ASPECTS OF NITROGEN METABOLISM IN RUMINANTS IN HEALTH AND DISEASE

by

Linda Mary Taylor, B.Sc.

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SUMMARY

Studies in this thesis investigated various aspects of nitrogen metabolism in ruminant livestock. Nitrogen balance and digestive efficiency studies were conducted in order to obtain indicators of the apparent efficiency of absorption and retention of dietary nitrogen, while studies using radioisotopes provided more detailed information on the metabolism of nitrogen.

During the course of the work described here techniques were developed for the continuous intravenous and intraruminal infusion of substances into cattle and sheep respectively. A simplified polythene chute system to facilitate the collection of low dry matter calf faeces was also developed.

Section I

In this section the current literature relating to the use of exogenous beta-adrenergic agonists in farm livestock is reviewed. These substances, termed 'repartitioning agents' alter the partitioning of nutrients such that lean tissue accretion is increased at the expense of fat deposition. This may produce the additional benefits of improved food conversion efficiency and daily liveweight gains. Possible modes of action and adverse effects on meat quality are discussed.

Experimental work (Experiment I) describes nitrogen balance and digestive efficiency studies conducted with young steers given repartitioning agents. Ten castrated male Friesian calves (c. 120 kg liveweight) were restrained in metabolism stalls for fifteen weeks during which time they received continuous intravenous infusions of the beta-agonists clenbuterol (1 mg/day), bitolterol (1, 5, and 25 mg/day) and physiological saline (0.9% w/v). The experimental design was that of two simultaneous 5 x 5 Latin squares and each calf received each treatment for a period of three weeks. Separate digestibility and nitrogen balance studies were conducted during the second and third weeks of each period. Neither beta-agonist had any significant effect on voluntary feed intake, digestive efficiency or faecal nitrogen excretion. Urinary nitrogen excretion was significantly ($P<0.001$) reduced by clenbuterol (1 mg/day) and bitolterol (25 mg/day) giving rise to significant ($P<0.01$) increases in nitrogen retention. The effect of treatment on final carcass composition could not be

differentiated as the different treatments were incorporated in a Latin square design. However the external appearance of treated steers displayed more musculature in the hind quarters than that of similar animals maintained on the same diet as possible replacements suggesting an improvement in the lean percentage of the carcass.

Section II

This section is concerned with the pathophysiological consequences of gastrointestinal nematode infection in cattle. In Western Europe the most abundant species are Ostertagia ostertagi and Cooperia oncophora which are typically located in the abomasum and small intestine respectively. The life cycle and pathogenesis of these nematodes is described together with the pathophysiological changes which precipitate clinical disease.

Three separate studies investigated the pathophysiological and parasitological consequences of gastrointestinal helminth infection established using multiple inoculations of either fresh or cold-conditioned O. ostertagi larvae or a mixed infection of O. ostertagi and C. oncophora. In each study a morantel sustained release bolus (MSRB) was administered to one group of infected calves in order to limit larval establishment (Jones, 1983).

The experimental design for each of the studies followed that of Armour et al (1987a). Twenty-five castrated male Friesian calves (c. 120 kg liveweight) which had been reared under helminth-free conditions were allocated to three treatment groups.

Group A calves (clean) were maintained free of parasites. Five were used as control animals for digestion and nitrogen economy studies and a further two for radioisotopic investigations.

Group B calves (MSRB) were each given a morantel sustained release bolus (Paratect, Pfizer) and were subsequently dosed daily per os for six weeks with either 2000 fresh O. ostertagi, 2000 cold-conditioned O. ostertagi or 2000 O. ostertagi and 10,000 C. oncophora infective larvae. Five calves were allocated to digestion studies, two to pathophysiological investigations using radioisotopes and two necropsied 21 and 42 days after the initial infection.

Group C calves (infected) were similarly treated, without prior MSRB administration, with five calves allocated for digestion studies, two for radioisotopic work and two for necropsy during the course of the trial (days 21 and 42).

Following an acclimatisation period of one week in metabolism stalls digestive efficiency and nitrogen balance data was obtained from four calves in each of the treatment groups clean, MSRB and infected during days 14-21, 35-42, 56-63 and 77-84. Radioisotopic investigations using tritiated water (TOH) to measure body water metabolism, and ^{125}I-albumin and $^{51}CrCl_3$ to measure albumin metabolism and leakage of plasma into the gastrointestinal tract were conducted during days 35-47 and 70-82. All calves were weighed regularly and blood and faecal samples were obtained at weekly intervals for biochemical, haematological and parasitological analyses. Remaining calves in metabolism and isotope studies were necropsed on day 84 for parasitological examination of the digestive tract.

