VFA concentrations influences meal size. Where rapid and large changes in VFA concentrations occur, such as with schedule fed ruminants, VFA may control intake to some extent.

Glucose and free fatty acids

Glucose does not appear to be a suitable cue for meal patterning in the ruminant. Blood glucose levels are not related to spontaneous meals (Chase, Wangsness and Martin, 1977; de Jong 1981a,b) and even where severe and unphysiological hypoglycaemia or hyperglycaemia have been reported, these conditions failed to affect meal patterns (de Jong, 1986). No good evidence exists for the involvement of glucose in negative feedback pathways controlling feed intake in ruminants (Forbes, 1986).

Free fatty acid (FFA) may have a role in regulating meal size. However the conflicting results and evidence of hunger in fasted animals, despite elevated plasma FFA concentration, make a major role for FFA in regulation of intake questionable (de Jong, 1986). Free fatty acid concentrations increased during spontaneous meals in cattle (Chase, *et al.*, 1977) but in fasting ruminants fell with feeding (Thye, Warner and Miller, 1970). However decreases in feed intake have been associated with experimental elevation of plasma FFA concentrations in sheep (de Jong, 1986).

Summary

Current evidence is conflicting for a role for metabolites in meal regulation. A role for VFA and free fatty acids appear more probably than one for at least peripheral glucose levels.

2.4.2.2 Hormones

Anticipation of and the initiation of feeding results in the release of gut and pancreatic peptides associated with digestion, absorption and nutrient metabolism. Considerable research has centred on the role of these peptides in feed intake regulation.

In addition to nervous pathways, hormones may be essential links in the physiological control of feed intake, acting as messengers which can respond to changes in the nutrient status of the body. For hormones to be involved in the regulation of feed intake, they must meet two criteria. Firstly, infusion of physiological levels of a hormone must change feed intake and, secondly, a suitable antagonist should block the feed intake response to the hormone. The following sections will concentrate on hormones for which a link with the regulation of feed intake has been demonstrated.

Pancreatic hormones

Insulin

Ingestion of feed causes increases in plasma insulin concentration, suggesting a potential role in intake regulation. However insulin levels are not at their lowest levels immediately prior to feeding and rise in response to eating, suggesting it is unlikely insulin has a controlling role in the initiation of meals. In both schedule (Evans, Buchanan-Smith and M^{ac}Leod, 1975) and free-fed (Chase *et al.*, 1977) ruminants, eating induces a small but significant rise in plasma insulin.

Although evidence suggests a role for insulin in regulation of short term control of feed intake, the wide fluctuations in plasma insulin concentrations which occur due to factors such as stress and exercise make it doubtful that insulin could exert a very precise control. Insulin could function as a long term regulator of intake and it is known that for both ruminants and monogastrics a positive correlation exists between degree of adiposity and mean plasma insulin concentration (de Jong, 1987). In baboons (Woods, Lotter, M^{c}Kay and Porte, 1979) and rats (de Jong, 1987) experimental elevation of CSF insulin concentration reduced feed intake, causing a consequent decrease in body fat content. Such evidence does not yet exist for ruminants.

Reductions of plasma insulin levels in *T. colubriformis* infected sheep are also observed in pair-fed controls, suggesting an insulin response to reduced feed intake rather than an insulin-mediated response (Titchen, 1982a,b).

Other pancreatic hormones

Glucagon secretion is stimulated in ruminants by gastrointestinal hormones, the autonomic nervous system and various nutrients (e.g. VFA) (de Jong, 1982; Peters *et al.*, 1983). Plasma glucagon concentration increased in response to schedule feeding (Bassett, 1972; Ostaszewski and Barej, 1979 *cited by* de Jong, 1987), whereas spontaneous feeding by goats caused only small increases in glucagon concentration (de Jong, 1981a) suggesting a possible role in meal regulation.

Current evidence supports a potential role for glucagon in regulation of short term satiety in both monogastrics and ruminants, whereas a role in long term control of intake appears to be unlikely.

Neuropeptide Y and Peptide YY

Of the pancreatic polypeptide-like hormones, both neuropeptide Y (NPY) and peptide YY (PYY) are potent stimulators of feed intake in the rat (Clark *et al.*, 1984; Stanley and Leibowitz, 1984; Stanley *et al.*, 1985) and sheep (Miner *et al.*, 1989) when administered into a lateral ventricle of the brain. In sheep 3 nmol of NPY will further stimulate intake in hungry animals and animals satiated by rumen distension (water filled balloons) or by prior injection of propionate (Miner *et al.*, 1990). Stanley *et al.* (1985) found repeated daily injections of NPY into the cerebral ventricle of rats increased daily feed intake and live-weight gain and the animals became obese. NPY is the only hormone known to have this action (Williams *et al.*, 1991).

Recently NPY has been suggested to have a role in energy balance by not only stimulating energy intake but also reducing energy expenditure in the rat (Williams *et al.*, 1991).

Gastrin

Pentagastrin (an active form of gastrin) depresses feed intake in sheep, whether given into the jugular vein (Grovum *et al.*, 1974) or hepatic portal vein (Anil and Forbes, 1980). Increases in plasma gastrin concentration have been recorded with feeding of milk to calves or lambs (Bloom and Polack, 1978; Reid *et al.*, 1984), yet in adult sheep plasma gastrin increases in response to restricted feeding, rather than *ad libitum* feeding (Titchen and Reid, 1986). In fasted sheep, with an isolated abomasal pouch abomasal acid concentration increased in response to exogenous pentagastrin (M°Leay and Titchen, 1977) but acid concentration was reduced in the abomasum if pentagastrin was administered after feeding. Gastrin has a direct effect on these cells to stimulate gastric acid secretion as well as strongly potentiating the action of histamine on parietal cells (Uttenthal, 1985). Gastrin also has long term effects on the GIT. These effects are principally trophic effects that play a part in the adaptation of the gut to longer term changes in intake or to compensate for pathological damage (Uttenthal, 1985).

Gastrin is one of the few hormones implicated in intake depression in parasite infected animals. Fox, Gerrelli, Pitt, Jacobs, Hart and Simmonds (1987) found a single dose of 100 000 *Ostertagia ostertagi* elevated blood gastrin concentration from day 17 of infection to a 7-fold increase on day 28. A later trial (Fox *et al.*, 1988) which utilised a trickle dosing regime also recorded elevated plasma gastrin concentrations, however the level of elevation was diet dependent, with infected animals on a hay diet having twice the increase

that was apparent with a concentrate diet. Similarly in sheep infected with *T. circumcincta*, rats infected with *T. spiralis* or *T. taeniaeformis* and pigs infected with *T. ransomi* blood gastrin levels were elevated (Titchen, 1982a,b). However sheep infected with the small intestinal parasite *T. colubriformis* showed a fall in plasma gastrin levels, possibly due to increased release of duodenal peptides which inhibit gastrin secretion (Titchen, 1982a,b).

This evidence suggests gastrin may have a role in feed intake depression associated with abomasal parasites. The trophic effects of gastrin on intestinal mucosa may be an adaptive mechanism allowing the host to better withstand the effects of worm induced mucosal damage.

Cholecystokinin

CCK is the gut/brain hormone for which there is the most compelling evidence for a role in feeding behaviour (Gibbs *et al.*, 1973a, b), as a satiety hormone involved in meal termination. CCK, classically a gastrointestinal hormone, appears to have an equally important role in the brain (Dockray, 1987). CCK peptides and their receptors have specific regional distribution in the brain (Rehfeld, 1978) with the highest concentration in the cerebral cortex but also being present in the periaqueductal grey matter, dorsomedial hypothalamus and hippocampus.

Exogenous CCK or its analogues administered peripherally before or during feeding may reduce intake in sheep (Grovum, 1981; Hondé and Buéno, 1984). However in other cases no effect of peripheral CCK on feed intake in sheep has been found (Baile and Della-Fera, 1984). Grovum (1981) did observe a markedly disturbed gut motility pattern following treatment of sheep with CCK which depressed intake and de Jong (1986) concluded that this alteration of gut motility suggested non-specific intake depression rather than a CCK mediated effect.

More consistent intake depression occurs with central administration of CCK in sheep, where CCK produces a dose-related reduction in feed intake in fasted (Della-Fera and Baile, 1979) and feed restricted sheep (Buéno *et al.*, 1983) with no accompanying abnormal behaviour. Further, continuous infusion of a competitive antagonist of CCK receptors, increased feed intake by 50 % compared with controls and a 2 h infusion of CCK antibodies into the lateral cerebral ventricle of sheep approximately doubled feed intake (Della-Fera and Baile, 1981). These studies in sheep where exogenous CCK seems to work centrally

but not peripherally suggest central but not peripheral CCK receptors are important in the control of feed intake.

The hypothesis that CCK is released during eating and serves as a short-term satiety factor deserves serious consideration. In monogastrics peripheral CCK released from the small intestine and most likely peripherally located CCK receptors stimulate satiety (Smith and Gibbs, 1979) with the emphasis appearing to be on circulating CCK as the signal while in ruminants there is a strong case for central or brain CCK and its receptors being important. CCK release from the small intestine in response to a meal activates afferent vagal circuits that result in the release of CCK at the paraventricular nuclei, thus eliciting satiety in monogastrics (Peiken, 1989).

CCK is also one of the few factors which has been implicated in parasite induced feed intake depression. Symons and Hennessy (1981) found plasma concentration of CCK (as measured by a bioassay) rose as feed consumption fell. Plasma CCK concentration and feed consumption both returned to pre-consumption levels within 6 days of anthelmintic treatment. The authors also reduced intake in non-infected sheep in the first 10 minutes following intravenous infusion of 150-300 ug of CCK. They concluded anorexia may be due to or mediated by a high concentration of CCK.

Opioids

Endogenous opioids are some of the few compounds suggested to have a role in the expression of eating behaviour. Endogenous opioids have a number of roles, including: nociception, regulation of cardiovascular and respiratory systems, behavioural patterns, homeothermy, appetite, thirst and various endocrine functions (Olson, Olson and Kastin, 1986).

Exogenous opioids increase short-term feed intake in sheep. Continuous lateral cerebral ventricular injection with 26 nmol/minute of Dala2 met5 enkephalinamide, a long-acting peptide, increased feed intake in sheep five-fold in the first 60 minutes of feeding (Baile *et al.*, 1981). However, minor changes in structure of these peptides have major effects because Dala2-leu5-enkephalin decreased feeding in sheep (Della-Fera and Baile, 1984). Continuous lateral ventricular injection of various dynorphin-A peptides increased feed intake in a dose dependent manner (Baile *et al.*, 1987). Again peptide structure appeared to be important with dynorphin B administration not affecting feeding. To establish whether

the response to these peptides was opioid mediated, sheep were injected intravenously with a 0.125 mg/kg bolus of naloxone 15 minutes prior to the lateral ventricle opioid injection (0.65 nmol/min DALA dynorphin(1-13) (Baile *et al.*, 1987). Under these conditions, naloxone blocked the feeding response to opioid by 50 %, yet it had no effect on intake when given alone. The authors suggested alteration of the level of opioid agonist and antagonist could fully block the feeding effects. A similar response was observed following i.v. injection of 3,4 dihydroxyl phenyl-3-4 dimethylpiperidine propiophenone maleate, also a specific opioid antagonist (Baile, *et al.*, 1981).

Further evidence for a role of opioids in hunger signalling in sheep was the identification of immunoreactive met-enkephalin and dynorphin neurons throughout the hypothalamus (Marson *et al.*, 1986) and reported elevation in endogenous plasma opioid levels in 4 hour fasted sheep compared with satiated controls (Scallett *et al.*, 1985).

In summary, evidence suggests opioids may have a role in hunger or meal initiation. Opioids appear to act at multiple sites with a number of receptor classes and may act via several independent mechanisms. Depressed voluntary feed intake reported in sheep infected by GIT parasites may result from disturbances to the opioid axis.

Summary

The role of hormones in regulating feed intake is not as well defined in ruminants as it is in the laboratory rat. CCK, insulin, glucagon and gastrin have suggested satiety roles in intake regulation. Some experimental evidence exists for gastrin and CCK having potential roles in parasite-induced anorexia. NPY, PYY and the opioids have been found to be potent stimulators of feed intake in the sheep as well as having a number of other functions. The physiological significance of many of these hormone effects remain to be fully understood.

2.5 Potential negative feedback pathways for influencing feed intake

The presence of feed in the gut and associated changes in digesta flow and products of digestion may result in satiety or negative feedback signals.

Although receptors present in the buccal cavity and throat are important in an animal's sensory perception of food, the suggestion that jaw fatigue causes reduced intake and cessation of feeding does not appear likely (Campling and Balch, 1961; Forbes, 1986)

because ruminants continued to eat for extended periods when ingested feed was removed via a gastric fistula.

Early studies which established the relationship between feed intake and rumen capacity (Balch and Campling, 1962) implied a role for mechano or stretch receptors in feed intake regulation in ruminants. In support of this, Egan (1972) found adding water-filled balloons to the rumen depressed feed intake, with a compensatory increase in the capacity of the rumen evident only when sheep were fed a concentrate diet and not with forage feeding.

In schedule fed sheep and cattle, meal eating decreased linearly with increases in the volume of a water filled balloon occupying space in the rumen (Campling and Balch, 1961; Adams and Forbes, 1981). Although a role for such stretch receptors has been demonstrated in schedule fed herbivores, the importance of these receptors is unknown in free fed animals.

Signals of digesta reaching the duodenum and jejunum may be mediated by stretch, osmoreceptors or chemoreceptors (Forbes, 1988). In pigs, evidence supports a role for osmoreceptors in meal termination. Houpt *et al.* (1983a) infused glucose or sodium chloride into the duodenum of pigs shortly after the start of spontaneous meals and found the resulting reduction in meal size was proportional to the osmolarity of the infusion solution. Non-absorbed solutions were less effective, suggesting the osmoreceptors were not on the intestinal surface (Houpt *et al.*, 1983b) but local anaesthetic blocked the effects, suggesting the receptors were not deeper than the mucosal layer of the intestine.

Using sodium chloride to increase the osmolarity of rumen fluid will reduce feed intake (Ternouth and Beattie, 1971), an effect which is blocked by local anaesthetic. However de Jong (1981a) found no correlation between feed intake and osmolarity but did find a relationship between feed intake and sodium or potassium concentration of the rumen fluid. Osmoreceptors within the GIT may have a satiety role in some species. Many effects which have been attributed to stimulation of chemoreceptors may be due to osmotic effects rather than specific chemical effects on the gut wall. Ternouth and Beattie (1971) and Phillip *et al.* (1981) found an inverse relationship between feed intake and osmolarity of the rumen liquor. Similarly an intragastric infusion of electrolytes delayed feeding in horses (Ralston and Baile, 1983). However Kato *et al.* (1979) found no correlation between osmolarity in the reticulo-rumen and feeding but did find a significant relationship between intake and sodium or potassium concentration, similar to the findings of de Jong (1981a). This

suggests concentration of specific ions rather than the resulting osmotic concentration of the rumen may have a role in feed intake.

Digestion of feed results in changing concentration of various end-products of digestion in the GIT and any of these may generate signals which influence the termination of a meal. The potential role of glucose, VFA and FFA as feedback signals was discussed earlier.

2.6 Liver

The role of the liver in the regulation of feed intake remains controversial (Forbes, 1982; de Jong, 1986). Most substances absorbed from the GIT pass through the liver making it strategically placed to monitor uptake from the GIT and contribute to regulation of feed intake.

Many early studies of the role of the liver as a regulator of feed intake are probably of limited value because hypertonicity of infusion solutions used was probably responsible for observed changes in feed intake (de Jong, 1981a). In two studies where this was not the case, propionate infused into the hepatic portal vein of sheep decreased feed intake (Anil and Forbes, 1980) whereas a similar infusion had no effect on feed intake in goats (de Jong 1981b). Anil and Forbes (1984) provided evidence for involvement of sensory nerves (vagal and splanchnic nerves) in the transmission of information from the liver to the brain.

2.7 Integration of feed intake

Both short term or meal regulation and longer term feed intake regulation appear to result from central (brain) integration of feedback signals. Within the brain, several areas may be involved in intake regulation, with the hypothalamus having a pivotal role in the integration and transmission of hunger signals.

The hypothalamus

Historically the hypothalamus has been considered to play a central role in the regulation of feed intake (Morley, 1980). The hypothalamus receives inputs from metabolic, hormonal, and cortical sources describing the nutritional status of the animal and has overall responsibility for co-ordinating this complex sensory input and to determine outputs such as hunger or satiety. In the last few decades a rich peptidergic network in the hypothalamus

has been described in which there is a delicate balance between various neurotransmitters involved in the hypothalamic control of feed intake (Morley 1980).

2.8 Conclusions

Subclinical GIT parasite infection reduces voluntary feed intake of grazing sheep. The type and number of larvae ingested as well as the nutritional status of the sheep affects the degree of anorexia which occurs.

Parasite infection elevates gut tissue metabolism through increased endogenous losses and reabsorption. Protein synthesis is energetically costly and increases nutrient demand at a time when anorexia is reducing nutrient intake. They also may contribute to osmolality changes in digesta. The role of these changes in precipitating feed intake depression is unknown.

Absorption of P has been found to be impaired during GIT parasite infection and may have a role in feed intake depression.

Parasite penetration, migration and feeding result in many histological changes of the gut tissue. The effects of these changes on the onset of feed intake depression are not known. Histological changes may have a direct effect on feed intake due to reduced production of neural or hormonal signals from the mucosal tissue, causing loss of receptors from the tissue. Alternatively histological changes may potentiate signals arising from damaged receptors. Stimulation of other pathways not normally associated with feed intake regulation may have a role during parasite infection, for example signals of pain, inflammation, distension or disturbances to flow or osmotic balance may be occurring.

In normal animals the regulation of feed intake is a complex, probably energy driven system which is integrated centrally to drive appetite. The central integrator, probably located in the hypothalamus, responds to central hormonal and neurotransmitter stimuli as well as peripheral feedback messages. Peripheral signals of the energy transactions within tissues and reticulo-rumen function are a combination of neural and hormonal signals, the liver and vagi probably carry out integration and transmission of many of these signals.

Parasite effects on the host are fairly well understood however little work has been

undertaken on the interaction or involvement of these effects with the accompanying feed intake depression. Such data are important for fully understanding the physiology of host response to parasites to aid in developing non-anthelmintic based methods of managing losses associated with parasite infection and to provide a model for further developing our understanding of feed intake regulation in ruminants.

CHAPTER 3

General materials and methods

All experimentation was carried out under the guidelines and with the prior approval of the Lincoln University Animal Ethics Committee.

3.1 Recording of feed intake.

i. Daily feed intake

Feed residuals (hereafter refusals) were removed from bins at 0700-0730 daily, weighed and bulked by animal for later dry matter determination. Feed intake was calculated by difference. *Ad libitum* feeding was maintained by offering 120 % of feed consumed in the previous 24 h.

ii. Short term feed intake

Refusals (i.e. feed which had not been eaten) were removed daily at 0730 h and fresh feed offered in a single feed at 0930 h. This meant that immediately prior to feeding, all animals were fasted for 2 h to standardise the time of the first meal. Feed bin weights were recorded, then the pre-weighed feed added to the bin and bin plus feed weight recorded. Following feeding at time 0, the bin and feed were weighed at 10, 20, 30, 40, 50, 60, 75, 90, 105 and 120 minutes then hourly for the next six hours and finally 22 h after feeding.

To determine short term feed intake, bins were individually removed from pens and taken to scales for recording before being returned to the animals. This process took 20-50 seconds to complete. Animals were accustomed to this procedure in the early stages of the experiment, and settled into the routine within 4-7 days of daily recording of intake. Experiments were performed only when the animals appeared unconcerned about the presence of the operators. The same 2-3 operators, to which the animals were accustomed, were used throughout the experiment. Animals were fed initially and feed intakes were recorded in a set order to keep the interval between feeds constant for each animal.

Feed and bins were weighed on an electronic balance (Sartorius 3815 MP, 1.6 000 - 16 000 or Mettler Pk 36 Max 30 000 g). Accuracy of the balance was checked daily using a standard weight prior to weighing and on at least 2 other occasions.

3.2 Measuring live weight

During all trials (except, Chapters 7, 9 and 10) animals were weighed on a portable load cell attached to a portable weighing crate (Warp 4, Rathgens Scales Ltd, Christchurch, N.Z., electronics by Wormald Vigilant Ltd, accuracy 100 g). Each animal was weighed twice or until the difference between weights was not greater than 200 g. A standard weight (26 kg) was used prior to and after weighing to establish the accuracy of the balance on the day. The balance could not be calibrated so on occasions where the standard weight was more than $\pm$ 200 g different from 26 kg, live weights were adjusted accordingly.

In the final series of studies an electronic platform load cell (Sauter Multirange, Max 240 kg, accuracy 10 g) attached to a portable data recorder was used. The number of weighings and acceptable variance were set manually for this system (200 weighings or $\pm$ 100 g used).

3.3 Dry matter (DM) determination

A single feed offered sample was collected daily, thoroughly mixed and subsampled for weekly dry matter determination. Feed refusals were bulked separately for each animal and subsampled by animal for determination of weekly dry matter. The weekly samples were subsampled, weighed and air dried at 80°C for at least 48 h before being reweighed.

A daily sample of feed offered was bulked for laboratory analysis at the completion of the trial.

3.4 Surgery

Duodenal cannulation

All animals were deprived of both feed and water for 24 h prior to surgery. Wool on the neck and a large midside patch on the right side of the sheep were removed before surgery using a standard shearing handpiece.

Ten minutes prior to induction of anaesthesia, animals received 1 ml of atropine solution (0.6 mg/ml atropine sulphate, M^{c}Gaw Ethicals N.Z.) by subcutaneous injection to reduce salivation and prevent ventricular fibrillation during surgery. The animal was then anaesthetised with 6-10 ml of sodium pentobarbitone 60 mg/ml (Nembutal, Sanofi, Techvet

Laboratories, N.Z.) injected intravenously.

Animals were placed left side down on the surgery table and positioned so that the head hung slightly downwards to allow saliva to drain from the mouth. The remaining 2-3 mm of wool left by the handpiece on the midside patch was removed using electric clippers. A dilute solution of cetrimide and chlorhexidine (Savlon, Coopers Animals Health Ltd, N.Z.) was used to disinfect the exposed skin, followed by painting with an alcoholic solution of iodine. A sterile surgery drape was placed over the disinfected area.

A right side mid-flank laparotomy (as described by Hecker, 1974) was performed by making a 6 cm lateral incision through the skin and subcutaneous fascia in the mid-flank region, half way between the costal arch and the anterior pelvic bone. Entry through the external oblique, internal oblique and the transverse abdominal muscles and the peritoneum was made by blunt dissection and enlarged as necessary by judicious tearing. This technique was found to lessen the likelihood of post-operative herniation (A.S. Familton pers. comm.). Any severed blood vessels which bled persistently were clamped and tied off. Allis forceps were used to secure the muscle layers either side of the incision. The section of the duodenum to be cannulated (approximately 10 cm distal to the pylorus) was located and the digesta in the tract gently forced away from the cannulation site. Two bowel clamps were positioned to prevent re-entry of digesta into the cannulation site. An elliptical purse string suture (ellipse size 20 mm x 4 mm) using polyglactin suture (Vicryl J.345, Ethicon, Johnson and Johnson, U.S.A.) was placed in the mucosa of the intestine and a single incision made within the suture. Scissors were then used to lengthen the incision, with care taken not to cut the suture. The internal flange of the cannula (Plate 1) was inserted into the visceral lumen, using Allis forceps to hold the duodenal wall. The purse string suture was then tightened and tied.

The barrel of the cannula was exteriorised by making a separate stab entry between the last two ribs using scissors. Artery forceps were passed through the stab entry and the barrel of the cannula pulled through the hole. Care was taken when pulling the barrel against the abdominal wall to ensure no intestine was looped between the flange and the wall. The external flange was placed on the barrel of the cannula and oxytetracycline powder (Terramycin, Pfizer Laboratories Ltd, N.Z.) applied to the wound. The laparotomy was closed using a continuous suture technique. One layer of suture was used to close the peritoneum and muscle layers, then the skin closed using horizontal mattress sutures of

polyglactin suture (3.5 metric with cutting needle, Vicryl J-616, Ethicon Inc., U.S.A.).

Immediately following surgery all animals received long acting antibiotic (3 ml Penstrep L.A., A.S.Rosco, Veterinary Ethicals Ltd., 100 000 iu procaine penicillin, 100 000 iu benzathine penicillin & 250 mg dihydrostreptomycin per ml) injected subcutaneously.

Abomasal cannulation

Pre-operative procedures were the same as those described above for duodenal cannulation and the surgical technique was similar to that described by Hecker (1974).

A ventral paracostal laparotomy was performed by making a 6 cm incision through the skin and subcutaneous fascia behind the right costal margin, near the midline to expose the abomasum. Cannulation was undertaken using a similar technique to that described for duodenal cannulation, again a purse string suture was used to place the cannula (Plate 2) approximately 8-10 cm from the pylorus. The cannula was exteriorised in the costal region using the same technique as for duodenal cannulation. A continuous suture was used to close the peritoneum and muscle layers, then the skin closed using horizontal mattress sutures (as above).

Immediately following surgery all animals received long acting antibiotic (3 ml Penstrep L.A., A.S.Rosco, Veterinary Ethicals Ltd., 100 000 iu procaine penicillin, 100 000 iu benzathine penicillin & 250 mg dihydrostreptomycin per ml) injected subcutaneously.

Cannulation of a lateral cerebral ventricle

All lambs were deprived of feed for 24 h and water for 12 hours prior to surgery. Ten minutes prior to anaesthesia, lambs were injected intramuscularly with 1 ml atropine sulphate (0.6 mg/ml Atropine, M°Gaw Ethicals N.Z.) to reduce salivation and prevent ventricular fibrillation during surgery. Animals were lightly anaesthetised using 1-3 ml of xylazine hydrochloride (Rompun, Bayer N.Z. Ltd) injected intramuscularly and placed in a sitting position on the table.

The scalp was clipped and 2 ml of local anaesthetic (Xylocaine, Astra Australia) injected intra and sub dermally into the scalp in the region of the incision. The scalp was thoroughly cleaned with a dilute solution of cetrimide and chlorhexidine (Savlon, Coopers Animal Health Ltd, N.Z.), then painted with an alcoholic solution of iodine.

A midline incision was made through skin and down to the skull in the sagittal plane between the eyes and the ears. The skull was cleaned of adhering tissue and a 1.5 mm hole drilled through the frontal bone of the skull with an electric drill, at a point 8-10 mm lateral to the point of Bregma. A specially prepared stainless steel cannula (G.S. Spencer pers. comm.) (Plate 3) was inserted through the hole into the brain until cerebrospinal fluid flowed back through the cannula (a depth of approximately 15 mm). A second hole was drilled partly into the skull and a small stainless steel screw (1.5 mm x 5 mm) screwed into the skull. The cannula was then cemented into place using methacrylate bone cement (surgical Simplex P, Howmedica, London) which covered the screw to lock the cannula to the skull. A stainless steel stylet was inserted into the cannula to prevent infection and maintain patency. Oxytetracycline powder (Terramycin, Pfizer Laboratories Ltd, N.Z.) was applied to the wound area and the incision closed over the cemented area using horizontal mattress sutures of polyglactin suture (3.0 metric with cutting needle, Vicryl, Ethicon Inc, U.S.A.). Animals then received 500 000 units of procaine penicillin and 500 000 units of benethamine penicillin subcutaneously (Propen L.A., Glaxo N.Z.). For the duration of the experiment animals received 3 ml of this antibiotic 2 x weekly. To minimise damage to the cannula protruding above the scalp, a piece of 50 mm diameter polyvinyl chloride water pipe was sutured (at 3 points) to the scalp immediately after surgery (Plate 4).

Post-operative care

Animals were placed in a sitting position supported by the wall of the pen and checked regularly until they had returned to full consciousness and were standing. Animals were offered fresh water and chopped lucerne hay. If any oedema occurred during the 72 h following cannulation of duodenum or abomasum, the external flange of the cannula was loosened to reduce pressure on the tissue. It was subsequently tightened as the oedema subsided. Two to four days after surgery the area around the cannula and laparotomy wound was dusted with diazinon powder (Fly Strike Powder, FIL Industries Ltd, N.Z.) to prevent fly strike. Fly strike treatment was continued on a weekly basis for the remainder of the experiment.

Plate 1. An illustration of the cannula inserted into the duodenum in Chapter 5. The red bung was inserted into the cannula to seal the cannula.

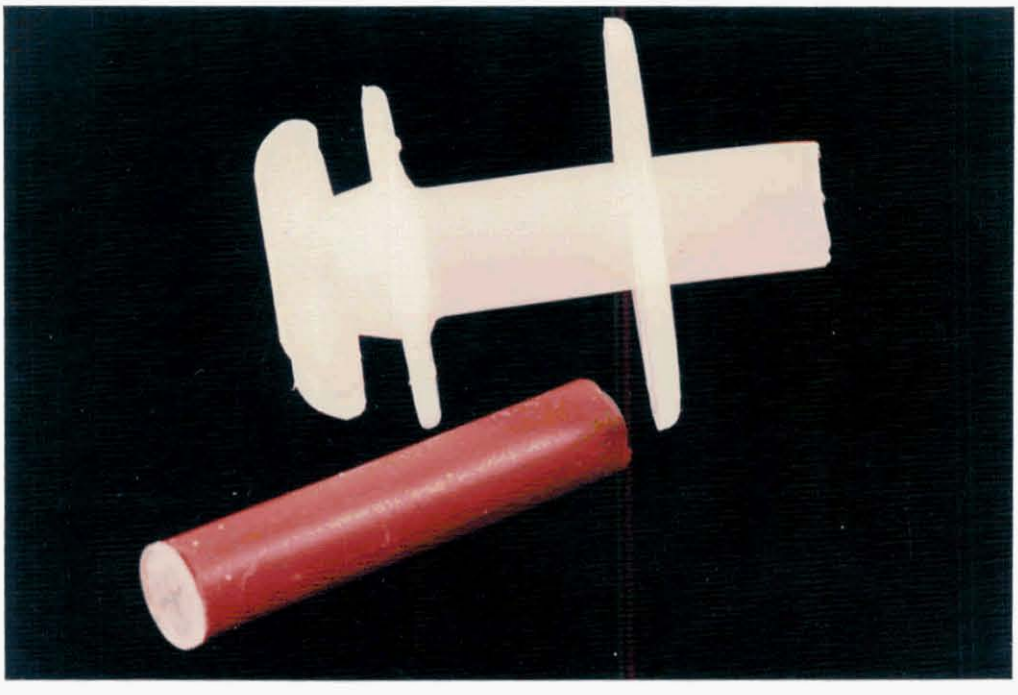


Plate 2. An illustration of the cannula inserted into the abomasum in Chapter 7. The red bung was inserted to seal the cannula.

Plate 3. An illustration of the cannula inserted into the lateral cerebral ventricle of lambs in Chapter 10. The cannula on the right has the stainless steel stylet removed.

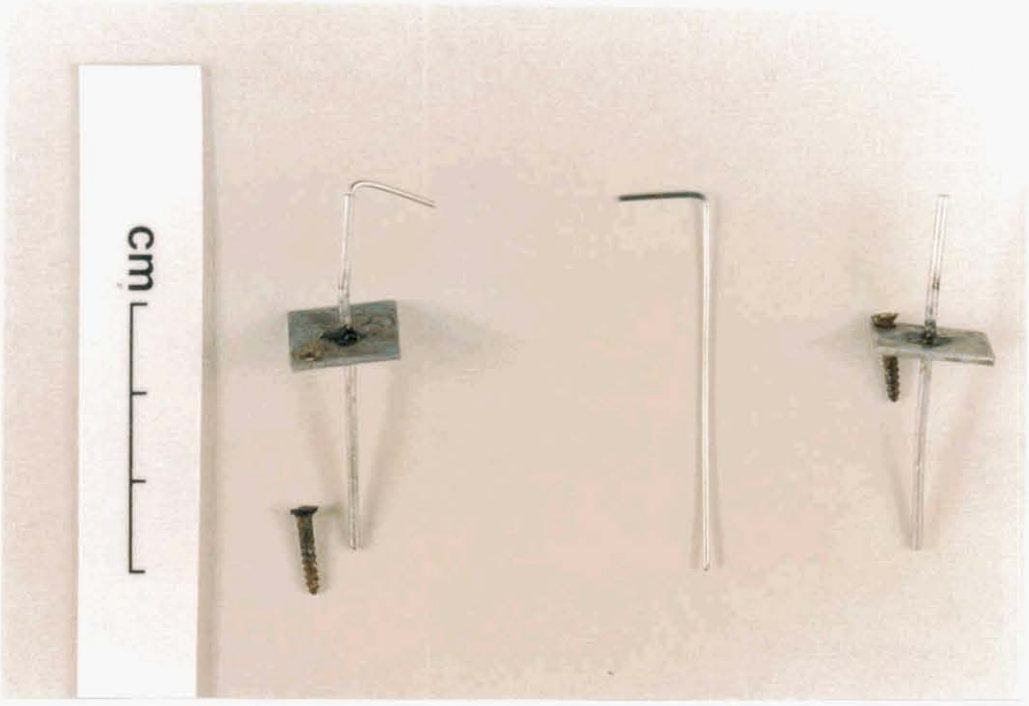


Plate 4. A lamb fitted with a lateral cerebral cannula and the protective tubing attached.

3.5 Jugular catheterisation

For infusion of the CCK blocker described in Chapter 7 a cannula was inserted into an external jugular vein. Wool was clipped from an area over the left and right jugular veins and the skin disinfected with a 30 % cetrimide and chlorhexidine solution (Savlon, Coopers Animal Health Ltd, N.Z.) Using a method similar to the Seldinger technique (Hecker, 1974) a 14 G * 5 cm introducer (Plate 5) (Intravenous catheter placement unit, Insyte, Deseret Medical Inc., Becton Dickinson & Co., U.S.A.) was inserted into a vein. The needle was removed and the introducer used to place 6 of a 7 cm length of medical grade vinyl tubing (1.00 mm I.D 1.5 mm O.D., Dural Plastics and Engineering, NSW, Australia) into the vein. Following removal of the introducer, the exposed end of the vinyl tubing was immediately plugged with a cut-down (20 mm) bluntened needle (19 G Terumo Corporation, Japan) and a Surflo injection plug (Terumo Medical Corporation, Elkton, MB, U.S.A.) (Plate 5). A narrow piece of tape (Leucoplast 1.25 cm, Beiersdorf, AG, Hamburg) was attached to the tubing and sutured to the skin to secure the cannula (Plate 5). A large piece of tape (Sleek, 5 cm, Smith and Nephew Ltd, Hull) was applied to the whole area using adhesive (Selleys Supa-glue Gel, Selleys Chemicals, Auckland, N.Z.) so only the plug was visible. This reduced accidental removal of the cannula by the animal during feeding. The cannula was flushed twice daily with sterile isotonic phosphate buffered saline solution, using 50 USP units/ml of sodium heparin (Heparin (mucous) B.P., Leo Pharmaceutical Products, Ballerup, Denmark) as an anticoagulant. Animals were injected subcutaneously with 3 ml of antibiotic (Penstrep LA, 150 mg procaine penicillin and 141.5 mg benethamine penicillin/ ml, Pitman Moore, N.Z.) following cannulation.

3.6 Parasitological techniques

Parasite dosing

Lambs in the infected groups were dosed *per os* with on average 4 000 third stage larvae of the small intestinal parasite *T. colubriformis* per day. Larvae were administered 3 times per week (8 000 on Mondays, 8 000 on Wednesdays and 12 000 on Fridays), in order to simulate chronic infection (R. M^{c}Anulty pers. comm.)

The dosing regime was intended to induce chronic parasite infection in the lambs, comparable to that caused by the daily ingestion of a small number of infective larvae experienced by growing lambs in the field (Anderson, 1972; Gibson and

Parfitt, 1972; 1973).

Larvae were dispersed onto moist filter papers which were rolled into 'bullets' suitable for administration with a veterinary tablet gun.

Faecal egg counts

On a weekly basis, faeces were removed manually from the rectum of each lamb and processed immediately to determine faecal egg concentration by a modified M^{c}Master method (M.A.F.F. 1979).

Faeces were first thoroughly mixed using the brass inner plunger of the faecal pelleter, forced into the pelleter chamber, and flattened off. The pellet (1.7 g) was discharged into a clean dry 51 ml jar by depressing the inner plunger. Up to 10 ml of tap water was then added to the jar and samples stored at 4 °C for at least 24 h before counting.

Prior to counting the jars were filled to a volume of 51 ml with a saturated sodium chloride solution (to suspend parasite eggs in solution) and mixed with a stirrer attached to an electric drill for 26 seconds. A single aliquot of sample was pipetted and the first chamber of a M^{c}Master counting slide was filled, the pipette was then revolved through 180° and the second chamber filled (0.15 cm^3 in each chamber).

The dilution effects of 1.7 g faeces in 51 ml solution and a 0.30 cm^3 aliquot means that each egg counted here is equivalent to 100 eggs per gram in the faeces.

Parasite Culture

A monospecific infection of *T. colubriformis* was generated in male sheep. Sheep were housed indoors, dosed with anthelmintic (200 mg/kg LW ivermectin, Ivomec, MSD Agvet N.Z.) and injected with corticosteroid (40 mg methylprednisolone acetate, DepoMedrol, UpJohn, Auckland N.Z.) to suppress immunity temporarily, 10 days before a single oral dose of 30-40 000 *T. colubriformis* larvae (third stage). Total faecal collection (Plate 6) was usually undertaken from week four to five of infection when large numbers of eggs were evident in faeces. Fresh faeces were placed in floating tanks in a climate controlled room to promote larval development (M^{c}Master mass larval culturing system) (Plate 7). Larvae hatching from faecal eggs migrated out of the tank into the aerated water surrounding the tank and gradually sank into an attached column. Larvae were removed by opening the tap

at the base of the column and collecting water from the column. Larvae were immediately stored at 4 °C until 24 h before use for trickle infection of sheep.

3.7 Weaning

Lambs were used in all the trial work undertaken. Mixed sex Coopworth lambs used in the first trial were from Lincoln University Ashley Dene Farm. The lambs used in remaining trials were all from the Lincoln University Research Farm and were female Coopworth or Coopworth X Dorset Down. Each year lambs were early weaned between 10 and 20 October, at which time they were six to seven weeks of age. Lambs were selected to have an average live weight of 17.5 to 18 kg at weaning. Following weaning all lambs were ear tagged and weighed. All lambs were vaccinated against black leg, pulpy kidney, malignant oedema, tetanus and black disease (Coopers Multine, Pitman-Moore, N.Z.) and orally dosed with ivermectin (200 mg/kgLW Ivomec MSD Agvet, N.Z.). The same vaccine was used at 3 monthly intervals for the duration of the experiment.

Following early weaning, lambs were reared in a minimal parasite environment until approximately 25 kg live weight. During this period animals were weighed fortnightly and treated with anthelmintic (25 mg/kgLW fenbendazole, Panacur Sheep Drench, Hoechst Ltd, Pitman-Moore N.Z.) to minimise development of parasite resistance. A brief outline of weaning practice used in each year is given below.

Year 1 (Appendix 4)

Following weaning all lambs were grazed on pasture which had not been grazed by parasite susceptible animals for 12 months. Animals were drenched fortnightly with anthelmintic whilst grazing the pasture.

Year 2 (Chapter 4)

Following weaning, all lambs were grazed on a lucerne pasture, which had not been grazed by any stock for the preceding 5 months. Lambs were grazed on the lucerne pasture for 6 weeks, orally dosed with anthelmintic at 2 weekly intervals (25 mg/kgLW fenbendazole, Panacur, Hoechst Ltd, Pitman-Moore, N.Z.) to minimise infection with parasites and gradually introduced to a pasture supplement (barley). All animals were moved indoors in early December 1987 and fed barley (200 g/head) and lucerne hay (*ad libitum*) until 20 January 1988. Over a period of 1 week the diet was changed to a complete pelleted ration

(Appendix 1.) and after 4 weeks of *ad libitum* intake on this diet, animals were restricted to 1.2 kg/head/day (1.5 x maintenance) to restrict live-weight gain.

Year 3 (Chapter 5,6 and 8)

Following weaning lambs were placed in a paddock which had been "spray fallowed" in the early winter to kill all vegetation. There was no pasture on offer so animals were run in an extensive feed-lot environment. Ryegrass straw and mineral blocks were available *ad libitum* and lambs were fed daily a complete pelleted ration (Appendix A.2), which was gradually increased to ensure 1-2 kg live weight gain per week. Animals were weighed weekly and drenched with anthelmintic (25 mg/kgLW fenbendazole, Panacur, Hoechst Ltd, Pitman-Moore, N.Z.) fortnightly for the duration of time in feed lot.

Year 4 (Chapter 7 and 9)

Lambs were moved to the Lincoln University cropping farm after weaning and strip grazed on "new grass". This pasture had been sown the previous autumn (7 months earlier) after a crop rotation and had not been grazed by any stock. Pasture larval counts during grazing showed no evidence of larval contamination on the pasture. The lambs were break fed with a new break being offered every 7 days. As the lambs settled and began to gain weight, the break size was reduced and a complete pelleted ration offered on a daily basis. When lambs reached an average live weight of 25 kg, they were transferred indoors into individual pens.

Year 5.

Following early weaning, lambs were housed indoors in pens (8 animals per pen) on a deep litter system. Initially animals were offered chaffed meadow hay *ad libitum* for 10 days. In the following period a complete pelleted ration (Appendix 2) was gradually introduced up to approximately 400 g/head/day. This was supplemented with ryegrass straw chaff (*ad libitum*). Animals were weighed weekly and moved if necessary to maintain similar size and rate of live-weight gain in each pen. Lambs were moved into single pens 4 weeks after weaning on approximately 15 November.

3.8 Statistical analysis

In all experiments, treatments were allocated in a replicated Latin square, and it was planned to use analysis of variance (ANOVA) or multiple analysis of variance (MANOVA) for statistical analysis of the feed intake data. A depression in feed intake was not recorded in

some lambs, following parasite infection, which were excluded those lambs from the treatments and all analysis and led in most cases to an unbalanced experiment.

The cumulative feed intake response of lambs to parasite infection and various treatments was investigated. Using cumulative feed intake data precluded the use of MANOVA because the variables were highly correlated. Orthogonal contrasts and ANOVA were not used because the data did not meet the assumptions of normality underlying these tests (Steel and Torrie, 1980) even following transformation of the data.

A number of models including linear, quadratic and piecewise linear models, were fitted to the data from Chapter 9 to test the effects of parasites and treatments on the cumulative intake curves. This approach was found to be unsuitable for 2 reasons. Firstly the cumulative intake curves were not of a consistent form making curve fitting very difficult. The lack of consistent form resulted from the response of animals to treatments imposed. Particular difficulties were encountered where feed intake was depressed, for example following naloxone treatment, and where very rapid feeding occurred following fasting and brotizolam treatment. Secondly where a model fitted much of the data, biological interpretation of the results was difficult.

The final approach was to use non-parametric or distribution-free statistical testing. The Sign test, Friedmans test and an ANOVA of ranked data were evaluated using the data from Chapter 9 with the cumulative feed intake data being analysed at each time period recorded. The Sign test was of very limited use in analysis of the present study and although Friedmans test could be used, it did not handle missing data points well. In addition Friedmans testing was difficult to use tests for further comparison of differences between treatment means. A non-parametric approach using ANOVA of the ranked data was selected as a suitable method of analysis of the cumulative feed intake data which did not fit the assumptions of a standard ANOVA. A non-parametric analysis is less powerful and more conservative than similar parametric tests. Non-parametric testing was carried out on the cumulative feed intake data at each time period recorded.

Plate 5. An illustration of the equipment used for jugular catheterisation of lambs in Chapter 7. From top left; suture with tape attached, Surflo injection plug, bluntened needle, vinyl tubing and the commercial introducer.

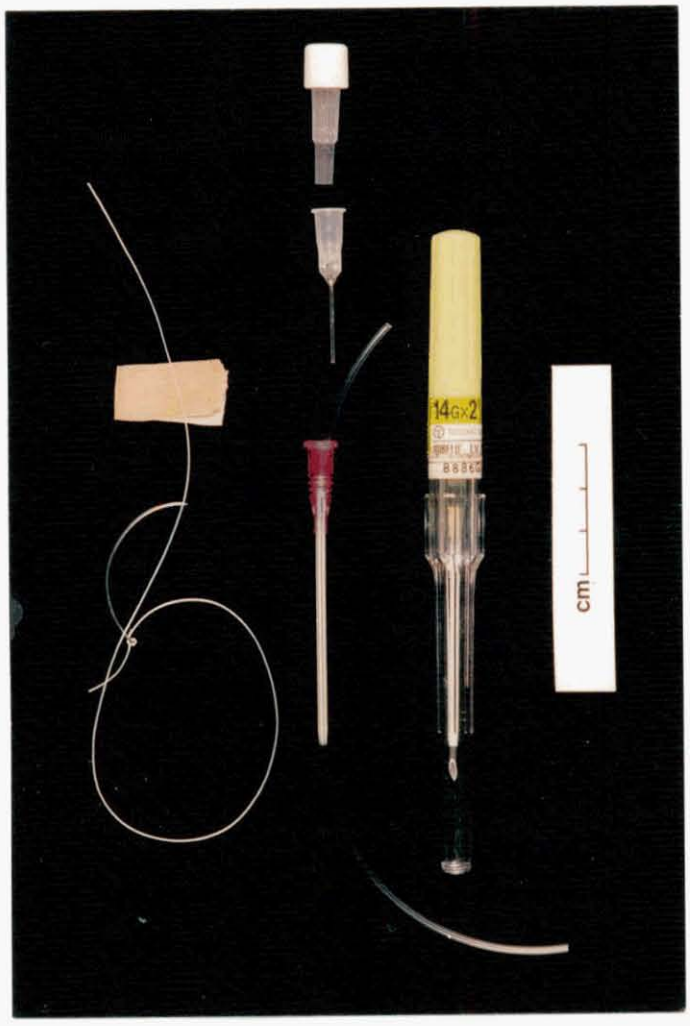


Plate 6. An illustration of the jugular catheter assembled as it was in the lamb.