Daily inoculation of calves with 2000 O. ostertagi larvae (Experiment IIa) produced mild diarrhoea in infected untreated animals. These calves did not become inappetent and there were no significant differences in daily liveweight gain, digestive efficiency or nitrogen economy between treatment groups. However radioisotopic studies revealed increased faecal clearances of both ^{125}I-albumin and $^{51}CrCl_3$ and an elevated catabolic rate of albumin for infected compared to clean control calves particularly during the first recording period. Small adverse changes in pathophysiological values were apparent when comparing MSRB treated and clean control calves.

A preliminary study (Experiment IIb) demonstrated that short-term storage of O. ostertagi infective larvae at $4^{o}C$ resulted in an interruption in normal parasitic development akin to Pre-Type II ostertagiasis. Calves inoculated with 2000 cold-conditioned O. ostertagi larvae (Experiment IIc) displayed no symptoms typical of O. ostertagi infection and there were no significant differences in nitrogen economy or pathophysiological parameters between treatment groups. Interestingly the overall apparent digestibility of each of the major proximate feed fractions was significantly reduced for clean control compared to infected and MSRB treated calves. This may be attributed to interference with rumen volatile fatty acid and abomasal HCl production by fenbendazole which was used at fortnightly intervals in this study in order to ensure that the clean calves remained parasite free.

A concurrent infection of O. ostertagi and C. oncophora (Experiment IId) produced clinical symptoms in infected untreated calves which ranged from a reduced rate of eating and mild diarrhoea in

some individuals to almost complete inappetence, lethargy, profuse watery diarrhoea and a dramatic liveweight loss in others. Symptoms subsided towards the end of the trial but the appearance of the infected calves was greatly inferior to that of the clean and MSRB treated groups at necropsy on day 84. Examination of digestive efficiency data revealed a consistent and significant depression in the apparent digestibilities of the crude protein, ether extract and gross energy fractions for infected compared to clean and MSRB treated calves. A significant ($P<0.01$) reduction in nitrogen retention for infected calves during days 35-42 and 56-63 was attributable to increases in both faecal and urinary nitrogen loss. Pathophysiological parameters measured using radioisotopes revealed marked disturbances in the metabolism of infected, and to a lesser extent MSRB treated calves compared to clean controls which persisted until the end of the experiment. For infected calves elevated faecal clearances of both ^{125}I-albumin and $^{51}CrCl_3$, and consequent increases in the catabolic rate of albumin were associated with hypoalbuminaemia and hypoproteinaemia.

Parasitological examination of the digestive tracts at necropsy revealed a higher establishment of O. ostertagi when given as a mixed rather than a monospecific infection in both infected and MSRB treated calves. Larger and more sustained elevations in plasma pepsinogen levels provided evidence of more persistent abomasal damage for the concurrent infection. Comparison of data obtained in this series of studies and that from a similar study using a monospecific C. oncophora infection (Armour et al, 1987a) reveals that the adverse effects of O. ostertagi and C. oncophora in combination far exceed those of either monospecific infection. This is possibly attributable to the enhanced establishment achieved by both species, together with the greater total area of mucosal damage and consequent reduction in the ability of the gastrointestinal tract to compensate for localised damage.

Prior administration of a MSRB limited larval establishment, the anthelmintic effect apparently being directed against adult worms. This resulted in a marked reduction in faecal egg output compared to infected controls.

Section III

The epidemiology of O. ostertagi and C. oncophora, together with that of the sheep nematode Ostertagia circumcincta are described. Measures for the prophylaxis and treatment of helminth disease in both cattle and sheep are discussed with particular emphasis on the avoidance of high levels of pasture larval contamination.

The experimental work employed a modification of the system developed for the continuous intravenous infusion of beta-agonists into cattle to investigate the effects of titrated doses of intraruminally adminstered levamisole hydrochloride on O. circumcincta infection in lambs (Experiment III). Thirty-six parasite naive crossbred lambs (c. 35 kg liveweight) were allocated to six equal treatment groups. Lambs in groups 1-5 were daily dosed per os with 4000 O. circumcincta infective larvae for 21 days while those in group 6 were maintained as a parasite-free control. In addition lambs in groups 2-5 received infusions of levamisole hydrochloride in solution in physiological saline at rates of 1.0, 1.5, 2.0 and 3.0 mg/kg/day for 24 days commencing on the first day of larval inoculation. Lambs were housed in metabolism stalls and separate digestibility and nitrogen balance studies were conducted during days 0-7, 7-14, 14-21 and 21-28. Half of the lambs in each treatment group were necropsied on day 24, and the remaining lambs on day 28 for parasitological examination of the digestive tract.