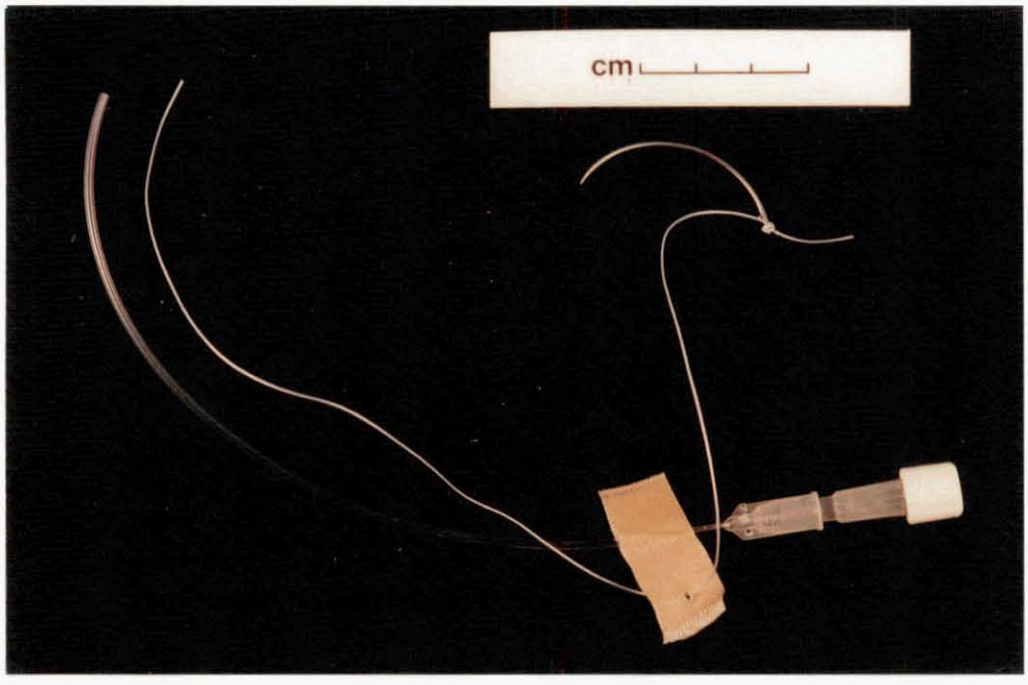
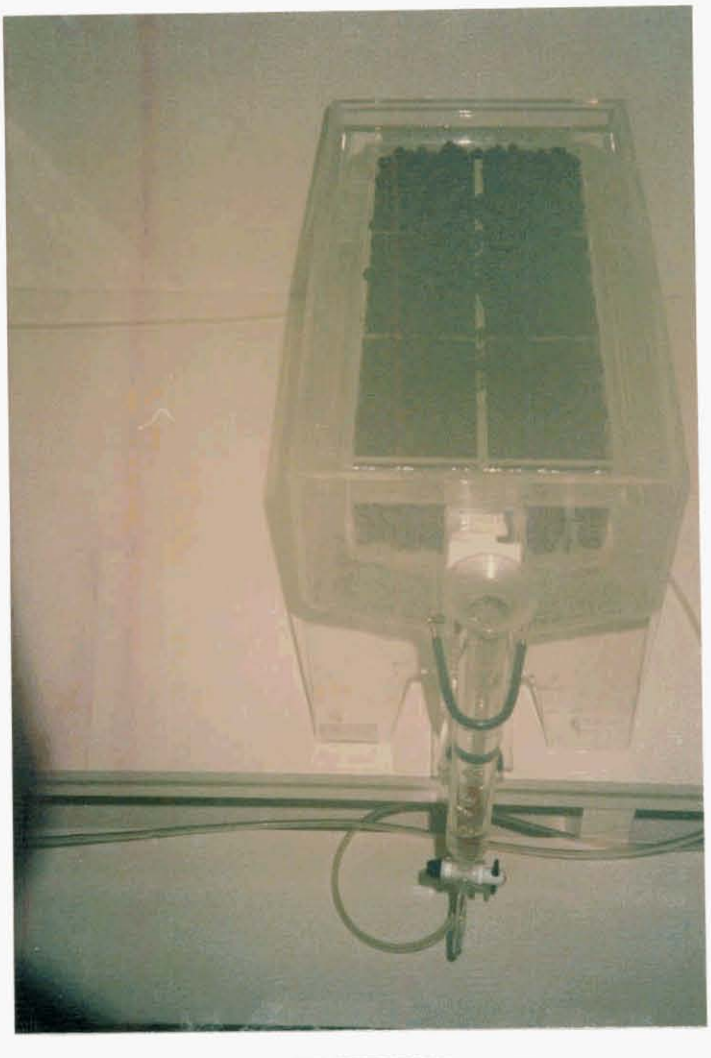


Plate 7. An illustration of total faecal collection for parasite culture.

Plate 8. An illustration of a mass larval culture tank used for culturing parasite larvae.



CHAPTER 4

The effect of infection by the gastrointestinal parasite *T. colubriformis* on short term feed intake in lambs and of some pharmacological agents on feed intake depression.

4.1 Introduction

In studies of intake regulation in sheep and other species, hormones, agonists and antagonists of hormones and neurotransmitters and other agents have been examined for their effects on a single meal or short term feed intake. Studies of short term intake are useful for testing potential regulatory mechanisms in single meals. They can utilise single small doses of test compounds and avoid much of the variability associated with measurement of daily intake, which is influenced not only by meal size but also by the number of meals consumed. Results from a pilot trial revealed significant differences in short term intake (frequent recording over 8 h) between infected animals whose intake was depressed and their non-infected controls.

The current study utilised parasite infected lambs with intake depression as an animal model to investigate some of the pathways which have been postulated for control of feed intake. This model enables the screening of pharmacological compounds with known agonistic or antagonistic actions to elucidate pathways involved in depression of feed intake. Two mechanisms were examined, *viz.* pain and/or discomfort and CCK, for their role in feed intake depression.

Extensive invasion and disruption of intestinal mucosa may manifest itself in pain, inflammation and/or generalised discomfort which could directly or indirectly cause feed intake depression. It can be argued that this may be the mechanism which operates in parasitised sheep. The first approach used here was to block the effects of pain and discomfort by using codeine phosphate, an opioid derived analgesic. Codeine provides selective pain relief, leaving other senses intact, and is most effective in humans against dull continuous pain as would probably occur in parasitised animals. The second approach used was to inhibit prostaglandin production by using indomethacin. Indomethacin has antipyretic and effects resulting from the inhibition of prostaglandin production and an analgesic effect, which would be beneficial where pain accompanies an inflammatory response, as may be occurring in parasitised lambs. The influence of these compounds on short term intake was

monitored in the present study.

Peripheral CCK levels appear to be elevated during intake depression in parasite infected lambs (Symons and Hennessy, 1981). This finding could be investigated further using a potent blocker of CCK receptors to temporarily antagonise peripheral CCK receptor activity. The short term feed intake response was monitored following treatment.

4.2 Materials and Methods

Sixteen Coopworth x Dorset Down cross ewe lambs, part of a larger group, were weaned at approximately 17 kg live weight onto lucerne pasture which had not been grazed over the winter period (5 months) (Section 3.7). On 26 April 1988 animals were paired by live weight and one of each pair randomly allocated to an infected or non-infected group (n=8). Infected animals received 4 000 *T. colubriformis* larvae/day administered orally as bulked doses 3 times/week (Section 3.6) whereas non-infected animals received only moistened filter paper tablets. Initially (weeks 0-4), each non-infected animal was fed the amount consumed by its infected pair in the previous 24 h period.

A method of short term intake recording was developed based on the feeding regime used by Baile *et al.* (1979) (Section 3.1). Treatments were administered when intake depression of at least 30% had occurred in the parasitised group.

Each pair (1 infected and 1 non-infected) received each of the 4 treatments (control, CCK antagonist, codeine phosphate or indomethacin) in a randomised Latin square design which was repeated once (replicated) approximately 8 weeks after the initial infection.

The treatments were:

1. CCK antagonist. A CCK antagonist (L364-718, Merck Sharp and Dohme, U.K.) at a dose of 0.1 mg/kg LW dissolved in 0.5 ml of dimethyl sulphoxide (DMSO) was used. Four sheep doses of L364-718 were placed in a sterile 10 ml glass beaker with 2 ml of DMSO and mixed thoroughly. A sterile 1 ml syringe and a 20 G 2.5 cm needle were used to deliver 0.5 ml of L364-718 solution which was injected subcutaneously 15 minutes prior to feeding, into an area on the medial surface of a fore leg which had been cleaned thoroughly.

2. Codeine phosphate. Codeine phosphate (120 mg, 60 mg tablets, Codeine Phosphate

Tabs BP 1973, Douglas Pharmaceuticals Ltd., N.Z.) was administered orally (2 tablets) to animals using a veterinary tablet gun, 3 h prior to feeding and again 1 h after the start of feeding.

3. Indomethacin. Indomethacin (100 mg Indocid supplied as 50 mg tablets, Merck, Sharp and Dohme N.Z. Ltd.) was administered orally (2 tablets) using a veterinary tablet gun 3 h prior to feeding and again 1 h after the start of feeding.

4. Control. The control treatment was 0.5 ml of DMSO injected as outlined in 1 and sham dosing with the veterinary tablet gun to mimic treatments in 2 and 3.

Following the completion of the treatment phase, all animals were dosed orally with anthelmintic (200 ug/kg ivermectin, Ivomec, MSD Agvet N.Z. Ltd). Short term feed intake patterns were again recorded (Section 3.1) 7-14 days after anthelmintic treatment. This was following full recovery of daily intake in previously infected animals to preinfection levels.

4.3 Results

Two of the 8 infected animals failed to show consistent feed intake depression during the time frame of the experiment. These animals plus their non-infected pair were excluded from the study.

Parasitology

Faecal egg counts recorded during the experiment are shown in Figure 4.1. *T. colubriformis* eggs first appeared in the faeces at the end of week 3 and the peak concentrations were also observed at the end of week 3. A second smaller peak was recorded during week 9. No parasite eggs were found in samples from non-infected animals during the experiment. The experimental treatments were imposed between weeks 8 and 12 of the trickle infection.

Live weight

For the first 6 weeks of infection, there was very little difference in live weight between the infected and non-infected animals (Figure 4.2). However, over the next 4 weeks the non-infected animals gained on average 203 g per day whilst the infected group gained only 70 g per day. During the final weeks of infection, weight gains were similar, but infected

animals remained on average 4 kg lighter than non-infected animals.

Daily feed intake

Mean daily feed intake (7 days) for infected and non-infected animals is shown in Figure 4.3. Non-infected animals initially were pair fed with infected animals. When feed was offered *ad libitum* (weeks 4-6), intake of the non-infected animals exceeded that of infected animals, this increase peaking 10 to 12 weeks after the commencement of infection. Mean daily feed intake fell gradually from week 4 in the infected group and was significantly lower than in the non-infected group from week 7 of infection ($p < 0.05$).

Short term feed intake

Mean cumulative feed intake for infected and non-infected lambs during weeks 8-12 is shown in Figure 4.4. During the period of intake depression (weeks 8-12) feed intake was significantly depressed in the infected group at each time period recorded over the 8 h. Both groups consumed a single large meal lasting 40-60 minutes immediately following feeding. During this first meal, infected and non-infected groups consumed 20 % and 22 % respectively of the cumulative intake over the 22 h period.

Figure 4.5 shows the cumulative feed intake (g/kg $LW^{0.75}$) for the infected group alone prior to and during parasite induced intake depression (weeks 8-12) and again following intake recovery after anthelmintic treatment. The short term cumulative feed intake was significantly less at all recorded time intervals during intake depression in infected animals (weeks 8-12) but had returned to the pre-infection level within 2 weeks of anthelmintic treatment.

Treatment effects

Short term feed intake patterns for infected and non-infected groups following sham, codeine phosphate, indomethacin or CCK antagonist treatment are shown in Figures 4.6 and 4.7. As indicated earlier, parasite infection had a significant effect on short term feed intake ($0.01 < p < 0.05$) at all time periods tested. A significant treatment by parasite interaction occurred during the first 5 h of feeding ($0.01 < p < 0.05$). This interaction was due to a tendency for codeine, CCK antagonist and indomethacin to reduce intake over the first 5 h of feeding in the non-infected animals.

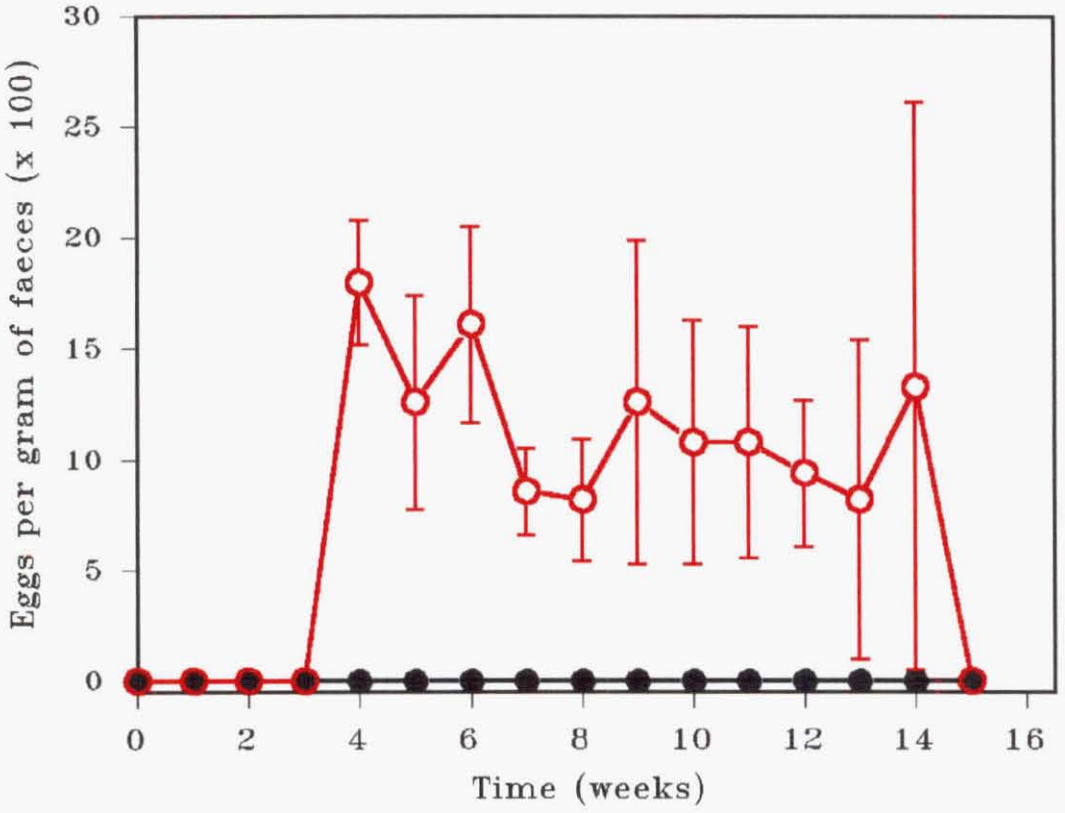


Figure 4.1 Mean (± s.e.m) number of *T. colubriformis* eggs per gram of faeces in infected (o) and non-infected (●) lambs sampled once per week (n=6).

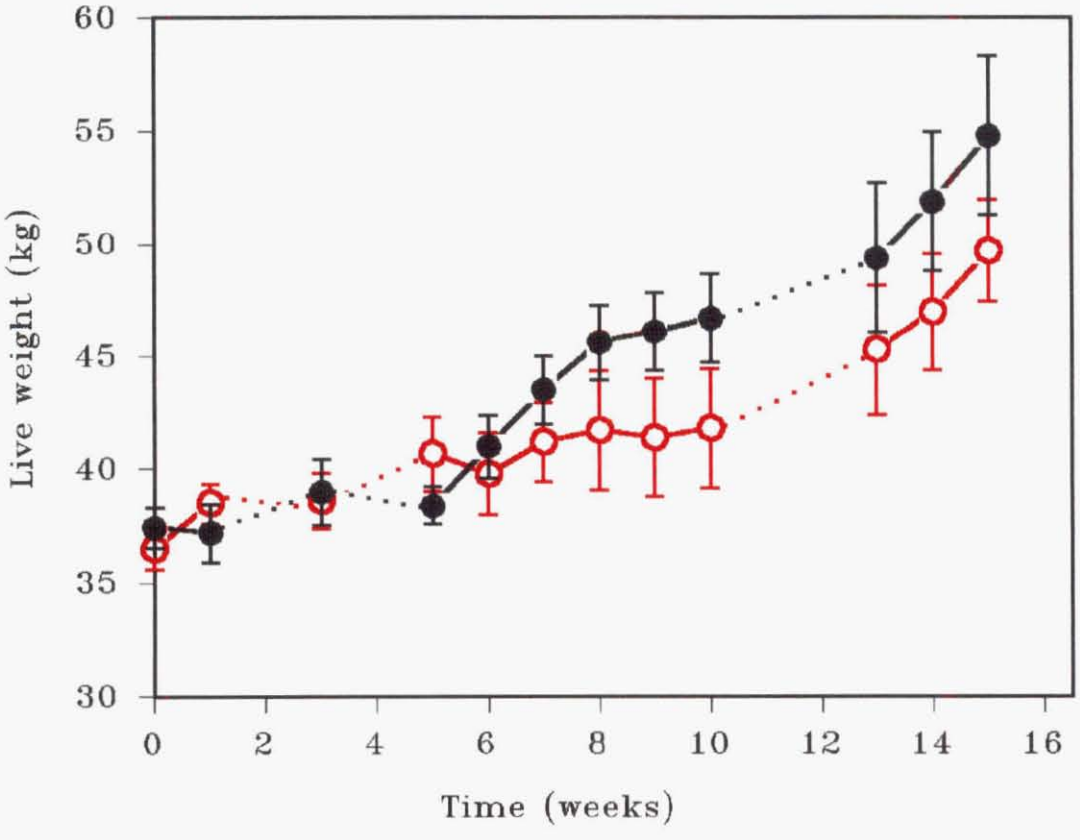


Figure 4.2 Mean live weight (± s.e.m) for infected (o) and non-infected (●) lambs during the experiment (n=6).

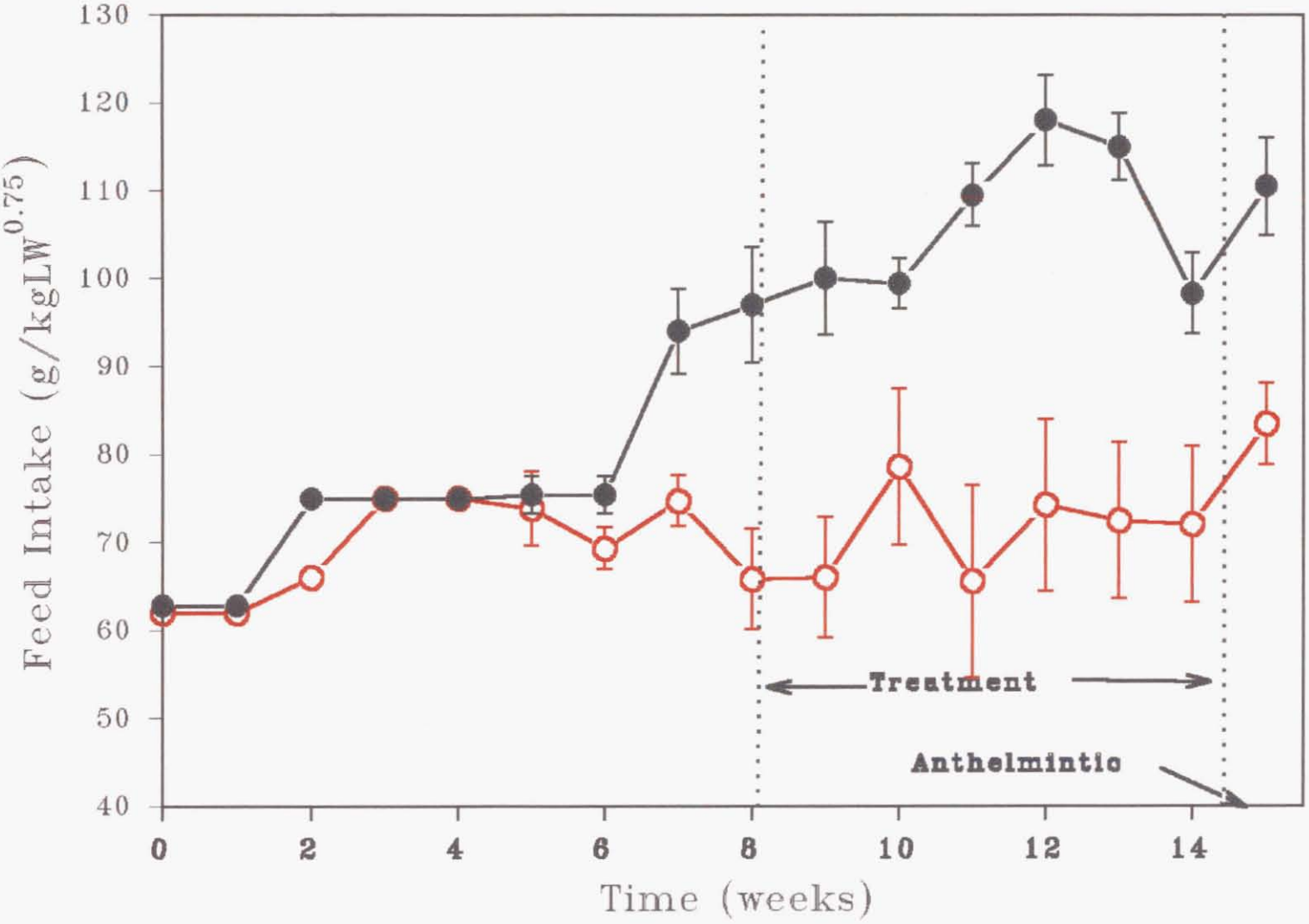


Figure 4.3 Mean daily feed intake (± s.e.m) for infected (○) and non-infected (●) lambs during the experiment (n=6).

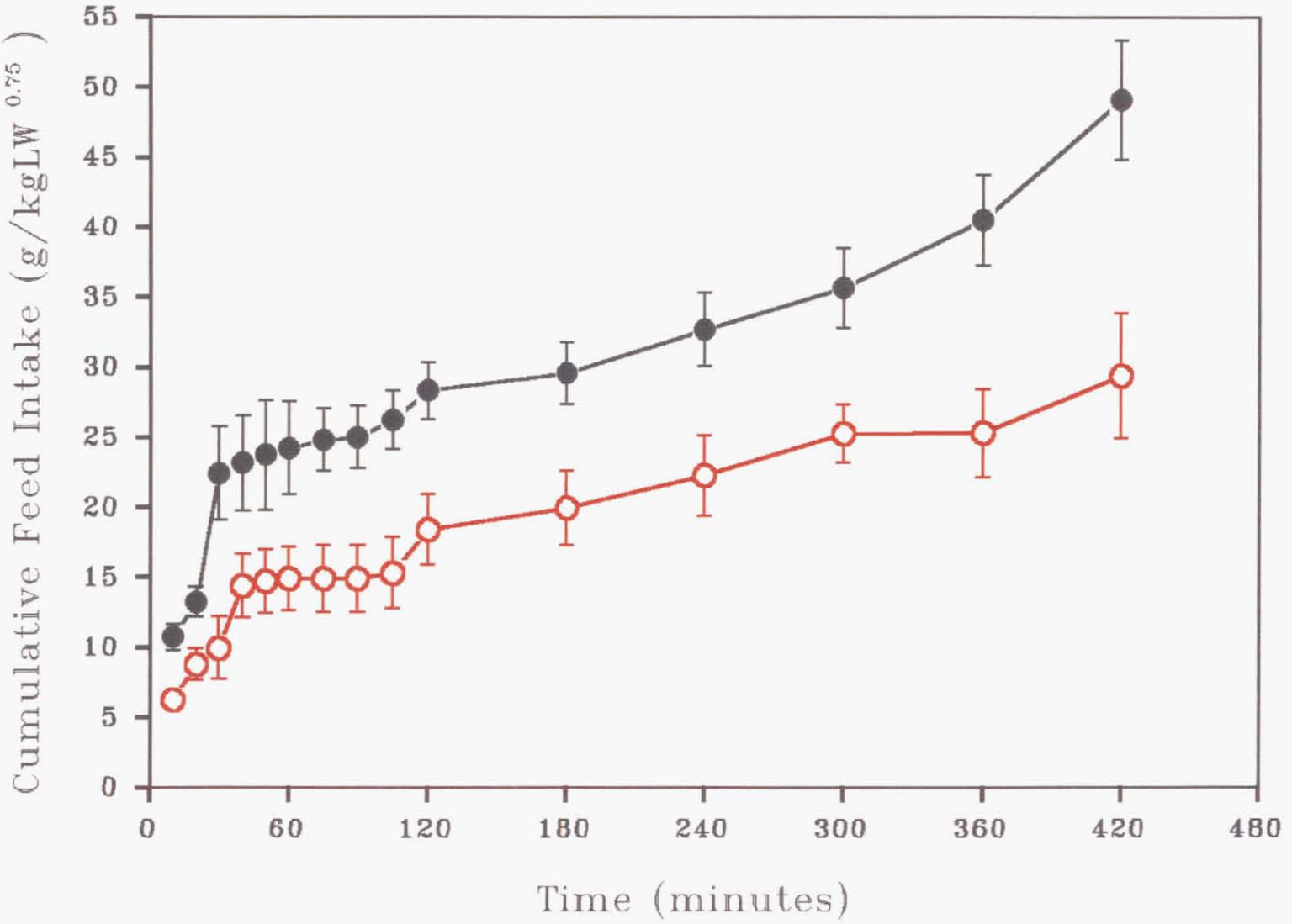


Figure 4.4 Short term cumulative feed intake (± s.e.m) for infected (○) and non-infected (●) lambs fed once per day (n=6).

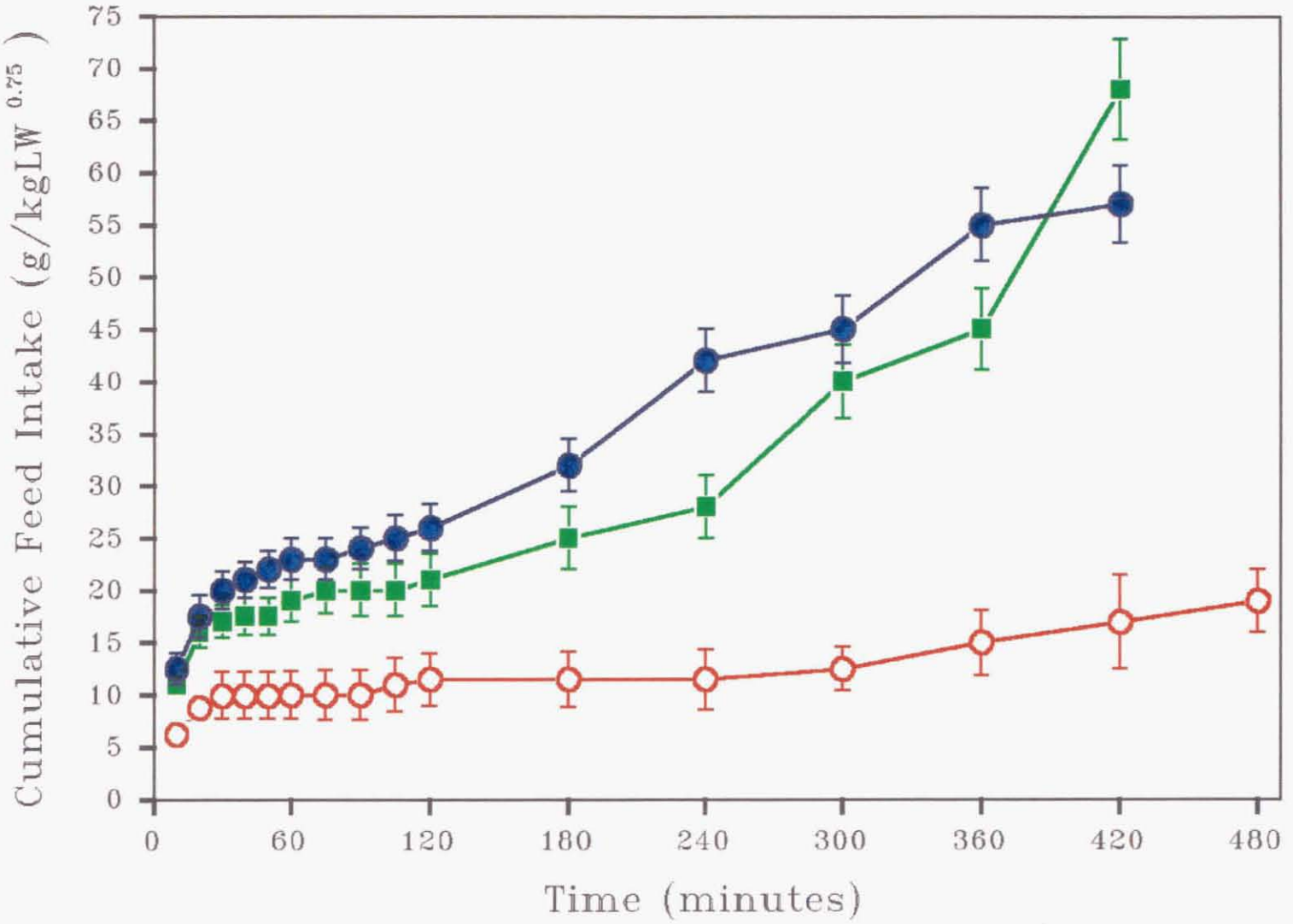


Figure 4.5 Short term cumulative feed intake (± s.e.m) for lambs prior to parasite infection (■), during parasite-induced anorexia (○) and following feed intake recovery after anthelmintic treatment (●), fed once per day (n=6).

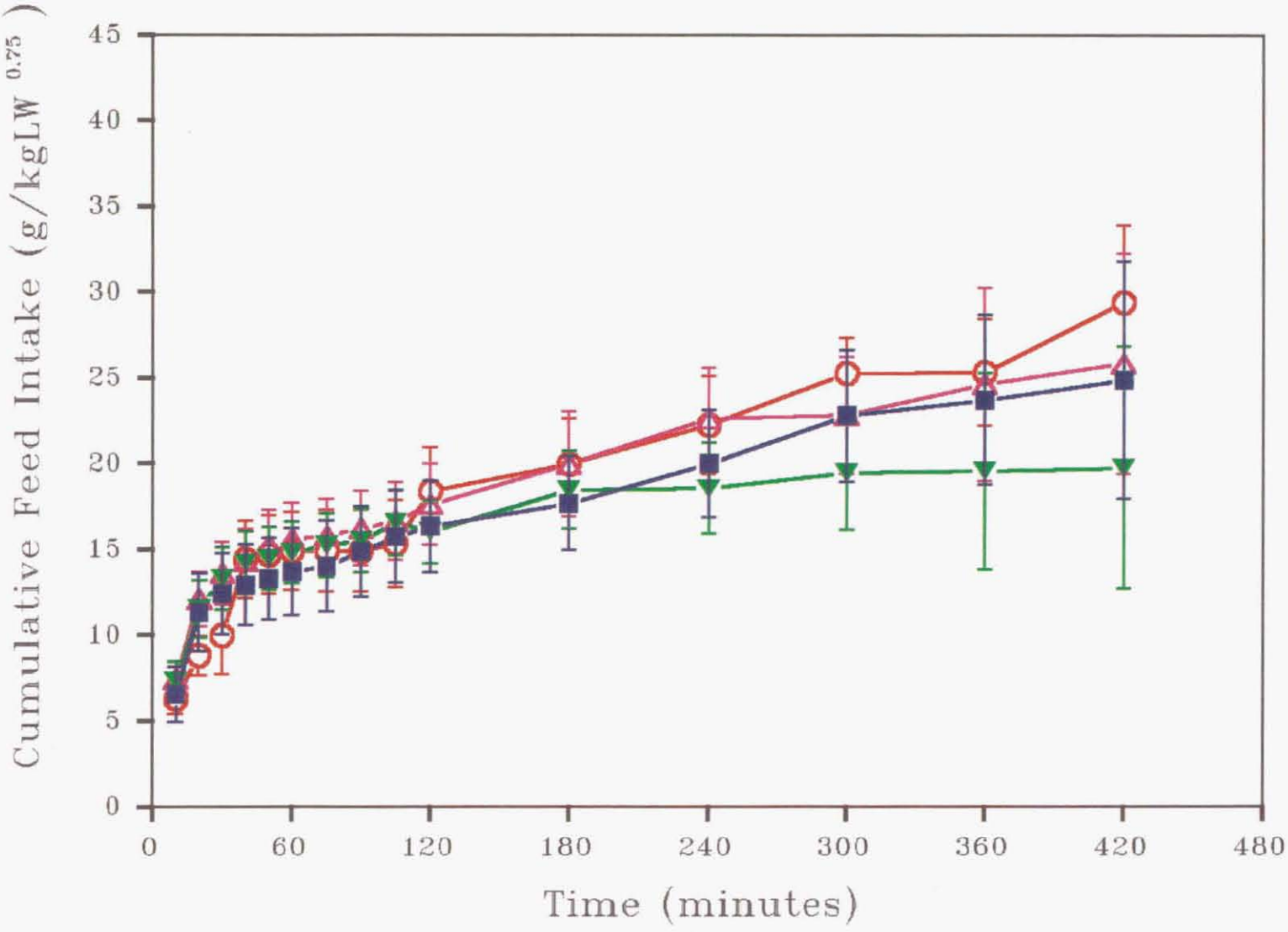


Figure 4.6 Short term cumulative feed intake (± s.e.m) for infected lambs treated with no injecton (o), indomethicin (△), codeine phosphate (▼) or CCK antagonist (■) and fed once per day (n=6).

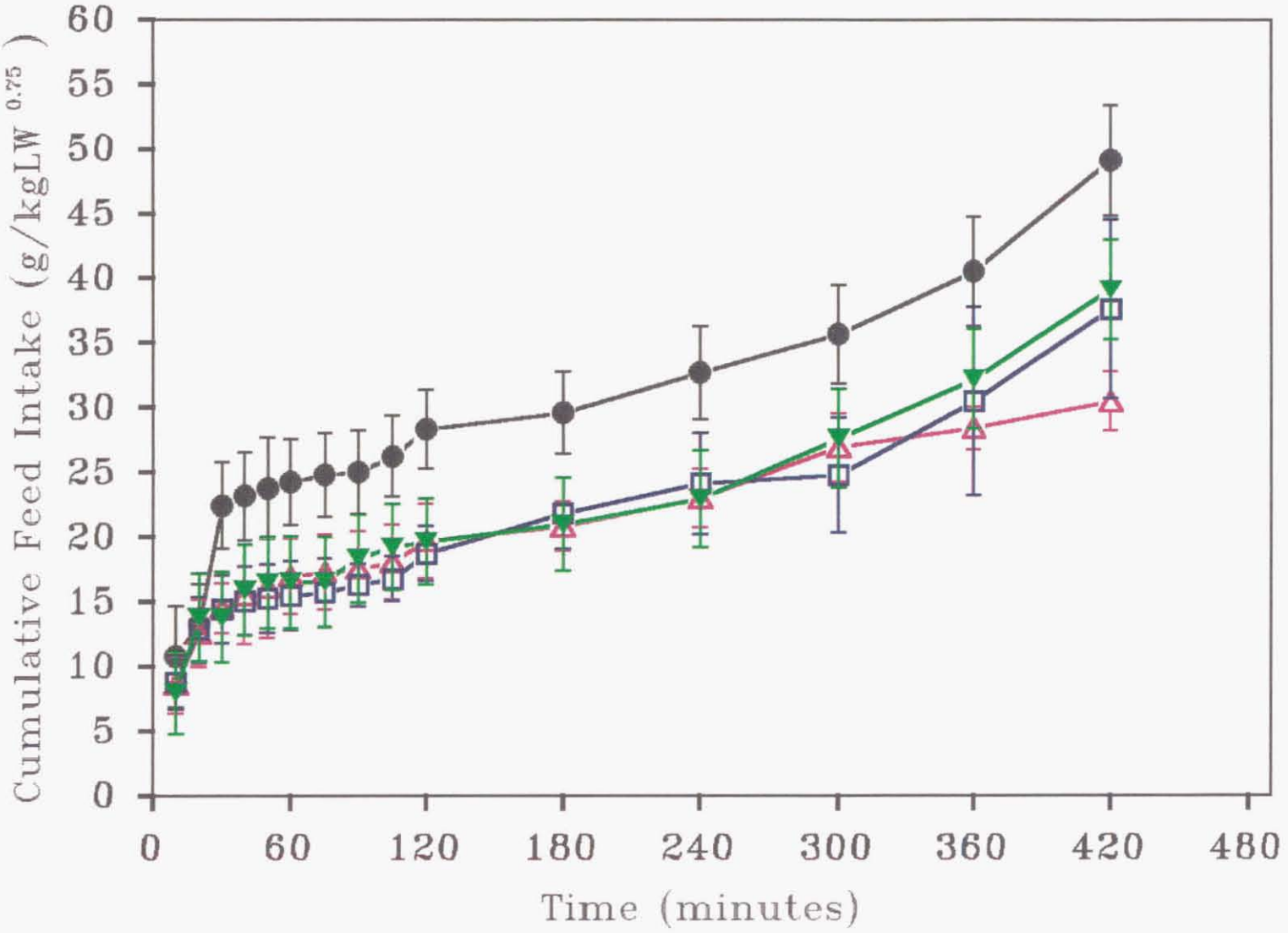


Figure 4.7 Short term cumulative feed intake (± s.e.m) for non-infected lambs treated with no injecton (●), indomethicin (△), codeine phosphate (▼) or CCK antagonist (□) and fed once per day (n=6).

4.4 Discussion

These results show that depression of daily feed intake in parasitised lambs can be detected by changes in the rate of food intake rather than the pattern of food intake which was similar for both groups. This can be readily seen in Figure 4.3. Whilst it was not possible to determine accurately individual meal size and pattern of meal distribution the cumulative feed intake curve provides a good description of what is occurring and is a suitable model with which to investigate the influence of treatments on short term feed intake. This approach is necessary because many compounds act only for a short period and their influence may not be detected in the daily feed intake. The results indicate that cumulative feed intake during parasite infection was depressed at all time intervals in the short term (0-8 h after feeding) as well as daily. It was apparent with this pelleted diet that there was a single large meal when food was offered which terminated for all animals between 40-60 minutes later. This suggests that at least in the present study the cues for initiation and cessation of a meal were similar for both parasitised and non-infected lambs. As cumulative food intake was depressed in parasitised animals it further suggests that rate of food intake differed between groups. An interesting observation in this regard is that within 1 h both groups had consumed 20-22 % of their daily food intake. These results clearly show for the first time how depression in average daily food intake arises.

The short term feed intake model used here provides a useful method for examining pathways which may be involved in signalling intake depression by using pharmacological agents to block or potentiate these pathways. One test is whether the daily intake and cumulative intake curves are reversible and the evidence here shows this is the case. Prior to parasite infection the mean short term cumulative intake of the infected animals was the same as that of the non-infected group. However, following the onset of appetite depression associated with subclinical parasite infection, short term cumulative feed intake fell in the infected group at all time periods recorded. After anthelmintic treatment the time to full feed intake recovery varied with individuals from approximately 4 days to 7-10 days. Generally the greater the degree of inappetence suffered by the individual the longer the time until full recovery of intake was observed. Short term intake recording following the recovery period showed cumulative intake patterns had returned to preinfection levels. The cumulative intake data recorded here demonstrate the depressive effects of parasitism on feed intake and the total recovery of appetite following removal of the parasites by anthelmintic therapy. This clearly reveals the reversible nature of the intake depression pathway and also

suggests that learned taste aversion is not a dominant feature of infection induced anorexia.

The failure to detect any response to the codeine treatment can be interpreted to suggest peripheral pain receptors are not mediators of feed intake depression in infected animals. Codeine is an opioid derived analgesic, which relieves pain in a large number of species and conditions (Jaffe and Martin, 1980). Codeine should have effectively blocked any pain in the parasite infected animals. However, the nature of the experiment, where only one dose level of treatment was given and one route of administration was used, means that the lack of response to codeine has other possible explanations. For instance it may have been due to lack of absorption or an insufficient dose of the drug. Codeine is readily absorbed from the gastrointestinal tract and doses of 100-120 mg are sufficient to produce analgesia in most species, with peak analgesia occurring 1-2 h after administration and lasting for 4-5 h (Jaffe and Martin, 1980). Codeine is generally most effective in relieving continuous dull pain and it is suggested here that, if pain is involved in parasite-induced intake depression, then the gradual onset of feed intake depression suggests it may be of the continuous dull sort. It may be that the dose used in the present experiment was too low. Although 120 mg of codeine phosphate is usually sufficient to elicit analgesia in most species, it would have been preferable to have tested higher doses as well.

It is likely that the intestinal mucosa of infected animals was inflamed, but the lack of response to treatment with indomethacin suggests that the inflammatory response is not the sole instigator of intake depression. Nevertheless similar problems exist when interpreting the lack of response to indomethacin as there were with codeine. Indomethacin blocks the production of prostaglandins. Prostaglandins cause anorexia in rats (Levine and Morley, 1981) while in both sheep (Baile *et al.*, 1981) and rats anti-inflammatory and antipyretic drugs (dipyrone and indomethacin respectively) attenuated endotoxin induced anorexia. In sheep infected by abomasal parasites, Dakkak (1986) found stimulation of bicarbonate transport to be indicative of elevated prostaglandin activity (PGE_2). Daily infusion of aspirin into the abomasum to inhibit prostaglandin release resulted in larger and earlier maturing populations of worms than in untreated infected animals (Dakkak and Daoudi, 1986, cited by Dakkak, 1986). Infusion of a prostaglandin precursor, arachacidonic acid, aggravated the functional problems within the gut of the host associated with parasite infection and led to the elimination of a large proportion of the worms after 48 h (Dakkak and Daoudi 1986, cited by Dakkak, 1986). This suggests that prostaglandins and/or other eicosoids are elevated during parasite infection.

Indomethacin is a potent inhibitor of prostaglandin synthetase thus reducing prostaglandin production within a tissue. However it does not inhibit production of other eicosoids such as leukotrienes so the role of other eicosoids cannot be eliminated. Therapeutic treatment with indomethacin reduces 70-98 % of prostaglandin turnover in man and all other species tested (Vane, 1978). Indomethacin is rapidly and almost completely absorbed by the GIT following oral ingestion, with peak plasma levels occurring within 1-4 h (Woodbury, 1970). The treatment regime used in the present study should have resulted in peak plasma levels of indomethacin at the time of feeding.

All treatments caused some intake depression in the non-infected group. This response is difficult to explain but may be attributable to 3 of the 6 control animals having very low intakes initially. Low feed intake immediately after feeding may have arisen from operator differences in the use of the tablet guns, or in handling of the animals. The depression may also have been due to the treatments being used. However there was no clear pattern to the incidence of low intakes and no problems were encountered in preliminary testing of dose rates in non-infected animals.

There was no effect of the CCK antagonist, L364-718, on short term feed intake in either the infected or non-infected animals. In pigs L364-718 had no effect on the intake of a normal diet (Rayner and Gregory, 1989), but by contrast in both the rat (Shillabeer and Davidson, 1984) and mouse (Silverman, Bank and Lendvai, 1987) L364-718 increased intake. The present results shift the evidence for a role of CCK towards the conclusion of Baile and Della-Fera (1984) that in sheep, unlike some other species, peripheral CCK levels do not appear to be important in regulating meal size. Nevertheless the same degree of caution in relation to dose level and availability of the agent should be applied to these interpretations as for the compounds described above.

L364-718 is a competitive CCK antagonist with no agonist activity but with high affinity and selectivity for peripheral CCK receptors (Chang and Lotti, 1986). Current evidence would support the finding that in healthy animals peripheral CCK would be low prior to a meal but may rise as the meal progresses.

In the current study there was interest in whether peripheral CCK concentrations increased in infected animals, as suggested by Symons and Hennessy (1981). Lack of other studies using L364-718 in sheep and the limitation of only 1 dose level in the current study means

that future studies should use a continuous infusion of the antagonist or a dose response study. The use of a subcutaneous injection as the means of delivery presents difficulties in determining whether the antagonist was reaching the receptors rapidly. A small trial was undertaken at the conclusion of the current trial to test the effectiveness of subcutaneous L364-718 in blocking exogenous CCK-induced feed intake depression. The study was inconclusive because it was impossible to obtain repeatable intake depression following intravenous injection of CCK (Appendix 6).

Administration of either a CCK antagonist, an opioid based analgesic or anti-inflammatory drugs, failed to attenuate parasite induced anorexia in the current trial. Only single dose rates were used for each of the drugs so further study where the compounds are used intravenously or intraduodenally is necessary to avoid the possible effects of rumen microbes on activity of these drugs.

The model developed here detects differences in short term feed intake following the establishment of parasite infection. The recovery to eating patterns following anthelmintic treatment provides further evidence of the temporary/reversible nature of parasite induced intake depression.

CHAPTER 5

An investigation of the role of duodenal discomfort and osmoreceptors in parasite induced feed intake depression.

5.1 Introduction

If the depression in feed intake is the result of painful stimuli from the intestine, then blockade of pain receptors in this organ with analgesic drugs or preventing transmission of sensory signals by use of local anaesthetics should result in a temporary increase in feed intake.

At the body surface pain is perceived directly via free nerve endings, however internally, such as within the intestine, such perception may be more indirect and be a response to chemical stimuli. Numerous chemoreceptors, including glucoreceptors, amino acid receptors, pH receptors and osmoreceptors have been identified in the GIT of the sheep (Forbes, 1986). All are connected to unmyelinated small diameter nerve fibres (C fibres). Application of local anaesthetics to the mucosa abolishes the response of these receptors to their respective chemicals (Mei, 1985). Local anaesthetics prevent both the generation and the propagation of the nerve impulse, principally by blocking the large transient increase in the permeability of the cell membrane to sodium ions (Ritchie and Greene, 1980). This suggests that the effects of chemical changes induced by the presence of parasites may be temporarily abolished by local anaesthetics.

Mucosal mechanoreceptors which discharge upon stroking of the mucosa and muscular tension receptors are both stimulated by contraction or distension of the viscera, and are present in the duodenum (Mei, 1985). These receptor classes may be affected by parasitism, passage rate of digesta, tissue swelling at the sites of invasion by worms, distension or other pathological changes in the small intestine. Their activity should be blocked by application of local anaesthetic.

Intestinal receptors play a role in the control of gastrointestinal motility, intestinal circulation, absorption and exocrine and endocrine secretions. The multifunctional role of chemoreceptors and their abundance suggest that some are capable of acting as nociceptive nerve endings, which upon stimulation by physical or chemical agents cause pain. Visceral

pain is often associated with excessive tension on nerve endings in smooth muscle (Keel *et al.*, 1982) as would occur in the inflamed intestines of parasitised sheep. Ulceration probably causes pain due to the tissue destruction which occurs, this pain may be increased by the action of acidic gastric juices on exposed sensory nerve endings. Inflammation may also contribute to the pain experienced as inflammatory-response products are associated with hypersensitivity to pain of the inflamed area (Keel *et al.*, 1982).

Local anaesthetics suppress the inhibition of feed intake resulting from glucose infusion in pigs (Houpt, 1982; Gregory, McFayden and Rayner, 1987), presumably by blocking chemoreceptor activity. This finding provides support for the idea that a local anaesthetic should be able to block pain felt by parasitised animals.

An opioid-derived analgesic such as codeine provides selective relief for dull continuous pain and may provide relief for parasitised animals.

Most animals become satiated (end meal) before complete absorption of all the nutrients from a meal can occur. A possible feedback pathway for regulating appetite could utilise osmoreceptors of the anterior intestine (Hunt, 1980). Satiety may occur for instance whenever the duodenal osmoconcentration exceeds body fluid tonicity (M^{c}Hugh, 1979). Intragastric and intraduodenal infusions of hypertonic solutions into pigs restrict meal size (Houpt, 1982). Local anaesthetics may inhibit the satiety effects of hypertonic solutions by preventing sensory neurons or cells from signalling changes in osmoconcentration. More recent findings (Gregory *et al.*, 1987) propose that intake control is not limited to just duodenal receptors but to receptors throughout the small intestine.

Osmolality of digesta in the GIT usually fluctuates around 200 to 300 mosmols (isotonic c.a. 330 mosmols/l), although, as in the case of adult ruminants on a dry feed, it may increase to 585 mosmols (Maloiy and Clemens, 1980).

Inflammation at the site of GIT infection may alter intracellular permeability causing disturbances in osmotic balance which may affect feed intake. Worms can also modify physiological function causing altered mucous secretion, increased plasma protein loss and desquamation of gut epithelium, any of which may change the osmolality of duodenal fluid. Elevated osmolality of intestinal fluid may trigger osmoreceptors to send neural or hormonal signals to the hypothalamus to induce satiety. These potential feedback mechanisms would

probably act via receptors associated with the duodenum where the adult *T. colubriformis* worm resides. An analgesic and a local anaesthetic could be used to identify the role of possible pathways in depression of feed intake in these circumstances. In experiment 1 (Chapter 4) oral administration of 120 mg of codeine phosphate did not elevate cumulative feed intake in parasite infected lambs but there was no guarantee that the codeine actually reached the site of damage. This may have been because it was administered orally and absorption was affected by rumen conditions. This concern can be overcome by direct infusion of the drug into the duodenum. To avoid these complicating factors in the present study, both an analgesic (codeine phosphate) and a local anaesthetic (xylocaine) were infused into the duodenum.

This study had 3 objectives. Firstly, it aimed to determine if there were any changes in osmolality of duodenal fluid as sub-clinical *T. colubriformis* infection established and developed. Secondly, it aimed to study the role of duodenal pain and osmoreceptors using intraduodenal infusions of an analgesic and a local anaesthetic. Finally, following anthelmintic treatment and full feed intake recovery in the infected group, absorbed and non-absorbed hypertonic solutions were injected intraduodenally to study their effects on feed intake and determine the effectiveness of a local anaesthetic at blocking osmoreceptors in the duodenum. The study used the model of short term cumulative feed intake developed in Chapter 4 as a means of assessing treatment effects.

5.2 Materials and Methods

Animals

Twelve lambs, part of a larger group born in September 1988, were early weaned at 7 weeks of age and reared in parasite free conditions (Section 3.7). On either April 11 or April 18, 1989 all animals were fitted with a T-shaped cannula into the duodenum (Section 3.4). Following this surgery all lambs were returned to paddocks for at least two weeks, during which time a 3 ml subcutaneous injection of antibiotic (Penstrep L.A., 100 000 iu procaine penicillin, 100 000 iu benzathine penicillin and 250 mg dihydrostreptomycin/ml, A/S Roscoe Veterinary Ethicals Ltd N.Z.) was administered every 3 days. Once a week for the remainder of the trial the area of skin around the cannula was cleaned, clipped and dusted with diazinon powder (Fly Strike Powder, FIL Industries Ltd, N.Z.) to prevent fly strike.

The lambs were moved indoors to individual pens on 30 April 1989 and randomly allocated on the basis of live weight to an infected group (n=8) and a non-infected group (n=4). Trickle infection of the infected group with *T. colubriformis* larvae started on 30 April with each animal receiving 28 000 larvae per week, administered in three doses; 8 000 on Mondays and Wednesdays and 12 000 on Fridays for the duration of the trial (Section 3.6). All animals were initially fed at maintenance to restrict live-weight gain and to aid in the rapid establishment of a pathogenic parasite infection. In a group from the same pool of animals where parasite dosing had commenced 8 weeks earlier establishment of infection had been poor, so the decision was made to restrict the feed intake of animals in the present study.

Three weeks after the commencement of parasite treatment, faecal sampling for determination of faecal egg counts commenced. Samples of faeces were obtained once per week and faecal egg counts determined by the McMaster technique (Section 3.6). Following 5 weeks of infection all infected animals had low faecal egg counts and displayed no sign of sub-clinical parasite infection. To promote the establishment of a satisfactory infection, feed on-offer was further restricted to 450 g per head per day for a 14 day period and all animals treated with 20 mg of methylprednisolone (0.5 ml Depo Medrol, Upjohn) on 2 occasions 7 days apart. This occurred during weeks 6 to 8 from the start of trickle infection, the feed restriction to stress the animals and the glucocorticoid to inhibit temporarily any immune response to developing parasite infection. Feed intake was then gradually increased to *ad libitum* levels in all animals during weeks 8 and 9 of the trickle infection. During this period animals became accustomed to having feeds removed for weighing at regular intervals (Section 3.1).

Duodenal osmolality

Over the 9 week infection period, prior to treatments being imposed, a duodenal digesta sample (20 ml) was taken weekly for determination of osmolality. Each sample was taken by allowing 'free flow' from the duodenal cannulae into a polythene bag attached to the duodenal cannula with a Gray clip (acetal resin snap-on tubing clip, Gallankamp). The samples were centrifuged at 2 500 rpm for 20 min, supernatant decanted and stored frozen for analysis at a later date.

Following onset of feed intake depression in infected animals (9 weeks), further duodenal samples were collected prior to feeding and after the first meal (45 min) on both control and

treatment days (see experimental design section). Again duodenal samples were centrifuged at 2 500 rpm for 20 min and the supernatant collected and frozen for later analysis. Supernatant samples were thawed in a water bath (20 °C) before analysis. This technique had been checked previously to confirm that the procedure did not affect the osmolality of the sample. Osmolality of digesta samples was determined using the freezing point depression method (Fiske Osmometre, Uxbridge Mass, U.S.A. 3203226).

Experimental procedure

i. Pain blockade

When feed intake depression of at least 20 % had occurred in the infected animals, the experimental treatments commenced. This was 10 weeks from the start of trickle infection. A replicated Latin square design was used to test the effect of 4 treatments on feed intake of both infected and non-infected animals. These were:

1. control - no injection.
2. phosphate buffered saline solution - 50 ml, infused via cannula into the duodenum 15 min before feeding.
3. local anaesthetic - 50 ml xylocaine hydrochloride (1 % solution Lopaine in sterile distilled water, Lopaine 2 % Troy Laboratories Pty Ltd), infused via cannula into the duodenum 15 min before feeding.
4. analgesic - 50 ml solution of codeine phosphate (360 mg in phosphate buffered saline) infused via cannula into the duodenum 15 min before feeding.

The Latin square design was repeated so animals received all treatments twice during the period 7 to 17 August 1989. Treatments were administered every second day to minimise carry-over effects.

All treatments were administered via the duodenal cannula using a 60 ml syringe attached to a duodenal stopper, which had a 10 cm section of silicone rubber tubing (I.D. 4 mm O.D. 6 mm, Silastic, Dow Corning Corporation, Michigan, U.S.A.) threaded through it and secured in place with silicon rubber adhesive (Dow Corning Corporation, Michigan, U.S.A.). This apparatus prevented backflow of injected material out of the cannula. The 50 ml treatment solution was warmed in a water bath (37 °C) prior to infusion and injected slowly into the cannula. 25 ml of saline solution followed the injection to wash the

treatment solution out of the cannula and the modified injection tube was clamped off and left in place for 2 h. No spillage of duodenal contents occurred.

ii. Osmoreceptor stimulation plus blockade

Experimental treatments commenced on 28 September 1989, 4 weeks after anthelmintic treatment to infected animals (29 August), 10 animals received the following treatments via the duodenum in a repeated Latin square allocation of treatments;

1. control - 250 ml saline.
2. absorbable hypertonic solution - NaCl 5 ml/kg W of a 6.5 % solution.
3. non-absorbable hypertonic solution - mannitol 5 ml/kg LW of a 40 % solution.
4. NaCl (as in ii) + 100 ml 1 % local anaesthetic (20 mg/ml lignocaine hydrochloride, Lopaine 2 % Troy Laboratories Pty Ltd).
5. mannitol (as in iii) + 100 ml 1% local anaesthetic (as above)

Treatments were applied 5-10 min prior to feeding. Where treatment included local anaesthetic, the anaesthetic was infused into the cannula prior to infusion of the hypertonic solution. Feed was then offered at t_o and intake recorded as in Section 3.1.

Animals were weighed weekly prior to feeding and feed sampling carried out as per Section 3.2 and 3.3.

Statistical analysis

Feed intake recorded at each time period was analysed using one-way analysis of variance (Minitab v. 7.2, Minitab Inc.) and general linear model (GLM) for unbalanced designs. Student's *t*-test (Minitab v. 7.2, Minitab Inc.) was used for comparison of mean osmoconcentrations.

5.3 Results

A failure to display consistent feed intake depression resulted in 4 infected animals being removed from the trial and their data excluded from all analysis. Treatment and analysis was undertaken on 4 infected and 4 non-infected animals.

Parasitology

Mean faecal egg numbers recorded during the experiment are shown in Figure 5.1. *T. colubriformis* eggs first appeared in the faeces after the sixth week of infection and peak egg numbers were evident after week 11 of infection. There was considerable individual variation in faecal egg numbers.

Live weight

Mean live weights for infected and non-infected animals are shown in Figure 5.2. Mean live weight of infected lambs showed little change up to week 6 of infection then increased over the remainder of the experiment although live weight remained approximately 5 kg less than that of the non-infected animals. Throughout the experiment, non-infected lambs gained weight steadily.

Daily feed intake

Average daily feed intakes (7 day) are shown in Figure 5.3. Up to week 6 intake was restricted, thereafter, feed was offered *ad libitum*. Following removal of intake restriction, intake increased rapidly in both groups, however, after 9 weeks intake fell in infected animals and remained depressed for the next 3 weeks. After 12 weeks of infection intake gradually increased in infected animals.

Short term feed intake

Mean short term cumulative feed intakes are shown in Figure 5.4. In infected animals intake was depressed at all time periods by approximately 40 % ($p < 0.05$). All animals initially ate rapidly, with infected animals terminating their first meal 30-40 min after feeding and non-infected animals terminating theirs about 10 min later.

Treatment Effects

i. Effect of parasite infection and feeding on duodenal osmolality.

Mean osmolality of duodenal fluid collected from lambs before and immediately after feeding is given in Table 5.1.

Table 5.1 Mean (± s.e.m) osmolality of duodenal digesta fluid for infected and non-infected lambs fed once per day and sampled prior to feeding or at the termination of the first meal (n=4).

	Mean osmolality (mosmols/l)	
	Prefeeding	Post feeding
Infected	250 ± 14.5	260 ± 14.5
Non-infected	240 ± 6.7	260 ± 6.7

Treatment with local anaesthetic or analgesic had no effect on the osmolarity of the duodenal fluid, so results were pooled. There was no change in duodenal fluid osmolarity with parasite infection or following feeding (Table 5.1).

ii. Injections of analgesic and local anaesthetic to infected and non-infected animals.

Figures 5.5 and 5.6 show feed intake for infected and non-infected groups following intraduodenal treatment with saline solution, codeine phosphate and local anaesthetic. Only parasite infection had a significant effect ($0.01 < p < 0.05$) on intake by causing intake depression in the infected group at all time periods analysed. Treatment with intraduodenal analgesic or local anaesthetic did not attenuate this feed intake depression in the infected group. Similarly the treatments had no effect on intake in the non-infected group.

iii. Intraduodenal infusion of hypertonic solutions to non-infected animals only.

Intraduodenal infusion of hypertonic mannitol or NaCl solutions prior to feeding significantly reduced feed intake ($0.001 < p < 0.05$) for the first 3 h of feeding (Figure 5.7). Intake depression of around 70-80 % occurred immediately after treatment, with individuals eating nothing or a small amount in the first 10 min of feeding, then no feed for the next 1.5 h. The intake depressive effects of the treatments lasted for 1.5 h and was followed by a period of rapid intake. Between 1.5 and 3 h after feeding on control treatment days animals ate 6.65 g/kg $LW^{0.75}$, whereas following mannitol, NaCl, mannitol + anaesthetic or NaCl + anaesthetic they ate 17.2, 13.3, 21.2, and 17.1 g/kg $LW^{0.75}$ respectively. These intakes being significantly higher ($p < 0.05$) than those of the controls. By 4 h after feeding, effects of the treatments on cumulative feed intake were not evident.

Local anaesthetic did not block the intake depressive effects of either mannitol or NaCl, the cumulative intake in both cases was not different from that when the hypertonic solutions were given alone.

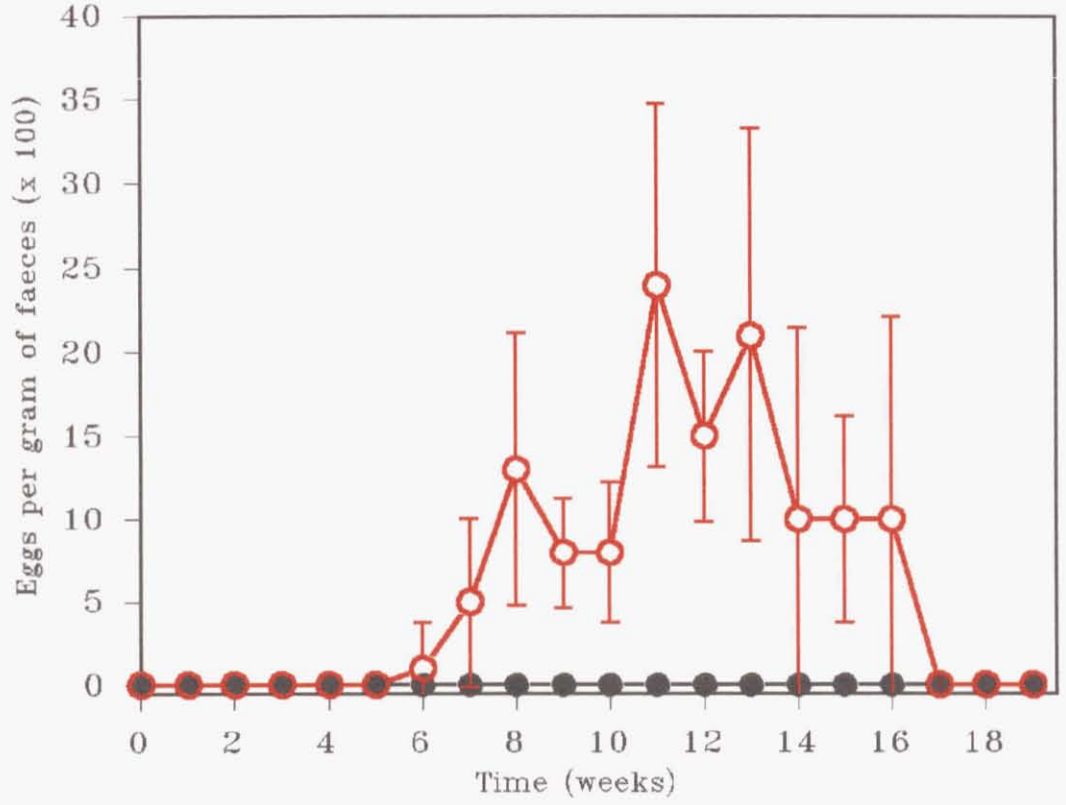


Figure 5.1 Mean (± s.e.m) number of *T. colubriformis* eggs per gram of faeces in infected (○) and non-infected (●) lambs sampled once per week (n=4).

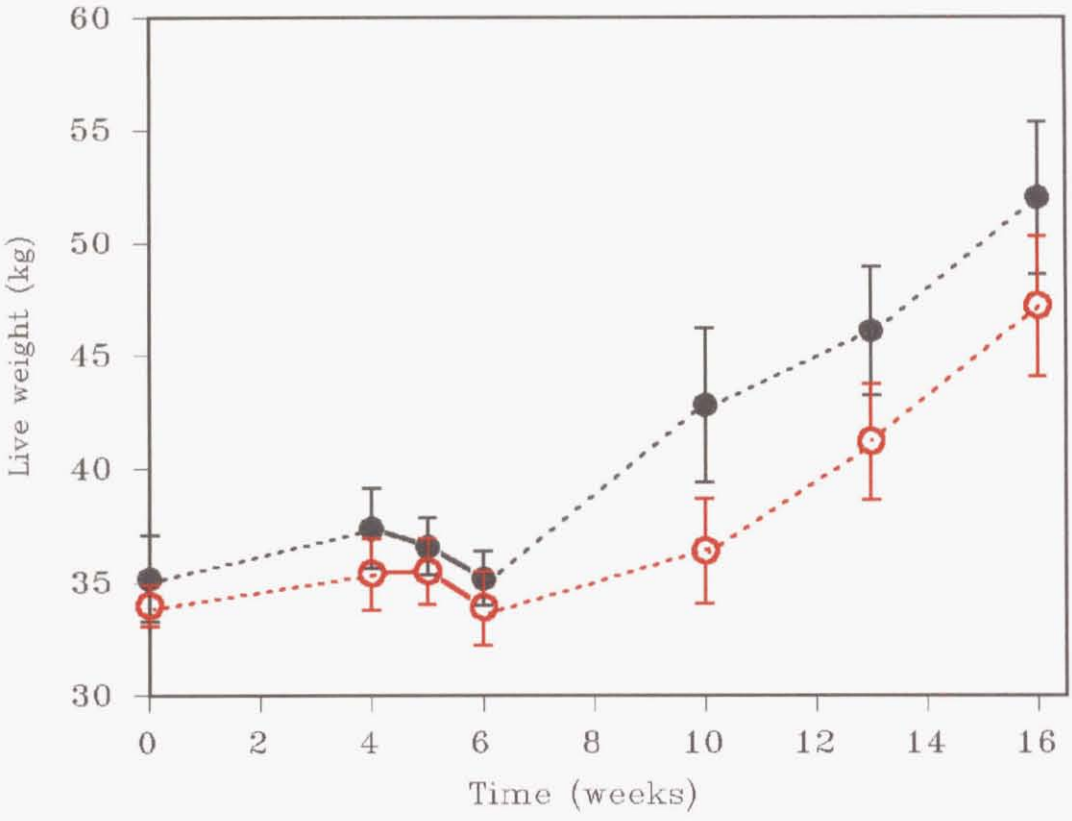


Figure 5.2 Mean live weight (± s.e.m) for infected (○) and non-infected (●) lambs during the experiment (n=4).

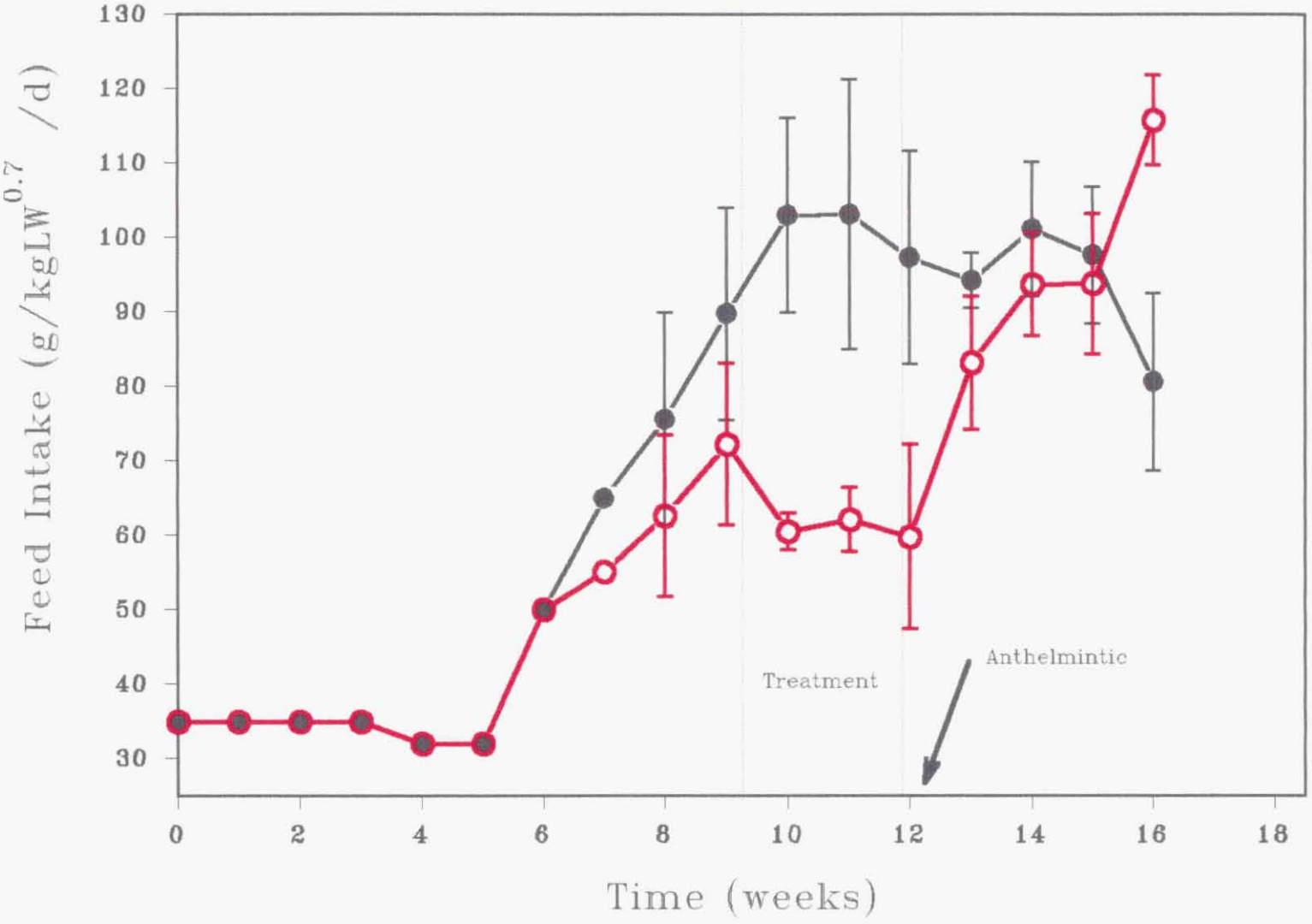


Figure 5.3 Mean daily feed intake (± s.e.m) for infected (o) and non-infected (●) lambs during the experiment (n=4).

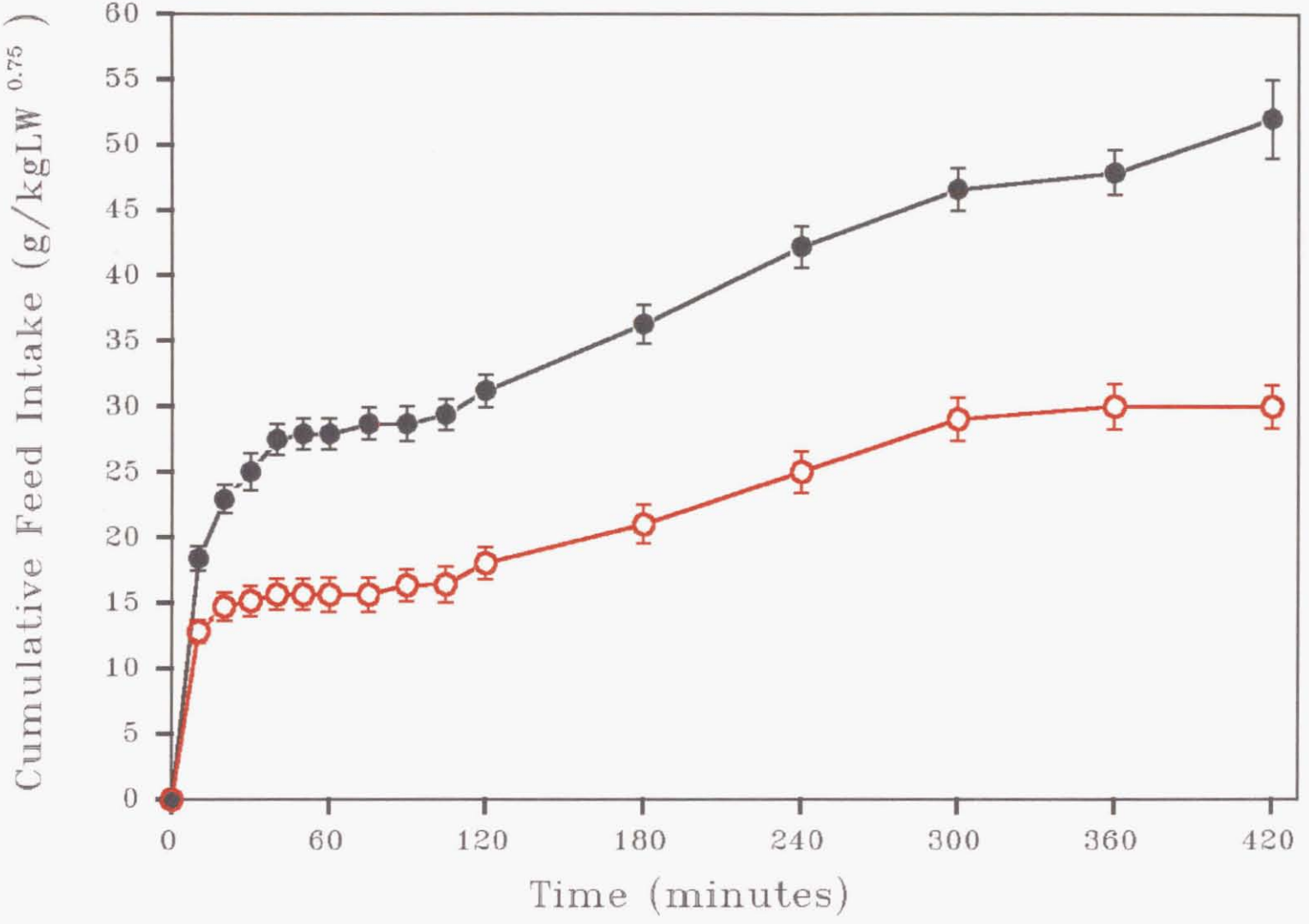


Figure 5.4 Short term cumulative feed intake (± s.e.m) for infected (○) and non-infected (●) lambs fed once per day (n=4).

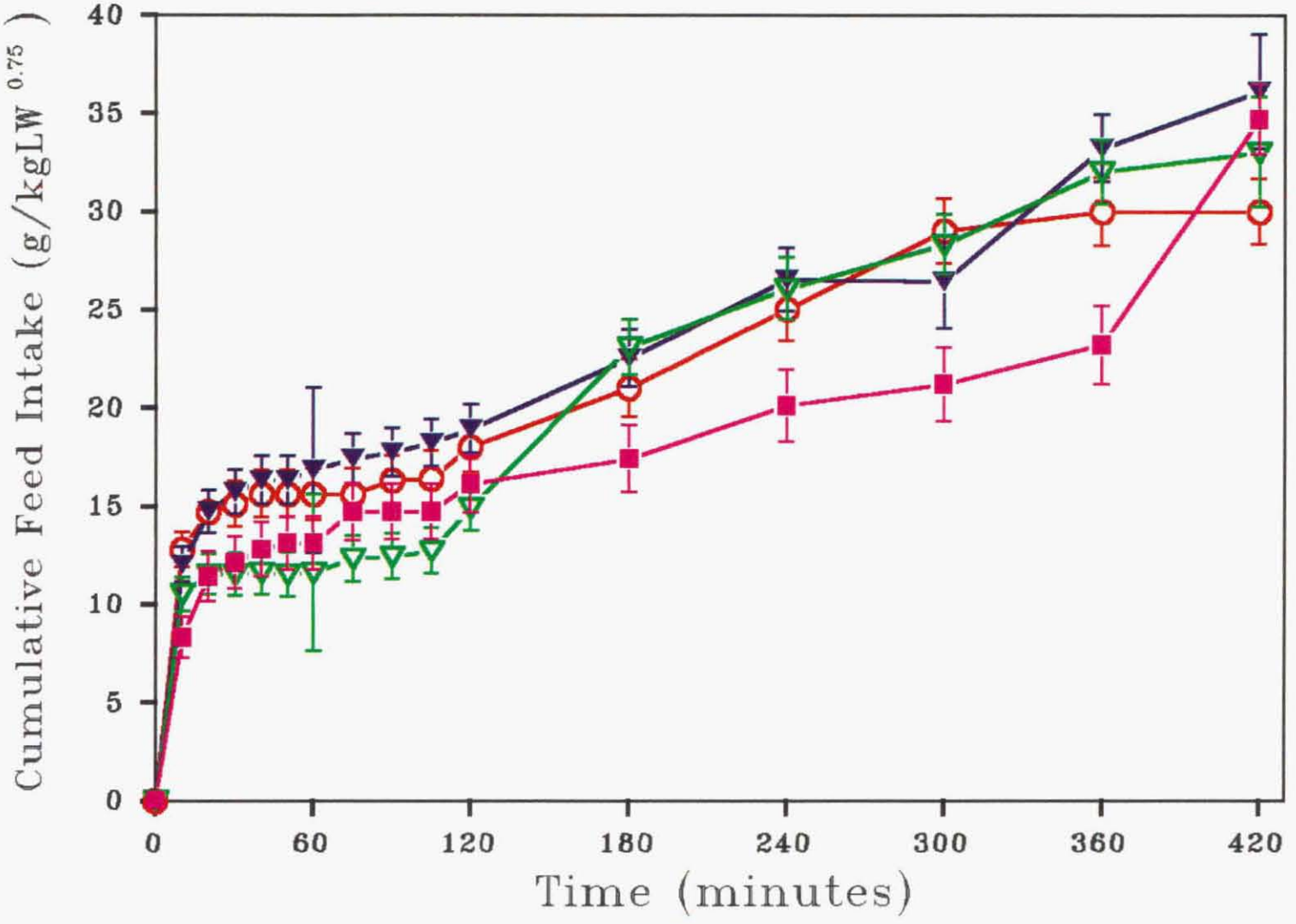


Figure 5.5 Short term cumulative feed intake (± s.e.m) for infected lambs treated with no injecton (○), saline (▼), codeine phosphate (▽) or anaesthetic (■) and fed once per day (n=4).

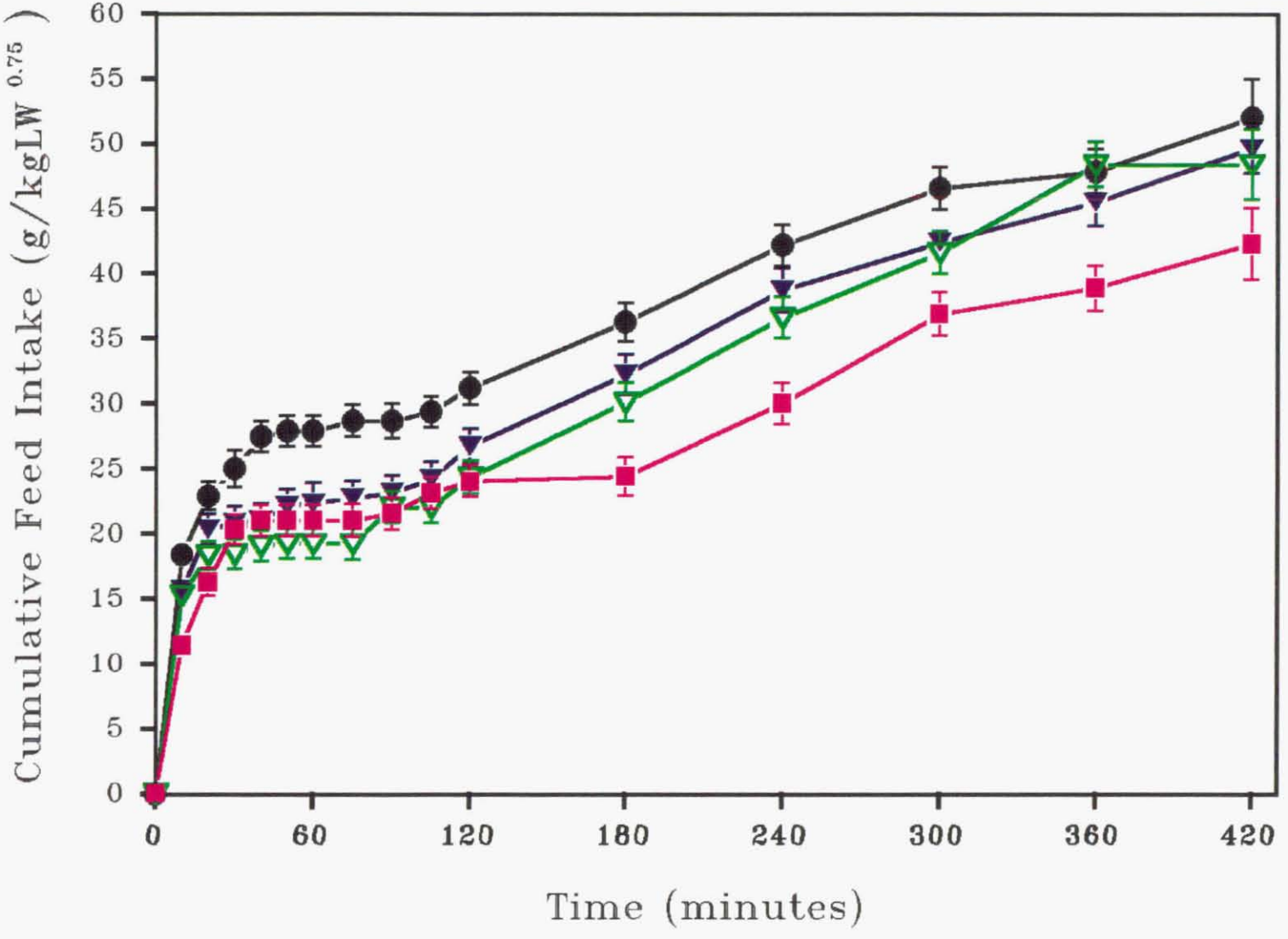


Figure 5.6 Short term cumulative feed intake (± s.e.m) for non-infected lambs treated with no injecton (●), saline (▼), codeine phosphate (▽), or anaesthetic (■) and fed once per day (n=4).

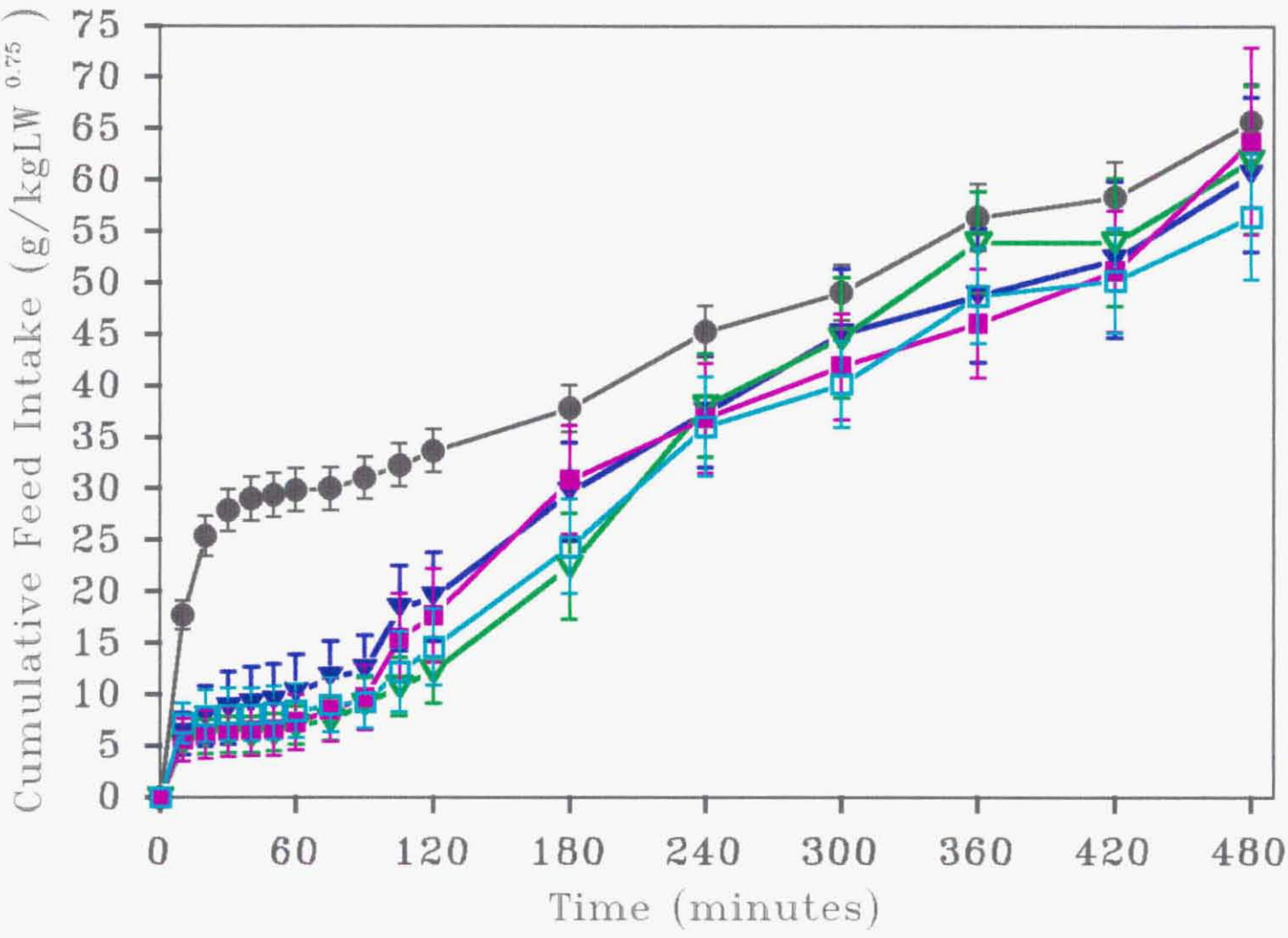


Figure 5.7 Short term cumulative feed intake (± s.e.m) for non-infected lambs treated with no injecton (●), NaCl (▽), mannitol (▼), NaCl plus anaesthetic (□) or mannitol plus anaesthetic (■) prior to feeding (n=10).

5.4 Discussion

Some difficulty was experienced in the establishment of pathogenic parasite infections. The initial larval preparation appeared to have a poor pathogenicity, possibly due to lengthy storage of the larvae or repeated passage of the same culture over several years to obtain larvae (R.W M^{c}Anulty, M. Bown pers. comm.). To minimise the likelihood that the initial exposure to larvae may compromise development of infection with the new larval culture, all animals were treated with a corticosteroid, to suppress the immune system.

Intake depression occurred 10 weeks after commencing infection, somewhat later than the 6 weeks usually recorded by other authors (Sykes and Coop, 1977) but similar to earlier studies (Chapter 4). Intake depression in the infected group was similar to that reported for *T. colubriformis* infections (Sykes, 1982; Holmes, 1986). However there was no clear fall in live weight in the infected animals in the current trial, rather their weight remained unchanged while the non-infected animals continued to grow.

Establishment and onset of *T. colubriformis* infection did not affect the osmolality of the duodenal fluid in infected animals. This was despite a number of samples from infected animals having mucus and a strong green colouration present in the digesta collected. Changes to mucosal function and increased leakage of albumin into the digestive tract might be expected to increase osmolarity. Flow of digesta from the abomasum even in infected animals is of the order of 120-180 ml/h (see Chapter 7) so any localised changes would have to be marked to overcome the diluting effect of digesta flow. Difficulties existed with the collection of duodenal samples for analysis. Initial plans to pass silicone rubber tubing into the cannula and down the duodenum some 20-50 cm, were not successful, so a free-flow sample was collected. The duodenal cannula was placed in the anterior duodenum, approximately 10 cm from the pylorus. Although 90 % of *T. colubriformis* worms are in the anterior third of the small intestine, the cannula was close to the pylorus and would be likely to be cranial to the locality of a large percentage of the adult worm population. Despite this, many samples from infected animals were obviously different in appearance to those from non-infected animals. Furthermore, there was no change in duodenal osmolarity following feeding. In pigs, changes in duodenal osmolarity have been recorded 8 min after feeding (Houpt, Houpt and Swan, 1983b). The presence of a rumen reservoir may well buffer against major changes in duodenal osmolarity in the case of ruminants such as sheep. The osmolarity recorded here (approximately 250 mosmol/l) was somewhat lower than the

465, 585 and 564 recorded in duodenums of sheep, camels and cattle by Maloiy and Clemens (1980). These differences can arise from species differences or dietary effects.

Sampling coincided with the termination of the first meal (45 min after feeding) and it may be concluded that satiety was not associated with osmotic changes in the anterior duodenum or in digesta from the abomasum in either infected or non-infected animals. Elevated osmotic pressure stimulates intestinal receptors to inhibit feed intake in humans (Hunt, 1980) and pigs (Houpt, Anika and Houpt, 1979; Houpt, Baldwin and Houpt, 1983a; Houpt *et al.*, 1983b; Gregory *et al.*, 1987). In the pig, digesta arrives at the intestine 12 min before the termination of the meal, suggesting osmoreceptors are not the only regulators of meal size (Houpt *et al.*, 1983b). Gastric distension may have an important inhibitory role in this situation (Gregory *et al.*, 1987). In ruminants osmoreceptors in the reticulo-rumen may have a role in intake regulation (Carter and Grovum, 1990). Prior to feeding rumino-reticular fluid is hypotonic to plasma, 247 $\pm$ 18 mosmol/kg (Engelhardt and Hauffe, 1975), the precise level being a function of diet type and its fermentation characteristics. A post prandial rise in the osmolarity of rumen fluid occurs with the rate and extent depending on the diet, amount consumed per unit time, activity of ruminal microbiota and water intake (Carter and Grovum, 1990) so these changes may have a role in the termination of meals.

There appears to be little in the literature on the role of duodenal osmolarity in meal regulation in ruminants. No changes in duodenal osmolarity following feeding were observed in the present experiment. Hypertonic solutions injected into the duodenum caused a dramatic and transient depression in intake, but the effect was not blocked by local anaesthetic. The apparent inability of the local anaesthetic to reverse this response is interesting. Mannitol is not absorbed in the intestine and its osmotic effect would be expected to act on receptors at or near the mucosal surface whereas NaCl is absorbed by the intestinal mucosa and could affect deeper receptor sites. For the first 30 min sheep were prevented from drinking water. This led to some distress in a number of instances, not unexpectedly, as the hypertonic solution would lead to considerable movement of extracellular water into the gut and could stimulate a strong thirst. Concentration and levels of local anaesthetic and the hypertonic solutions were similar to those used successfully in pigs to block responses to hypertonic solutions (Houpt *et al.*, 1983b). This finding suggests intestinal osmoreceptors are not present in sheep. The hypertonic solutions (approximately 1 000 mosmol/l) could have caused a rapid movement of fluid into the intestine possibly reducing blood volume which may have caused a centrally mediated intake depression or

stimulated osmoreceptors. Alternatively, the intake depression following hypertonic solutions recorded here may not be due to specific osmoreceptors but due to generalised malaise caused by the massive movement of fluids within the gut resulting from the infusion of the hypertonic solutions into the duodenum.

Local anaesthetic did not increase feed intake in infected animals. Xylocaine is absorbed very rapidly from the GIT tract and peak anaesthetic effects should be felt within 2-5 min, with the duration of the effect 30-45 min. Pain felt at the mucosal surface could be expected to be alleviated by local anaesthetics, but, pain caused by pressure or distortion of adjacent structures may not be alleviated (Smith and Aitkenhead, 1985). Pressure or distortion of adjacent structures in the duodenum may occur in infected animals and maybe this mechanism is not blocked by the anaesthetic hence intake depression prevailed. Local anaesthetics have been used successfully in the rumen of sheep (Baile and Forbes, 1974) and in the intestine of pigs (Houpt *et al.*, 1983a,b) to block receptor function and attenuate the intake depressive effects of VFA and hypertonic solutions respectively. Interpretation of the lack of response here is confounded by the local anaesthetic not blocking intake depression caused by infusion of hypertonic solutions.

Blocking continuous dull pain by using an opioid-derived analgesic intraduodenally did not successfully elevate feed intake in infected animals. This result confirms the earlier finding (Chapter 4) of no attenuation of intake depression when codeine was administered orally. Codeine phosphate is a highly soluble salt which should have been absorbed rapidly and its analgesic activity could be expected to have a duration of 4-5 h, sufficient time to elicit an eating response. This evidence indicates that dull pain associated with GIT parasites is unlikely to have a major role in feed intake depression.

There were no changes in the osmolality of duodenal digesta in this experiment during the establishment or onset of a subclinical *T. colubriformis* infection of the small intestine. Parasite induced feed intake depression was not blocked by local application of either an opioid analgesic or a local anaesthetic. Any local pain at the infection site does not appear to be an important mediator of the observed anorexia.

In conclusion, feed intake depression could not be alleviated by either blocking duodenal pain receptors using an opioid derived analgesic or by using a local anaesthetic to block duodenal osmoreceptors. Furthermore, feed intake could be depressed in healthy animals by

infusing hypertonic solutions but the presence of osmoreceptors remains uncertain because of an inability of the local anaesthetic to block the effects of the hypertonic solution.

CHAPTER 6

The effect of varying levels of peripheral CCK blockade on feed intake in parasite infected lambs.

6.1 Introduction

No increase in feed intake of infected lambs following a single subcutaneous injection of the peripheral CCK antagonist L364-718 was found in the experiment reported in Chapter 4. Conclusions were limited by the fact that the dose and route of administration had to be assumed as suitable to elicit a short term eating response. It is proposed here that use of intravenous administration and the formulation of a dose-response relationship should help to refute many of the limitations of the earlier approach.

The CCK receptor antagonist loxiglumide (CR1505), a highly potent and competitive antagonist of peripheral CCK, was used in the current study. A proglumide derivative, loxiglumide is from a chemically different family to the asperlycin derived L364-718 used in the earlier study but is also a potent CCK antagonist and can be administered intravenously.

Specific CCK receptors have been localised in different areas of the GIT and have a role in gut motility, function and feed intake regulation in a number of species (Smith and Gibbs, 1984). Unlike the case in other species, the role of peripheral CCK receptors in intake regulation in sheep remains controversial. Baile and Della-Fera (1984) concluded, following considerable work in the area, that in sheep peripheral CCK receptors are not as important for regulating intake as they are in other species. However differences may occur in parasite infected lambs. Symons and Hennessy (1981) found elevated CCK levels in infected animals associated with the period of intake depression, and the plasma concentration of CCK fell following removal of worms by anthelmintic treatment. Elevated peripheral CCK concentration could depress feed intake directly or indirectly by slowing gut movement, and although not a factor in normal control of intake may operate as a safety mechanism in cases of disturbance to the interior mileau such as occurs with parasite infection.

This study investigated the involvement of peripheral CCK action in feed intake depression by using a continuous infusion of a potent peripheral CCK antagonist (loxiglumide).

6.2 Materials and Methods

Animals

A pool of Dorset Down-Coopworth cross ewe lambs were early weaned (see Section 3.7) in October 1988. Sixteen of these lambs were moved indoors into individual pens on 10 April 1989 and offered a complete pelleted ration (Appendix 1). Larval dosing of infected lambs (n=8) commenced on 10 April 1989 (Section 3.6), non-infected animals (n=8) were dosed with filter tabs only.

Feeding

For the first 3 weeks of trickle infection, non-infected and infected animals were offered 80-85 % of maintenance metabolisable energy (ME) requirements. Intake gradually increased so that after 4-5 weeks of infection all animals were offered *ad libitum* rations. Animals were fed a complete pelleted ration (Appendix 1). Feeding procedures, sampling and analysis were as described in Section 3.3.

Parasite infection

As in Chapter 4 problems encountered with establishment of infection led to all animals receiving 20 mg methylprednisolone (0.5 ml Depo Medrol, Upjohn Ltd.) on 2 occasions 7 days apart during weeks 4 and 5 of infection. A culture of *T. colubriformis* larvae was obtained (Dr T. Watson, Ruakura Agricultural Research Centre) in the hope that it would have greater pathogenicity than the local culture and animals were infected with the new culture from week 3.

Treatments

The trial was run as a replicated duplicate Latin square design (8 infected animals paired with 8 non-infected animals receiving 2 replicates of each of 4 treatments). Immediately prior to feeding animals were injected i.v. with 0, 5, 10 or 20 mg loxiglumide per kg live weight via a temporary indwelling catheter (16 G x 5 cm, I.D. 1.3 x 51 mm, Surflo I.V catheter, Terumo Corporation, Tokyo, Japan). Loxiglumide was supplied in sterile glass vials containing 0.54 % loxiglumide in a phosphate buffered saline solution. Zero dose (0 mg) consisted of phosphate buffered saline solution only.

Prepared solutions were drawn from sterile vials into 60 ml syringes. The syringe was attached directly onto the catheter and the solution infused steadily into a jugular vein.

Statistical analysis

Analysis of variance for unbalanced data was carried out on ranks of cumulative intake data at each time period (non-parametric testing) and least squares differences used to establish differences between means (SAS v 6.1, SAS Institute Cary N.Y., U.S.A.). Live weight, weekly intake and faecal egg counts were analysed using Student's *t*-test (Minitab v 7.2).

6.3 Results

Two of the infected animals failed to develop consistent feed intake depression within the duration of the experiment so were excluded, along with their non-infected pair, from treatment and all data analysis.

Parasitological

Faecal egg counts recorded during the experiment are shown in Figure 6.1. Eggs first appeared in faeces after week 3 of infection and remained high for the duration of the experiment. No eggs were detected in faeces of non-infected animals during the experiment. All animals were treated with anthelmintic at week 7 of infection, and egg numbers fell after drenching.

Live weight

Mean live weights of infected and non-infected animals during the experiment are shown in Figure 6.2. Following the removal of feeding restrictions the live weight of the non-infected animals continued to increase and these animals gained approximately 480 g/day, while the infected animals gained very little weight (90 g/day). As a result, by the end of the experimental period non-infected animals were 5-6 kg heavier than the infected group.

Daily feed intake

Mean ($\pm$ s.e.m) daily feed intakes over 7 day periods are displayed in Figure 6.3. Following removal of intake restrictions, feed intake in the non-infected group rose rapidly to approximately 110 g/kg $W^{0.75}$/day then fell slightly to 90-100 g/kg $W^{0.75}$/day. Intake in the infected animals dropped immediately and remained depressed (24-28 %) for approximately 5 weeks, until 7-10 days after anthelmintic treatment, then increased gradually and recovered fully by week 11.

Short term feed intake

Short term intake for infected and non-infected animals recorded during weeks 5-7 of infection is shown in Figure 6.4. Parasite infection reduced short term voluntary feed intake by between 34 % (360 min) and 50 % (40-50 min), with the average intake depression being 43 % during the period recorded (0 to 8 h).