No clinical signs of O. circumcincta infection were seen in any of the lambs and there were no statistically significant differences in nitrogen economy between treatment groups. However over the whole 28 day period the apparent digestibilities of the dry matter, crude protein, crude fibre and ash proximate fractions were significantly ($P<0.05$) reduced for lambs receiving 3.0 mg levamisole hydrochloride/kg/day, compared to the clean control. The apparent digestibilities of the dry matter and crude fibre were also significantly ($P<0.05$) reduced for infected compared to clean control lambs. Higher daily doses of levamisole resulted in increased reductions in worm burdens at necropsy compared to the infected control but it seems likely that levamisole, like the substituted benzimidazoles, interferes with ruminant digestion when used continuously at levels approaching that required for efficient prevention of parasite establishment.

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A. Experimental Animals and Management

1. Bovine studies

Castrated male Friesians which had been reared indoors were used for the bovine studies described in this thesis. Each separate study involved a separate group of animals, but all groups had an initial mean liveweight of approximately 120 kg and an initial mean age of between three and four months. For each study animals were allocated to treatment groups on the basis of liveweight such that the mean liveweight and the liveweight range was similar for all treatments. Initially calves were loose housed together in a strawbedded pen and individually fed using head locking feeders. During the parasitological studies described in Section II, clean control calves were maintained in a separate building from calves on infected treatments to avoid possible cross contamination via soiled bedding material. However during experiments IIb and IIc both clean and infected calves were housed together and the parasite-free status of the clean calves was maintained by regular anthelmintic treatment.

During metabolism studies calves were accomodated in standard metabolism stalls while those maintained as possible replacements remained in the loose housed accomodation.

The experimental diet consisted of a grass/cereal mix/mineral complete cube preparation ('Superstar Cubes', Hamlyn Milling, Balgarvie Mill, Scone, Perthshire). In order to provide controlled intake of long fibre this was supplemented by barley siftings. Griseofulvin (Fulcin, Coopers Animal Health) was also admixed in the diet for seven days prior to the commencement of each experiment to control ringworm. Ringworm lesions present during the experiments were treated using topical applications (Mycophyt, Gist-Brocades and Imaverol, Janssen). All calves were vaccinated against infectious bovine rhinitis (Imuresp, Smith-Kline) on arrival.

2. Ovine studies.

The study described in Section III was conducted using parasite naive crossbred lambs of approximately 35 kg liveweight, which had been reared indoors. The lambs were randomly allocated to treatment groups on the basis of liveweight and sex such that the distribution on each treatment was similar. Initially all animals were housed in individual peat bedded floor pens for 14 days prior to the commencement of the experiment during which time lambs in four of the treatment groups were

surgically fitted with indwelling ruminal catheters. Catheterized lambs and male lambs from the remaining two treatments were subsequently moved to metabolism stalls for nitrogen balance studies, digestive efficiency studies being conducted on all lambs. No possible replacements were maintained.

The experimental diet consisted of grassnuts and water was available ad libitum.

B. Digestibility and Nitrogen Balance Studies

1. Collection of urine and faeces

During digestibility and nitrogen balance studies animals were normally restrained in metabolism stalls. Sheep were fitted with a conventional body harness. Faeces from males was collected in a rubber bag and that from females was collected in a nylon mesh bag which allowed the through-passage of urine. Urine passed through the mesh floor of the metabolism stall and for males was funnelled through a glass wool filter into a collection jug. Urine from female sheep was not collected.

Calves were fitted with a specially designed body harness. Faeces was collected in a polythene lined canves bag or by a polythene chute system which increased collection efficiency during periods when parasitised animals were diarrhoeic. Urine passed through the slatted metal floors of the metabolism stalls onto sloping polythene-lined metal trays and into collection receptacles. Rubber cow matting was fitted at the front of the metabolism stalls to help maximise calf comfort.