Treatment effects

Figure 6.5 shows the short term cumulative feed intake for infected and non-infected animals following intravenous injection of 0, 5, 10 or 20 mg of loxiglumide per kg live weight before feeding. There was no significant effect of the 3 levels of loxiglumide on cumulative feed intake at any of the recorded time periods. In all cases the resulting feed intake was not significantly different from that of the saline (control) treatment in both groups of animals.

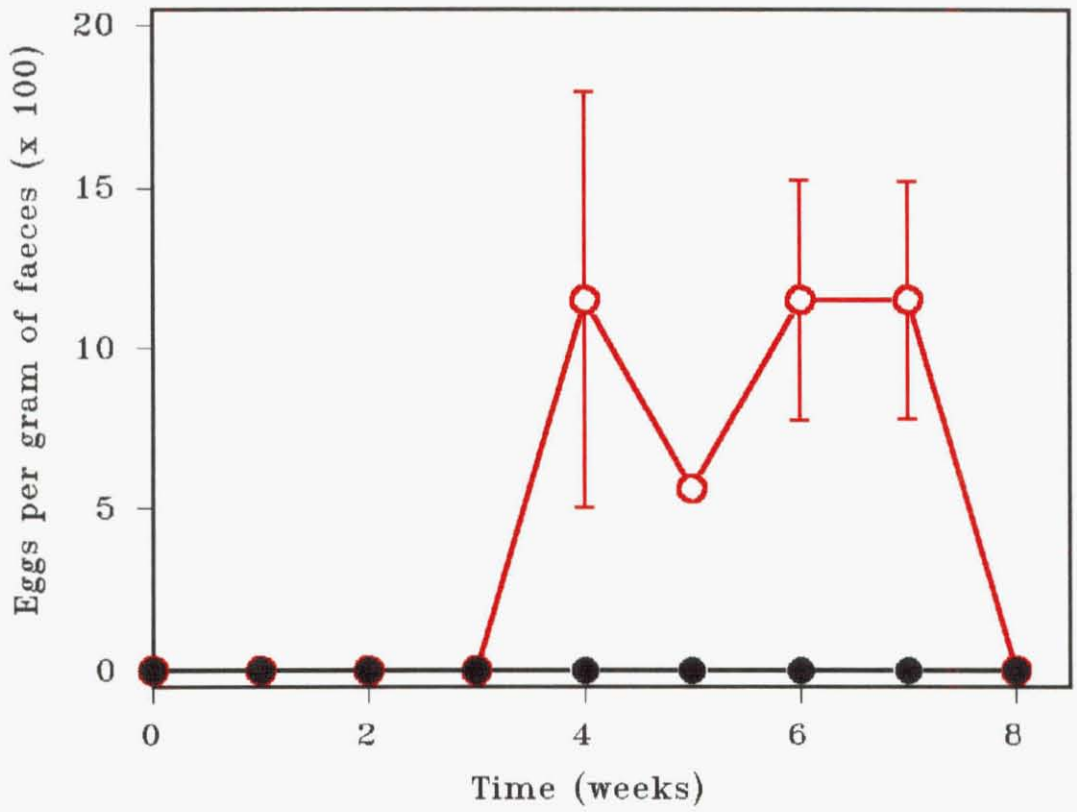


Figure 6.1 Mean (± s.e.m) number of *T.colubriformis* eggs per gram of faeces in infected (○) and non-infected (●) lambs sampled once per week (n=6).

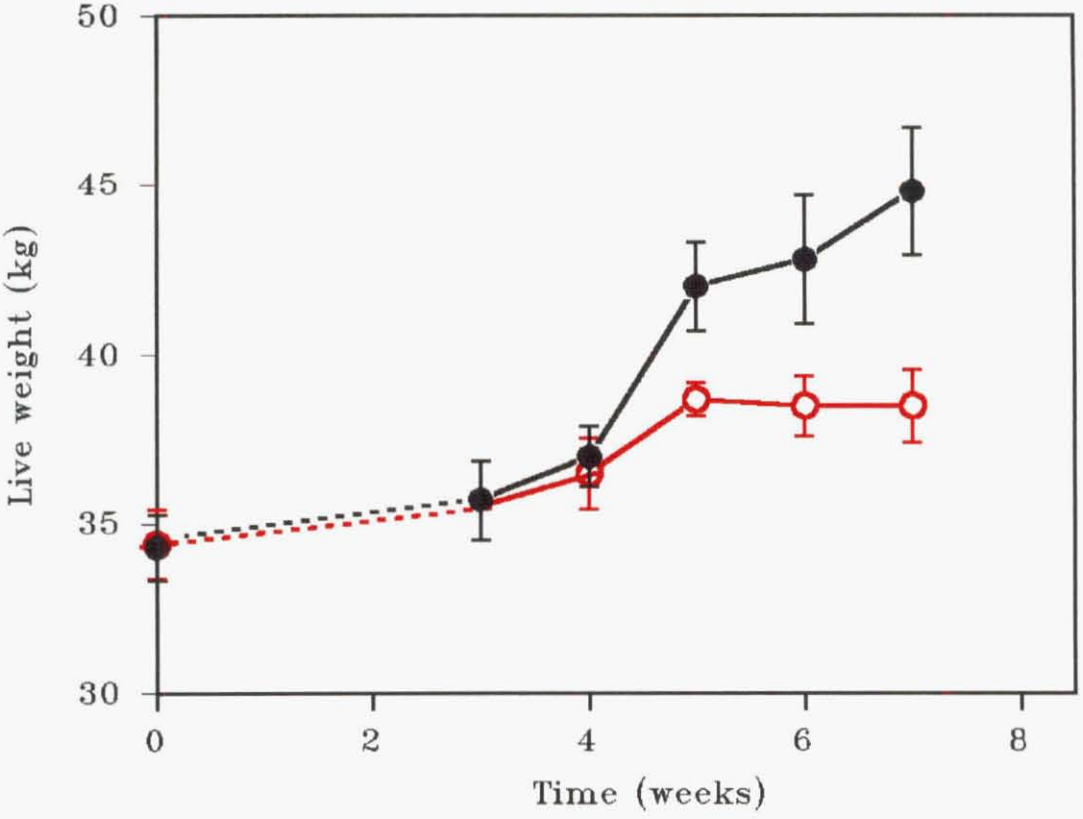


Figure 6.2 Mean (± s.e.m) live weight of infected (○) and non-infected (●) lambs during the experiment (n=6).

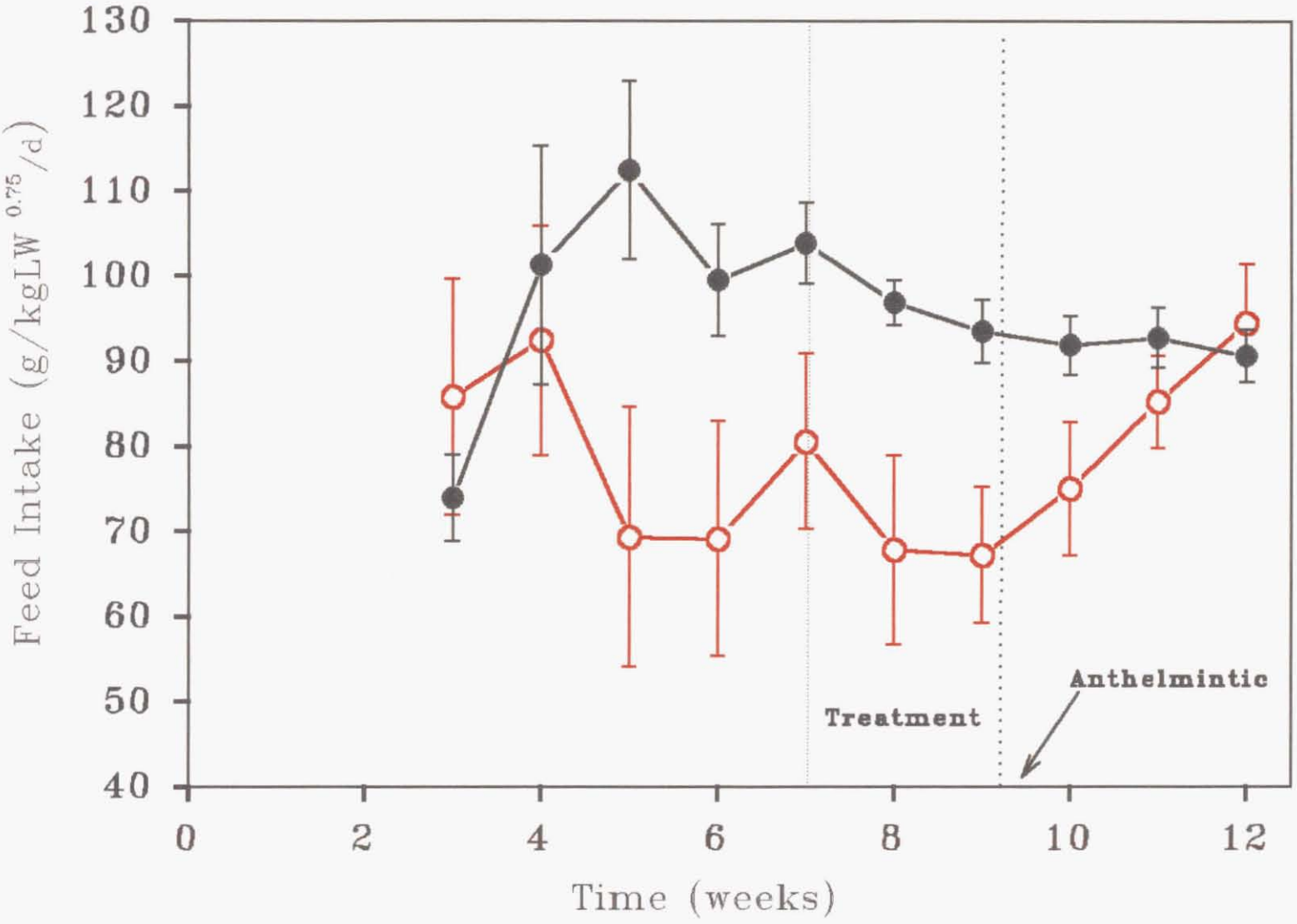


Figure 6.3 Mean daily feed intake (± s.e.m) for infected (○) and non-infected (●) lambs fed once per day (n=6).

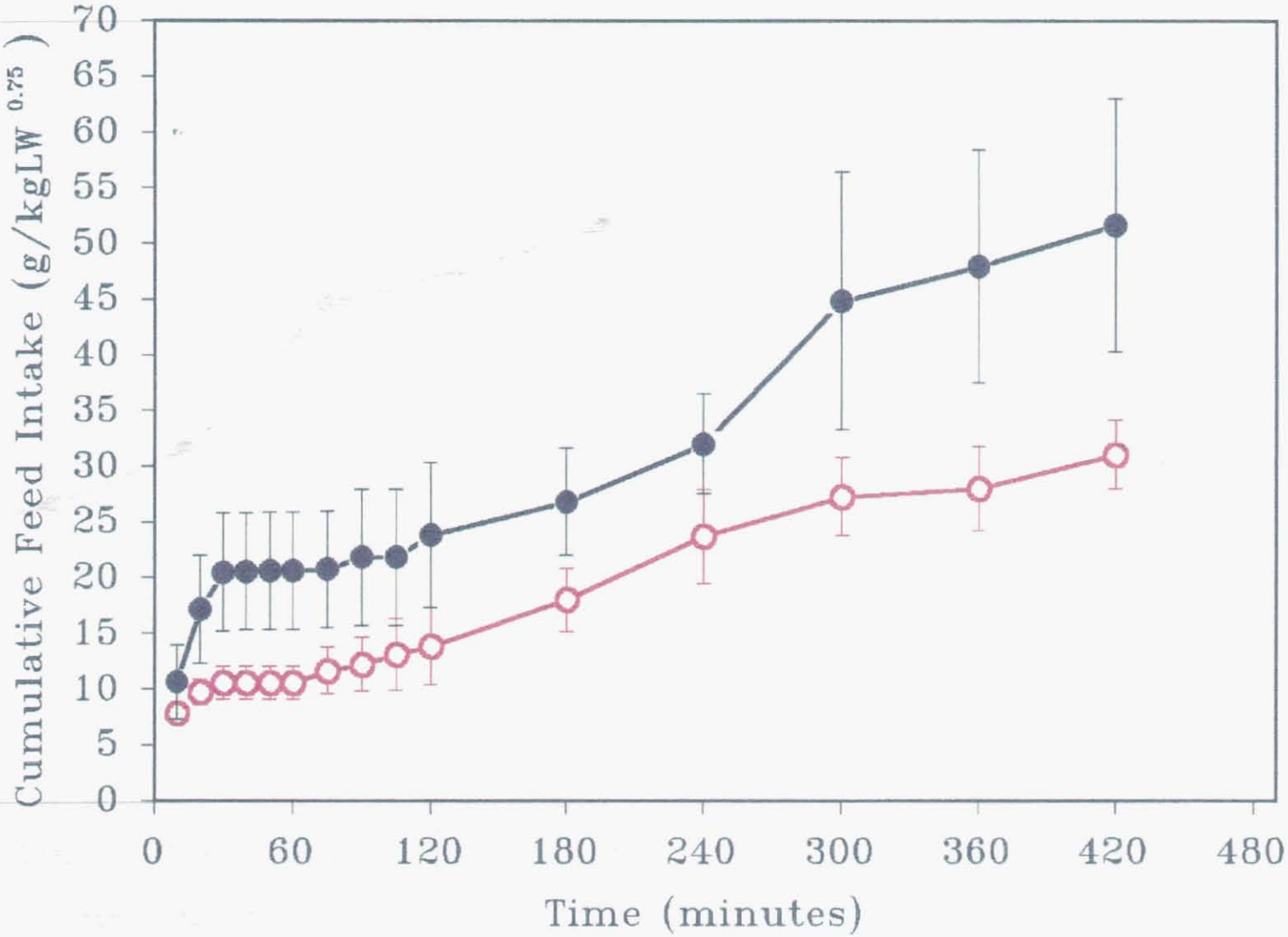


Figure 6.4 Mean (± s.e.m) short term cumulative feed intake for infected (○) and non-infected lambs (●) fed once per day (n=6).

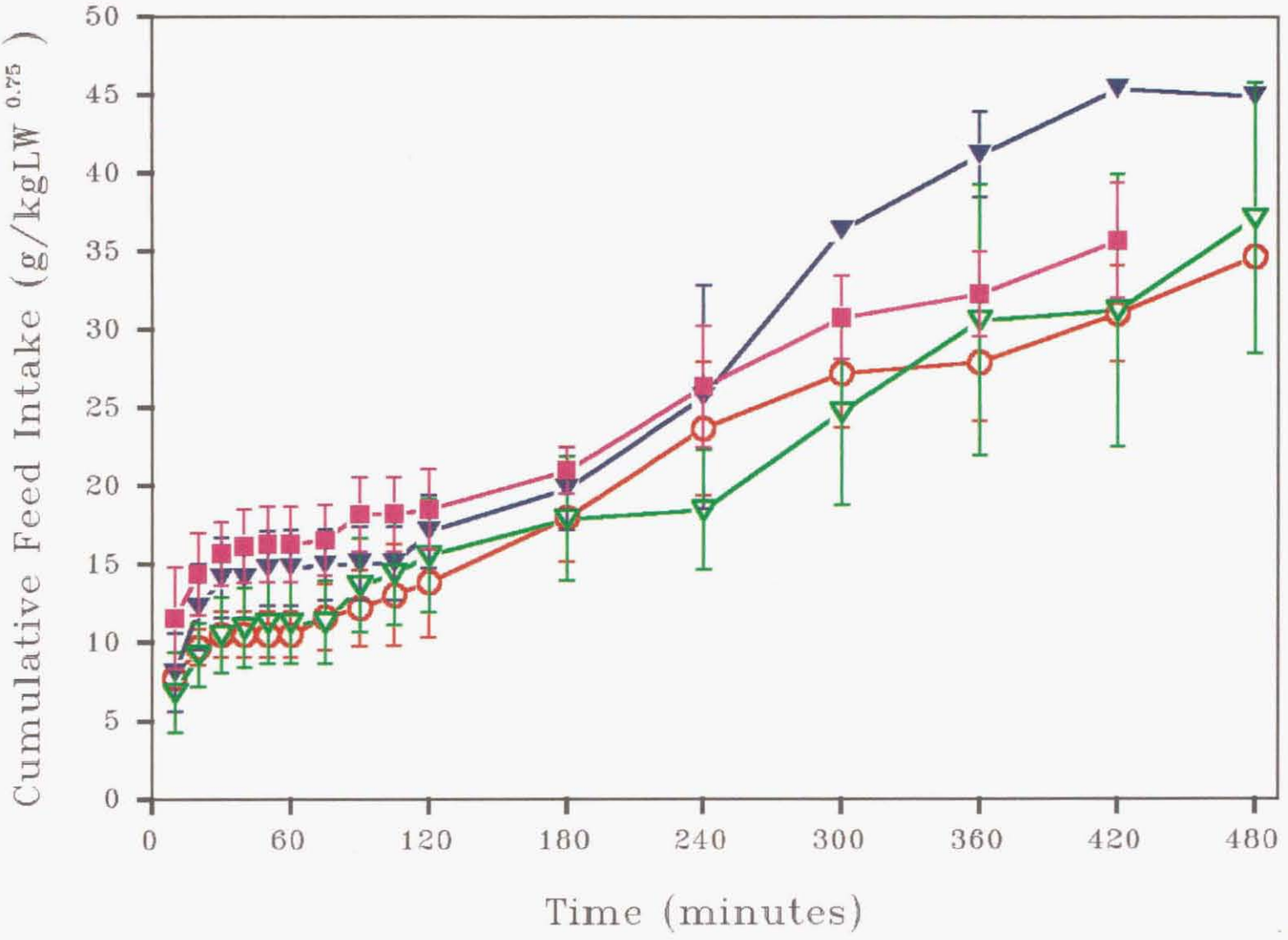


Figure 6.5 Mean (± s.e.m) cumulative feed intake for infected lambs injected intravenously with saline (○), or 5 (▽), 10 (▼) or 20 (■) mg of loxiglumide immediately before feeding (n=6).

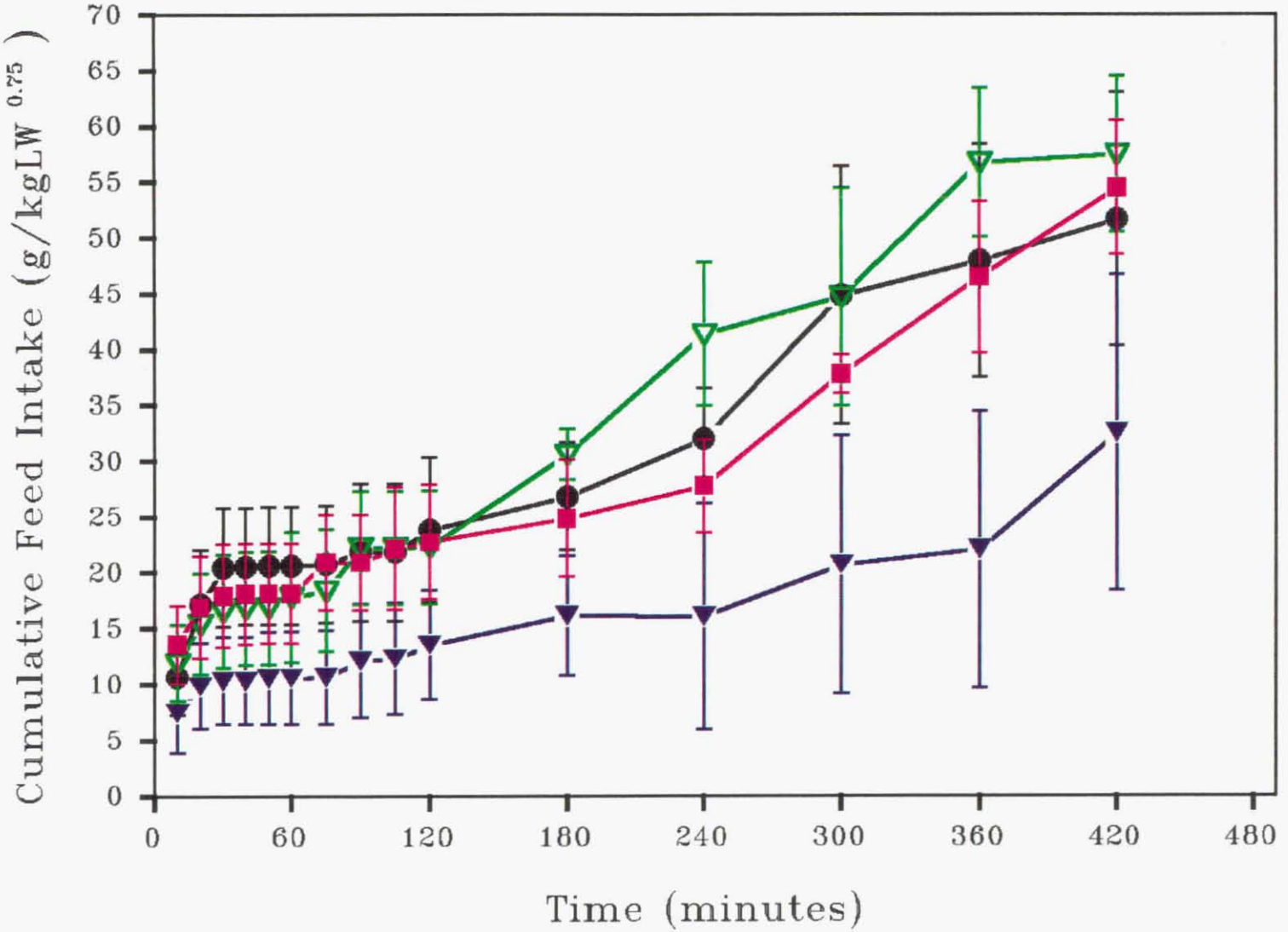


Figure 6.6 Mean (± s.e.m) cumulative feed intake for non-infected lambs injected intravenously with saline (●), 5 (▽), 10 (▼) or 20 (■) mg of loxiglumide immediately before feeding (n=6).

6.4 Discussion

There was no change in feed intake in either parasite infected or non-infected animals at any of the 3 levels of loxiglumide used.

Trickle infection with *T. colubriformis* resulted in a 24-28 % depression in daily feed intake, characteristic of that reported by others (Steel *et al.*, 1980; Sykes and Coop, 1976) and similar to other experiments reported in this study. Live-weight gain was also depressed by infection, again a common feature of such infections (Holmes, 1985) and of the studies described in earlier chapters.

Loxiglumide has been used frequently as a research tool to aid in understanding the role of CCK in gastrointestinal function and a number of disease conditions (Setnikar *et al.*, 1987b). However, there are no published reports of its use in sheep. An attempt to validate its use in sheep in conjunction with this trial (Appendix 6) was thwarted by lack of consistent and reproducible depression in short term intake in sheep injected intravenously with CCK-8 to test the antagonist effects of loxiglumide. In man loxiglumide pretreatment is a powerful means of blocking exogenous CCK effects on gastric acid secretion, gut emptying (Konturek *et al.*, 1990) and gall bladder contraction (Malesci *et al.*, 1990) as well as blocking of post prandial effects of a rise in endogenous CCK (Fried *et al.*, 1991; Schwarzendrube *et al.*, 1991). Similarly single doses of loxiglumide block exogenous CCK effects on gall bladder contractions in rats (Reidelberger *et al.*, 1991) and in opossum (Hanyu, 1991). In man loxiglumide has a ½ life of approximately 4.8 h and does not interfere with the distribution and metabolism of CCK (Jebbink *et al.*, 1990).

These results support the conclusions of Baile and Della-Fera (1984) that unlike many other species, peripheral CCK does not appear have a meal intake regulating role in sheep. The current trial was limited by my inability to test the effectiveness of loxiglumide on peripheral CCK (Appendix 6) However, the response of the intake depressed infected animals in this study was of particular interest. If, as Symons and Hennessy (1981) suggested, elevated peripheral CCK levels were depressing feed intake, then loxiglumide should have blocked these effects at least in the short term. But there was no change in feed intake in infected animals even at doses of 20 mg/kg, which is almost double the dose used orally in humans to cause total inhibition of gall bladder contraction (Corazziari *et al.*, 1990).

Several experimental limitations restrict the conclusions from this experiment. Firstly, loxiglumide use has not been reported in sheep nor validated for use in this species. Secondly, single doses of loxiglumide were used here whereas a continuous infusion would have ensured a constant availability of loxiglumide. Finally, if the effects of elevated CCK are indirect, for instance reduced feed intake is a consequence of slowing gut emptying, the short term reversal of this effect may not have been of sufficient intensity or duration to elicit an intake response detectable by the model used here. Notwithstanding these limitations, a role for peripheral CCK receptors in intake depression appears unlikely. Further work is required to address the limitations discussed above.

CHAPTER 7

A study of the effect of a peripheral cholecystokinin antagonist on feed intake and abomasal emptying in lambs infected with *Trichostrongylus colubriformis*.

7.1 Introduction

Disturbances to gut motility have been observed during clinical *T. colubriformis* infections (Buéno *et al.*, 1975; Buéno *et al.*, 1982; Wiesbrodt & Castro, 1977) but the role of these disturbances in feed intake depression have not been studied. The effects of subclinical parasite infection on gut motility in lambs have not often been reported where account is taken of the lowered feed intake in infected animals (Roseby, 1973). In one trial, Gregory *et al.* (1985b) recorded increases in small intestinal transit time, abomasal volume and half time of marker clearance, associated with reductions in abomasal emptying in lambs infected with *T. colubriformis*. The parasites caused a progressive inhibition of motility and slowing of digesta flow which were more severe than could be explained by a reduction in feed intake alone, and appeared to be due to a failure of transit through the duodenum and upper jejunum.

Altered secretion of gastrointestinal hormones such as CCK may have a role in changing gut motility or even reducing feed intake. Symons and Hennessy (1981) recorded elevated levels of plasma CCK in infected lambs which preceded the fall in feed consumption and postulated this elevation to be due to the presence of parasites in the duodenum. *T. colubriformis* worms reside in the proximal small intestine, the site of the greatest number of CCK-secreting cells (Polack *et al.*, 1975), so could stimulate CCK release directly or secreting cells could respond to the worm-induced changes in intestinal motility reported by Gregory *et al.* (1985b).

The possible role of CCK in changes in intestinal motility which accompany worm infection has not been investigated. A problem with CCK studies has been the lack of a reliable assay to measure changes in endogenous CCK secretion. An experimental approach which has been available for nutritional and physiological studies on the role of CCK has been the use of CCK antagonists. Loxiglumide (CR1505), a pentanoic acid derivative ($C_{21}H_{30}Cl_2N_20_5$) (Setnikar *et al.*, 1987a) is a CCK antagonist. It is a potent, specific and competitive antagonist of CCK receptors in both *in vitro* and *in vivo* studies (Setnikar *et al.*, 1987b). In

in vivo studies loxiglumide was on average 100 times more potent than the classic CCK blocker proglumide (Setnikar *et al.*, 1987b) thus it provides an effective means for blocking peripheral CCK activity.

The hypothesis under test here is that a change/disturbance to the function of intestinal secretory cells due to worm infestation or the physical presence of the worm, will promote increased secretion of peripherally active CCK. Previous trials described in this thesis, using single bolus injections of loxiglumide, failed to show any changes in short term feed intake in infected lambs. This study was undertaken to determine if feed intake could be temporarily elevated and/or the rate of abomasal emptying increased in infected animals by the provision of a plateau level of loxiglumide in their blood.

7.2 Materials and Methods

Animals

Sixteen Coopworth x Dorset Down ewe lambs were weaned at seven weeks of age (17 $\pm$ 1.5 kg) (Section 3.7).

Surgical preparation

On 20 and 21 of November 1989, T-shaped cannula were inserted into the abomasum, approximately 10 cm cranial to the pylorus (Section 3.4).

Administration of parasites

At approximately 4.5 months of age (25 kg live weight) the lambs were treated with anthelmintic (ivermectin 200 mg/kg Ivomec, MSD Agvet N.Z.) and housed in individual pens. At this stage 8 lambs were randomly allocated to parasite infection and 8 to control groups. Infected animals were each dosed orally *per os* three times per week with approximately 9 400 infective *T. colubriformis* larvae (equivalent to 4 000 larvae/lamb/day) for the duration of the trial.

Faeces were taken manually from the rectum of each lamb on a weekly basis, and processed immediately to determine faecal egg concentration (Section 3.6). Animals were weighed weekly at 0800 h prior to feeding except during treatment runs.

Feeding

Animals were offered a complete pelleted ration (Appendix 2). Feed refusals (hereafter refusals) were removed daily at 0730 h and all animals deprived of food for 2 h, to standardise the time of the first meal at 0930 h (Section 3.1).

During the first 3 weeks individual non-infected lambs were pair-fed with an infected animal (i.e. offered the amount consumed by its pair on the previous day). After 3 weeks feed offered to non-infected lambs was increased in a stepwise manner until sheep refused to eat 10-20 % (by weight) of the feed offered. During this period, animals became accustomed to feed intake recording (Section 3.1).

Experimental procedures

On 6 January 1990 all lambs were transferred to metabolism crates. The trial commenced on 15 and 22 January (3 infected and 3 non-infected on each date), when an in-dwelling catheter was inserted into a jugular vein of all animals (Section 3.5).

Infusion of CCK antagonist

The experiment was run in two parts:

Part 1

Infected and non-infected lambs were infused with loxiglumide for 140 minutes. Loxiglumide powder was added to sterile phosphate buffered saline solution which had been adjusted to pH 7.6 to increase solubility, then filtered through a bacterial filter (0.2 μm cellulose acetate filter, Advantec, Toyo, labdisc disposable 50 mm unit). The infusion was via gravity feed, using a sterile commercial solution administration set (2COOO1 s Solution Administration Set, Travenol, Travenol Laboratories INC U.S.A.) which had a drip chamber and a friction wheel to adjust flow rate (Plate 9). Initially 1 ml/min of the 10 mg/ml solution was administered for 10 min to approximate a 30 mg/kg LW/h infusion, to produce plateau levels in the blood. The infusion rate was reduced to 0.3 ml/min (approximately 10 mg/ kg/ h) for the remaining 2 h, as had been advised to maintain a steady state level of loxiglumide in circulation (L Rovati, Rotta Research Laboratory, pers. comm.). Flow rates were monitored at least every 10 min and altered as required by adjusting the rate of flow into the drip chamber. During connection and disconnection of the infusion lines aseptic conditions were maintained. The hub of the needle was swabbed

with a 70 % ethanol solution and the stopper cleaned then placed in the ethanol solution whilst the infusion was running. Sheep were fed following the initial 10 min infusion and intake recorded as described in Section 3.1.

Part 2

In Part 2, the procedure was carried out as for Part 1 except that a single dose of indigestible radioactive marker, the ^{51}chromium complex of ethylenediaminetetra-acetic acid (10 ml solution ^{51}Cr EDTA; Downs and MacDonald, 1964; Amersham Australia Pty. Ltd) was injected into the abomasum 45 min after feeding, a time which corresponded to the end of the first eating period. Injection of 10 ml ^{51}Cr EDTA was calculated to deliver 100 000 counts per min of tracer into the abomasum. The initial and final syringe weights were recorded and the exact infusion weight calculated. ^{51}Cr EDTA was injected through the abomasal cannula using a 10 cm long piece of slightly curved but rigid polyvinyl chloride tubing (I.D.= 4 mm ; O.D.= 6 mm) with a rubber stopper fitted on the syringe end. This allowed the operator to inject the solution upwards into the body of the abomasum and prevented any spillage. Abomasal samples were then collected at 15, 30, 45, 60, 75, 90, 105, 120 and 135 min following injection by placing a plastic collection bag over the cannula and carefully removing the stopper. Abomasal motility caused a flow of digesta through the open cannula. Each sample was immediately mixed thoroughly and a 10 ml aliquot taken for determination of radioactivity using an Autogamma scintillation spectrometer (1282 Compugamma Universal Gamma Counter; LKB Wallac). The samples and standards were stored at 4 ^{o}C until counting, which was within 4 days of collection.

Statistical methods

Cumulative feed intake data did not meet the assumptions of normality, so a general linear mode (GLM, SAS 6.1, SAS Institute, N.Y.) for unbalanced data was carried out on the ranked values (non-parametric analysis) and least square differences used to test differences between means. Rank values have no biological meaning, so data are presented as raw means. Means of abomasal volume, flow, retention time and T 1/2 were tested using GLM and a Duncan's new multiple range test for differences between individual means.

Calculation of abomasal volume, flow and retention time

Marker analysis was carried out using regression analysis on the natural logarithms of the adjusted counts.

$$k\ (time^{-1}) = 1/_{retention\ time} = 0.693/\ [T½]$$

where k is the slope of the plot of the natural log concentration of the marker *vs* time (fractional outflow rate, ml/min), retention time is the average time the marker remains in the abomasum and T½ is the time at which half of the marker remains in the abomasum.

Abomasal volume and flow were found using the following :

$$Volume = Dose/[marker]\ t_0$$

Where dose is the total counts/min of standard x volume of marker injected (i.e. total counts injected) divided by the concentration of marker in the abomasum at the time of injection (t_0). This is found by extrapolating the regression of the natural logarithm of the ^{51}Cr counts vs time back to the origin (intercept of Y axis) to give the concentration at time 0 (Faichney, 1975).

7.3 Results

Nine weeks after the start of larval dosing 2 infected animals had failed to show significant depression in feed intake, so these and their pair-fed controls were excluded from all experimental treatments and analysis.

Live weight

Live weights of infected and non-infected animals during of experiment are shown in Figure 7.2. Up to week 3 the mean live weight of infected and non-infected animals was similar. After week 3, which coincided with all animals being offered *ad libitum* feed, mean live-weight gain of non-infected lambs was greater than that of infected lambs. A consistently greater live-weight gain in the non-infected animals resulted in a final live weight some 11 kg heavier ($p < 0.001$) than the infected animals (live-weight gains of 16 vs 5 kg for non-infected and infected animals respectively).

Faecal egg counts

Mean faecal concentration of trichostrongylid eggs are given in Figure 7.1. Eggs first appeared in the faeces of the infected lambs during the third week of infection and faecal concentration peaked during week 6 of infection. Faecal egg concentration fell gradually from week 6 to week 10, at which time all animals were treated with anthelmintic. No parasite eggs were detected in faecal samples for non-infected animals during the

experiment.

Daily dry matter intake

Mean daily voluntary feed intakes, calculated each week, are shown in figure 7.3. Up to week 3 intake of the non-infected animals was restricted to that of an infected pair.

To allow for differences in live weight as the experiment progressed, intake was expressed per kg metabolic live weight ($LW^{0.75}$).

Parasitism caused a reduction in mean daily intake from the fourth week of infection. The mean reduction in voluntary intake was 36 %, with a maximum reduction of 53 % occurring during week 7. From weeks 3-6 of infection, mean daily intake of infected lambs fell steadily to the minimum value at week 7. Thereafter to the end of the trial, daily intake gradually increased in the infected group. There was considerable variability in both the degree of intake depression suffered by individual lambs and the timing of the onset of the depression.

Short term feed intake

Short term feed intakes for infected and non-infected animals are presented as cumulative means in Figure 7.4. Parasite infection depressed short term feed intake at all recorded time periods ($p < 0.001$) by between 33 % (10 min) and 49 % (8 h). The pattern of eating was not markedly different during the 8 h day (Figure 7.4) i.e. the initiation and termination of meals did not differ greatly. The reduction in cumulative feed intake was reflected by a lower rate of consumption (Table 7.1).

Table 7.1 Rate of feed consumption (g/ $kglw^{0.75}$ / min) over the first, second and subsequent hours (2-8 h) of feeding for parasite infected and non-infected lambs fed once per day (t_0) (n=6).

	0-1 h	1-2 h	2-8 h
Infected (I)	0.27 ± 0.003	0.05 ± .003	0.03 ± .004
Non-infected (N)	0.47 ± 0.003	0.16 ± 0.004	0.06 ± 0.004
% depression (N-I/N *100)	43	69	50

The 69 % depression which occurred between 1 and 2 h after feeding was significantly greater than that recorded over 22 h (36 %), 0-1 h (43 %) or 2-8 h (50 %) ($p < 0.05$).

Effect of CCK antagonist loxiglumide on feed intake

The effect of either intravenous saline or loxiglumide on short term feed intake is presented in Figure 7.5. Cumulative intake data are presented here (Fig 7.5) as raw means because the rank transformed data have no biological meaning and are hard to interpret visually.

Loxiglumide did not elevate short term feed intake in either the infected or the non-infected groups and there was no effect of the infection status of the animals on the response to loxiglumide, so data were pooled for analysis. Analysis of variance of the rank transformed, pooled data revealed a significant depression ($p < 0.05$) in feed intake following loxiglumide infusion, the depression occurred only at feed intake recordings 20, 75, 105 min, 4 and 6 h after feeding. Except for the depression following loxiglumide infusion at 20 min, depression at the other time periods occurred only in the non-infected animals during the second phase of the study when the ^{51}Cr was being injected. When account of this was taken in the model for injection of ^{51}Cr, no differences in intake due to loxiglumide were detected.

Effect of infection status on marker retention and flow.

Table 7.2 Mean ($\pm$ s.e.m) value of the fractional outflow rate (k; ml/min) of marker from the abomasum of infected and non-infected lambs following intravenous infusion of saline (control) or the CCK receptor antagonist loxiglumide (n=6).

Infected		Non-infected	
saline	loxiglumide	saline	loxiglumide
2.2 ± 0.17^{a}	2.5 ± 0.14^{a}	2.8 ± 0.12^{b}	2.8 ± 0.14^{b}

*superscripts with different letters indicate significant differences ($p < 0.05$)

The rate of disappearance of the radioactive marker from the abomasum, as estimated by the slope of the log concentration of the marker (k), was significantly depressed in infected

lambs ($p < 0.05$). Treatment with loxiglumide had no effect on the value of k in either infected or non-infected groups.

Effect on abomasal volume

Parasite infection reduced abomasal volume of lambs by 60 % (162 ml and 67.3 ml for non-infected and infected animals respectively) (Table 7.3). Treatment with the CCK antagonist loxiglumide did not affect the calculated value of abomasal volume in either infected or non-infected groups.

Table 7.3 Mean (± s.e.m) abomasal volume (ml) for infected and non-infected groups following infusion with either saline (control) or CCK antagonist (loxiglumide) (n=6).

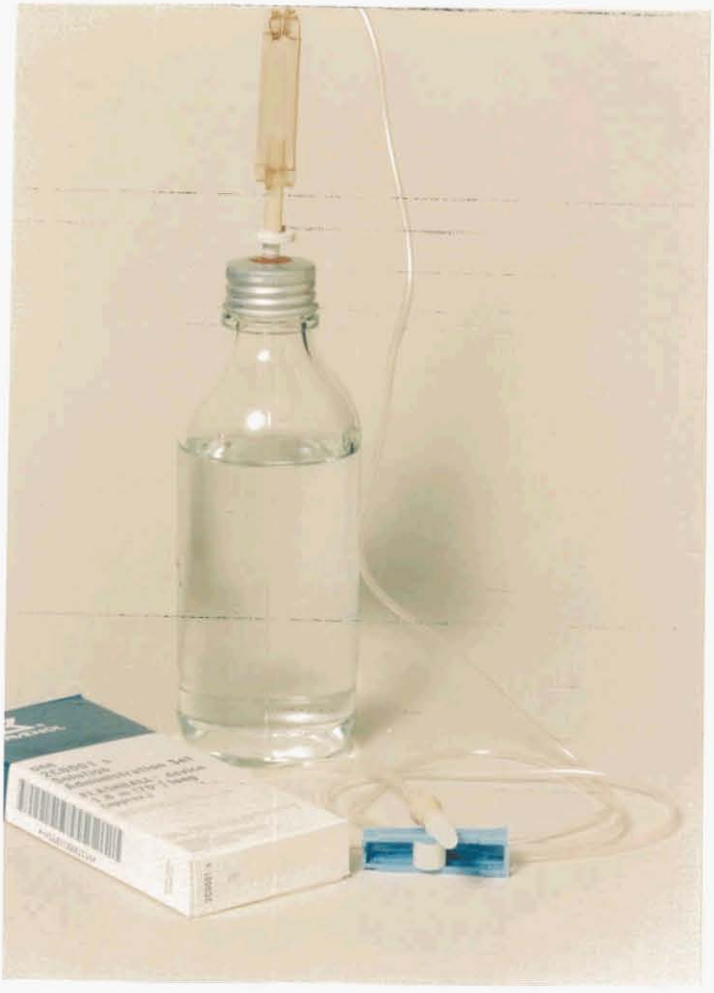
infected		non-infected	
saline	loxiglumide	saline	loxiglumide
67.3 ± 11.30[a]	79.8 ± 18.49[a]	162.2 ± 24.20[b]	167.4 ± 27.89[b]

*superscripts with different letters indicate significant differences ($p < 0.05$)

A further experiment (Appendix 3) measured abomasal emptying in lambs offered a range of levels of feed intake and found a strong correlation between both abomasal volume and flow rate and level of feed intake, while the level of feed intake was not correlated with T ½ or retention time of the marker in the abomasum.

Reanalysis of abomasal volume and flow rate data from the earlier experiment with infected and non-infected animals, using the feed intake of individual lambs on the day prior to recording abomasal emptying as a covariate, showed that all differences in abomasal volume between infected and non-infected groups were due to the different levels of feed intake of the groups.

Plate 9. An illustration of the gravity-feed infusion technique used to infuse the loxiglumide.



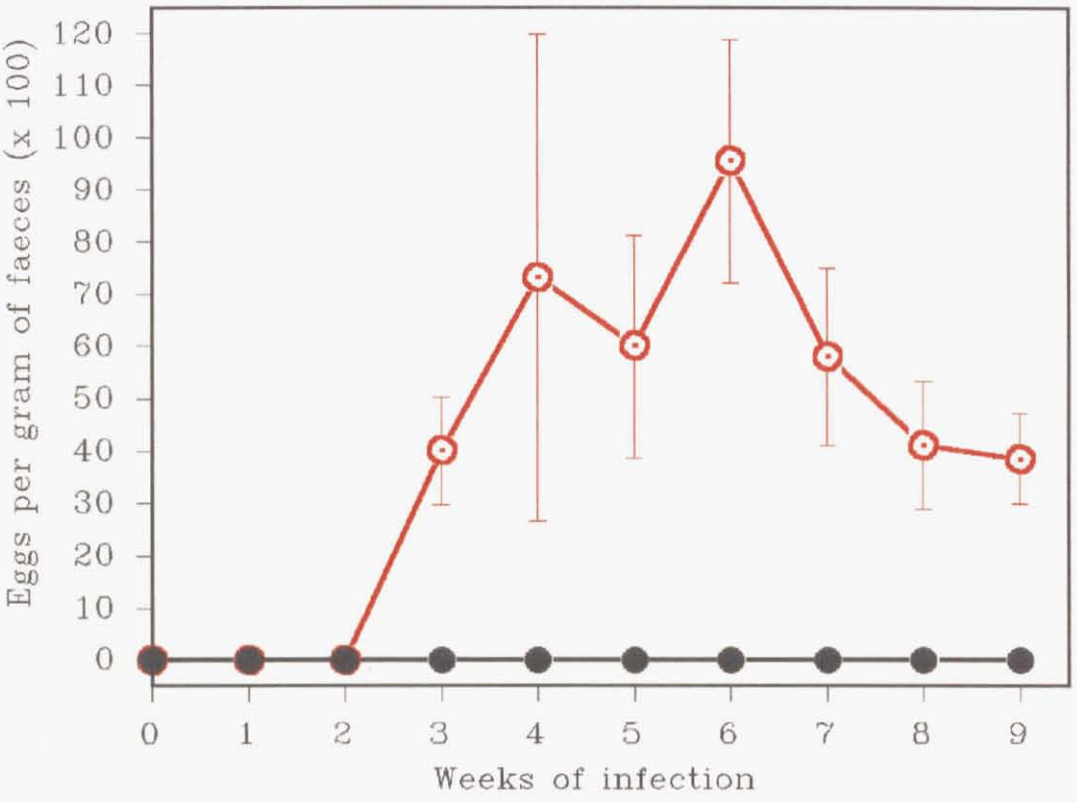


Figure 7.1 Mean (± s.e.m) number of *T. colubriformis* eggs per gram of faeces in infected (⊙) and non-infected (●) lambs sampled once per week (n=6).

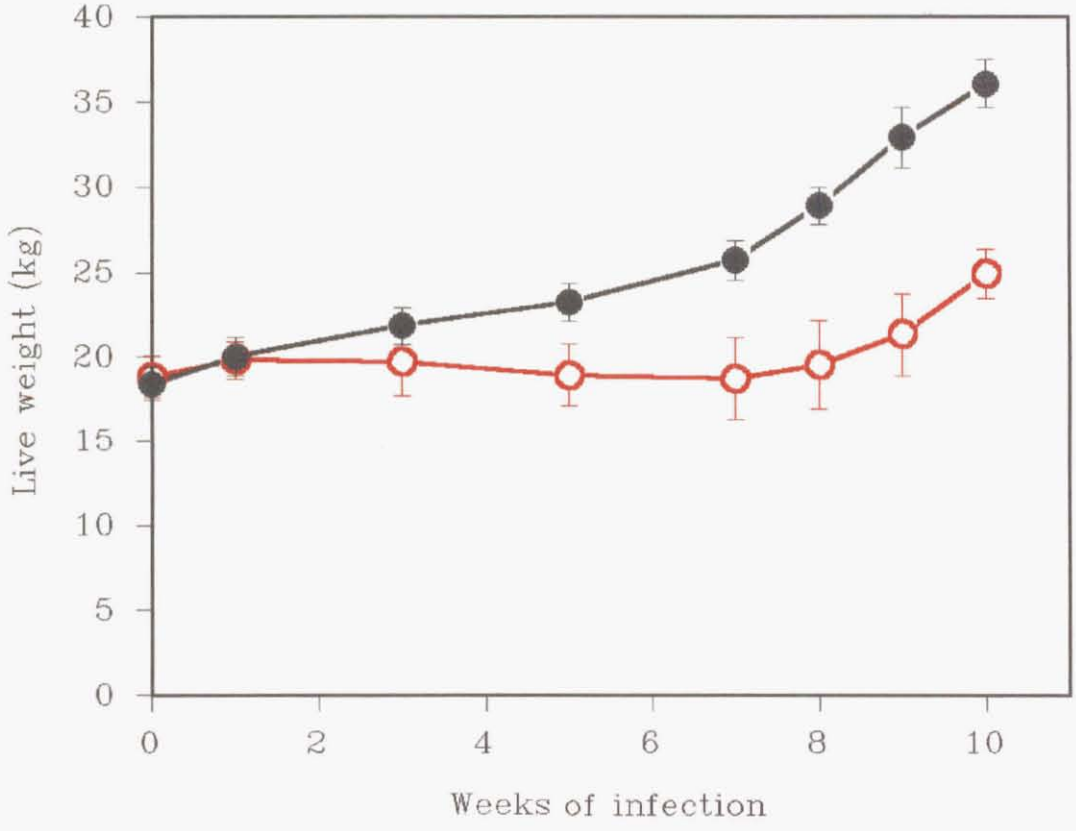


Figure 7.2 Mean (± s.e.m) live weight for infected (⊙) and non-infected (●) lambs during the experiment (n=6).

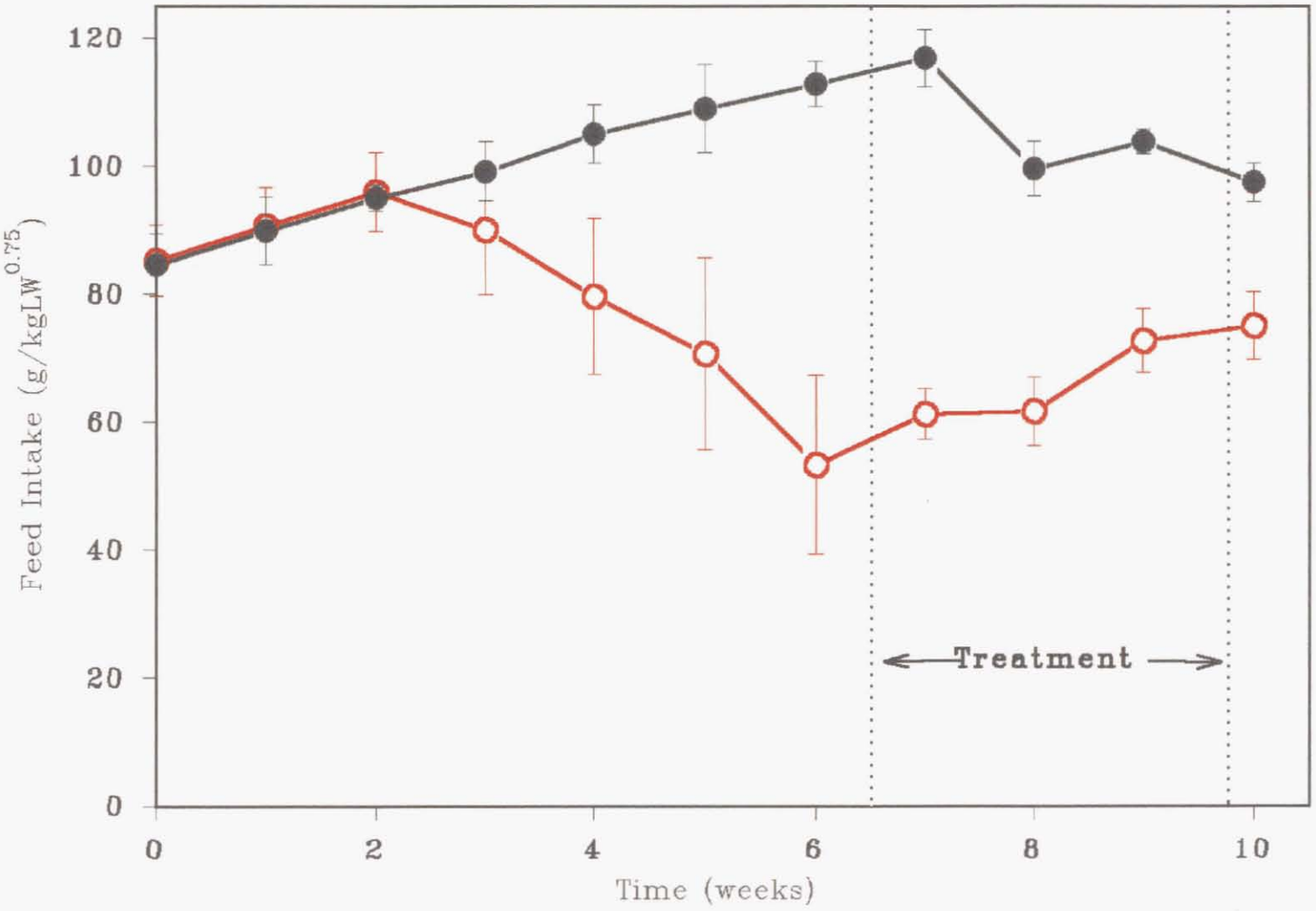


Figure 7.3 Mean daily feed intake (± s.e.m) for infected (○) and non-infected (●) lambs during the experiment (n=6).

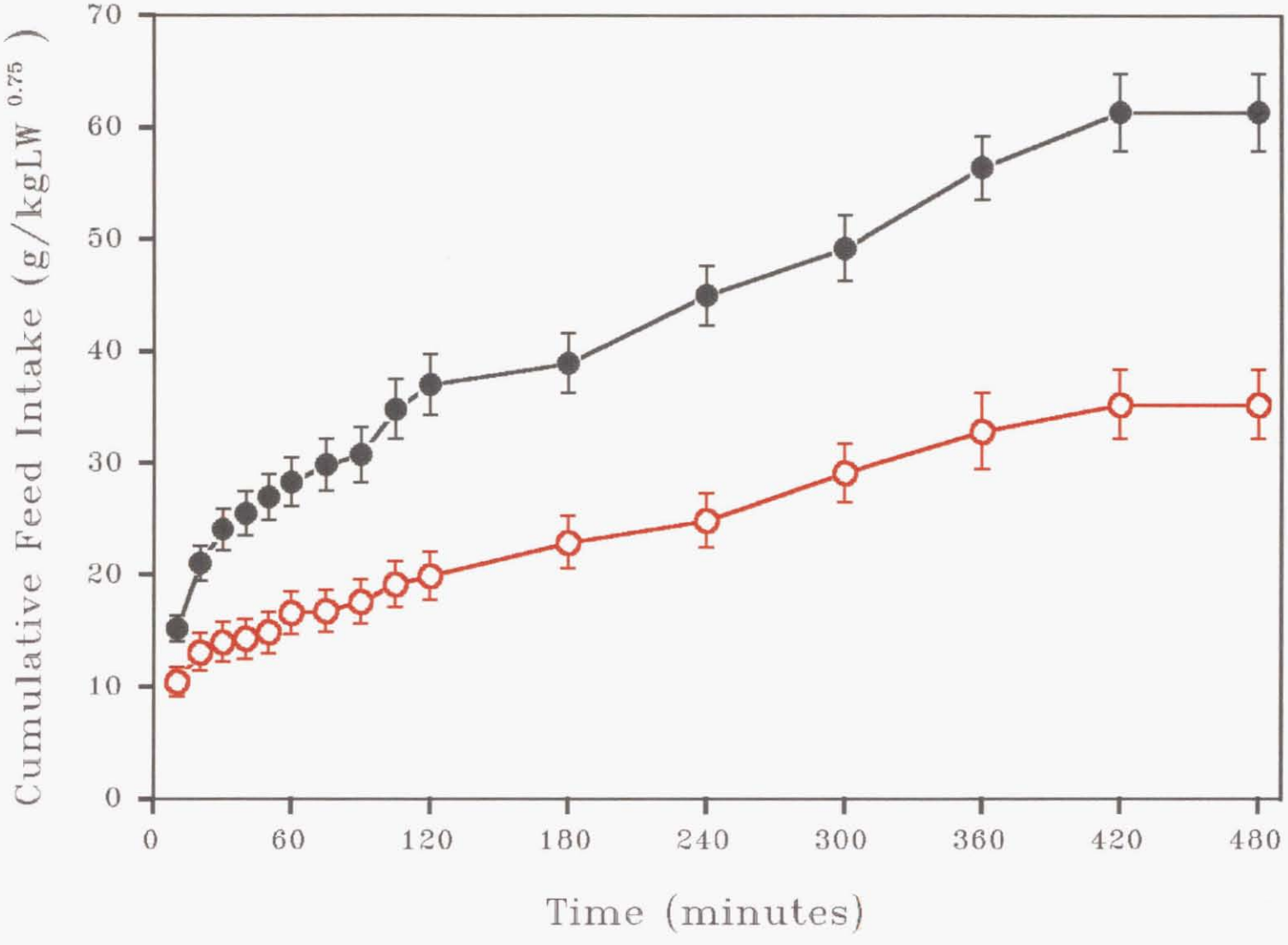


Figure 7.4 Mean (± s.e.m) short term cumulative feed intake for infected (◎) and non-infected (●) lambs fed once per day (n=6).

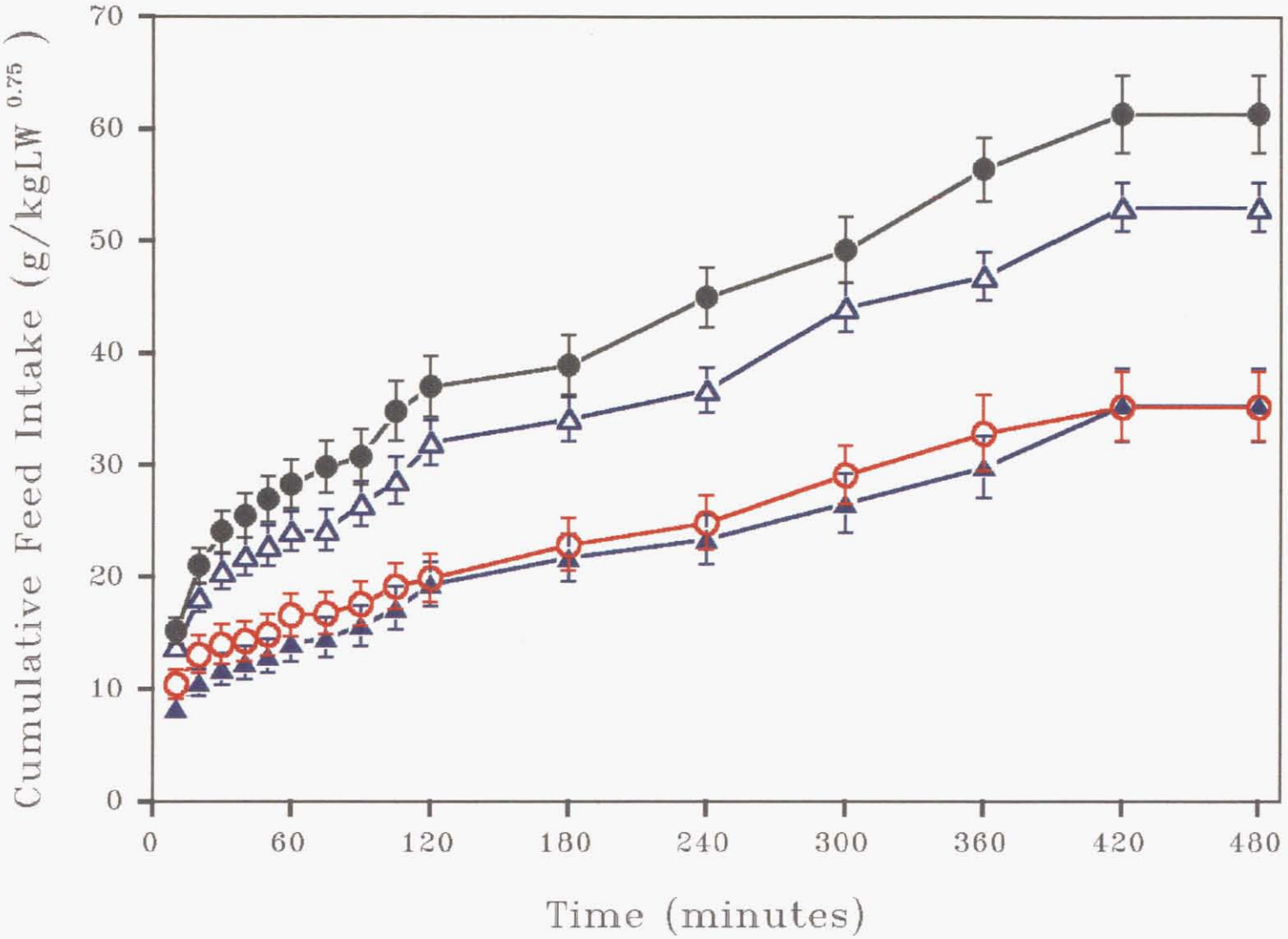


Figure 7.5 Short term cumulative feed intake (± s.e.m) for infected and non-infected lambs intravenously infused prior to and for the first 2 h of feeding with saline (infected ○; non-infected ●) or the CCK antagonist loxiglumide (infected ▲; non-infected △) and fed once per day (n=6).

7.4 Discussion

A 36 % reduction in feed intake is within the range characteristic of sheep infected by the gastrointestinal parasite *T. colubriformis* (Dargie, 1980; Sykes, 1982; Holmes, 1985) but in the present studies feed intake depression was first evident at week 5, rather than weeks 3-4 when depression in feed intake is commonly detected (Sykes, 1982) and later than the appearance of eggs in the faeces of the infected animals which occurred during week 3. However, both the level of feed intake depression and onset of depression here were similar to those in other experiments in this study.

Short term feed intake expressed as intake per kg of metabolic live weight ($LW^{0.75}$) was depressed by, on average, 41 % over the 8 h measurement period. This correlated well with the daily intake depression of 36 % but was consistently 5 % higher suggesting that in the once-a-day feeding situation, where a large component of the daily intake is consumed over a relatively short period, the depression of intake of infected animals is more pronounced. The cumulative intake graph (Figure 7.4) does not suggest major changes in the eating pattern of infected animals compared with their non-infected counterparts. Differences in the rate of intake observed between 60 and 120 min implies the infected animals either had a longer inter-meal interval between the first and second meals or, if the inter-meal interval was the same, then their rate of feed consumption was further reduced. A study of the intake curves for individual animals suggests the former is true, with the infected animals having more time intervals with no changes in feed intake, apparently due to the first meal being terminated earlier in infected animals.

Unfortunately it was not possible to record meal duration, but animals were observed to terminate the first meal 40-50 min after feed was offered, drink water then a non-eating phase occurred. Meals occurred at intermittent intervals throughout the remainder of the day. In the infected animals it was difficult to observe changes in meal duration. Animals appeared to be feeding, yet when feed bin weight was next checked, no changes in weight would be evident. By comparison non-infected animals tended to be more decisive, finish eating, move immediately to the water trough then move back to feeding or to sit and little time was spent at the feed bin without feeding.

Both groups of lambs continued to gain weight during the experiment though the non-infected group grew on average 3 times faster than the infected group. Other workers have

found the presence of gastro-intestinal parasites causes a fall in the rate of live-weight gain in infected animals (Coop, Sykes and Angus, 1976), or a live-weight loss (Steel *et al.*, 1980) during infection. The comparatively moderate increase in live weight in the infected group can be attributed to their significantly lower feed intake during the experimental period. The *ad libitum* feeding regime offered to both groups of animals makes it impossible to separate any effects of parasitism on live weight change from differences in live weight due to different levels of feed intake. In several cases (Bown, 1986; Coop *et al.*, 1976; 1977 and Sykes and Coop, 1976; 1977) restricting the intake of non-infected animals to that of infected animals has produced a greater fall in live weight gain in the infected animals suggesting parasitism *per se* depresses live-weight gain.

Short term feed intake in parasite infected animals was not increased by continuous infusion of a powerful peripheral CCK receptor antagonist (loxiglumide). This was despite the tendency of infected animals to terminate meals earlier than the non-infected animals. Likewise there was no increase in short term intake in the non-infected animals. If the loxiglumide was blocking peripheral CCK activity then these results would suggesting peripheral acting CCK is not a factor in parasite induced feed intake depression or a regulator of meal termination in healthy sheep fed once-a-day. As was discussed in Chapter 6 this may lend further support to the findings of Baile and Della-Fera (1984) that unlike some monogastric species, peripheral CCK action does not appear to be involved in individual meal termination. Alternatively loxiglumide may have blocked satiety effects of CCK, without changes in intake being recorded because other biological regulators of short term intake become important when the effects of one system are removed. The findings of the present study do not rule out the possibility that loxiglumide was not blocking receptor activity, but findings in a later study (Chapter 10) support the activity of loxiglumide in sheep.

Unexpectedly, there was a tendency for loxiglumide to reduce short term feed intake, but only intermittently. This depression in intake at 75, 105, 240 and 360 min occurred in non-infected animals during the second part of the experiment. The observed depression of intake occurred when animals were receiving a single abomasal injection of ^{51}Cr EDTA 45 minutes after feeding, with abomasal digesta samples collected every 15 min over the next 2.5 h. Lambs became accustomed to digesta collection and were observed to be not stressed by sampling. They were generally required to stand during collection, and this may have disturbed subsequent eating patterns resulting in the observed significant feed intake

depression. Accounting for the chromium injection in the analysis of variance model did not alter the finding that there was no significant intake stimulation due to loxiglumide treatment.

There are no reports in the literature of loxiglumide causing feed intake depression. In the present work intake was depressed with loxiglumide compared to saline infusion, so the infusion *per se* does not appear to be the cause. Clinical trials have recorded incidences of local reaction to the loxiglumide infusion when the concentration was over 0.05 % in humans (L.Rovati, Rotta Res. Lab., pers. comm.). In this case a concentration of 0.05 % was used. This may have caused some localised discomfort, particularly given the relatively long and repeated nature of the infusions. There was no intake depression due to loxiglumide in the first part of the experiment and the observed depression did not occur at all time intervals, further supporting the conclusion that the intake depression was associated with abomasal sampling.

Interpretation of the lack of response of animals to loxiglumide infusion was limited by the inability to measure blood CCK or loxiglumide levels. In a preliminary study to validate the use of loxiglumide to block peripheral CCK in sheep, difficulties were encountered with using exogenous CCK (Appendix 6). Exogenous CCK administration in lambs immediately prior to feeding failed to produce repeatable feed intake depression, and prevented testing of the effectiveness of the loxiglumide. However, extensive experiments in rats and humans have shown the effectiveness of loxiglumide in antagonising CCK receptors (Setnikar *et al.*, 1987b).

GIT parasite infections can alter gut motility and increase abomasal volume (Gregory *et al.*, 1985b) but here abomasal volume reduced by 60 % in infected animals. This difference was fully accounted for by the differences in voluntary feed consumption between the 2 groups. Abomasal volumes even in the non-infected animals were much lower than those reported by Gregory *et al.* (1985a,b) (162 ml vs 300-900 ml). A large component of this difference was probably due to differences in feeding regimes between the two experiments. In the present study, to facilitate testing of pharmacological agents, sheep were fed once per day, whereas Gregory *et al.* (1985a) used a continuous feeding regime which produces more steady state rumen conditions and differences in the pattern of digesta flow from the gut. For instance sheep fed 3 times per day have approximately double the abomasal outflow of digesta compared with sheep fed the same amount of feed once per day (Harrison & Hill, 1962;

Gregory *et al.*, 1985a) so it could be expected that major differences in abomasal volume between the experiments may reflect different feeding regimes. This does not explain the lack of effect of *T. colubriformis* infection on gut emptying recorded here, by comparison, Gregory and co workers found approximately 20 % increase in abomasal volume over that expected for the level of feed intake in the infected group. The nature of the outflow from the abomasum on once daily feeding may have masked any parasite induced effects on increases in abomasal volume and emptying which were reported by Gregory *et al.* (1985b).

As discussed above, large differences in abomasal volume occurring in the present work were attributed to the differing levels of feed intake recorded. When animals were fed a number of levels of intake encompassing the range of intakes recorded during this study, a strong linear relationship was established (Appendix 3) between level of feeding and both abomasal volume and flow, also reported by Gregory *et al.* (1985a). There was no relationship between marker retention time or T 1/2 and level of feeding, also in agreement with Gregory *et al.* (1985a). Considerable variability within and between animals occurred in the present experiment. Gregory *et al.* (1985a) partially overcame this problem by recording abomasal emptying on twelve occasions which was sufficient to reduce the within animal variability but not the between animal variability whereas in the current study emptying was recorded in each animal only twice at each feeding level.

A continuous infusion of CCK antagonist loxiglumide had no effect on retention time, T 1/2, volume, or flow of the abomasal contents, in either the infected or non-infected group. T 1/2 values did not differ with either infection status, treatment, or level of feed intake but did fall within the range of 17-42 min reported by Grovum and Williams (1973) and Gregory *et al.* (1985a). Loxiglumide infusion at 30 mg/ kg LW/ min for 10 min and then at 10 mg / kg LW/ min for the first 2 hours of feeding should have been more than adequate (L Rovati, Rotta Res. Lab., pers. comm.) to achieve a plateau circulating level of antagonist and block any CCK induced changes in abomasal emptying in infected animals. Loxiglumide is a competitive antagonist of CCK receptor activity with an affinity for peripheral receptors 19 000 times greater than that of the model CCK antagonist, proglumide (Setnikar *et al.*, 1987a). Loxiglumide and a closely related antagonist (CR 1409) block exogenous and endogenous CCK activity in a number of species (Makovec *et al.*, 1986) and should have blocked peripheral CCK activity here. In addition these receptor antagonists are effective at blocking exogenous CCK activity in a wide number of *in vitro*

situations. However, to detect changes due to loxiglumide infusion this model required the CCK antagonist to change short term intake or change abomasal emptying so that it was detected in the 0.75 to 2.5 h period digesta samples were collected in, or that antagonist induced changes in abomasal emptying brought about detectable changes in intake later in the day. Any delayed effects of loxiglumide on emptying or feed intake may not have been detected by the current model.

Further to the earlier findings that a single dose (Chapter 4) or a range of doses of loxiglumide (Chapter 6) did not change short term feed intake in infected animals, the present experiment has shown that maintaining plateau levels of loxiglumide over the first two hours of feeding also did not enhance feed intake in lambs suffering anorexia. This study provides initial evidence to discount a role for the elevated peripheral CCK being the primary disturbance to feed intake in infected lambs either directly or indirectly via CCK effects on gut emptying. In light of conflict over the effect of *T. colubriformis* on gut emptying between this study and that of Gregory *et al.* (1985b), further studies under a variety of feeding regimes are needed to investigate the effects of parasite infection on gut emptying.

CHAPTER 8

The effect of varying levels of a centrally active benzodiazepine on feed intake in infected lambs.

8.1 Introduction

The short term feed intake model developed in the study described in Chapter 4 provides a useful method for investigating a variety of potential pathways involved in parasite induced feed intake depression by using various pharmacological agents to block or stimulate potential pathways.

In studies described so far in this thesis, parasite infected animals have not shown any increase in feed intake following blockage of pain receptors, peripheral CCK receptors or prostaglandin production (Chapters 4-7). The diazepam family of chemicals contains members which enhance appetite in many species (Baile and M^{c}Laughlin, 1979). Stimulation of food intake by such compounds has been reported in humans, rats, cats, dogs, pigs, sheep, cattle and horses (Baile and M^{c}Laughlin, 1979). Benzodiazepine induced hunger is intense and will suppress many inhibitory effects on intake such as those due to amphetamines, taste aversion, heat stress, anorexia, bulk limitation or disease (Baile and M^{c}Laughlin, 1979) so may temporarily overcome the effects of parasite infection on intake.

This study used brotizolam (a benzodiazepine compound with central satiety blocking activity) to stimulate feed intake in sheep following infection with *T. colubriformis*.

8.2 Materials and Methods

A pool of Dorset Down-Coopworth cross ewe lambs were early weaned (see Section 3.7) in October 1988. Sixteen of these lambs were moved indoors into individual pens on 1 March 1989 and fed a complete pelleted ration. Parasite infection commenced on 1 March 1989 (Section 3.6) for the infected group (n=8).

Feeding

For the first 3 weeks of trickle infection non-infected and infected animals were fed at 90-95 % of maintenance energy requirement. Intake was then gradually increased so that

following 5 weeks of infection all animals were receiving rations *ad libitum*. The animals were offered a complete pelleted ration (Appendix 2). Feeding procedures, sampling and analysis were as described in Section 3.1.

Parasite infection

Problems encountered with establishment of infection led to all animals receiving 20 mg methylprednisolone (0.5 ml Depo Medrol, Upjohn Ltd.) on 2 occasions, 7 days apart, during weeks 7 and 8 of infection. A culture of *T. colubriformis* was obtained from a source external to Lincoln University (Dr T. Watson, Ruakura Agricultural Research Centre) and animals infected with the new culture from week 7 in the hope that this strain would have improved pathogenicity.

Experimental design

The experiment was a replicated duplicate Latin square design (8 infected animals paired with 8 non-infected animals receiving 2 replicates of each of 4 levels of brotizolam). The same procedure was then repeated with all animals receiving all levels of brotizolam at the termination of the first meal (45 min after feeding).

Infected and non-infected animals received 0, 2, 4 or 8 mg of brotizolam/animal immediately prior to feeding or 45 min after feeding. Brotizolam (Mederantil, Boehringer Ingleheim, N.Z.) was administered by very slow injection into a jugular vein. To prevent ataxia care was taken not to occlude the vein during injection.

Statistical analysis

Analysis of variance for unbalanced data was carried out on ranked cumulative intake data at each time period (non-parametric testing) and least squares differences were used to establish significance of differences between means (SAS, v. 6.0, SAS Institute, Cary N.Y., U.S.A.). Live weight, weekly intake and faecal egg counts were analysed using Student's *t* test (Minitab v. 7.2).

8.3 Results

Four of the infected animals failed to develop consistent feed intake depression within the duration of the experiment. These animals and their non-infected pairs were excluded from treatment and statistical analysis.

Parasitological

The faecal egg counts during the experiment infected and non-infected groups are shown in Figure 8.1. Eggs first appeared in the faeces of infected animals after week 3 of infection and their number increased slightly to a maximum after week 6 of infection. No eggs were detected in the non-infected animals during the experiment. Egg counts were generally very low throughout the trial.

Live weight

The mean live weights for infected and non-infected animals during the experiment are shown in Figure 8.2. Live weight increased in both infected and non-infected groups and was not affected by parasite treatment.

Daily feed intake

Means ($\pm$ s.e.m) of daily feed intake over 7 day periods for animals in both infected and non-infected groups are given in Figure 8.3. Intake depression had developed by week 9 in the infected group, with the maximum intake depression being recorded after 14 weeks of infection when voluntary feed consumption was 27 % lower than in the non-infected group.

Short term feed intake

The short term cumulative feed intake for infected and non-infected groups recorded during weeks 9-14 of the experiment are shown in Figure 8.4. Parasite infection reduced short term voluntary feed intake by between 34 % (360 min) and 50 % (40-50 min) with the average intake depression being 43 % during the period recorded (0 to 8 h).

Effects of brotizolam

i. Brotizolam administered before feeding.

Cumulative short term feed intake for infected and non-infected animals treated with 0, 1, 2 or 4 mg brotizolam per kg live weight is shown in Figures 8.5 and 8.6. There was a significant treatment by parasite interaction ($0.001 < p < 0.05$) for the first 4 h following feeding except between 30-50 min ($p < 0.1$). An interaction resulted from an elevation of feed intake in infected animals following treatment with all 3 levels of brotizolam. There was no significant effect of treatment on the non-infected animals ($p > 0.05$). Although 4 mg of brotizolam elevated feed intake in the non-infected animals from 1 to 3 h after feeding by between 23 and 37 %, the variability of this response (CV @ 1 h = 37 %) precluded it from being significant. By comparison all 3 levels of brotizolam increased feed intake in the

infected animals although there was no difference in the intake response between doses (Table 8.1). Brotizolam did not elevate daily (22 h) feed intake in either group.

ii. Brotizolam administered 45 min after feeding.

Treatment with 1, 2 or 4 mg of brotizolam following the first meal (45 min after feeding) resulted in a second meal immediately following the injection which markedly increased ($p < 0.01$) 1-2 h intake compared with the feed intake of animals receiving saline alone (0 mg brotizolam) in infected and non-infected groups (Table 8.2 and 8.3). Brotizolam administered 45 min after feeding elevated total daily intake in the infected group using 1, 2 or 4 mg but only 1 and 4 mg elevated daily intake in the non-infected group.

Table 8.1 Mean (± s.e.m) cumulative feed intake (g/kg $LW^{0.75}$) over the first 8 h of feeding of infected animals following treatment with 0, 1, 2 or 4 mg of brotizolam injected i.v. immediately before feeding (n=4).

Cumulative feed intake				
	0 mg	1 mg	2 mg	4 mg
10 min	8.1 ± 1.19	15.6 ± 1.70	14.4 ± 2.34	18.8 ± 2.54
30 min	10.9 ± 1.75	25.8 ± 4.16	23.9 ± 4.19	28.3 ± 4.66
60 min	11.3 ± 1.22	30.4 ± 3.51	32.1 ± 3.52	37.9 ± 5.49
8 h	34.1 ± 4.25	42.7 ± 2.68	42.5 ± 3.47	52.2 ± 6.2

Table 8.2 Mean (± s.e.m) cumulative feed intake (g/kg $LW^{0.75}$) of infected animals following treatment with brotizolam immediately prior to feeding (0 min) or at the termination of the first meal (45 min) (n=4).

Cumulative feed intake							
Level of brotizolam							
	0 mg	1 mg		2 mg		4 mg	
Time after feeding brotizolam administered							
	Control	0 min	45 min	0 min	45 min	0 min	45 min
1 h	11.3 ± 1.22	30.4 ± 3.51	34.0 ± 2.80	32.1 ± 3.37	24.0 ± 2.80	37.9 ± 5.49	47.5 ± 2.80
2 h	13.3 ± 1.95	32.0 ± 3.77	36.0 ± 2.80	32.9 ± 3.37	24.0 ± 2.80	38.1 ± 5.49	48.0 ± 2.80
8 h	34.1 ± 4.25	42.7 ± 2.68	46.0 ± 2.68	42.5 ± 3.47	40.5 ± 2.80	52.2 ± 6.20	57.5 ± 2.80
22 h	55.0 ± 2.60	50.0 ± 2.60	67 ± 2.60	57.0 ± 2.60	71.0 ± 2.60	54.0 ± 2.6	80.0 ± 2.80

Table 8.3 Mean (± s.e.m) cumulative feed intake (g/kg $LW^{0.75}$) of non-infected animals following treatment with brotizolam immediately prior to feeding (0 min) or at the termination of the first meal (45 min) (n=4).

Cumulative feed intake							
	0 mg	1 mg		2 mg		4 mg	
	Control	0 min	45 min	0 min	45 min	0 min	45 min
1 h	22.3 ± 1.36	23.70 ± 2.73	23.0 ± 1.60	22.7 ± 2.16	23.0 ± 1.60	35.2 ± 6.66	30.0 ± 1.60
2 h	24.50 ± 1.82	25.8 ± 3.49	25.0 ± 1.60	23.6 ± 2.17	24.0 ± 1.60	36.4 ± 6.58	30.0 ± 1.60
8 h	52.4 ± 2.60	42.6 ± 2.60	45.0 ± 1.60	43.5 ± 4.00	38.0 ± 1.60	43.5 ± 6.38	45.5 ± 2.80
22 h	71.0 ± 2.40	57.0 ± 1.60	78.0 ± 2.40	70.0 ± 2.40	71.0 ± 2.60	65.0 ± 2.4	80.0 ± 2.80

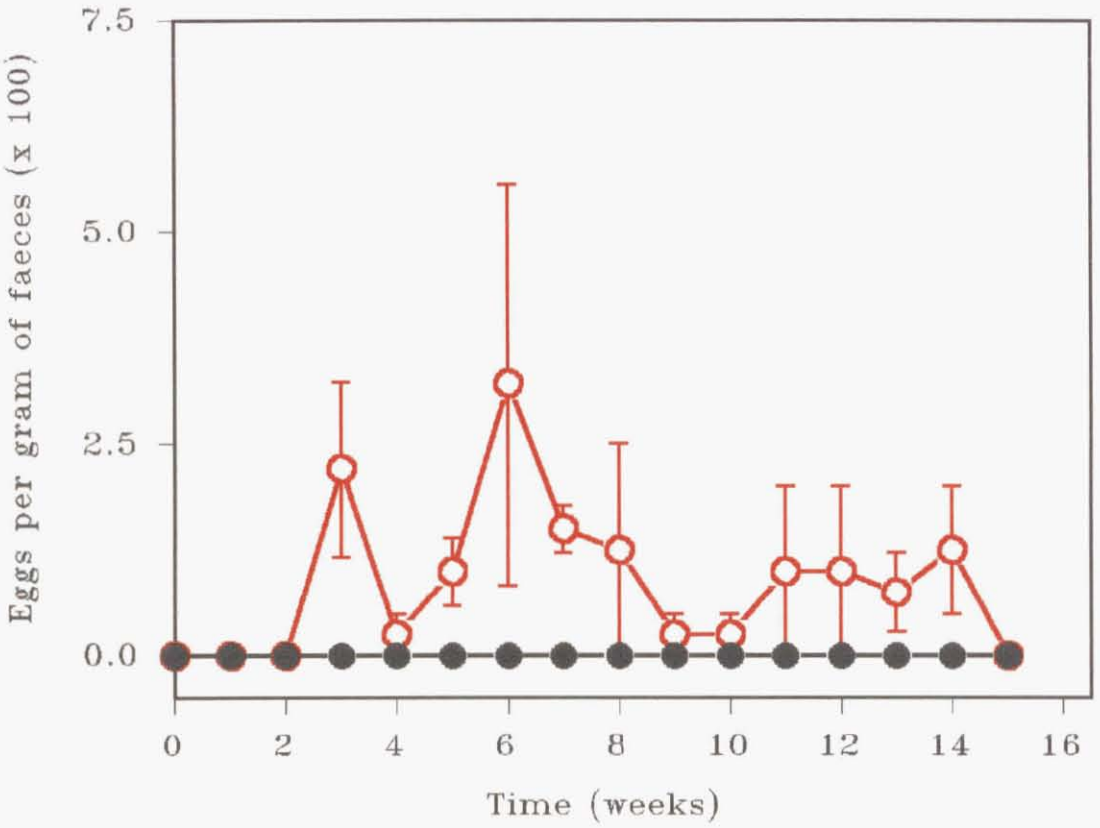


Figure 8.1 Mean (± s.e.m) number of *T.colubriformis* eggs per gram of faeces in infected (○) and non-infected (●) lambs sampled once per week (n=6).

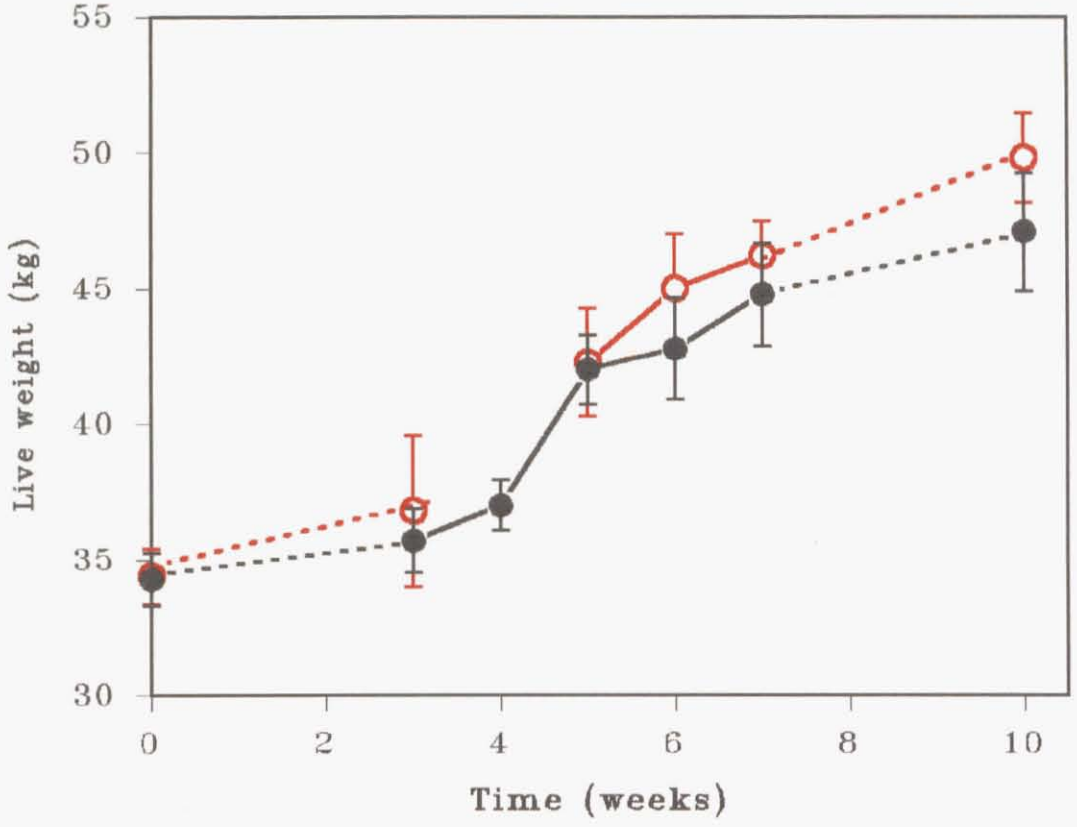


Figure 8.2 Mean (± s.e.m) live-weight of infected (○) and non-infected (●) lambs during the experiment (n=6).

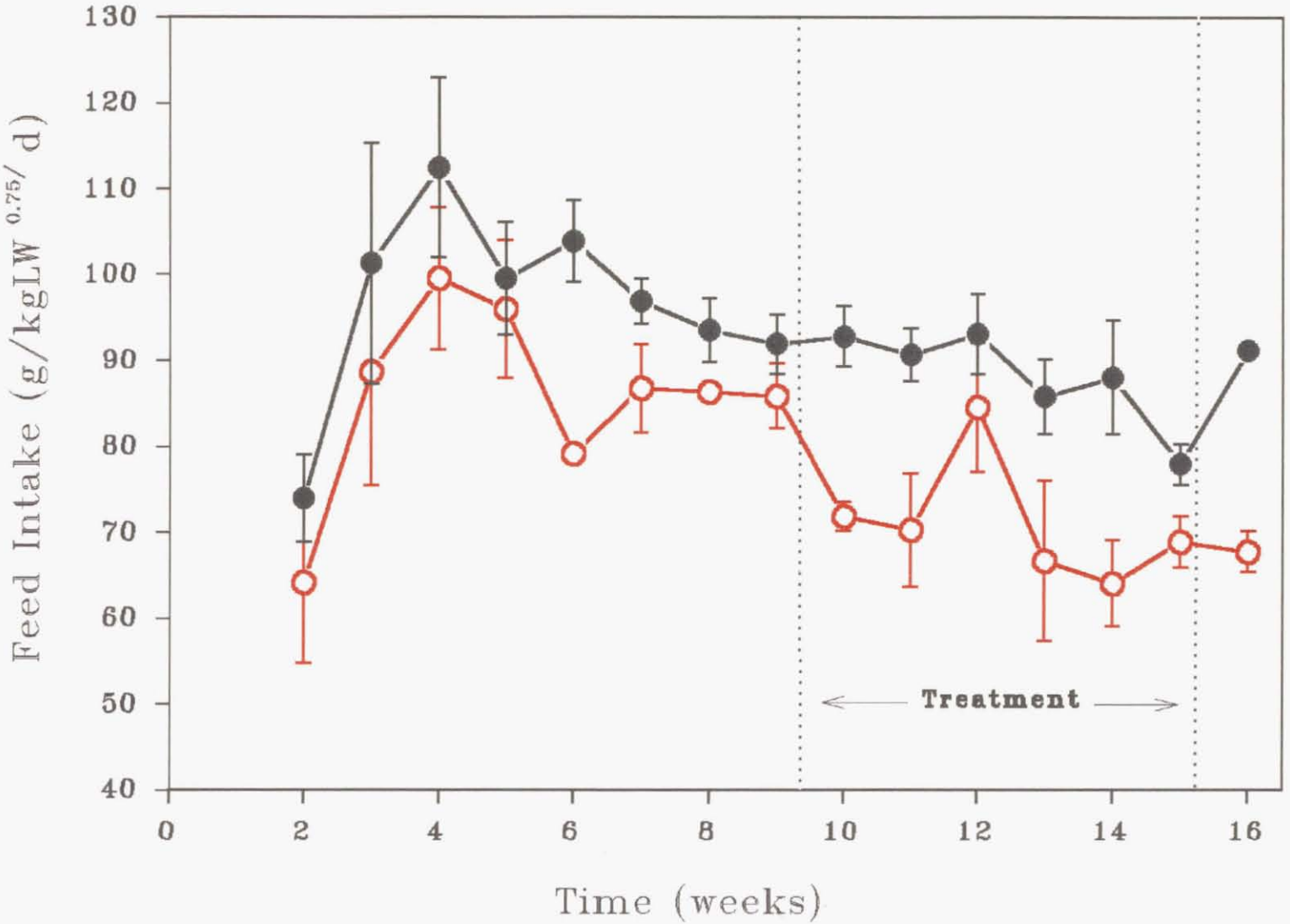


Figure 8.3 Mean daily feed intake (± s.e.m) for infected (◎) and non-infected (●) lambs fed once per day (n=6).

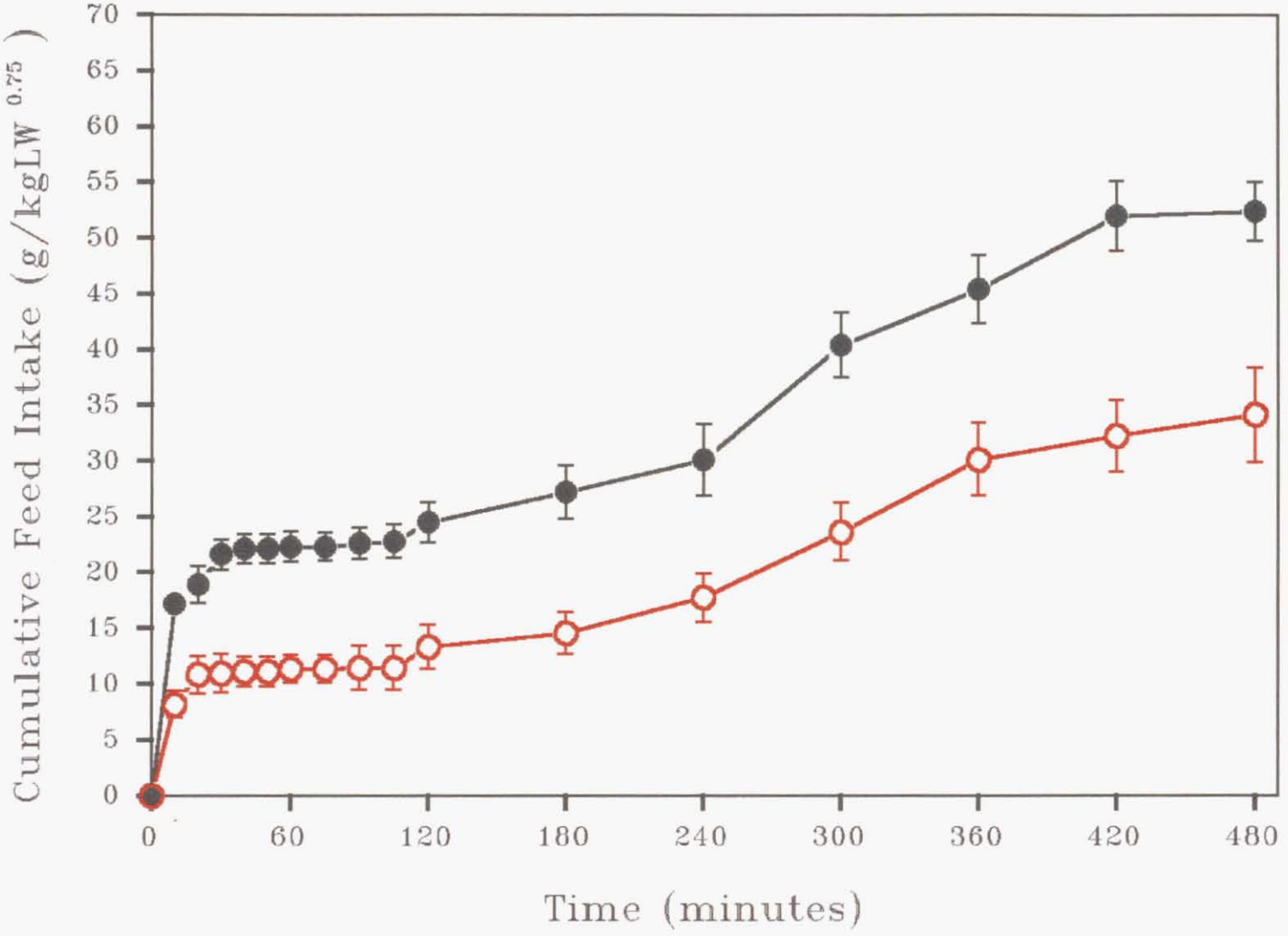


Figure 8.4 Mean (± s.e.m) short term cumulative feed intake for infected (○) and non-infected lambs (●) fed once per day (n=6).

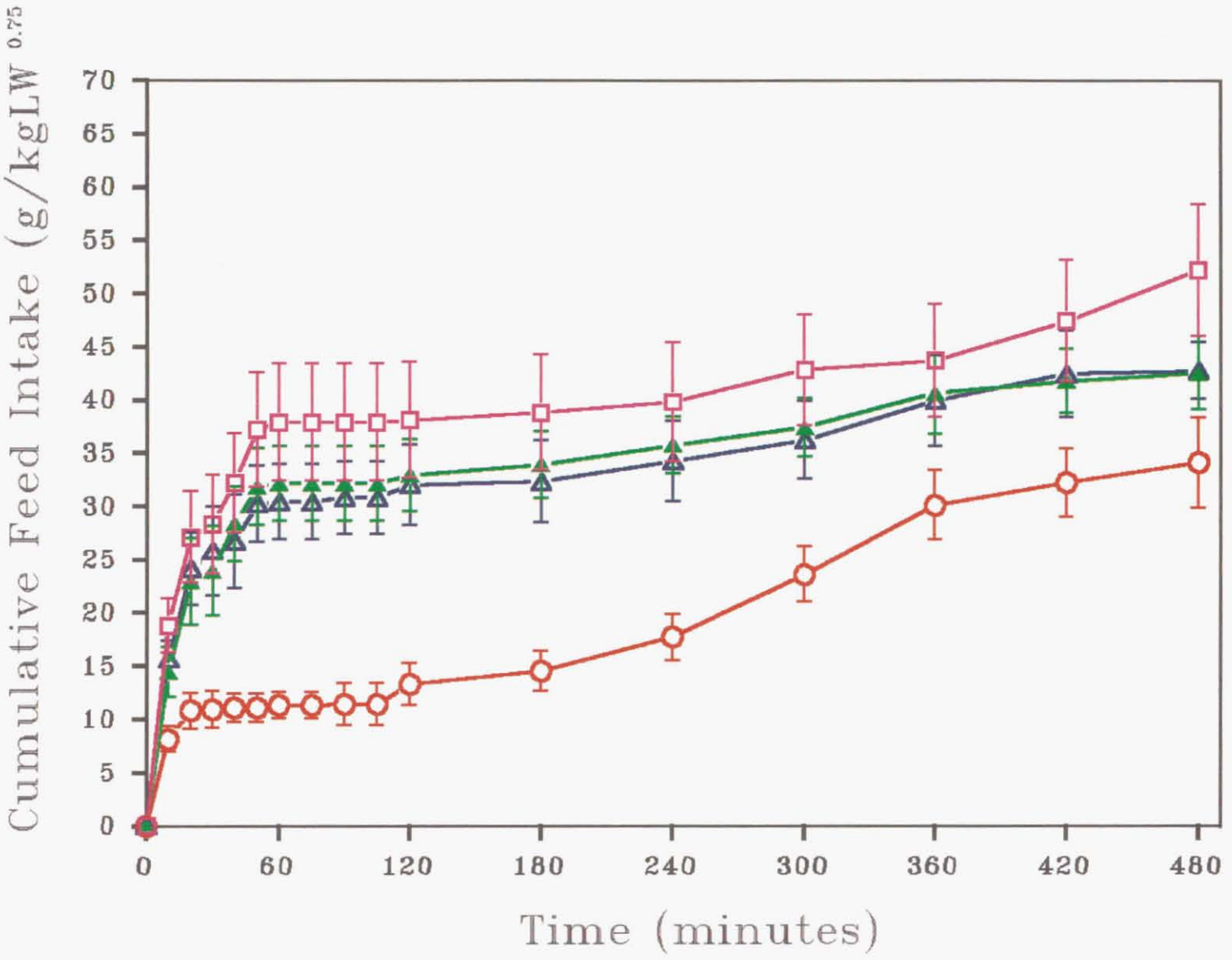


Figure 8.5 Mean (± s.e.m) cumulative feed intake for infected lambs injected intravenously with 0 (○), 1 (△), 2 (▲) or 4 (□) mg of brotizolam immediately before feeding (n=6).