2. The design of a novel calf body harness

The experiment decribed in Section I required calves to be fitted with an indwelling jugular vein catheter during metabolism studies. The calf harness shown in Figure 1 was designed for this experiment and was used subsequently. It had a minimum of strapping in the neck region and was made of leather with the exception of the wide canvas girth strap which provided the main support for the faecal collection bag. An important feature was the foam back pad positioned either side of the calf's spine, reducing pressure in this area. A canvas faecal collection bag was attached to the harness by means of a tie to the dorsal cross-over and two quick release clips attached to buckles on the lateral straps. The dorsal tie slightly increased the time

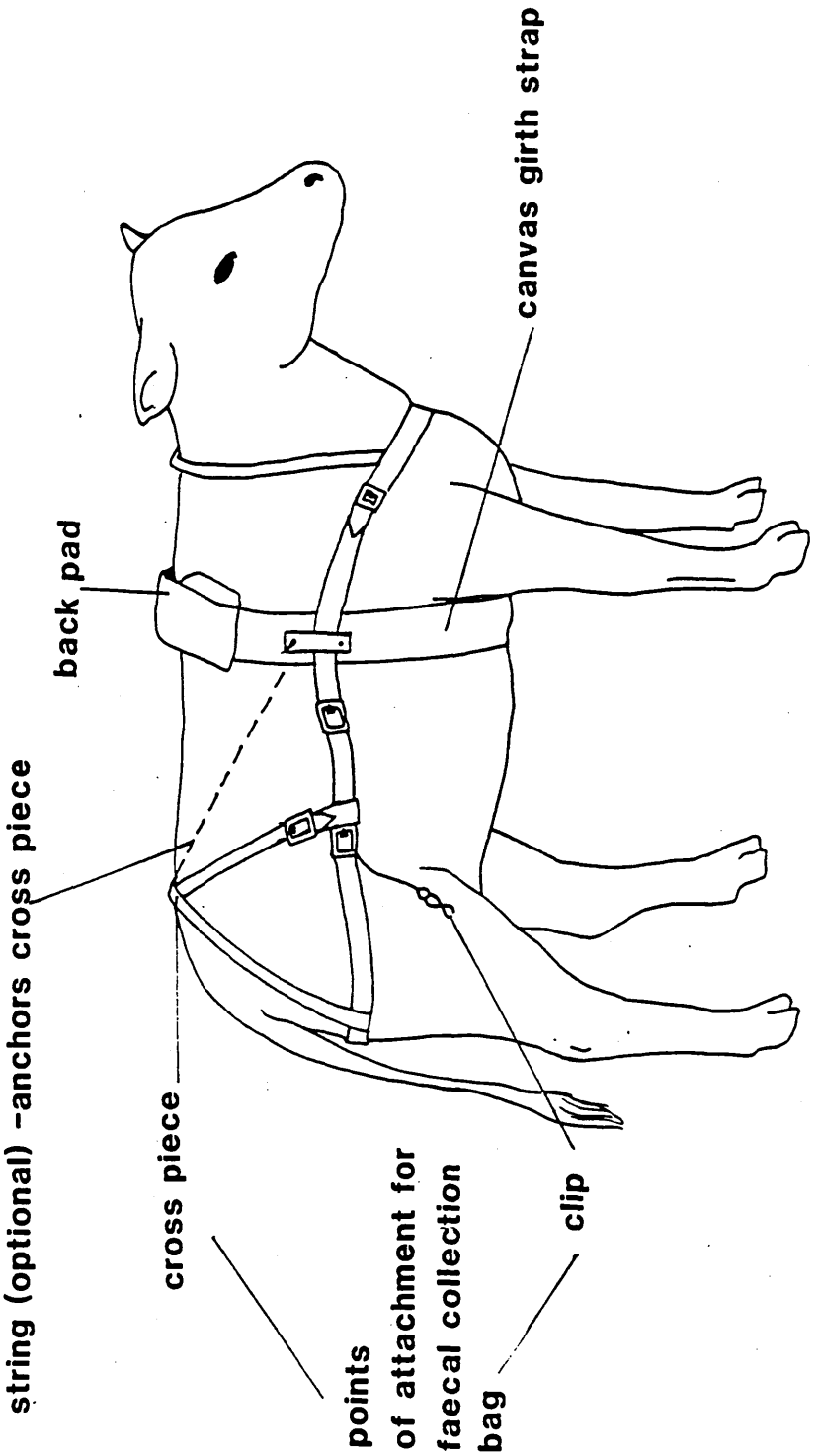


Figure 1. A novel calf body harness to facilitate simultaneous faecal collection and continuous intravenous infusion via the jugular vein.