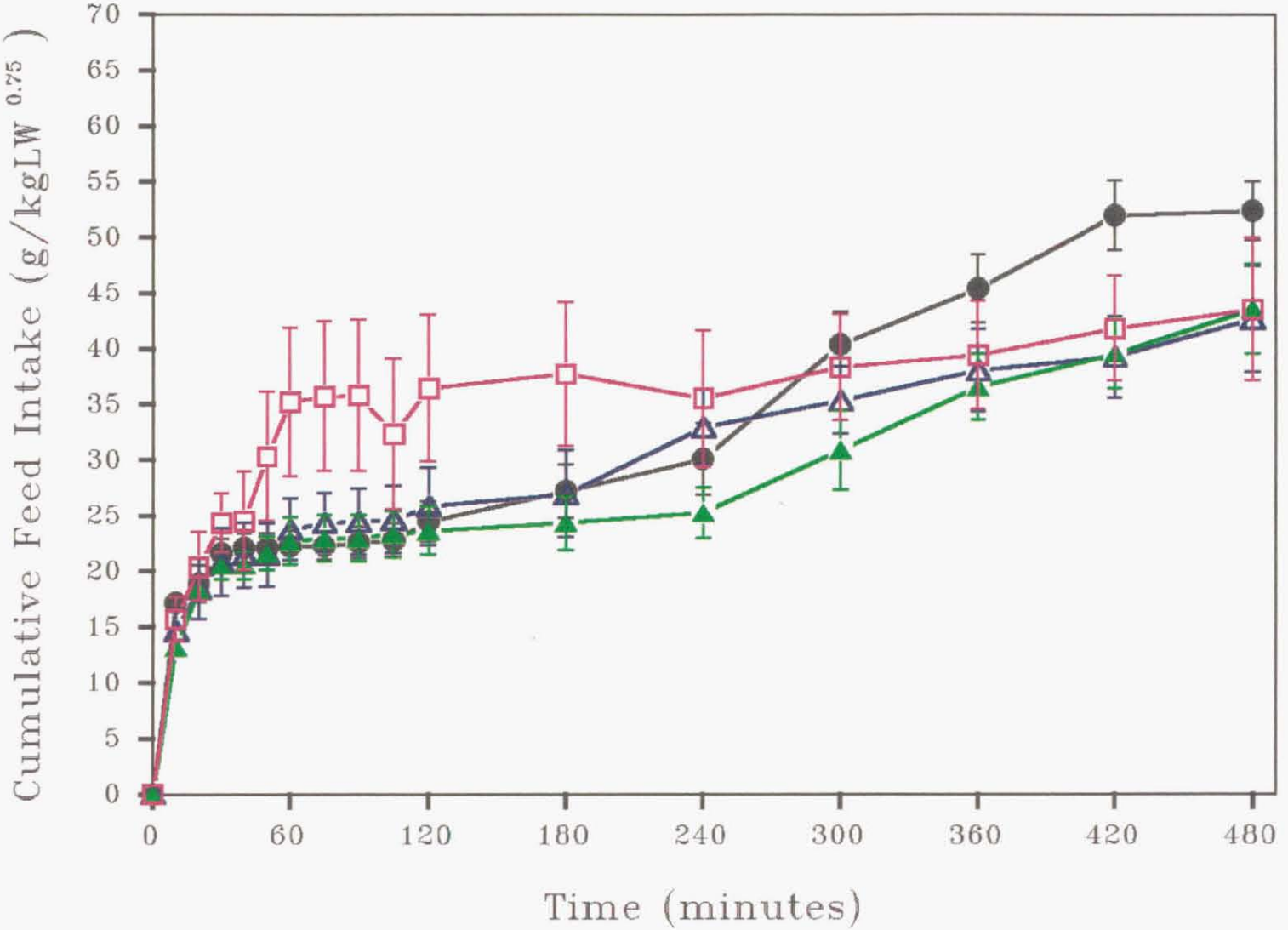


Figure 8.6 Mean (± s.e.m) cumulative feed intake for non-infected lambs injected intravenously with 0 (●), 1 (△), 2 (▲) or 4 (□) mg of brotizolam immediately before feeding (n=6).

8.4 Discussion

This study provides the first evidence that feed intake in parasite-infected lambs might be increased by the use of a satiety blocking agent.

Brotizolam elevated both short term and daily (22 h) feed intake when administered immediately after the first meal, while administration prior to the first meal elevated only short term intake (0-5 h). When brotizolam was administered just prior to feeding, the resulting cumulative feed intake curves were similar in magnitude to those of non-infected animals receiving saline. This finding of an increase in intake following administration of a benzodiazepine in infected animals suggests a central mediation of feed intake depression in parasite infected lambs. These results also suggest that the non-infected lambs were eating at a maximum rate which could not be increased by benzodiazepines.

The actual mechanisms involved in the response to brotizolam were not investigated in this experiment, and one cannot eliminate peripheral mechanisms being involved. However evidence from the current trial and the wide distribution of diazepam receptors in the brain, (including hypothalamic nuclei), of many species (Cooper, 1983) suggests peripheral and/or central mechanisms are integrated centrally to depress intake. Stimulation of diazepam receptors with benzodiazepine agonists elevates feed intake while inverse agonists decrease feed intake and antagonists block the effects of both agonists and inverse agonists on feed intake, suggesting diazepam receptors may have elicited the hyperphagic response recorded here. Other work reviewed by Baile and M°Laughlin (1979) and Cooper (1983) found benzodiazepines to mimic γ amino butyric acid (GABA-ergic) pathways but this response appears likely to be the mediator of motor incoordination which can occur following benzodiazepine administration and as such may not be of significance in the hyperphagic response to benzodiazepine.

Opioid pathways are another proposed pathway of response (Cooper 1983) by the animal to benzodiazepine administration. Naloxone (an opioid receptor antagonist) when used concurrently with benzodiazepines will block the induced hyperphagia suggesting benzodiazepines may act by releasing endogenous opioid peptides and/or their action at the receptors. If brotizolam acts via an opioid pathway, the results of the current study suggest a lack of hunger or a weak hunger signal, possibly due to lower than normal levels of

endogenous opioids, may be causing the reduced rate of feed intake recorded in parasite infected animals. If brotizolam acts to block a satiety signal as was concluded from early rat studies, then this implies a strong peripheral and/or central satiety signal may be operating. Further work to explore both these areas may provide valuable information of the mechanisms involved in parasite induced reduction in rate of eating. The current experiment did not investigate mechanisms of benzodiazepine action but further valuable information on mechanisms of benzodiazepine action in disease-induced anorexia could be gained by using parasite infected lambs and benzodiazepine agonists, antagonists and inverse agonists, and hunger/satiety agonists and antagonists like loxiglumide and naloxone.

Intense feeding occurred when brotizolam was administered prior to feeding, both the rate of feeding and the duration of the first meal were increased in the infected animals by 100 % and 30 min respectively. Intense feeding was uncharacteristic of infected animals which even following the 2 h feed deprivation period never showed a strong desire for feed, unlike the non-infected animals which were vocal and active prior to feeding. These observations support the earlier suggestion that a reduced hunger signal may be occurring in infected lambs during anorexia.

In a study similar to the present experiment, Van Miert, Koot and Van Duin (1989) administered a single injection of brotizolam before feeding to goats fasted for 2 h and recorded a 35 % increase in intake 30 min after feeding. Unfortunately no other time periods were reported. A lack of significant response in the non-infected animals in the present study was similar to that of Fanneau de la Horie and Vaugon (1986) who found cattle with a variety of gastro-intestinal disorders responded more to brotizolam than healthy animals. The lesser response by the control animals may also be attributed to these animals being closer to maximum potential physical rate of consumption. Thus in removing satiety blockage other factors, like rate of prehension, limited the intake response. It was interesting that where brotizolam was administered to non-infected animals after the first meal, daily intake was increased by some 12 % yet the same dose rates administered prior to feeding did not change daily intake. In these once daily fed animals, the control animals showed considerable hunger prior to fresh feed being offered and ate rapidly during the first 40-50 min resulting in a variable hyperphagic response to brotizolam. Treatment following the first meal effectively added another meal into the daily pattern. In this case a meal terminating satiety signal was overcome so the response was not constrained by physical factors such as may have been the case with brotizolam treatment prior to feeding.

Parasite infection produced a characteristic depression in daily intake, similar to that found by Steel *et al.* (1980) and reported in earlier experiments. In the current study parasite infection did not impede live-weight gain, unlike other reports of the effects of GIT parasite infection on live weight changes (Sykes 1982; Holmes 1986) and unlike earlier studies (Chapters 4-7) where infection restricted live-weight gain.

In conclusion, this study found stimulation of diazepam receptors (probably within the brain) prior to feeding temporarily restored short term feed intake in anorexic parasite infected lambs. Where treatment was administered immediately after the first meal, daily intake in these infected animals was increased. These results show that parasite infected lambs will respond to stimulation of receptors, probably located within the brain, associated with feeding. Further studies on the role of hunger and satiety pathways in mediating this intake depression are required.

CHAPTER 9

The effect of fasting and an opioid antagonist on feed intake in lambs infected by *Trichostrongylus colubriformis*.

9.1 Introduction

Work on the role of hormones such as CCK in parasite induced feed intake depression described in this thesis and in other studies has focused on the effects of enhanced satiety signals in parasite infected lambs. There has been little consideration of a possible effect of "reduced hunger" in infected lambs. In earlier studies of short term feed intake, parasite infected lambs consumed feed but were observed to lack a strong desire to seek feed, manifested in a reduced rate of intake over short time periods, suggesting that they may be experiencing a reduced hunger drive.

Some studies (Della-Fera and Baile, 1984) have implicated endogenous opiates as mediators of hunger signals in sheep. At least three families of opioid peptides have been defined, the enkephalins, endorphins and dynorphins. Feeding responses in many animals have been recorded with all three of these opioid families. Generally the magnitude of the response increases in the order B-endorphins, enkephalins and dynorphins but depends on the specific structure of each peptide (M°Laughlin and Baile, 1986). For example, central administration of met-enkephalin via a lateral cerebral ventricular cannula (i.c.v.) did not affect feed intake, however i.c.v. infusion of D-ala2-met5-enkephalinamide, which differs from met-enkephalin by only one amino acid residue, increased feed intake by 500 % in the first 60 min (Baile *et al.*, 1981). In contrast D-ala2-leu5-enkephalin (one more amino acid residue) decreased intake (Della-Fera and Baile, 1984), highlighting the importance of the specific structure of each opioid for these responses. Similarly various dynorphin A peptides increased feed intake in sheep in a dose-dependent manner following continuous i.c.v. injection (Baile *et al.*, 1987).

Further support for a role of opioids as mediators of hunger signals is the identification of immunoreactive met-enkephalin neurones throughout the hypothalamus of the sheep (Marson *et al.*, 1986), which is evidence that these peptides could act directly at the level of feed intake regulating centres.

Fasting or feed deprivation is a method employed by researchers to enhance hunger and is commonly used in the study of both hunger stimulating and satiety hormones. Elevated brain levels of hunger hormones such as opioids have been recorded in sheep following at least a 4 h fast (Scallett *et al.*, 1985), conversely low levels of satiety hormones like CCK have been associated with fasting in laboratory animals.

One method of establishing whether or not an eating response is opioid mediated is to determine if administration of the universal opioid antagonist naloxone, will block it. For this reason naloxone was used here in a preliminary study of the role of opioids in intake depression. Brotizolam was also used, as previous studies (Chapter 8) had shown it temporarily elevated feed intake in infected lambs, so it was included as a treatment here to check the reliability of the short term feed intake recording technique and to demonstrate that lambs in the study could respond to an intake stimulant (i.e. a positive control treatment). Fasting was included as a treatment, as a means for "naturally" enhancing hunger in both infected and non-infected lambs.

9.2 Materials and Methods

Animals

Twenty female sheep (Coopworth x Dorset Down) from the pool of lambs which had been weaned in October 1989 at 7 weeks of age (live weight 19 $\pm$ 2 kg, Section 3.7) were maintained indoors on a complete pelleted diet (Appendix 2). At approximately 12 weeks of age (25 kg live weight), animals were randomly allocated to parasite infected (n=10) and non-infected groups (n=10), balanced for live weight.

Infected lambs were dosed orally with 28 000 infective *T. colubriformis* larvae each week, administered 3 times per week (c.a. 9 300 larvae per dose) commencing on 29 January 1990. For the first 4 weeks of the trial non-infected animals were pair-fed with infected animals to minimise live weight differences between groups. In the first 2 weeks the pattern of short term feed intake was established for each animal by recording intake, following a 2 h feed deprivation period (Section 3.1).

Treatment commenced during weeks 7-8 of infection, when feed intake depression of at least 30 % occurred in all lambs of the infected group.

Experimental procedure

Treatments were applied in a factorially designed (2 x 2^3) regime to both infected and non-infected animals. Animals were fasted for 26 h or not fasted then infused i.v. with either saline vehicle, brotizolam or naloxone prior to feeding (see below).

		brotizolam
	fasted	saline
Infected		naloxone
		brotizolam
	non-fasted	saline
		naloxone
		brotizolam
	fasted	saline
Non-infected		naloxone
		brotizolam
	non-fasted	saline
		naloxone

Treatment allocation to infected and non-infected animals

Treatments were allocated in a randomised Latin square design, such that each infected or non-infected animal received all combinations of treatments once. The design was replicated once.

Fasted animals had free access to water but no feed for a 26 h period and non-fasted animals had *ad libitum* feed for 22 h followed by a 2 h feed deprivation prior to infusion of the test substances.

Brotizolam (Mederantil, Boehringer Ingleheim, N.Z.) was administered directly into a jugular vein by slow injection (2 mg in 2 ml) immediately prior to feeding (within 5 min). Naloxone (Sigma Chemical Co. St Louis, Mo, U.S.A.) was administered intravenously at a rate of 0.125 mg/ kg LW in a sterile 2 ml 0.9 % phosphate buffered saline solution (Appendix 5) 15 minutes prior to feeding. To minimise carry-over effects of either the drugs or the 26 h fast on recorded intake, treatments were applied on alternate days or after

a 2 day rest period when each animal had been fasted.

Sampling

Feed intake was recorded in the short term (0-8 h) and daily (22 h) on both treatment and rest days (Section 3.1). Faecal samples were collected once per week and the number of parasite eggs per gram counted (Section 3.6). All animals were weighed weekly (Section 3.2).

Statistical analysis

Statistical analysis was carried out using a non-parametric rank transformation of the intake data followed by analysis of variance for unbalanced data using the SAS statistical package (SAS 6.01 SAS Institute N.C., N.Y., U.S.A.)

9.3 Results

Parasitological changes

All eggs recovered from faeces were identified as those of *T. colubriformis*. Changes in mean faecal egg concentration of infected animals over the experiment are shown in Figure 9.1. Eggs first appeared in faeces 3 weeks post-infection and the number excreted peaked during week 4. Thereafter egg output tended to fall, with a sudden drop in output during week 8, but with a second peak occurring during week 9. No eggs were found in samples from non-infected animals during the experiment.

Live weight

Changes in mean live weight for infected and non-infected groups during the experimental period are shown in Figure 9.2. Prior to week 9 live weight gains of the 2 groups were very similar with mean weekly gains of 1.3 ($\pm$ 0.30) and 1.3 ($\pm$ 0.43) kg for infected and non-infected groups, respectively. From week 8 mean live weight of the infected group remained unchanged (31.0 $\pm$ 1.30) kg while the non-infected group continued to gain weight (1.3 $\pm$ 0.04 kg/week) for the remainder of the trial.

Feed intake

Mean weekly feed intake of infected lambs was lower than that of non-infected lambs (Figure 9.3). Feed intake of the infected lambs remained depressed for 5 weeks of the

experiment.

The maximum depression of 29 % was recorded during week 12 of infection.

Short term feed intake

Short term feed intake in infected animals was depressed relative to non-infected animals at all recorded time periods following a single daily feed (Figure 9.4). Average feed intake depression was 36 %, with maximum depression of 48 % recorded at 10 min and minimum depression of 25 % recorded at 7 h. The observed depression in intake tended to decrease as the day progressed.

Treatment effects

Fasting

A 26 h fast alone significantly ($0.01 < p < 0.05$) elevated feed intake in both infected and non-infected groups in the short term (100 % increase in intake at 120 minutes) and on a daily basis (12 % increase). Over the 8 h recording period the infected, fasted group ate only slightly less feed than the non-infected and fasted group (Figure 9.5). During the first 40 min of feeding the mean cumulative feed intake for the infected and fasted group was not significantly different from that of the non-infected non-fasted group. However, from 50 min to 7 h post feeding intake of the infected, fasted group was significantly above that of the non-infected non-fasted group ($p < 0.05$).

Naloxone

Naloxone depressed short term feed intake in both infected and non-infected groups following the 26 h fast (Figs 9.8, 9.9) and after the standard 2 h feed deprivation period (Figs 9.6, 9.7), except in the infected, non fasted group (Fig 9.6) where the slightly lower intake was not significantly different from the intake following saline solution alone ($p > 0.05$).

Where the groups were not fasted, naloxone-induced intake depression in the non-infected groups resulted in a cumulative intake curve which was the same as for the infected control treatment. Where both groups were fasted first, naloxone treatment appeared to depress feed intake more in the non-infected group, resulting in the infected and non-infected groups having similar intakes over the first 5 h following feeding.

Brotizolam

Brotizolam significantly elevated feed intake only in the infected animals.

In the non-fasted, infected animals, brotizolam increased the rate of feed intake by 88 % in the first hour (Figure 9.6). On average the increase during the first 3 h was 62 % for these infected animals. Following fasting in infected animals (Figure 9.8) brotizolam increased feed intake significantly during the period 1.75 h to 7 h after feeding ($p < 0.05$). In the non-infected group brotizolam tended to increase intake during the first 2 hours (Figure 9.9) but this increase was not significant ($p > 0.05$).

There were no significant parasite by treatment interactions revealed by analysis of variance on untransformed or rank transformed data.

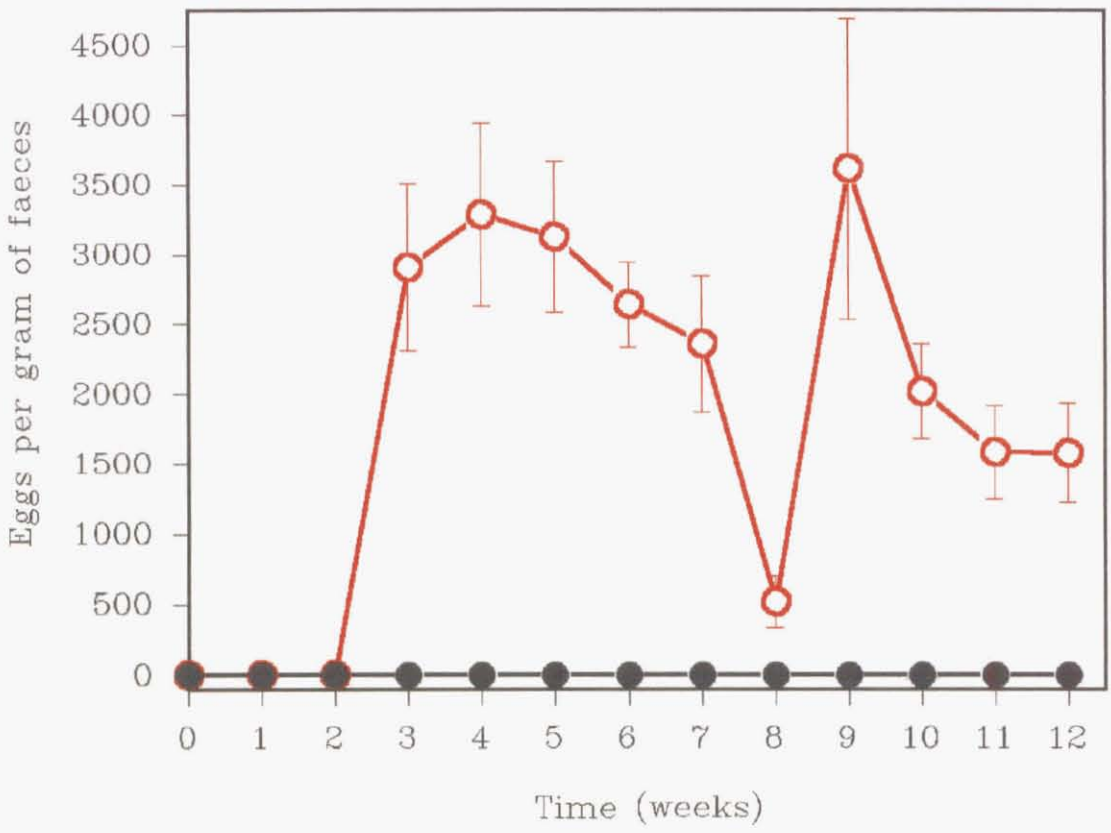


Figure 9.1 Mean (± s.e.m) number of *T. colubriformis* eggs per gram of faeces in infected (o) and non-infected (●) lambs sampled once per week (n=10).

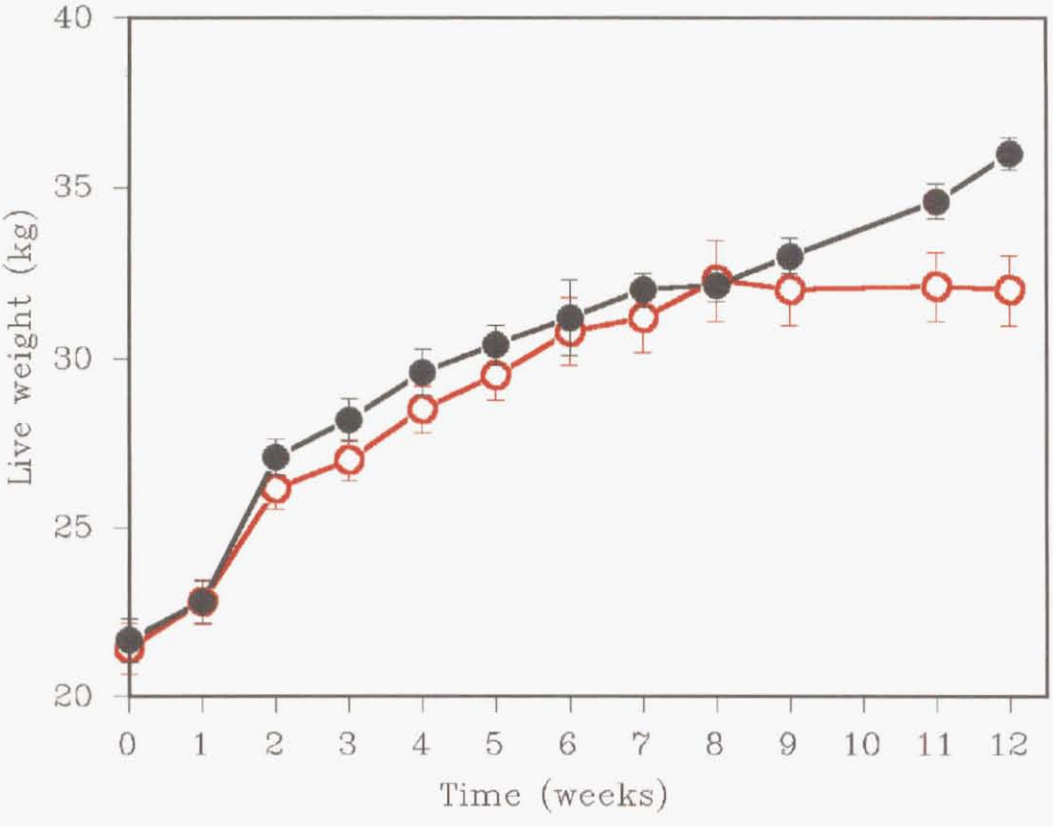


Figure 9.2 Mean live-weight (± s.e.m) for infected (o) and non-infected (●) lambs during the experiment (n=10).

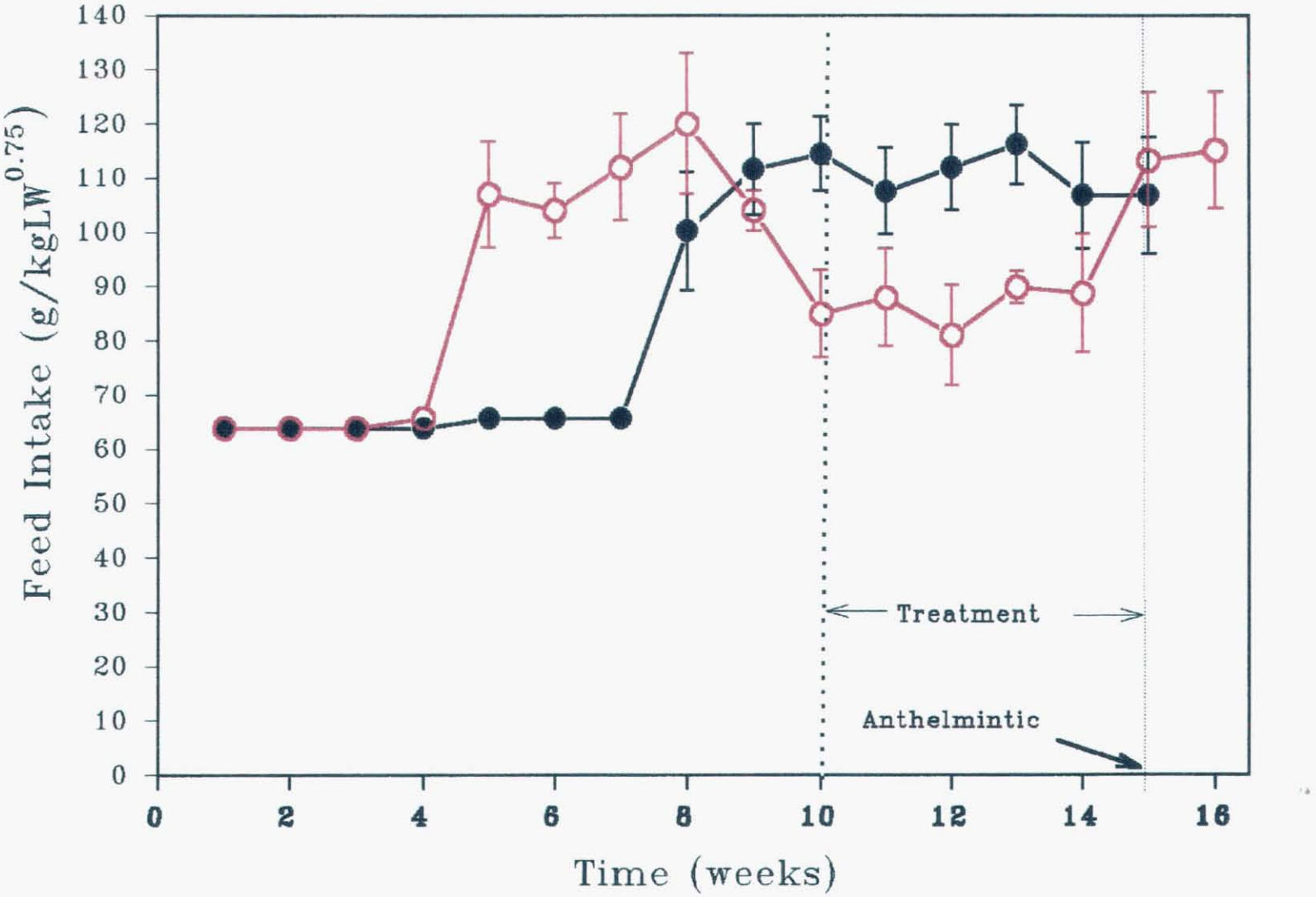


Figure 9.3 Mean daily feed intake (± s.e.m) for infected (○) and non-infected (●) lambs during the experiment (n=10).

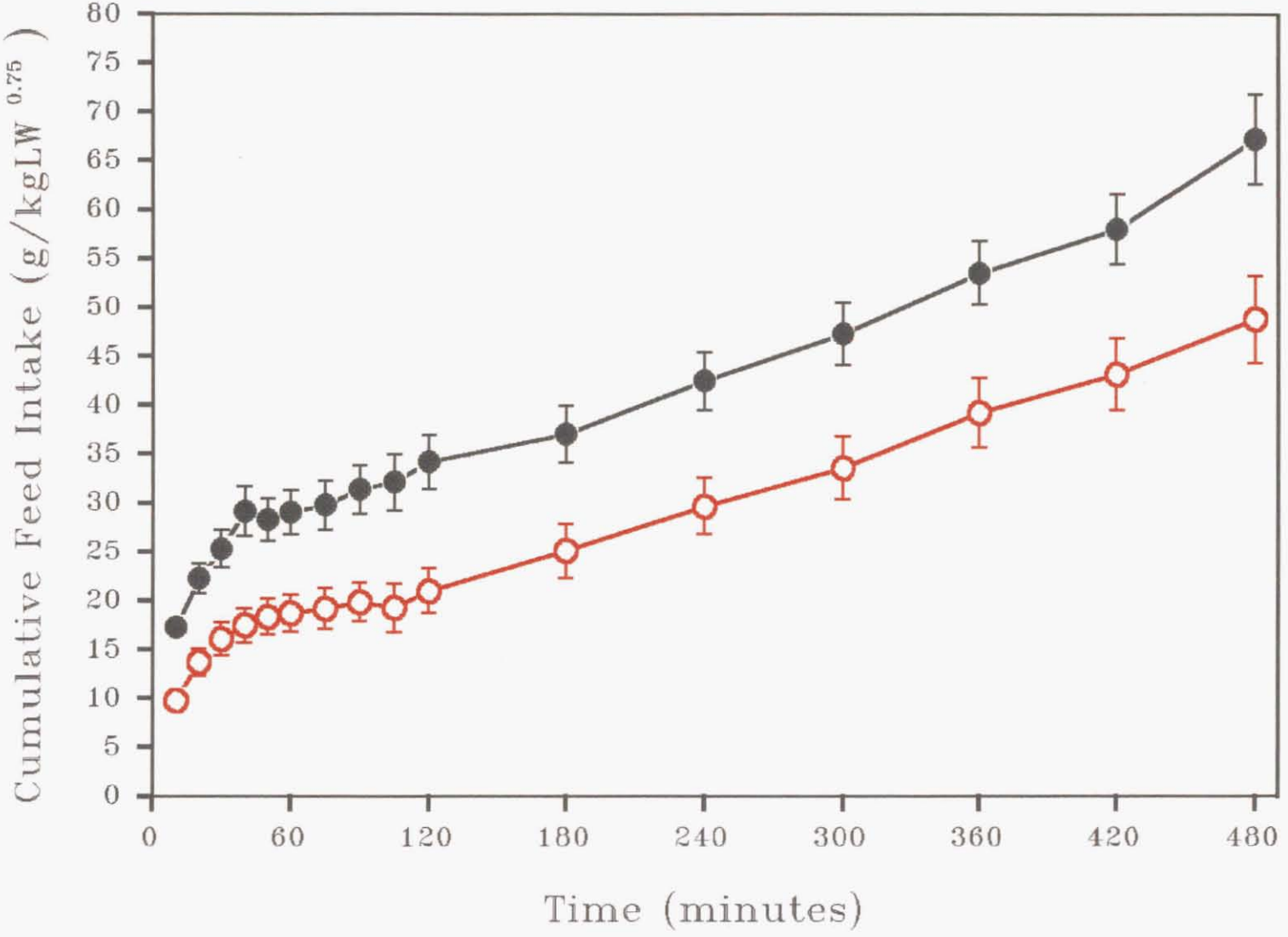


Figure 9.4 Short term cumulative feed intake (± s.e.m) for infected (o) and non-infected (●) lambs fed once per day (n=10).

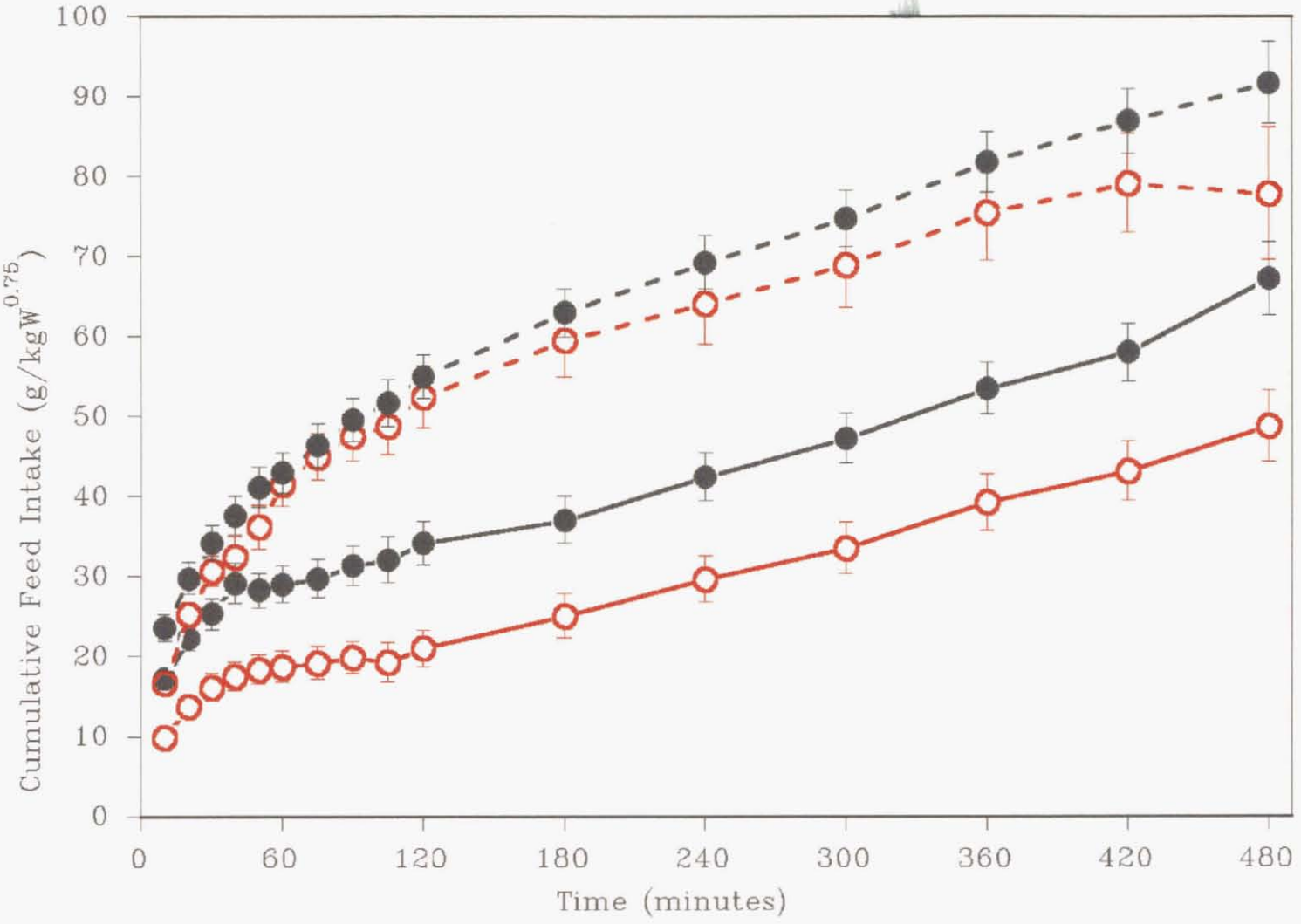


Figure 9.5 Short term cumulative feed intake (± s.e.m) for infected (○) and non-infected (●) lambs not fasted (—) or fasted for 26 h (- - -) prior to feeding (n=10).

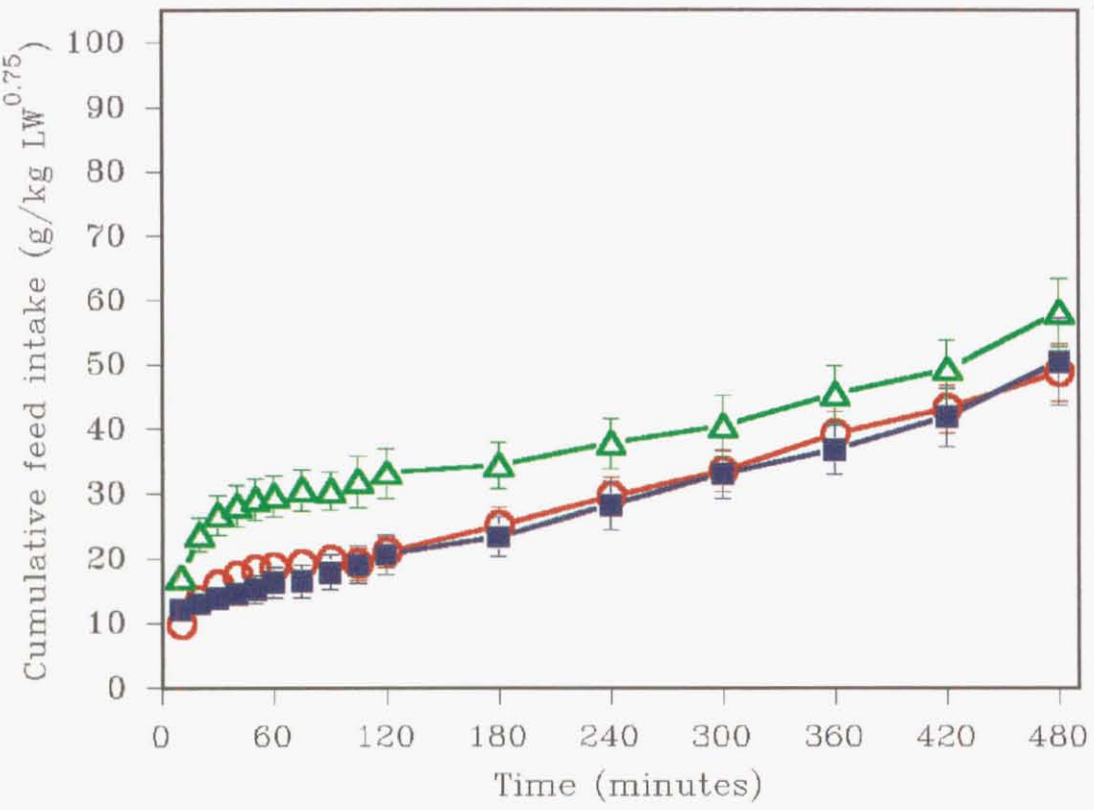


Figure 9.6 Mean (± s.e.m) cumulative feed intake over an 8 h period for infected lambs not fasted and treated with saline solution (control) (○), naloxone (■) or brotizolam (△) immediately before feeding (n=10).

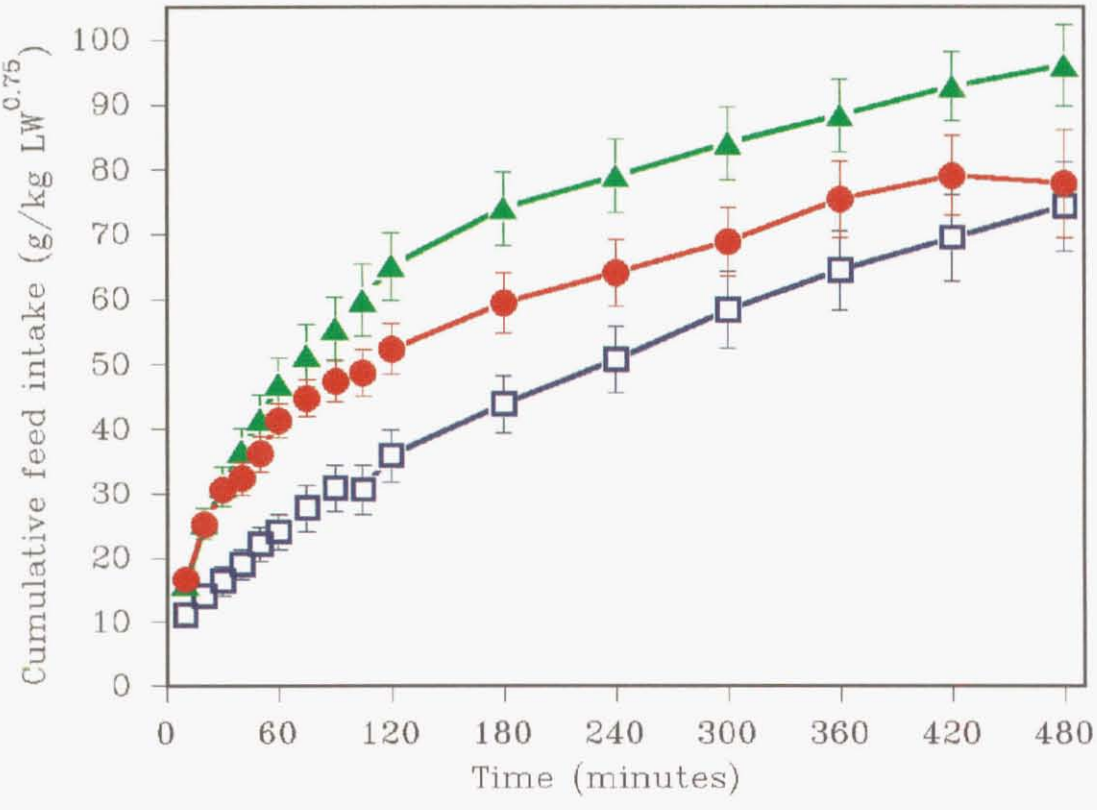


Figure 9.7 Mean (± s.e.m) cumulative feed intake over an 8 h period for infected lambs, fasted and treated with saline solution (control) (●), naloxone (□) or brotizolam (▲) immediately before feeding (n=10).

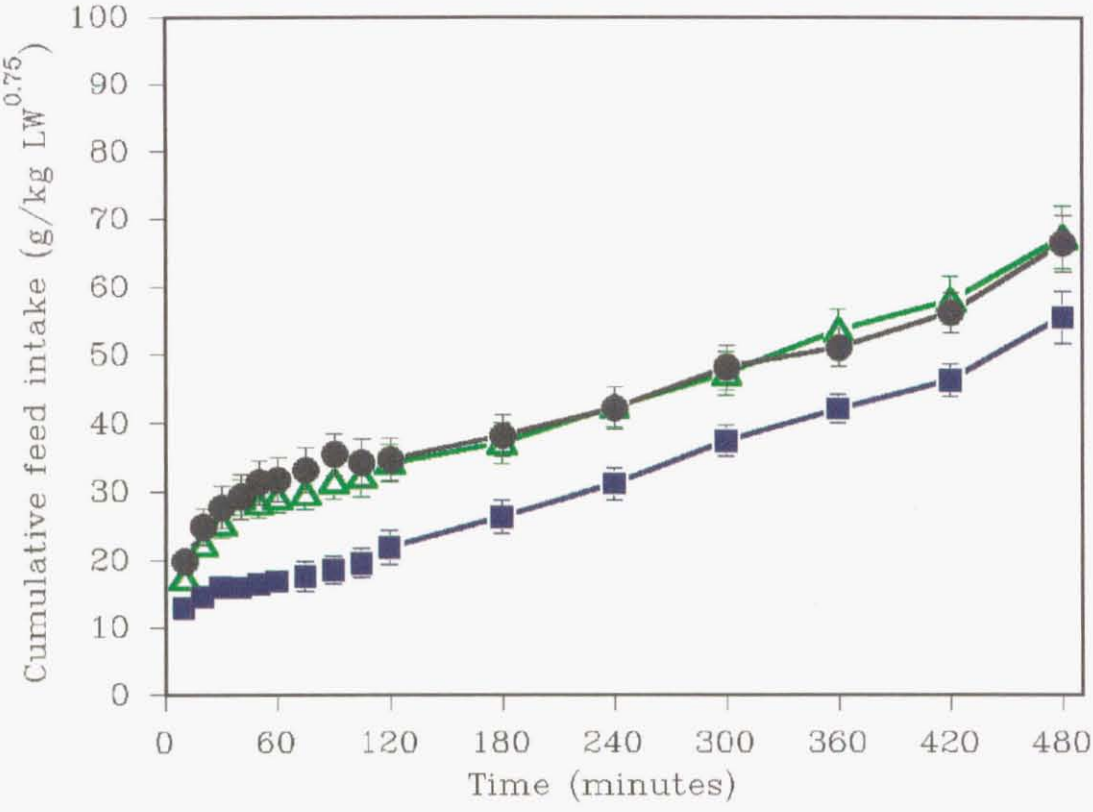


Figure 9.8 Mean (± s.e.m) cumulative feed intake over an 8 h period for non-infected lambs not fasted and treated with saline solution (control) (●), naloxone (■) or brotizolam (▲) immediately before feeding (n=10).

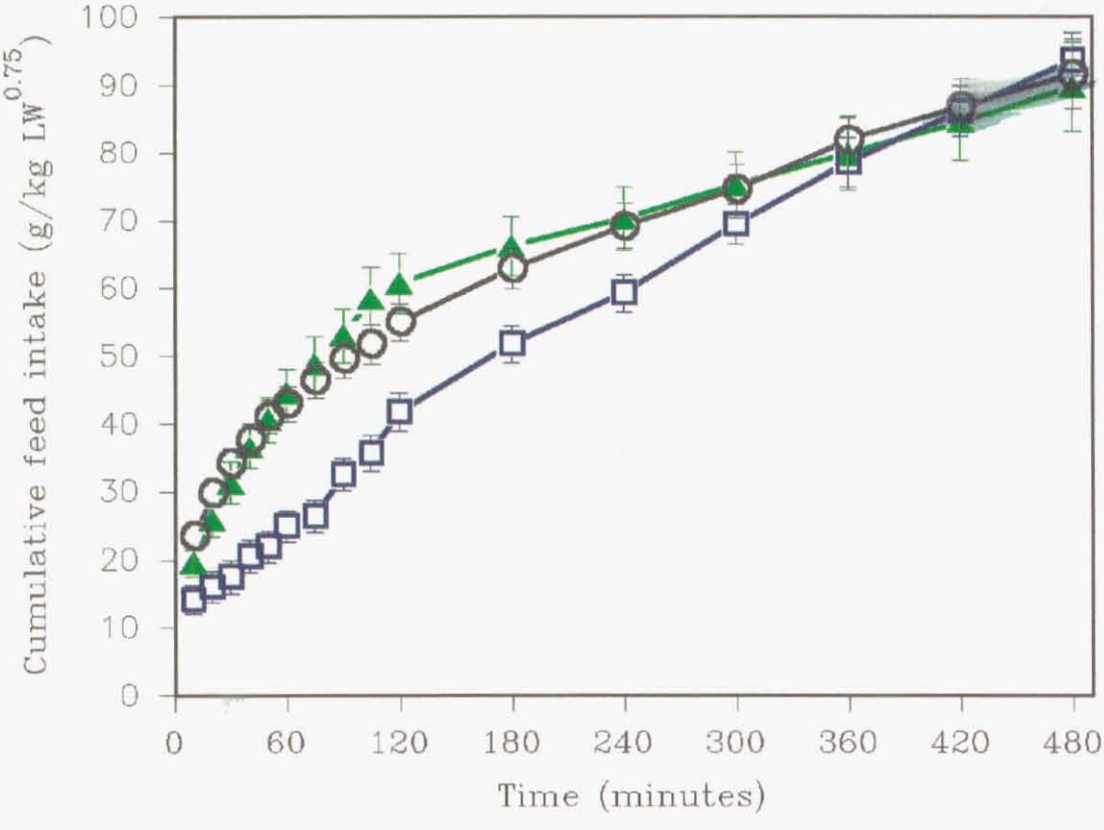


Figure 9.9 Mean (± s.e.m) cumulative feed intake over an 8 h period for non-infected lambs, fasted and treated with saline solution (control) (○), naloxone (□) or brotizolam (▲) immediately before feeding (n=10).

9.4 Discussion

These findings demonstrate that fasting will stimulate feed intake in lambs even when they are suffering inappetence due to parasite infection. Fasting was a powerful stimulant to both short term (100 % increase in intake by 120 min) and daily intake (+12 %). The increase in consumption rate from 0.25 g/kg $LW^{0.75}$/min to 0.42 g/kg $LW^{0.75}$/min would result in a fasted 30 kg lamb consuming 262 g DM more than a non-fasted lamb in the 2 h period following feeding.

An interesting result was the finding that a 26 h fast resulted in infected animals consuming a very similar amount of feed to the non-infected, fasted animals and, at least over 7 of the 8 hours recorded, more than the non-infected non-fasted animals. This means the signals resulting from the one day fast were sufficient, in the short term anyway, to override the parasite induced mechanisms which caused intake depression.

The strong desire of the animals to seek food and increased rates of consumption indicate an elevation of hunger following fasting. Feed consumption increases following central administration of opioids (Baile *et al.*, 1981; Morley, Levine, Yim and Lowy, 1983) so it can be argued that decreases in feed intake and blockage of opiate induced feeding following administration of opioid antagonists suggests opiates are involved in the appetite stimulating effects of feed deprivation. Also, naloxone treatment partially blocked the stimulatory effects of a 26 h fast suggesting a role for opiates in fasting-induced hunger. This is consistent with the findings of Scallett *et al.* (1985) who found elevated levels of brain opioids in sheep fasted for 4 h, compared with those of satiated sheep. In the present study, fasting was for considerably longer than 4 h which would favour further enhancement of hunger levels and presumably of hunger stimulating hormones. However, because endogenous hormone levels were not measured, information about the duration of opioid elevation is not available. Because naloxone crosses the blood-brain barrier (Jones and Richter, 1981) its site of action cannot be determined and the effect could have been mediated by peripheral and/or central opioid receptors.

Naloxone suppressed the intake stimulatory effects of a 26 h fast in both infected and non-infected animals, the suppression in the first 90 min resulted in consumption being equivalent to that of animals which were not fasted, and is further support for the role of opioids in hunger. Naloxone suppressed intake in control animals (non-infected and non-

fasted) in the present study, which conflicts with suggestions that naloxone will not affect intake in normally fed animals (Jaffe and Martin, 1980). Feed intake depression of 36 %, 45 % and 42 % at 30, 60 and 90 min, following naloxone treatment, recorded here in non-infected and non-fasted animals, is consistently higher than the 27 %, 25 % and 24 % depression recorded in the same time periods by Baile *et al.* (1981) who used 0.125 mg/kg of naloxone following a 4 h fast. Baile *et al.* (1981) fed their animals for an hour immediately prior to the 4 h fast whereas in the current study feed was on offer, except for the 2 h deprivation period, immediately prior to fresh feed being offered. In a free feeding situation, sheep will consume 16-18 meals per day (R. Weston pers. comm.) so the 2 h feed deprivation period used every day to standardise the timing of the first meal may have been sufficient to stimulate endogenous opioids and therefore hunger. Because of the relatively long term nature of this study lambs may have become conditioned to the offer of fresh feed at the same time daily and delayed eating in anticipation of fresh feed thereby enhancing hunger signals.

The non-fasted, infected animals showed no reduction in intake following naloxone treatment. The lack of response compared with the non-infected group under identical experimental conditions may be evidence for lower release of opioids and therefore less hunger in parasite infected lambs. It is tempting to conclude that opioid levels were lower in these infected animals because of the lack of effect of naloxone. Such a conclusion cannot be reached without measuring circulating levels of endogenous opioids. A possible explanation for the presumed low opiate concentrations in infected lambs could be increased levels of peptidases, enzymes which break down circulating opioids. The presence of these enzymes has been suggested as a reason for the conflicting results of trials using injection of opioids into peripheral blood (Rivére and Buéno, 1988). These peptidases are released from the intestines, so it is possible that parasite damage to the intestines may result in higher than usual levels of peptidases and therefore faster clearance of peripherally released opioids in infected animals. This argument would hold for peripherally circulating opioids. However if peptidases of intestinal origin were to affect opioid action centrally they would have to cross the blood-brain barrier and their ability to do this has not been established or rejected.

Brotizolam was used principally to demonstrate the short term responsiveness of both infected and non-infected animals to a feed intake stimulating challenge. This means that the intake depression observed here in naloxone treated lambs was achieved in animals with competent feed intake regulatory pathways, which increases the validity of the observation.

Brotizolam stimulated feed intake in the infected animals but no significant increases in intake were apparent in the non-infected animals following brotizolam treatment. This finding is not unexpected since other workers (Fanneau, de la Horie and Vaugon, 1986) have found that cattle suffering from gastro-intestinal disorders responded to brotizolam better than controls. Similarly in an earlier experiment (Chapter 8) non-infected animals did in fact respond to brotizolam treatment but the response was variable and much less than that observed in the infected animals.

Only fasting significantly increased daily food intake. A lack of effect of brotizolam on 24 h intake was also observed by Fanneau, de la Horie and Vaughan (1986) who concluded this was due to the short biological half-life (0.3 h) of the compound (Cooper 1983). Nevertheless increases in daily feed intake following brotizolam have been reported previously (Baile and M^{c}Laughlin, 1979; Breier, 1985). However, in both of these instances treatment was administered when animals were already satiated whereas in the present case animals had been deprived of feed for 2 h at the time of treatment and a significantly larger than normal meal occurred. In an earlier study (Chapter 8) daily feed intake was elevated by brotizolam only where brotizolam was administered after the first meal and again not where treatment was prior to feeding.

The 20 % reduction in voluntary feed intake in the infected group of lambs is of similar magnitude to the 16 % depression recorded by Sykes and Coop (1976) and Steel, *et al.* (1980) but quantitatively less than has been recorded in other experiments in this study. The trickle infection was obtained from a successful monoculture with no parasite eggs from another species detected in faeces during the experiment. The sudden drop in parasite egg numbers observed during week 8 appears likely to be due to a processing problem during the counting procedure, in this instance, a weak salt solution was thought to be the problem (R M^c Anulty pers. comm.). The comparatively mild effect on feed intake recorded here possibly reflects low pathogenicity of the larvae.

In conclusion, this study has established that parasite-induced depression of feed intake in lambs can be temporarily overcome by fasting or by central stimulation of feeding centres. In addition, the results show that fasting may produce its effects on intake by an opioid pathway and it is possible that this mechanism is impaired in parasite-infected sheep.

CHAPTER 10

The effects of central administration of CCK, opioids and their antagonists in feed intake depression in lambs infected by the gastrointestinal parasite *Trichostrongylus colubriformis*.

10.1 Introduction

In the previous studies described in this thesis I have been unable to demonstrate a role for peripheral CCK action in parasite induced feed intake depression. Using high affinity CCK receptor antagonists, both in single injection and continuous infusions, short term feed intake was not elevated in either infected or non-infected lambs. Similarly extensive studies on peripheral effects of CCK, (Baile and Della-Fera, 1984) suggest that even in "normal" control of intake in sheep, peripheral CCK does not appear to be as important a controlling hormone as in other species. This does not exclude the possibility of CCK acting centrally within the brain, either directly or indirectly, to influence feed intake.

Specific CCK receptors have been localised in the hypothalamus of the sheep (Marson *et al.*, 1986) and fasting decreased CCK content in this part of the brain (Morley, Levine and Knelp, 1981) so there is good evidence for an involvement of CCK in central control of intake. Nevertheless inconsistent results following injection of CCK into the brain of sheep has led to some controversy on its role in feeding behaviour. In a thorough study with sheep, Della-Fera and Baile (1979) showed that CCK injected into a lateral cerebral ventricle depressed feed intake in a dose dependent fashion, with the dose required to elicit satiety being directly related to the duration of the fast. The lowest dose was similar to normal concentrations reported for humans (Rehfeld and Kruse-Larsen, 1978) suggesting that the dose was physiological. Since then, these workers have shown that central administration of CCK antibodies increased short term intake in sheep (Della-Fera and Baile, 1981).

A significant feature of the changes to short term feed intake in parasite infected lambs is the reduction in the rate of feed consumption. Naloxone, a universal opioid antagonist, was found to reduce short term intake in fasted and non-fasted control animals but only in infected animals following a prolonged fast (Chapter 9). The apparent lack of hunger and lack of responsiveness to naloxone observed in parasite infected lambs after the usual 2 h

fast may be the result of a diminished opioid signal or response. This together with the successful stimulation of feed intake by use of a central acting compound (brotizolam) has provided an incentive to investigate the central involvement of opioids in intake depression. Synthetic opioid analogues tend to be less rapidly cleared from the system, and have been used successfully to elevate feed intake in a number of species, including sheep. An enkephalin analog, D-ala-2 met-5-enkephalinamide, injected into a lateral cerebral ventricle (26 nmol/min) increased intake by 346 % at 90 min while 51 and 102 nmol increased intake by 414 and 439 % respectively, an effect which was blocked by using the opioid receptor antagonist naloxone (Baile *et al.*, 1981).

The following experiments examined the role of centrally acting opioids and CCK in feed intake depression, by using both the active compounds and their receptor antagonists. These studies employed the same model of short term cumulative intake to identify a response as has been used in the studies described in earlier chapters.

10.2 Materials and Methods

Twenty five female lambs (Coopworth) were early weaned on 7 October 1990 (live weight 17 $\pm$ 2.5 kg) (Section 3.7). On 30 October animals were weighed, stratified on the basis of live weight, and allocated randomly within the live weight strata to a parasite infected or a non-infected control group (n=12). Lambs were moved into individual pens on approximately 15 November and into individual metabolism crates on the 25 November 1990.

Parasitology

Parasite dosing commenced on 7 November 1990. See Section 6 of general materials and methods (Chapter 3) for details.

Feeding

All animals were offered a complete pelleted ration (Appendix 2) from early November, with the chaffed meadow hay being gradually reduced over 7-10 days. Non-infected lambs were restricted to the average intake of the infected group for the first 3 weeks of the parasite dosing, then feed offered to non-infected animals was gradually increased so all lambs were receiving *ad libitum* rations by early December.

Surgery

All animals were fitted with a chronically implanted stainless steel cannula into a lateral cerebral ventricle (Section 3.4).

Animals spent 2 days in individual pens immediately after surgery. All commenced eating within 2 h of surgery and there were no apparent side effects of surgery.

Two days after surgery animals were returned to modified metabolism crates. Briefly, feed bins were placed inside the metabolism crates and the crate lined with 10 mm plastic mesh to reduce the likelihood of the cannula being damaged. Animals remained in visual contact with the surroundings.

On the same day, the 3 x weekly dosing of infected lambs with *T. colubriformis* was recommenced as outlined above. During this period lambs became accustomed to having feed removed for weighing at regular intervals, to being handled in metabolism crates, and to having tubing attached to their cannulae and tied into their fleece.

When feed intake of an infected lamb was depressed by 20 % from its pre-infection level, this lamb and its non-infected pair were started on the infusion treatment sequence.

Treatments

Treatments were randomly allocated in a replicated Latin square design and, unless stated otherwise below, infused into the lateral ventricle at a rate of 0.03 ml/min for 90 min, 30 min prior to feeding and for the first hour following feeding.

1. Control:	Sterile phosphate buffered saline solution pH=7 (Appendix 5).
2. CCK	CCK-8 dissolved (2.5 pmol/min) in sterile phosphate buffered saline solution.
3. CCK antagonist	0.54 % (w/v) of loxiglumide in 3 ml phosphate buffered saline solution.
4. CCK plus antagonist	Rates as above
5. Opioid agonist	0.102 pmol/min D-ala2-met5-enkephalinamide dissolved (2.0 μg/ml) in phosphate buffered saline solution.
6. Opioid antagonist	Naloxone 0.125 mg/kg LW (3.75 mg/lamb/day) in 2 ml

phosphate buffered saline. Administered in a bolus intravenous injection 15 min before feeding.

7. Opioid agonist plus antagonist

Rates as above

Treatments were administered on alternate days over 2 x 2 week periods.

Solution preparation

1. Phosphate buffered saline:

Prepared as per Appendix 5 and heat sterilised in sealed glass bottles in an autoclave for 20 min.

2. CCK agonist

48 μg CCK-8 ([Tyr(SO_3H)^{27}], fragment 26-33, product n° 9271, Sigma Chemical Co., St Louis, Mo, U.S.A.) was weighed on a Cahn Balance and dissolved in 250 ml of sterile phosphate buffered saline solution. 125 ml of this stock solution was aliquoted as a 200 % stock for use with loxiglumide and the remaining 125 ml was added to 125 ml of sterile saline solution to give 250 ml of a 100 % stock solution (0.096 μg CCK-8 /ml). Aliquots (10 ml) were placed in sterile 50 ml polyvinyl tubes and frozen at - 70 °C until required.

3. CCK antagonist

Loxiglumide (sodium salt of Loxiglumide, C_{21} H_{29} Cl_2 N_2 Na O_5, MW 483.37, Rotta Research Laboratorium, Monza, Italy) was dissolved in a sterile phosphate buffered saline solution at pH 7.6 to permit dissolution of loxiglumide. The solution was filtered through a bacterial filter (labodisc 50 mm disposable unit, 0.2 μm cellulose acetate; Advantec Toyo). Fresh stocks of the 0.54 % solution were prepared on a weekly basis and stored at 4 °C until required.

4. Opioid agonist

Methionine enkephalinamide (D-ala2-met5-enkephalinamide, acetate salt, MW 655.7, Product n° E 2006, Sigma Chemical

Co., St Louis, Mo, U.S.A.) was dissolved in sterile phosphate buffered saline solution. The opioid was difficult to solubilize, so the manufacturers recommendations were followed and approximately 0.5 ml of sterile dilute acetic acid (6 % glacial acetic acid) were added to the saline solution to promote dissolution. Aliquots (10 ml) were transferred to 50 ml plastic tubes and frozen at -70 °C until required.

Treatment Procedures

The treatment solutions were thawed at 4 °C overnight as required. Treatment solutions were drawn into sterile 3 ml syringes and loaded into sterile tubing (Silastic Medical-grade tubing I.D. 0.04 in O.D. 0.085 in, Dow Corning U.S.A.) infusion lines. The dead volume of the infusion lines was greater than 3 ml, so the lines were clamped to prevent the introduction of air bubbles, then sterile saline solution loaded into the line until the solution containing drug started to drip out of the infusion line. The line was then clamped and connected to a syringe containing 3 ml of sterile saline solution which was attached to the multichannel syringe pump (Sage Instruments Syringe Pump, model 355). The multichannel pump had been modified by attaching a perspex backing plate and a perspex syringe holder to enable the pump to hold 12 syringes (Plate 10). The required pump speed settings had been established in calibration trials using saline solution to determine the speed required to deliver 0.03 ml/min. There tended to be slight daily variations in the rate of pumping, so the infusion rate was checked every 15 min during the 90 min procedure and the pump rate adjusted, if required.

Once all the lines were loaded, they were connected to the animals using aseptic techniques. Briefly, the outer cannula, stylet and surrounding area were swabbed with 70 % ethanol solution. The stylet was carefully withdrawn from the cannula and the clamped infusion line swabbed with ethanol solution and attached (friction fit) to the cannula. The infusion line was tied into the wool at the base of the neck, over the shoulder and at least once on the lamb's back, to prevent chewing of the lines, and passed over the back of the metabolism crate. Finally the clamp was removed from the line and the pump switched on to start the infusion.

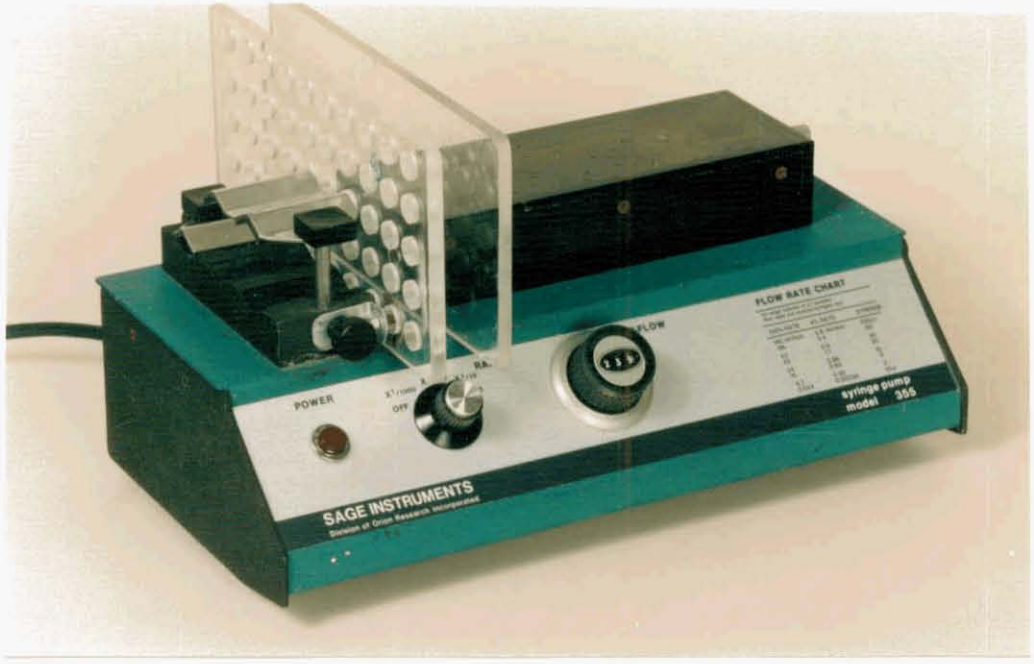
After 30 min of continuous infusion all animals were fed and intake recorded as described in

After 90 min of infusion the pump was switched off, the cannula area thoroughly cleaned with the 70 % ethanol solution, the line disconnected and stylet (freshly sterilised in an autoclave) replaced. All lines and syringes were flushed twice with deionised water then once with sterile saline solution before re-sterilisation.

Statistical analysis

Feed intake data recorded in this trial did not meet the assumptions of normality, so a non-parametric statistical analysis was undertaken. Initially an analysis of variance on rank transformed data was carried out using the SAS statistical package (SAS 6.06 SAS Institute Inc Cary, N.C., U.S.A.) on the complete model, including all treatments and both groups of animals. A significant model ($p < 0.05$) was found at all time periods, so the model was separated to investigate the two aims of the experiment namely 1. the role of central CCK and 2. the role of central opioids in parasite induced feed intake depression. A rank transformation and analysis of variance was repeated using data from the CCK, CCK antagonist and control for both groups and again for the opioid agonist, antagonist and control.

Plate 10. An illustration of the continuous infusion syringe pump used to deliver the intracerebral infusions in Chapter 10.



10.3 Results

One of the infected animals died prior to the start of treatments due to a clostridial infection, and one of the other infected animals failed to show any feed intake depression. These animals and their non-infected pairs were removed from all analysis.

Parasitology

The mean faecal egg concentrations of infected animals each week during the experiment are shown in Figure 10.1. Eggs first appeared in faeces after the 3 rd week of infection and output of eggs reached a peak during week 10 of infection. No eggs were detected in the faeces of the non-infected animals during the experiment.

Live weight

Mean live weights for infected and non-infected animals are shown in Figure 10.2. The risk of damaging the cerebral ventricle cannulae precluded weekly live weight recording being undertaken.

Daily feed intake

The mean daily feed intake (7 days) for infected and non-infected lambs during the experiment is shown in Figure 10.3. Following the period of restricted feeding, both groups had a rapid increase in daily feed intake. In the non-infected group, intake continued to increase until week 8 of the trial, thereafter it remained relatively constant and tended to decrease slightly. By comparison feed intake in the infected animals peaked in week 6, after which it fell and remained low for the next 8 weeks, and then appeared to increase in the final 2 weeks of infection.

Short term intake

The effect of *T.colubriformis* infection on short term feed intake recorded over an 8 h period is shown in Figure 10.4 for infected and non-infected animals. Parasitism significantly depressed feed intake at all recorded time periods ($0.05 > p < 0.001$) by between 22 % and 39 % of that consumed by the non-infected animals (per kg metabolic live weight $LW^{0.75}$).

When the recording period is divided into 3 sub periods: an initial meal (0-60 min), medium

term (1-2 h), and over the remainder of the measurement period (2-8 h), the reduction was similar in the 1st and 3rd intervals, but was considerably higher in the medium term (1-2 h) (Table 10.1).

Table 10.1 Feed intake (g/kg $LW^{0.75}$) (± s.e.m) and rate of consumption (g/kg $LW^{0.75}$/ min) for 1st 2nd and 3rd periods of feeding for infected and non-infected lambs fed once pre day (n=6).

	0-60 min		60-120 min		2-8 h	
	Intake	Rate	Intake	Rate	Intake	Rate
Infected (I)	17.1 ± 2.12	0.29	2.8 ± 1.69	0.05	14.1 ± 3.99	0.03
Non-infected (N)	21.9 ± 2.17	0.37	5.5 ± 1.71	0.09	22.7 ± 4.21	0.06
% Depression (N-I/N)*100	22		49		38	

Treatment effects

Opioid agonist and antagonist

Cumulative intake of infected and non-infected animals treated with the opioid agonist D-ala2-met5-enkephalinamide is shown in Figures 10.5 and 10.6. A continuous infusion of this opioid agonist for 30 min prior to feeding and for the first hour of feeding tended to elevate intake in the infected animals initially. However, this effect was not significant ($p > 0.05$) and there was no effect on short term feed intake in the non-infected groups.

A single bolus injection of the opioid antagonist naloxone 15 min prior to feeding depressed feed intake in the infected animals and intake remained depressed for 40 min ($p < 0.05$). Naloxone had no effect on short term intake in the non-infected animals (Figures 10.5 and 10.6).

Feed intake was not affected when naloxone was administered with the opioid (Figure 10.5).

CCK and CCK antagonist (loxiglumide)

CCK-8 infused alone for 30 min prior to feeding and for the first hour of feeding depressed intake in the infected animals. The resulting reduction in intake was significant from 20 min to 6 h after feeding ($0.05 > p < 0.001$) (Figure 10.7). CCK reduced short term intake over the 6 hours by 41 % to 55 %. CCK had no effect on intake in the non-infected animals (Figure 10.8).

The CCK receptor antagonist (loxiglumide) elevated feed intake in both infected and non-infected groups (Figure 10.7 and 10.8). Loxiglumide increased intake gradually, with the increase becoming apparent at 30 min ($p < 0.1$) and being significantly increased from 40 min through to 4 h in the infected ($0.08 > p < 0.001$) and non-infected ($0.05 < p < 0.001$) groups.

Following infusion of both CCK and loxiglumide, feed intake was intermediate between that of the control (saline) and the loxiglumide alone in both groups (Fig 10.7 & 10.8). In the infected group at 1.25 and 1.5 h the increase in intake above that of infected controls was approaching significance ($p < 0.1$) and was significantly different 1.75 h after feeding ($p < 0.05$). In the non-infected group cumulative intake was increased above that of controls from 40 min to 3 h after feeding ($p < 0.05$).

Table 10.2 Effect of Loxiglumide on mean cumulative feed intake (± s.e.m) and rate of intake for infected and non-infected animals in the first hour of feeding (0-1 h), medium term (1-2 h) and remainder of the recording period (2-8 h) following once-a-day feeding (n=6).

		Time since feeding (h)					
		0 - 1		1 - 2		2 - 8	
		Intake (g/kg $W^{0.75}$)	Rate (/min)	Intake (g/kg $W^{0.75}$)	Rate (/min)	Intake (g/kg $W^{0.75}$)	Rate (/min)
Infected animals	Loxiglumide	30.6 ± 5.54	0.51	7.8	0.13	12.2	0.033
	Saline	17.2 ± 1.49	0.29	2.7	0.045	18.9	0.052
Non-infected animals	Loxiglumide	35.9 ± 5.33	0.60	5.6	0.093	20.28	0.056
	Saline	21.9 ± 1.62	0.36	4.8	0.080	22.9	0.064

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Loxiglumide infusion increased the rate of eating during the first hour of feeding by 75 % in the infected animals and 67 % in non-infected animals compared to their respective control treatments (saline) (Table 10.2). The greatest effect was evident in the second hour of feeding when non-infected animals ate 16 % more feed and the infected 188 % more than in the same period following saline treatment. During the final period (2-8 h) the loxiglumide treatment effects were no longer apparent and both groups ate less feed compared with their respective controls, being 36 % and 13 % less for the infected and non-infected groups respectively. As a result of the early stimulation of intake, the cumulative intake for both groups was 30 % higher than control values at the end of the short term recording period (8 h). Infusing loxiglumide to the infected animals produced a 8 h cumulative feed intake not different from the non-infected control (saline) treatment so feed intake had been temporarily restored.

Between 1 & 2 h after feeding there was a significant parasite by treatment interaction ($p < 0.05$, except $p < 0.1$ at 1 ½ h). The interaction was due to the infected animals suffering significant intake depression following CCK-8 infusion when no effect was evident in the non-infected animals, and as a result of a greater response in the infected animals to the intake stimulating effects of loxiglumide.

Treatment effects on daily intake (22 h)

There was no significant effect of any treatment on total daily intake ($p > 0.05$).

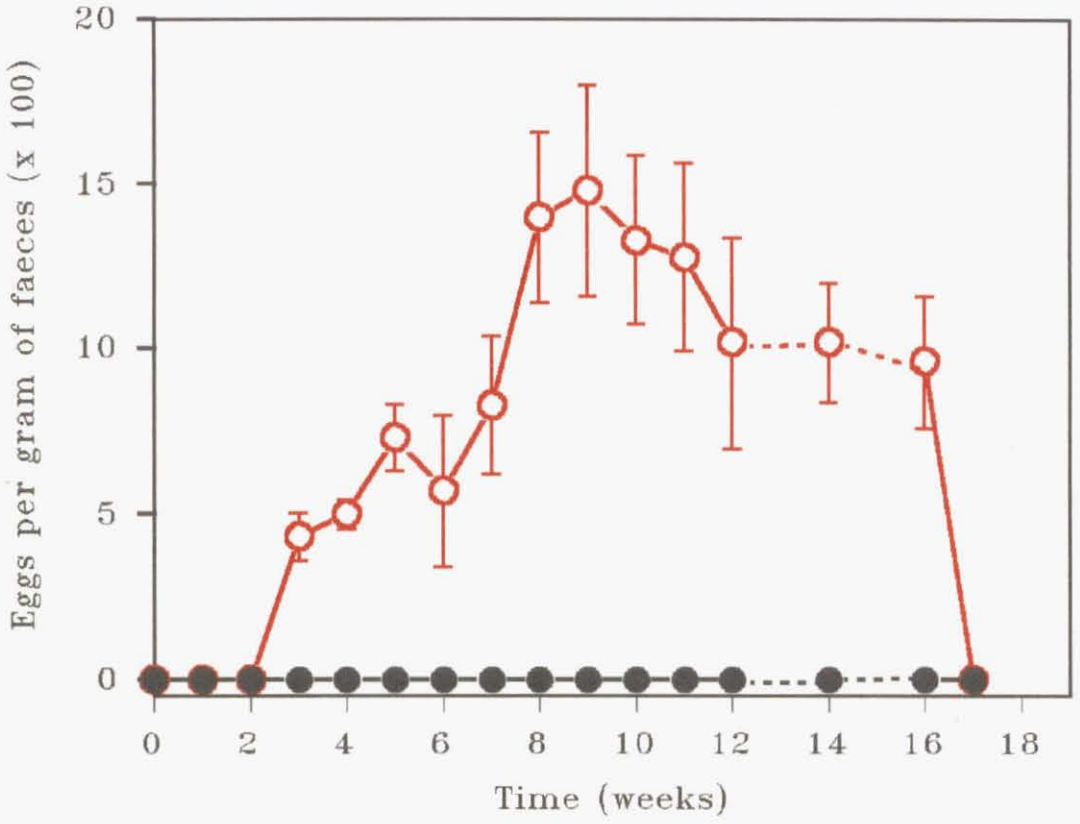

Figure 10.1 Mean (± s.e.m) number of *T. colubriformis* eggs per gram of faeces in infected (o) and non-infected (●) lambs sampled once per week (n=6).

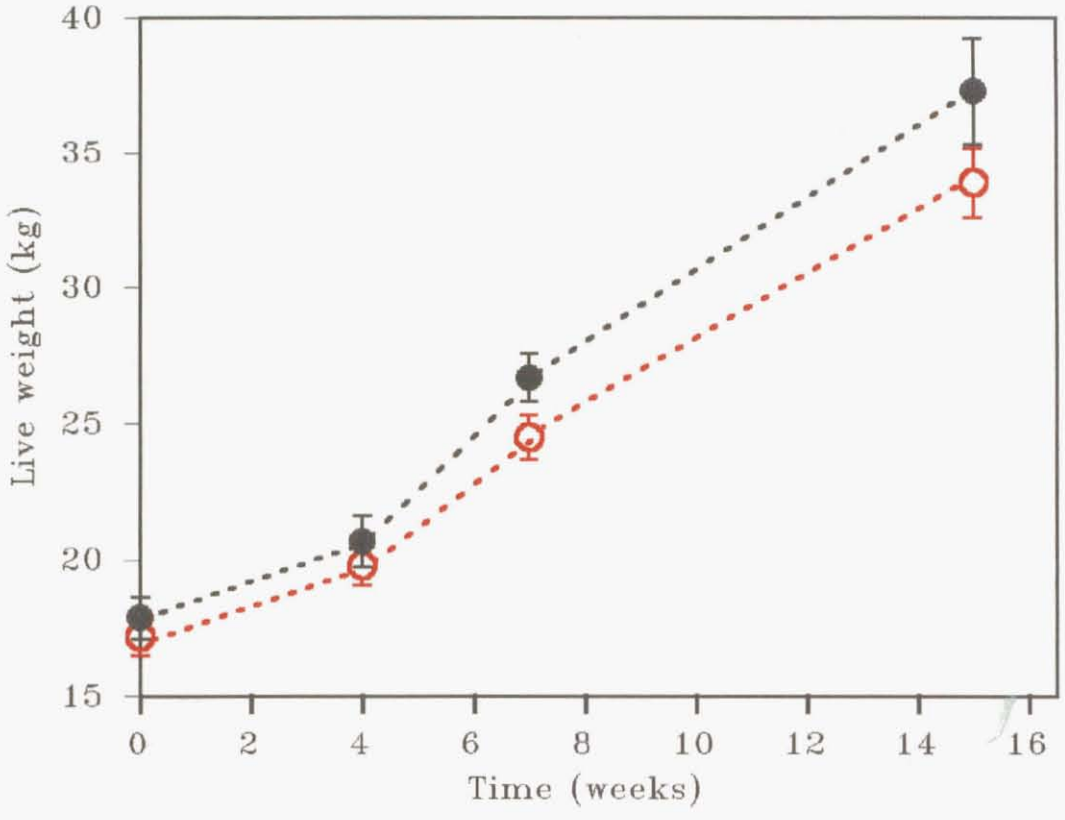

Figure 10.2 Mean live-weight (± s.e.m) for infected (o) and non-infected (●) lambs during the experiment (n=6).

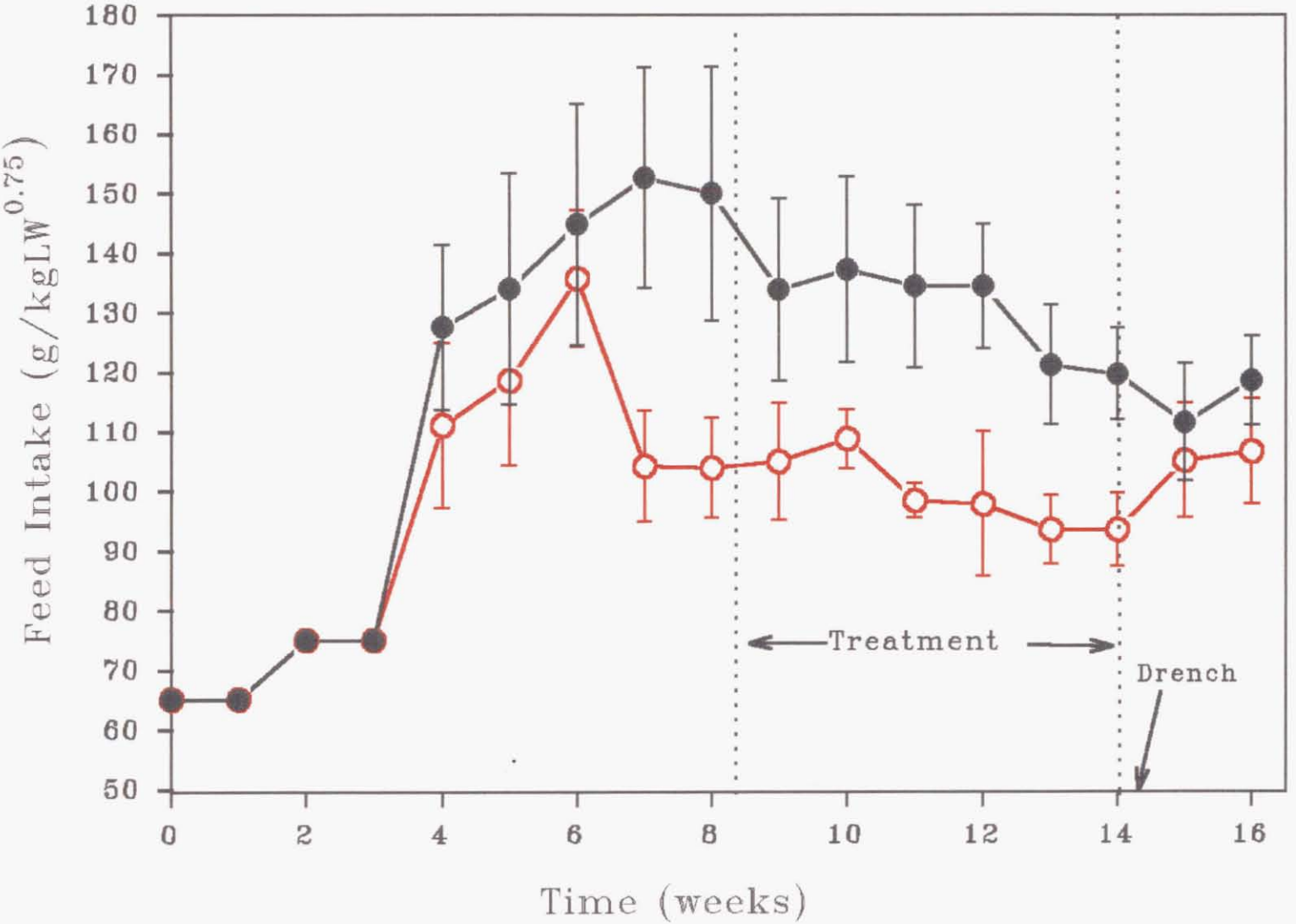

Figure 10.3 Mean daily feed intake (± s.e.m) for infected (O) and non-infected (●) lambs during the experiment (n=6).

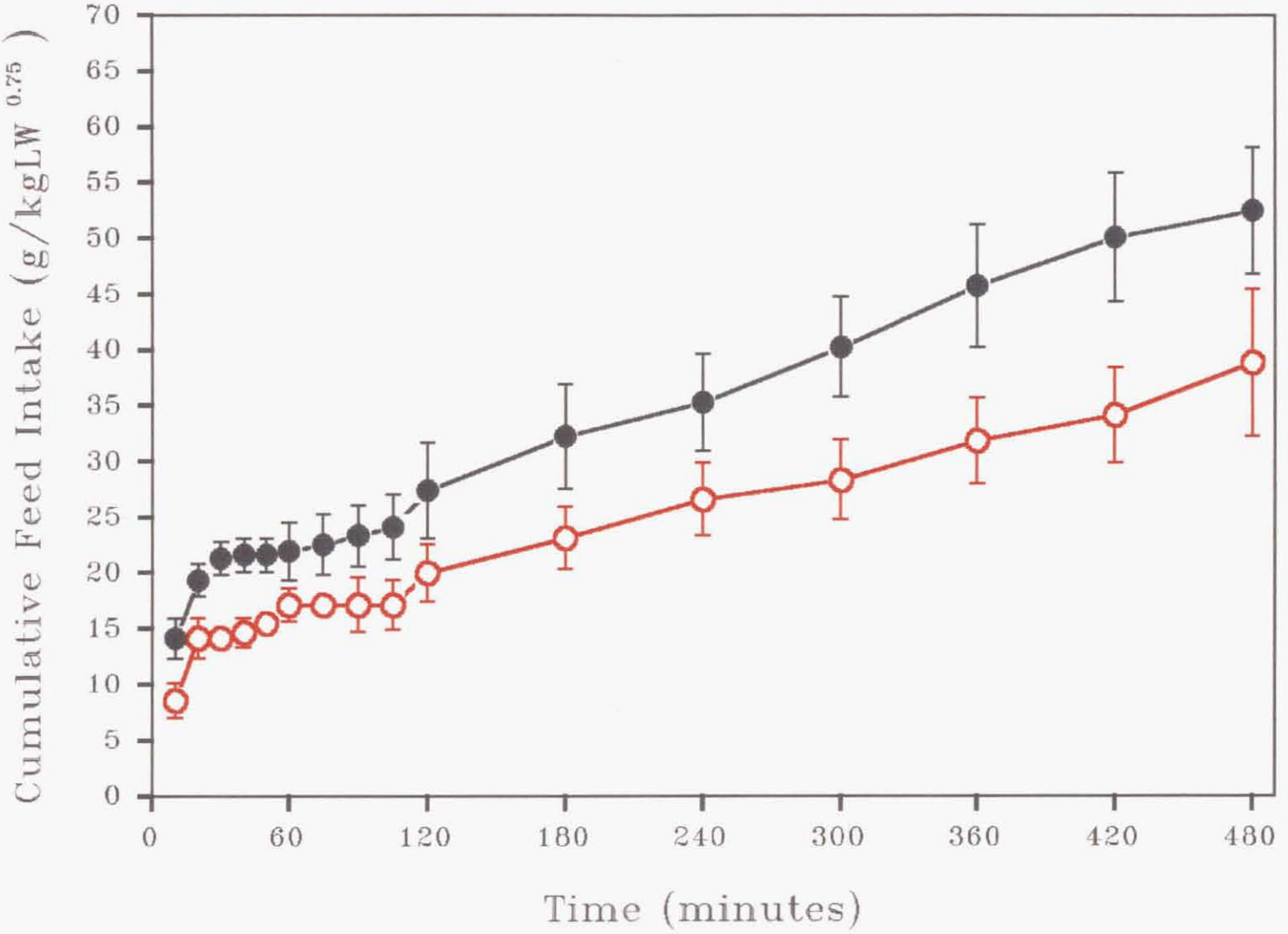

Figure 10.4 Short term cumulative feed intake (± s.e.m) for infected (○) and non-infected (●) lambs fed once per day (n=6).

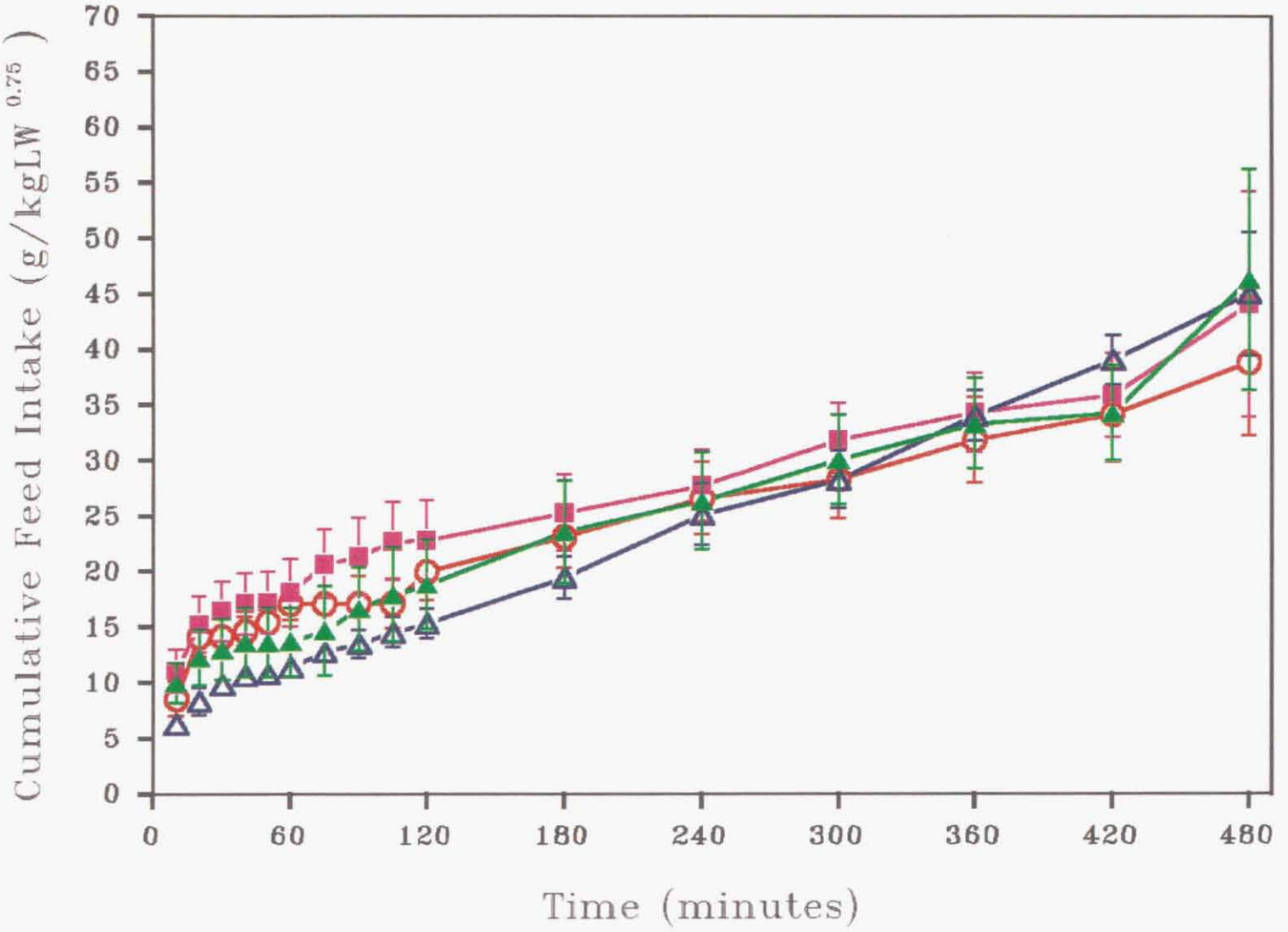

Figure 10.5 Mean (± s.e.m) cumulative feed intake over an 8 h period for infected lambs not fasted and i.c.v infused with saline (○), naloxone (△) opioid (■) or naloxone plus opioid (▲) for 30 min before and the first hour of feeding (n=6).

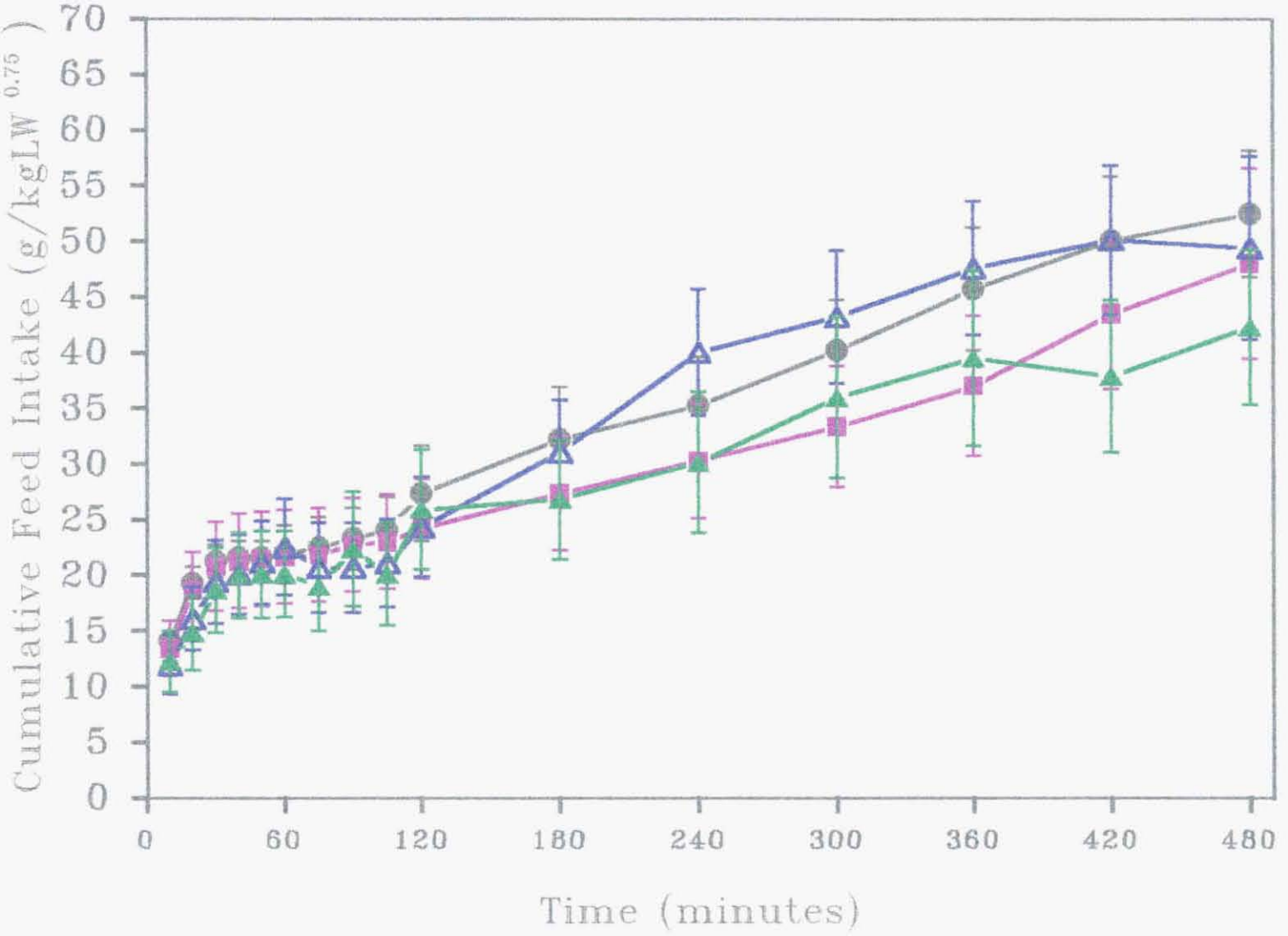

Figure 10.6 Mean (± s.e.m) cumulative feed intake over an 8 h period for non-infected lambs i.c.v infused with saline (●), naloxone (△), opioid (■) or naloxone plus opioid (▲) for 30 min before and the first hour of feeding (n=6).

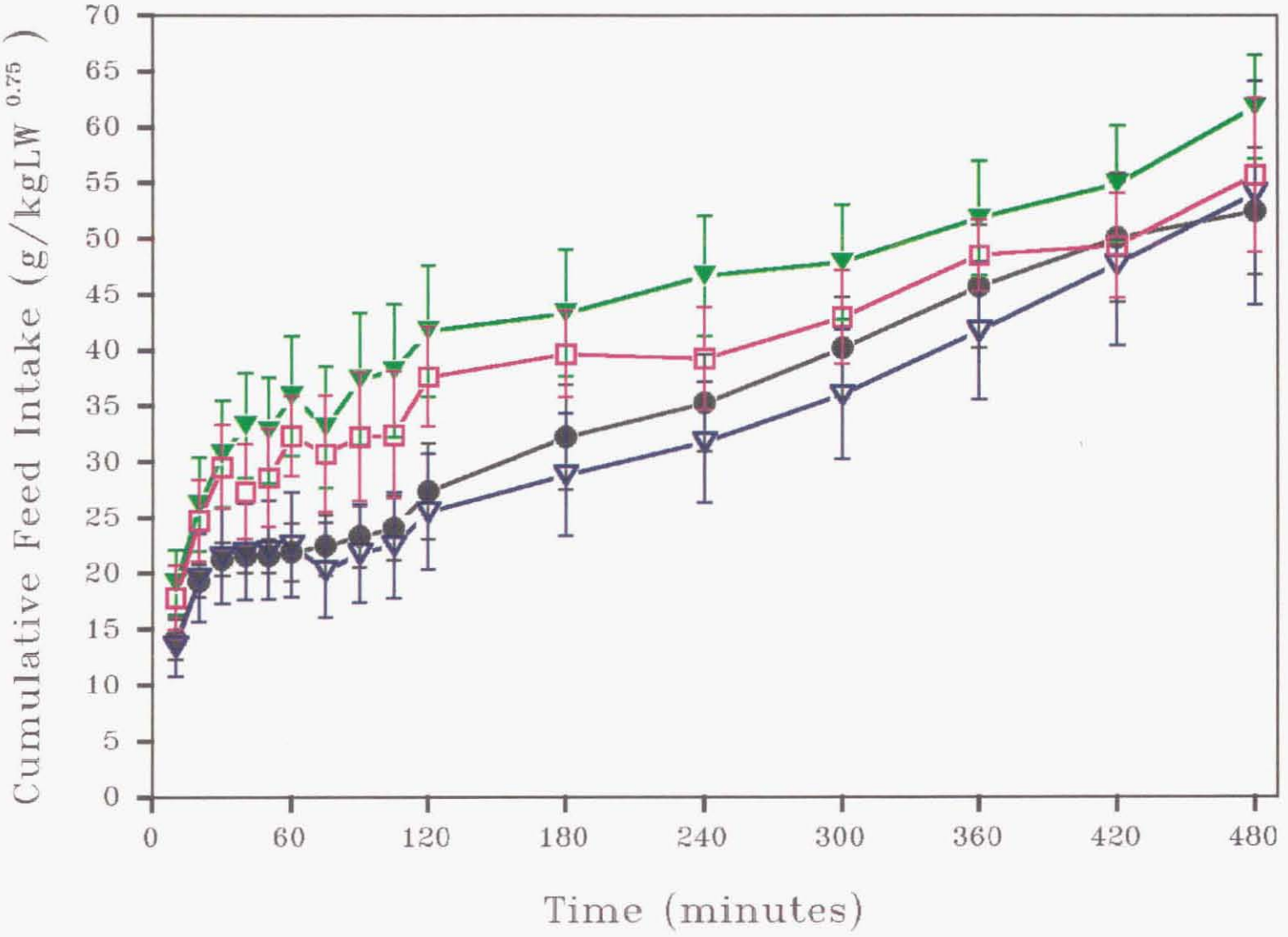

Figure 10.7 Mean (± s.e.m) cumulative feed intake over an 8 h period for non-infected lambs i.c.v infused with saline (●), loxiglumide (▼), CCK (▽) or loxiglumide plus CCK (□) for 30 min before and the first hour of feeding (n=6).