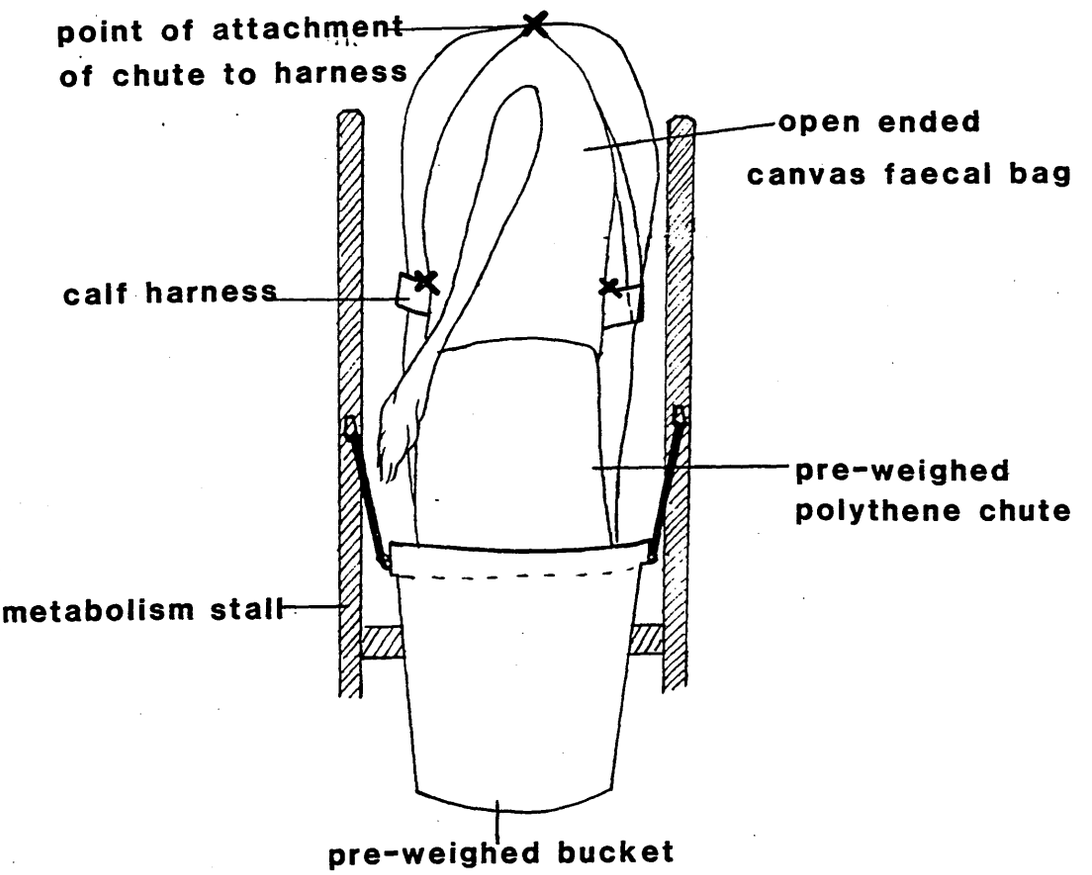


Figure 2. A polythene chute system for the collection of low dry matter calf faeces.

required to empty the collection bags compared to a three-clip attachment, but was necessitated because a dorsal clip supported the bag in a lower than ideal position.

3. An improved technique for the collection of low dry matter calf faeces.

Brockway (1979) first described a method for the collection of faeces from steers which utilized a long polythene chute in place of the conventional bag carried by the animal. The increasing weight of faeces collected in conventional bags contributes greatly to the causation of sores under the supporting harness and may also lead to a shifting of the harness resulting in spillage. These problems are exacerbated during the collection of low dry matter faeces. Thus in order to maintain collection efficiency, conventional faecal bags were replaced by a modification of this method during periods when calves became diarrhoeic.

The faecal collection chute is shown in Figure 2. The proximal part of the chute was comprised of a conventional canvas calf faecal collection bag and polythene liner, the bottoms of which were cut open. A second liner, cut square and open at both ends was taped onto the first liner forming the chute, the distal end of which rested on the bottom of a bucket suspended from the back of the metabolism stall. Suspension of the bucket in this manner allowed it to tilt if necessary when the calf lay down, ensuring that the bottom of the chute remained in situ. Attachment of the chute to the calf harness was as described for the conventional canvas bag with the addition of two elastic straps between the calf's back legs running from the bottom of the chute to a strap around the stifle region. These additional straps ensured good contact between the chute and the calf's skin under the anus and greatly improved faecal collection efficiency.