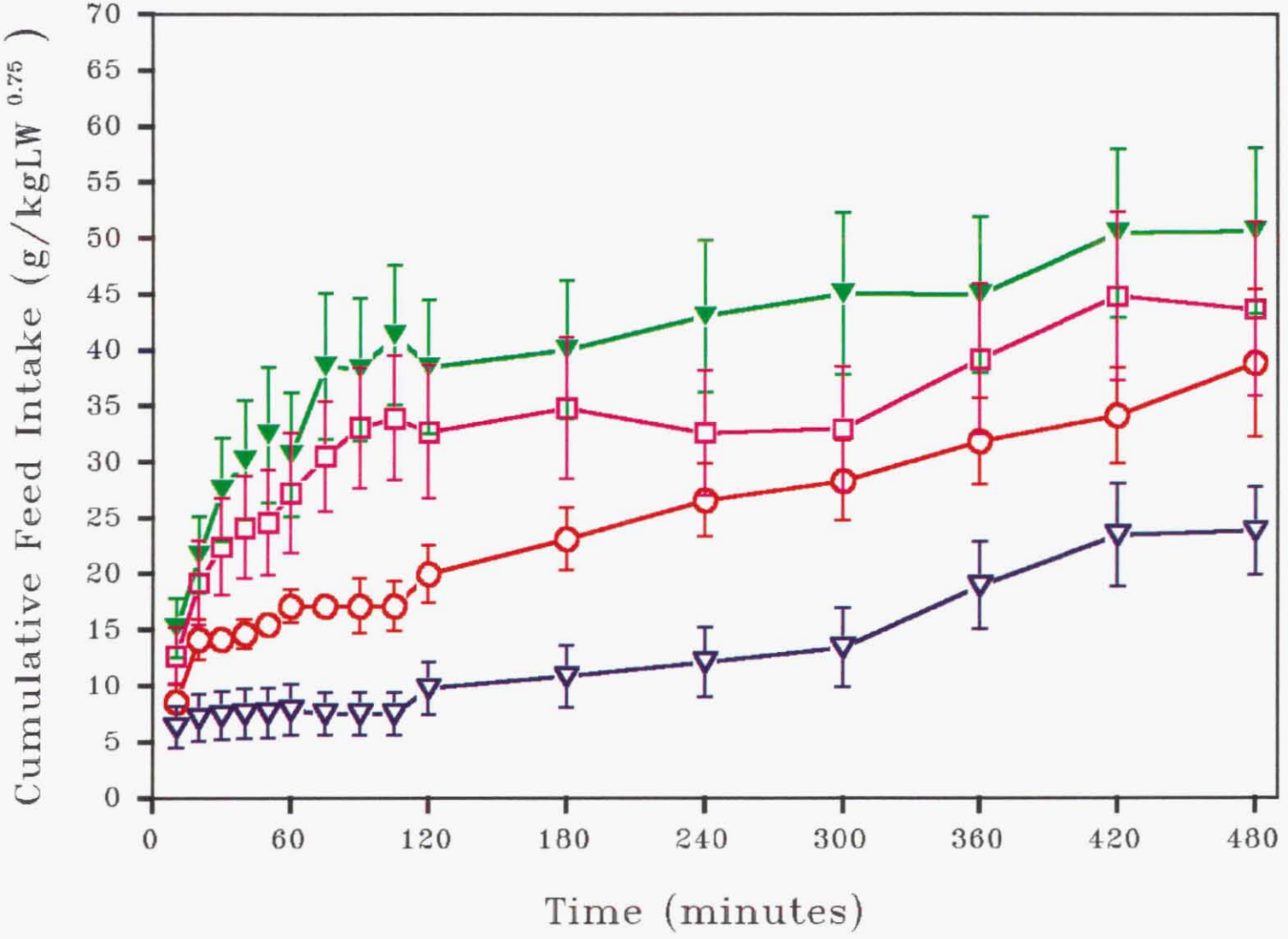

Figure 10.8 Mean (± s.e.m) cumulative feed intake over an 8 h period for infected lambs following i.c.v infusion with saline (o), loxiglumide (▽) CCK (▼) or loxigulmide plus CCK (▫) for 30 min before and the first hour of feeding (n=6).

10.4 Discussion

Increased feed intake by parasite infected lambs following infusion of the CCK antagonist, loxiglumide, in this study is the first evidence of a CCK receptor antagonist alleviating parasite induced intake depression in lambs. This is the second procedure by which intake was successfully elevated in parasite infected animals following the use of centrally active compounds (brotizolam and loxiglumide). These experiments provide evidence to support the view that central mechanisms have an important role in causing the feed intake depression, with the current study supporting an involvement of central CCK mechanisms.

A simultaneous infusion of CCK-8 and loxiglumide elevated feed intake, but the elevation was less than when loxiglumide was administered alone. Loxiglumide successfully blocked the intake depressive effects of exogenous CCK-8 and appeared also to block some endogenous CCK activity as well. Had the biological activity of loxiglumide and the CCK infused been comparable then the recorded feed intake should not have differed significantly from the control treatments. However it was difficult to equate the treatment level of loxiglumide in the present study with doses used in rats to block CCK activity (Setnikar *et al.*, 1987a) so a maximum recommended (0.54 % w/v) (L. Rovati, pers. comm.) infusion was used. No data on kinetics of loxiglumide activity are available thus the relative receptor on/off rate of loxiglumide or its clearance from the system cannot be speculated upon.

Increased cumulative intake in lambs following infusion of loxiglumide, which was recorded over the whole 8 h, is the result of changes in the pattern of eating during the first 2 h of feeding. The rate of feed consumption was elevated in both the first and second hours of feeding, but the most marked change occurred in the second hour when the rate of consumption was elevated by 188 % in infected animals and by 16 % in the non-infected group. Generally the second hour of feeding was observed to be a rest/rumination period, but loxiglumide induced an almost continuous eating period, contrasting with the control treatments where animals stopped eating 40-60 min after feeding with a second small meal between 1.25 and 2 h after feeding. Usually feeding was rapid for the first 20 min of feeding then declined to meal termination 40-50 min after feeding, possibly due to rising levels of endogenous CCK inducing satiety. Blocking this effect with the infusion of the CCK receptor blocker led to a prolonged first meal in both the infected and non-infected animals. Although there was a significant elevation of feed intake in the short term, this was not manifested in changes to daily intake in either group, possibly due to other

constraints to intake such as reticulo-rumen fill reducing intake in the latter parts of the day. This contrasts with the brotizolam treatment (Chapter 8) where the extra feed intake following injection of brotizolam after the first meal was maintained and evident in the daily feed intake.

It is tempting to conclude that central CCK activity is an important factor in meal termination in sheep and that elevated central CCK levels reduce meal size and resultant daily intake in intake depressed parasite infected sheep. Further work is required to draw such a conclusion. Offering feed to animals once a day changes eating patterns, from the 17 or 18 small meals spread throughout the day (Weston pers. com.) and the observed diurnal grazing pattern of sheep on pasture (Scott & Sutherland, 1981; Thomson, Cruickshank, Poppi and Sykes, 1985), to a large single meal immediately following feeding, then small meals throughout the day. It could be unwise to assume that the same meal control mechanisms exist in both situations. However, it would appear logical that they may. Thomson *et al.* (1985) found grazing sheep did not appear to regulate grazing pattern by reference to rumen fill, with the rumen reaching its maximum fill only at the end of the afternoon grazing, the intensity of both morning and midnight grazing being insufficient to markedly increase rumen fill. The authors concluded that models such as Forbes (1980) which incorporate physical, metabolic and hormonal regulators are required. Integrated models such as those of Forbes (1980) and Weston (1985) do accommodate hormonal regulators such as the role of CCK in the termination of individual meals. Studies are needed to provide data on levels of CCK, both central and peripheral, in sheep before and after spontaneous meals to understand the physiological significance of this hormone.

Loxiglumide is a pentanoic acid derivative which was originally developed as a therapeutic aid to manage CCK dependent disorders in man. Although loxiglumide has almost 30 times greater affinity for peripheral CCK than for central CCK receptors, its affinity for central receptors is still 19 000 times greater than that of proglumide, the original CCK antagonist (Setnikar *et al.*, 1987a). There is considerable evidence from studies on laboratory animals for the antagonistic potency of loxiglumide on CCK-8 induced stimulation of gastrointestinal tissue (Setnikar *et al.*, 1987b). There are no published studies of its effects on feed intake in sheep so comparisons with the present findings are not possible. Makovec *et al.* (1986) noted that a very similar antagonist (CR 1409) tended to increase feed intake in rats during testing of its ability to antagonise the intake depressive effects of CCK.

I.C.V. infusion of the CCK octapeptide was very effective in reducing feed intake in the infected animals only, with the depression resulting in a much smaller than usual first meal followed by an extended intermeal interval, lasting between 2-3 h after feeding. Intake was depressed by 39 % after 20 min, peaking at 52 % depression 5 h after feeding. Interestingly Della-Fera and Baile (1979), also using a 2 h feed deprivation and 2.5 pmol/min i.c.v. infusion of CCK-8 found that healthy animals ate no feed at all for the duration of the 3 h infusion and as little as 0.159 pmol/min depressed intake by 85 % during the infusion period. In the present study there was no effect of CCK at all in non-infected animals and where it was effective in the infected animals the maximum recorded depression was only 52 %. Conditioning to once-a-day feeding, as used in the present study, may lead to animals not eating for approximately 3 hours prior to feed being offered then having a large meal of fresh feed (Forbes, 1980). Cumulative intake curves show clearly the large initial meal the animals eat immediately following feeding, so these animals may have had a greater hunger drive than expected from a 2 h fast. However, CCK-8 depressed feed intake by 53 % in 8 h fasted animals during a 3 h infusion (Della-Fera and Baile, 1979), so it is somewhat surprising that a depression in feed intake in the non-infected animals was not seen during the 90 min infusion.

Differing responsiveness to CCK-8 infusion between infected and non-infected animals may be due to the infected animals having higher levels of brain/central CCK activity acting directly on satiety centres, with the result that the exogenous CCK was sufficient to precipitate a strong satiety signal and reduce intake accordingly, whereas in the non-infected animals central CCK levels/activity would have been low (Scallett *et al.*, 1985) and even with a relatively high level of infusion insufficient CCK was present to elicit satiety effects.

Some authors have suggested that general malaise may cause intake depression following CCK injection, especially since the historic lack of an effective means of measuring CCK has prevented an understanding of what are physiological levels of CCK to infuse. However, the finding here that a CCK receptor antagonist blocked the depressive effects of the CCK is evidence for the effect having occurred via a CCK receptor and not from undefined malaise. Further studies are needed in this area to record endogenous levels of CCK both peripherally and centrally in infected and non-infected lambs.

Infusion of the opioid agonist D ala2-met5-enkephalinamide tended to raise intake in infected

animals, but this difference was not significant. Baile and Della-Fera (1981) have found significant increases in intake following infusions of between 26 and 102 nmol/min. The authors found the increases in intake were large and lasted for up to 3 h. In the present study, the stock solution was inadvertently prepared to infuse 102 pmol/min instead of the 102 nmol used by other workers. As a result the lack of response appears likely to be due to insufficient elevation of central opioid levels. A tendency for the infected animals to eat more following infusion of the opioid agonist is encouraging, with some individuals seeming to respond well to the treatment. It would be useful to repeat this experiment using higher levels. Baile *et al.* (1981; 1987) elevated intake by 80-500 % over a 3 h period of which 90 min involved infusion of an opioid agonist in satiated sheep, a situation where opioid levels could be expected to be low. In healthy animals deprived of feed for 2 h, hunger levels would be expected to be high and even at the higher levels of infusion used by Baile it is difficult to predict if an eating response would have been observed. Here the real interest lay in the response of the parasite infected animals which even following a 2 h deprivation period did not appear hungry. The tendency of the opioid, at a level much lower than used by other workers, to increase intake in these animals suggest this study should be repeated using higher levels of opioids.

Naloxone will reduce short term intake in rats in a dose dependant manner and will reduce eating induced by food deprivation, glucose, insulin, a GABA agonist or adrenalectomy (Reid, 1985). Similarly in an earlier study (Chapter 9) 0.125 mg/kg naloxone depressed intake in healthy sheep for 90 min, while Baile *et al.* (1981) found doses as low as 0.033 mg/kg depressed intake in 4 h fasted sheep. By comparison naloxone had no effect on normal animals (non-infected) in the present study but depressed intake in the 2 h fasted infected animals for 40 min. The inconsistency between trials in the response of infected and non-infected animals to naloxone treatment is disturbing. When naloxone was first identified and used it was thought to be inert with no activity of its own except blocking opioid activity (Jaffe and Martin, 1980). There is now wide ranging evidence for it decreasing eating and drinking in a number of species (Reid, 1985). However it is not apparent whether these effects are via the opioid axis or are nonspecific and indirect nor why differences in response were recorded between trials.

T. colubriformis infection reduced daily voluntary feed intake from week 6 of infection. The onset and magnitude of the fall in intake was consistent with Chapters 3 and 4 and with published results of Sykes and Coop (1976). Likewise the depression in feed intake evident

over the 8 h recording period was similar to that recorded in Chapters 2 through 4. Parasite infection reduced the rate of consumption in lambs 1 to 2 hours after feeding, more than at any other time during the day, and similar to the findings of the earlier studies (Chapters 4-9).

In summary, central administration of a potent CCK antagonist blocked the effects of exogenous CCK in both infected and non-infected lambs. Furthermore the CCK antagonist administered alone significantly elevated feed intake in both groups of lambs. The present experiment reinforces a role for central or brain mechanisms in parasite induced feed intake depression. Further these results suggest CCK pathways are involved in integrating this effect.

Unfortunately the findings of the role for opioids in this study were inconclusive but the trends suggest more work should be undertaken.

CHAPTER 11

General Discussion

It was known from the outset of these studies that a major effect of internal parasites was a reduction in voluntary feed intake and that there needed to be a decision on the appropriate experimental method to characterise this, i.e. measure average daily feed intake or short term rate of feed consumption. Short term rate of intake was chosen for study here as this enabled identification of potential pathways and compounds and manipulation by various compounds or their agonists and antagonists, many of which have a short duration of action. Using a short term rate of intake model permitted the testing of a wide range of compounds or pathways which would not have been possible had average daily intake been investigated. This was considered to be much more beneficial in an initial investigation because so little was known about intake depression under parasitism. Only two studies (Symons and Hennessy, 1981; Fox *et al.*, 1987) have previously examined intake depression and they related CCK and gastrin respectively to depression in average daily feed intake. Using the average daily feed intake approach would have limited the study to a few "best bet" pathways whereas the approach using short term rate of intake in this thesis has enabled a number of pathways to be examined. It was hoped that this would rapidly identify promising pathways and open up this field of research.

For this approach to be successful a number of criteria had to be met. Firstly, learned taste aversion must not unduly influence the outcome of experiments. If, for example a compound did block an intake inhibitory pathway but learned taste aversion was a dominating factor then no increase in feed intake might be observed, leading to an incorrect rejection of any hypothesis. This is particularly important when using the short term rate of feed intake approach and arose from Keymer *et al.* (1983) who suggested that learned taste aversion was a real phenomenon in parasitised rats. The role of learned taste aversion was investigated in a preliminary experiment (Appendix 4) and found to be minor and thus unlikely to influence the results of future studies. Secondly, the mode of action of a compound should be known and must be effective in the sheep. In the present study this was a major difficulty as many of the compounds considered here were well documented for their effects on humans, rats or mice but not for sheep. In some cases the mode of action had to be inferred from the action in other species and/or their veterinary application e.g. codeine phosphate. To define clearly the role of a pathway in feed intake regulation it is

useful to both activate and suppress the pathway under investigation. This approach was tried here but met with variable success. An example was increased osmolality which clearly depressed feed intake in non-infected animals (Chapter 5) yet administration of a local anaesthetic did not block the effect of the hypertonic solutions. The use of the local anaesthetic in parasitised animals could not unequivocally be used to dismiss osmolality as a contributing factor to intake depression, although there is probably little debate about the effectiveness of local anaesthetics elsewhere in sheep. Similarly, CCK antagonists which are effective at blocking the effects of exogenous and post-prandial CCK on gut function in humans and rats (Setnikar *et al.*, 1987b) have not been widely used in sheep. Because the administration of peripheral CCK has been shown to have a variable effect on feed intake in sheep (Baile and Della-Fera 1984), administration of an antagonist under these circumstances does not clearly define the effectiveness of the antagonist, or, therefore the role of the pathway under investigation.

These examples serve to highlight some of the difficulties which were encountered during experimentation. It is important to realise with this experimental approach that certain criteria needed to be met and that these were not always achieved for a variety of experimental reasons although an attempt was made in all cases to test the effectiveness of compounds or action of pathways under investigation. In early trials some problems were experienced with the pathogenicity of the parasite larvae, with low faecal egg counts and slow onset of feed intake depression. Initially this was attributed to early exposure to parasites and the resultant initiation of immunity and to the high quality diet. Subsequently it appeared much of the problem was one of reduced larval vigour due to continued culture of the original strain and to the long storage time of larvae. Having outlined above the general philosophy of the approach, a discussion of the actual experimental results follows below.

If short term rate of feed intake was to be used as the response parameter for this experimental approach, there had to be clear-cut differences in short term feed intake between infected and non-infected animals. This was in fact the case (Figure 4.5). It was one of the initial findings described in this thesis and demonstrated for the first time that the rate of intake, particularly that observed in the first hour of feed consumption, was a major difference between infected and non-infected animals. The pattern of eating was similar but the rate at which animals consumed feed differed. Differences in short term intake was recorded in all subsequent experiments. Actual meal sizes and duration could not be

measured as appropriate equipment was not available but careful visual observation indicated that animals tended to start and stop eating at similar times. The magnitude of the depression in both daily and short term feed intake varied between animals and between and within experiments.

The procedure to determine cumulative rate of feed intake involved manually weighing feed bins at set times and initial training ensured animals became quite accustomed to this procedure. For the procedure to be valid, the depression in rate of intake had to be reversible. This was indeed the case in Chapter 4 where it can been seen that infected and non-infected animals had similar rates of intake in the preinfection period, the parasitised group were lower after about 6 weeks of infection and on administration of an anthelmintic their intake returned to the level of the non-infected group. This clearly demonstrated that feed intake depression under parasitism could be examined by the cumulative feed intake model and that differences were readily apparent in the first main eating period, i.e. approximately 1 h, and all subsequent periods recorded. Removal of the parasite reversed the short term depression of intake demonstrating that the presence of the parasite was instrumental in the feed intake depression. The fact that it took up to 10 days for intake to return to control levels after the parasite was removed suggests tissue repair was needed and that the presence of the worm *per se* and/or its secretions were not the only causative factors.

This model of cumulative feed intake to examine short term rate of feed consumption was then used throughout subsequent studies to examine the actions of various compounds on feed intake of infected and non-infected animals to determine which pathway(s) were involved. Identification of the pathway(s) might then provide suggestions for a means to block their action. If intake can be increased in parasitised animals, this is likely to minimise the reduction in animal performance observed in parasite infected animals and also hasten the development of immunity or "self cure"

Although the pattern of eating did not change markedly in infected animals, there was a significant reduction in the rate of feed consumption, which suggests a reduced hunger in the infected animals. Hunger was defined as a desire to consume feed with increasing hunger reflected in an increased rate of consumption and satiety as the termination of a meal, followed by non-feed seeking behaviour (rest, ruminate, socialise) and usually immediately preceded by a decreasing rate of feed consumption. Changes in meal patterns

due to disturbances to the intermeal interval would support a role for increased satiety causing a reduction in voluntary feed intake, but were only observed following some treatments. This interpretation is limited by an inability to continuously record feed intake and thus accurately define meal patterns. The reduced rate of consumption occurred throughout the day and appeared to be a greater contributor to reduced feed intake than changes to meal patterns.

The aim of the studies undertaken was to increase feed intake in the infected animals and thereby identify pathways with a potential role in feed intake depression for further study. A number of potential pathways were examined in both infected and non-infected lambs, a summary of the pathways examined is shown in Table 11.1.

Table 11.1 Pathways tested, pharmacological agents used and feed intake response (no=none, inc=increased, dec=decreased) of infected and non-infected lambs described in this thesis.

Pathway	**Pharmacological agent**	**Change to intake**	
		Non-I	Inf
Pain	oral codeine phosphate	no	no
	duodenal codeine P.	no	no
Inflammation	oral indomethacin	no	no
CCK	peripheral antagonist	no	no
	central antagonist	inc	inc
Osmolality	local anaesthetic	no	no
	hypertonic solutions	dec	-
Central	brotizolam	inc*	inc
Hunger	feed deprivation	inc	inc
Opioids	central opioid analogue	no#	no
	naloxone (antagonist)	dec	dec*

* response not observed in all experiments

level used may have been too low

Initial findings (Chapters 4-7) suggest that any one of a number of potential peripheral pathways, including changes to osmolality, gut emptying, pain and inflammation of the gut,

alone were not the trigger for anorexia in parasite infected animals. In regulation of "normal" intake it is highly improbable that a single pathway would regulate intake but the temporary/reversible nature of parasite induced intake depression suggests a single pathway may cause the depression. A number of peripheral mechanisms such as pain and inflammation may result in increases in central hormone or neurotransmitter levels. For example increases in the release of central CCK may occur in response to a number of peripheral signals and blockade of a single peripheral signal temporarily may be insufficient to elicit an initial short term feed intake response. Limitations of the experimental technique, where the action of a number of compounds which did not change feed intake could not be demonstrated unequivocally in sheep make it difficult to conclude that many of the pathways tested do not have a role. It can however be concluded that these compounds will not increase intake in parasite infected lambs.

Fasting, i.c.v. loxiglumide and brotizolam, all treatments which probably act centrally (brain), increased the rate of feed intake and duration of the initial meal in infected animals. Fasting and post meal brotizolam also stimulated daily feed intake in the infected animals. However, these treatments also elevated feed intake and meal duration in the non-infected animals so these findings are not unequivocal evidence for the pathways being those responsible for parasite induced intake depression. Changes in circulating metabolites (glucose, VFA, FFA) as a result of fasting were not investigated. Any changes may have directly or indirectly contributed to the increased hunger observed.

A comparison of the response of infected and non-infected animals to these intake stimulating compounds is shown in Figure 11.1a and b. Parasitism decreased the rate of feed consumption without major effects on the time to terminate the initial meal. All three treatments increased the rate of feed intake and duration of the first meal in infected and non-infected animals but the magnitude of the increase in rate of intake was on average three fold greater in infected animals than in non-infected animals (Table 11.2). The duration of the initial meal was 2-3 times longer in both infected and non-infected groups (Table 11.2).

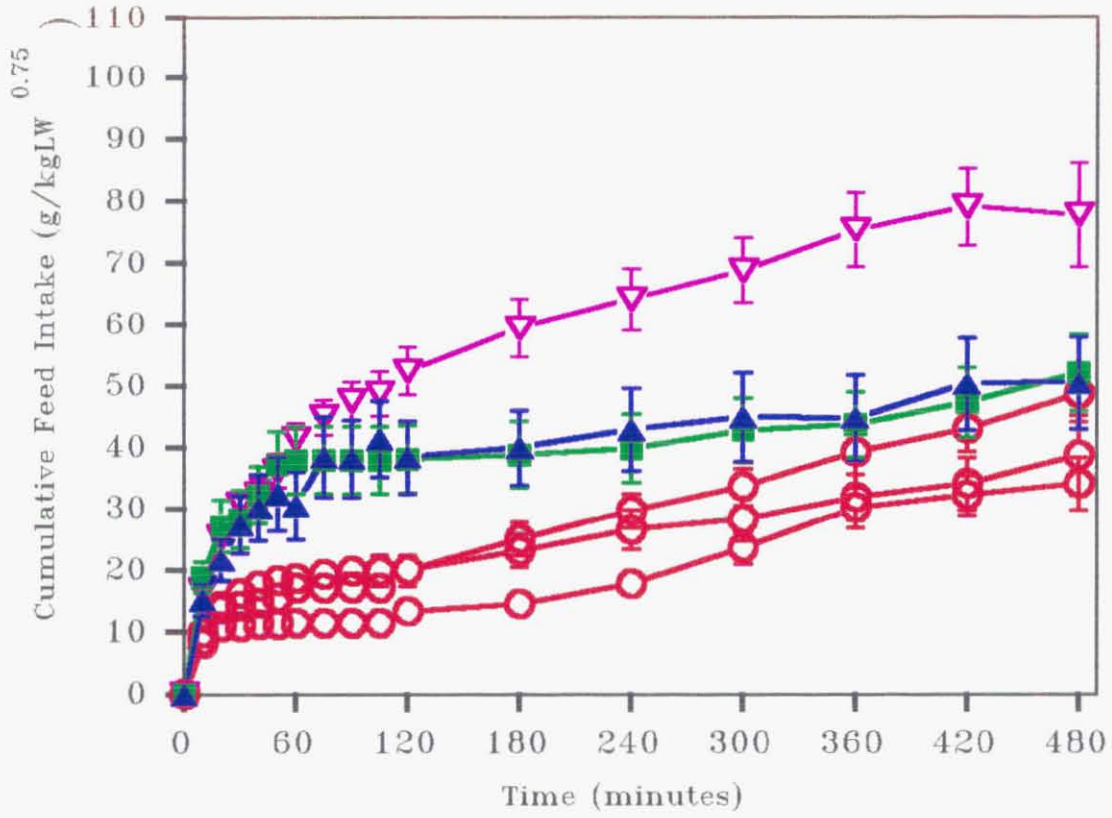

Figure 11.1a Mean (± s.e.m) cumulative feed intake of infected lambs, not treated ( ), fasted for 26 h ( ), injected with brotizolam immediately prior to feeding ( ) or infused with loxiglumide for 90 minutes ( ) (n=6).

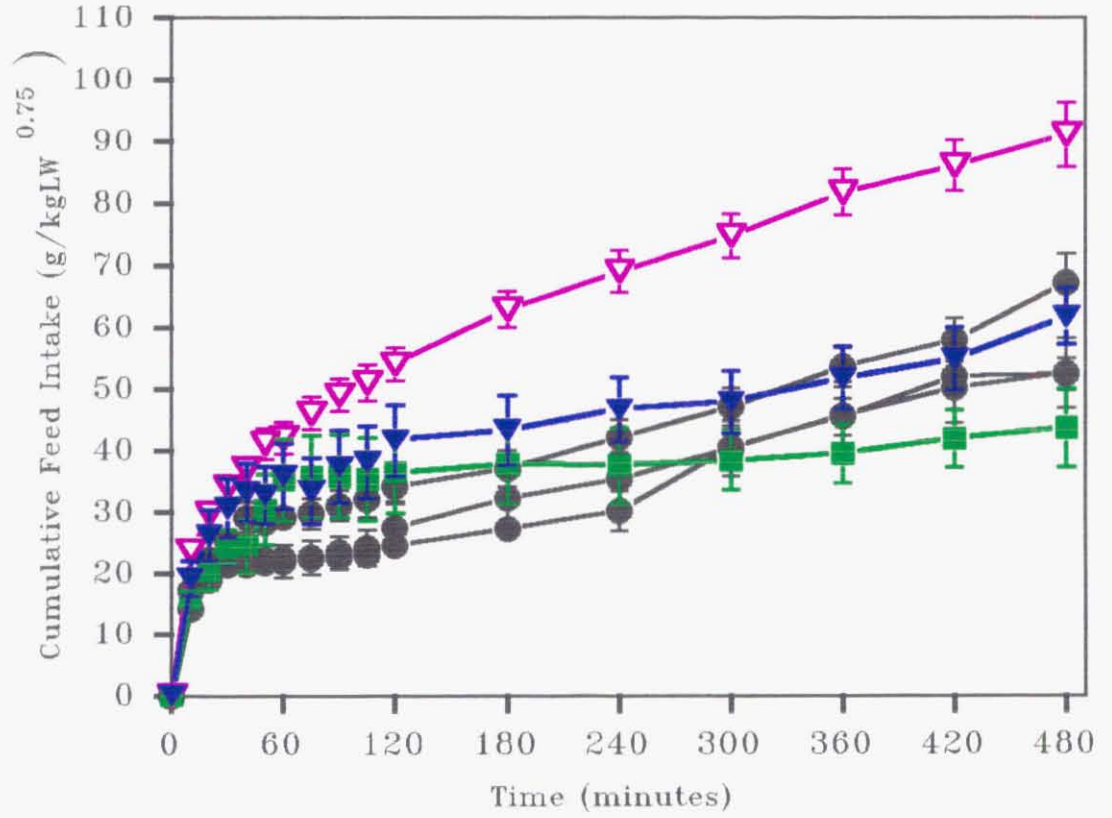

Figure 11.1b Mean (± s.e.m) cumulative feed intake of non-infected lambs, not treated ( ), fasted for 26 h ( ), injected with brotizolam immediately prior to feeding ( ) or infused with loxiglumide for 90 minutes ( ) (n=6).

Table 11.2 Change in feed intake (%) during the first hour of feeding and in the duration of the first meal (2 consecutive recordings with no change in cumulative intake) in infected and non-infected animals treated with i.c.v. loxiglumide, i.v. brotizolam, fasted for 26 h or not treated (control).

	% change in feed intake 1 h after feeding		Approximate duration of the first meal (minutes)	
	Infected	Non-infected	Infected	Non-infected
control	0	0	30	30-40
loxiglumide	178	58	60-75	50-60
brotizolam	235	58	60	?
fasting	121	44	90-105	90-105

However, despite differences in the magnitude of the response between groups, the absolute response in cumulative intake to treatments was very similar (Figure 11.1a and b) in infected and non-infected animals and peaked initially (60-90 minutes after feeding) at approximately 40 and 50 g/kg $LW^{0.75}$ following brotizolam, loxiglumide or fasting respectively in both infected and non-infected animals, suggesting other factors like prehension or chewing rate probably limit feed intake above this level. The nature of the response to i.c.v. loxiglumide and brotizolam was very similar. Both treatments increased the initial rate of consumption and duration of the first meal, without increasing daily feed intake. In contrast fasting appeared to increase the rate of eating over the whole day with no extended intermeal intervals being apparent.

The similar response by both groups to treatments which increased intake reinforces the view that a unique mechanism is not operating in infected animals to reduce feed intake but rather it is a disturbance of normal central (brain) control mechanisms. However the magnitude of the response by infected animals, which temporarily restored intake to pre-infection levels may indicate a role for central CCK receptors in parasite induced anorexia. Further, the intake depression caused by exogenous CCK was attenuated in both infected and non-infected lambs by i.c.v. loxiglumide, which supports a role for CCK receptors specifically rather than a generalised response. Loxiglumide (i.c.v.) not only increased the rate of consumption of feed but also appeared to increase the duration of the first meal. The response was greater in infected animals but the stimulation occurred in both groups and

suggests a potential role for central CCK in feed intake regulation rather than a specific parasite induced effect. Fasting temporarily restored intake in infected animals and naloxone treatment (an opioid antagonist) partially attenuated this response, supporting the view of reduced hunger in parasitised lambs. The response to naloxone suggests reduced circulating opioid levels may be the cause of the lack of hunger in infected animals. Changes in the rate of feed intake may be mediated by opioid pathways while changes to the onset of satiety may reflect the role of CCK receptors. An interaction of these pathways may be occurring, with CCK affecting the production or action of opioid mechanisms (Kumar, 1990).

If fasting is assumed to stimulate a hunger signal and both i.c.v. loxiglumide and brotizolam elicit responses via satiety signals, these seemingly conflicting results of both hunger and satiety signals acting centrally may reflect the effect of integration which leads to depressed rates of consumption. This mechanism may be the result of a balance between disturbances to both hunger and satiety mechanisms located centrally or reflect the interrelationship between hormonal pathways and would explain why treatments appeared to both increase aspects of hunger (rate of consumption) and reduce satiety signals (increase the initial meal length). The reduction in hunger and increase in satiety occurring centrally may be a response to both central and peripheral signals, with disturbances occurring to the action of one or several of these pathways in infected animals, resulting in anorexia.

This study did not address potential interactions between more than one pathway and interactions between pathways which alone did not change intake cannot be discounted. For example there is considerable research evidence for benzodiazepines and opioid activity being interrelated possibly via effects on GABA transmission (Cooper, 1983; Kumar, 1990)

In summary *T. colubriformis* infection reduced the rate of feed consumption as well as total daily intake, without changing meal patterns. A single peripheral mechanism appears unlikely to be the only trigger of feed intake depression in infected animals. Increasing the rate of feed consumption and meal duration by fasting animals or administering a central satiety blocker or CCK antagonist restored intake in the short term and suggests a number of signals, all acting centrally may be involved in mediating both the rate of intake and the onset of satiety.

In conclusion, feed intake appears to be regulated through the same mechanisms in infected and non-infected animals. However, intake in infected animals responded to a much greater extent when fasting, i.c.v. loxiglumide or brotizolam were employed. The findings suggest central CCK receptors are important in parasite induced anorexia, possibly by changing the onset of satiety or by interacting with endogenous opioids to reduce the rate of feed intake. Secondly, reduced endogenous opioids may be causing the reduction in the rate of feed consumption alone or as a result of other interactions. It was concluded that intake in parasitised animals could be increased to that of control animals by employing procedures and compounds thought to act on the hypothalamus.

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Appendix 1

Diet 1 (Propel)

Composition

Malt culms	50.0 %
Barley screenings	49.5 %
Vitamin/minerals	0.5 %

Analysis

% Dry Matter	89.0
Organic Matter	88.8 g/kg DM
Neutral detergent fibre (NDF)	40.6 g/kg DM
Fat	1.07 g/kg DM
Protein	18.3 g/kg DM

in vivo digestibility 72 %

Appendix 2

Diet 2 (Integrity Feeds)

Composition

wheat/barley straw	30 %
barley grain	49 %
field peas	20 %
vitamins/minerals	1 %

Analysis

	88/89	89/90	90/91
% Dry Matter	87.7	87.6	91.3
NDF (g/kg DM)	31.5	26.5	35.5
Fat (g/kg DM)	1.2	1.4	1.85
Protein (g/kg DM)	16.7	18.4	20.3
in vivo digestibility	71.7	72.0	73.6

Appendix 3

The effect of the level of feed intake on the rate of abomasal emptying in lambs fed once per day.

Introduction

The rate of passage of digesta through the GIT tract of sheep is strongly influenced by the level of feed intake in the individual. Consequently the retention time of markers in the GIT of sheep decreases as the level of feed intake increases (Grovum and Williams, 1973; 1977). This effect is most evident in the reticulo-rumen and hind-gut of the sheep (Grovum and Williams, 1977), areas having long retention times. Whilst the abomasum and small intestine with much shorter retention times are less affected by the level of feeding.

In Chapter 7, abomasal emptying was studied in infected and non-infected animals fed once per day. The hypothesis that parasite induced neural or hormonal effects might slow abomasal emptying, thus reducing digesta flow and feed intake, requires an understanding of the effect of feed intake on abomasal emptying. This study examined the relationship between level of feed intake and rate of abomasal emptying.

Materials and Methods

A group of 6 animals recently used in an experiment (Chapter 7) were used. All animals were treated with anthelmintic 4 weeks prior to commencement of study. Animals were offered 1600, 1200, 1000, 800, 600, or 400 g of the complete pelleted ration once per day. Each feeding level was offered for 10 days, then abomasal emptying measured as in Chapter 7. Abomasal emptying was recorded on 2 consecutive days in each animal at each feeding level.

Results

Table A 3.1 Mean (± s.e.m) retention time (RT), time for half the marker to leave abomasum (T ½), abomasal volume (Vol) and flow rate of digesta fluid from the abomasum in sheep with changing level of feed intake.

	Level of feed intake (g/day)					
	400	600	800	1000	1200	1600
RT (min)	44 ± 10.6	44 ± 10.3	46 ± 9.6	37 ± 10.2	42 ± 9.8	46 ± 11.2
Vol (ml)	48 ± 27.7	50 ± 27.0	55 ± 29.8	52 ± 30.0	110 ± 20.0	186 ± 38.1
Flow (ml/h)	60 ± 46.8	72 ± 46.8	72 ± 46.8	66 ± 52.2	174 ± 35.4	324 ± 58.8

There was generally a good linear relationship of the natural log concentration of marker (Chromium) *vs* time recorded in each animal at each level of feed intake. In the 8 measurements (out of 72) where a linear relationship was not recorded, these results were not used for further analysis. There was a significant relationship between abomasal volume and level of feed intake ($p < 0.01$) and between digesta flow rate from the abomasum and the level of feed intake ($p < 0.05$). No significant correlation was recorded between mean retention time or half-time of marker in the abomasum and feed consumption. The highest feeding level (1600 g) was close to *ad libitum* feed intake for these animals (90-98 %).

Discussion

Decreasing feed intake in lambs fed once per day led to significant decreases in both the flow of digesta from the abomasum and the volume of the abomasum. There was a tendency for T½ to decrease with increasing level of feed intake and the values fell within the range reported by Grovum and Williams (1973) of 17-42 minutes. Values recorded in the present experiment for abomasal volume and flow were much less than reported by

Gregory *et al.* (1985) at all feeding levels, probably reflecting the different feeding regimes which were described in Chapter 7 because animals were a similar live weight in both experiments.

There was considerable variation in all measured parameters of abomasal emptying between individuals, which was a similar problem encountered by other workers (Gregory *et al.* 1985), so the mean value for each parameter was calculated for individual animals, rather than pooling all results to determine responses.

Appendix 4

A study of the role of learned taste aversion in the reduction in voluntary feed intake associated with GIT parasitism.

Introduction

A reduction in voluntary feed intake is a common feature of parasite infections in many species (Symons, 1985), yet the mechanisms causing this reduction in feed intake remain poorly understood (Symons, 1985). Learned taste aversion may have a role in feed intake depression in ruminants. In rats infected by a single inoculum of *Nippostrongylus brasiliensis* (Nematoda), Keymer, Crompton and Sahakian (1983) found infected rats strongly preferred the diet not associated with infection when given simultaneous choice. Uninfected rats showed no preference and ate equal amounts of both flavoured diets.

Learned taste aversion occurs in ruminants and is an important survival mechanism for foraging ruminants (Provenza and Pfister, 1991). Learned aversion has been recorded as rapidly as within four hours of novel diet being offered (Provenza and Pfister, 1991). Preference for, or aversions to, foods may result from trial and error based on cautious sampling followed by nutritional and physiological consequences.

If intake depression of a particular diet is mediated via and/or prolonged by developed taste aversion to the diet associated with parasitic infection, then the feed intake response to blocking/potentiating a pathway may not be detected because of the dominant effect of learned taste aversion. The role of learned taste aversion in feeding preferences was examined in the present experiment.

Materials and Methods

Thirty lambs (Coopworth) were early weaned in October 1986 (Section 3.7) and grazed on "safe pasture" until March 1987. In March 1987 animals were house indoors in single pens, under continuous lighting. Preference ranking (pretreatment) for meadow hay and lucerne chaff was established by offering a free choice of chaffed meadow hay and chaffed lucerne hay *ad libitum*. Forages were offered in separate bins at either end of 3.5 m pens and alternated daily between bins to minimise any effect of bin location on forage

preference. Tap water was available at all times in the centre of the pen. Animals were weighed (Section 3.2), blood sampled by jugular venipuncture, and faecal sampled (during infection phase) (Section 3.6) weekly for the duration of the experiment.

After 14 days of *ad libitum* choice the animals were allocated on the basis of live weight to meadow hay or lucerne hay (n=15). Within each forage group animals were allocated to infected (n=10) or non-infected (n=5) treatments. Animals in the infected groups were dosed with *T. colubriformis* larvae (Section 3.6).

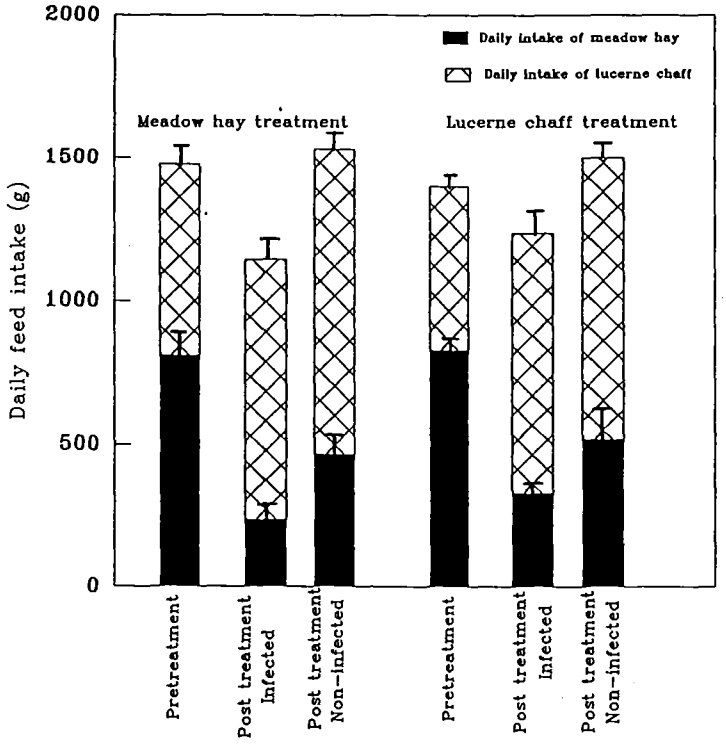
During the infected stage all animals were offered *ad libitum* only their allocated forage. Following the onset of feed intake depression of at least 25 % in the infected groups, all animals were treated with anthelmintic (200 mg/kg ivermectin, Ivomec, MSD Agvet N.Z.). The following day animals were again offered a free choice of meadow hay and lucerne chaff (post treatment preference ranking) as above for 4 weeks. Feed intake was recorded daily.

Results

Feed intake of meadow and lucerne hay before and following parasite treatment for all groups are shown in Figure A 4.1.

During the pretreatment preference ranking, both groups showed a slight, but not significant preference for meadow hay compared to lucerne chaff. Following the infection period all groups increased preference for and intake of lucerne chaff and reduced their intake of meadow hay. There was no effect of treatment or infection status on the change to selection ($p > 0.05$). Both groups which had been parasite infected tended to eat less over the day than the non-infected groups but this difference was not significant.

Figure A 4.1 Daily feed intake (± s.e.m) of lambs offered a free choice of meadow hay (■■) and lucerne chaff (⋇⋇), before infection (pretreatment) and following a period of parasite infection (infected group) or placebo (non-infected) and feeding on a single forage (meadow hay or lucerne chaff).



Discussion

There was no evidence of a consistent shift in feed intake in infected lambs to suggest learned taste aversion was operating to reduce feed intake in lambs infected by *T. colubriformis* on either of the diets tested. All groups decreased consumption of meadow hay in the second preference ranking test and there was an associated increase in intake of lucerne chaff. The forages were both from the same source and no changes in the quality of either ration were detected during the course of the experiment. However the results suggest a change in palatability of the forages may have occurred, with meadow hay becoming less palatable or the lucerne chaff becoming more palatable.

Previous studies using rats which suggested a role for learned taste aversion in parasite-

induced anorexia in rats (Keymer *et al.*, 1983) used a single large inoculation of parasites which would result in an acute infection and rapid onset of symptoms of infection. However the present experiment used trickle dosing and resulted in a relatively slow onset of subclinical infection (6-9 weeks) in the lambs. The gradual development of symptoms and anorexia would presumably be less likely to produce learned taste aversion than a sudden episode of malaise which would occur with acute infection or with aversive compounds (Provenza and Pfister, 1990).

In summary there was no evidence of learned taste aversion causing or prolonging intake depression in lambs suffering from subclinical *T.colubriformis* infection. With subclinical infection learned aversion does not appear to be a major component of the reduction in voluntary feed intake which occurs.

Appendix 5

Preparation of phosphate buffered saline solution

For 4 litres

NaCl	32.0 g
Na_2HPO_4(An)	1.82 g
KH_2PO_4	0.8 g
KCl	0.8 g

. . . add to 3.5 l distilled water and make up to 4 l

. . . stir for 10 min at 30 °C until dissolved

Appendix 6

A study of the effectiveness of loxiglumide and L364-718 (CCK antagonists) at blocking exogenous CCK-induced feed intake depression.

Introduction

The lack of a reliable and specific assay for endogenous CCK has limited our ability to understand the physiological role of CCK. Early work utilising a bioassay suggested elevated peripheral CCK levels may have a role in potentiating feed intake depression in lambs infected by gastrointestinal parasites (Symons and Hennessy, 1981). The development of potent, highly specific CCK receptor antagonists offers another approach to investigating the role of CCK in parasite infected lambs. Two CCK receptor blockers used in early studies in this thesis (Chapter 4 and 6) were developed for pharmacological use in treating CCK related conditions. L364-718 (Chang and Lotti, 1986) and loxiglumide (Setnikar *et al.*, 1987a,b) are potent, highly specific antagonists of peripheral CCK, as described earlier (Chapters 4 and 6).

These compounds were developed for use in the pharmacology and medical fields, so although tested extensively on laboratory animals and to a lesser extent on humans, their use in sheep had not previously been validated.

This experiment was undertaken to validate the use of the CCK antagonists in sheep, by using the antagonists to block the anorexic effects of exogenous CCK on short term feed intake.

Materials and Methods

Four ewe lambs, part of a larger pool of experimental animals were used. The animals were housed indoors under constant lighting in individual metabolism crates. All animals were accustomed to the metabolism crates and to removal of feed for determination of short term feed intake recording.

Animals were offered each of 4 treatments in a replicated Latin square design. Treatments were offered on alternate days only.

1) 5 ml phosphate buffered saline solution (control) administered by intravenous injection immediately prior to feeding.
2) 150 μg of CCK-8 (C-9271, Sigma Chemical Company, St Louis, MO, U.S.A.) in 5 ml of saline solution immediately prior to feeding.
3) 0.1 mg/kg L364 718 in 0.5 ml of dimethyl sulphoxide injected subcutaneously 60 minutes before feeding and 150 μg of CCK-8 (as above) immediately before feeding.
4) 10 mg/kg loxiglumide in phosphate buffered saline solution (0.54 % loxiglumide) administered by slow intravenous injection immediately prior to feeding and 150 μg of CCK-8 (as above) immediately before feeding..

Short term feed intake was recorded (Section 3.1) on treatment and rest days.

Results and Discussion

There was no effect of any of the treatments on short term feed intake. The use of the CCK antagonists in sheep was not successfully validated because CCK-8 administered peripherally did not depress short term feed intake.

Other work (Grovum, 1981) found exogenous CCK-8 administered peripherally to sheep depressed feed intake at a similar dose to that used in the present experiment but it was later suggested that changes to gut motility were responsible for the intake depression (de Jong, 1986). Della-Fera and Baile (1979; 1984) found exogenous peripheral CCK did not affect feed intake in sheep and the authors concluded that in sheep unlike in other species peripheral CCK may not have an intake regulatory role.

Peripheral CCK-8 did not depress feed intake in sheep in the present experiment. As a result the use of CCK antagonists L 364-718 and loxiglumide in sheep was not validated.