4. Daily routine during digestibility and nitrogen balance studies.

Digestibility and nitrogen balance measurements were conducted over seven day collection periods. Experimental animals were given their daily ration in two feeds, one at 07.30h and the other at 16.00h. Sheep feed was preweighed into paper bags in batches and stored in metal bins until required. Calf feed was weighed out directly at each feeding time. Any feed refusals were weighed and recorded. Water was available ad libitum.

Faecal collection bags were emptied at each of the feeding times and additionally at 21.00h for calves. Sheep faeces were emptied directly into a preweighed container. The output for each calf for each 24 hour period was collected in a small pre-weighed bucket and weighed daily at 09.00h before bulking in a large container. Where calves were fitted with faecal chutes the suspended bucket and chute were preweighed. The faecal output for each 24 hour period was collected directly and the chute was replaced daily at 09.00h at which time the bucket and faecal contents were removed for weighing and bulking. Liners in conventional calf faecal collection bags were replaced on every second day.

At each of the feeding times the filtered sheep urine collected in jugs below the metabolism stalls was emptied into bulk containers acidified with 100 ml 5N HCl. Jugs were replaced and stalls washed with minimal amounts of water, the washings being bulked together with the urine. Collection receptacles for calf urine were emptied at 07.30 h, 16.00 h and 21.00 h, the urine being filtered through glass wool into bulk containers acidified with 200 ml 5N HCl. Again stalls were washed with minimal amounts of water and the washings were bulked separately from the urine in containers acidified with 100 ml 5N HCl.

5. Preparation of feed, faecal, urine and washings samples for chemical analysis.

N loss ? + acid.

Random samples from each bag of feed used during each collection period were taken and stored together in a small sealed container until the end of that period when the composite sample was chemically analysed.

The bulked faeces obtained from each animal during each seven day collection period was thoroughly mixed and suitable aliquots were retained for analyses. Approximately 2 kg was retained for dry matter (DM), crude fibre (CF), ether extract (EE), ash and gross energy (GE) determination, and 200 g was made into a slurry with water and 4 ml toluene for nitrogen estimation (C.A.B., 1961).

Bulked urine samples for each animal were thoroughly mixed and two separate 200 ml samples were retained for nitrogen determination. Washings were similarly treated but only one 200 ml sample was retained.

C. Chemical Analyses of Feed, Faeces, Urine and Washings Samples

All the analytical methods used were officially established procedures i.e. MAFF et al 1973; Fertilizer and Feedingstuffs Regulations 1968, 1976 and 1982.

1. Dry matter (DM)

The dry matter in food and faecal samples was determined by heating known quantities (2 kg) in a hot air oven at 80°C for approximately 48 hours until a constant weight was attained. Dry material was ground using an 8 inch laboratory hammer mill with a 2 mm screen (Christy and Norris, England) and a sample of approximately 200 g was taken for subsequent laboratory analyses.

2. Nitrogen and crude protein

Total nitrogen (N) in dried feed, faecal slurries and urine samples was measured by an automated semi-micro Kjeldahl technique (Kjell-Foss Automatic 16210). For feed and faeces crude protein was calculated as the product of total nitrogen and 6.25.

3. Ether extract (EE), crude fibre (CF) and ash

The ether extract, crude fibre and ash contents of food and faeces were determined using standard methods (The Fertilizer and Feedingstuffs Regulations, 1975).

4. Gross Energy (GE)

The gross energy content of food and faeces was measured using a Gallenkamp automatic adiabatic bomb calorimeter equipped with a digital data systems calorific value microprocessor. Calibration was performed using benzoic acid (Thermochemical Standard, BDH). Both samples and benzoic acid were pelleted using a die operated by a hydraulic press.

5. Organic Matter (OM)

The organic matter content of food and faeces (g/kg) was calculated as the difference between the dry matter and the ash content (g/kg).

6. Nitrogen free extract (NFE)

The nitrogen free extract (g/kg) of food and faeces was calculated as the difference between the organic matter (g/kg) and the sum of the

crude protein, crude fibre and ether extract contents (g/kg).

D. Calculation of Digestibility Coefficients and Nitrogen Balance

'The digestibility of a food is most accurately defined as that proportion which is not excreted in the faeces and which is, therefore, assumed to be absorbed by the animal' (McDonald, Edwards and Greenhalgh, 1981). It is commonly expressed in terms of dry matter as a coefficient e.g. :-

$$\text{DM digestibility coefficient} = \frac{\text{amount of DM apparently absorbed}}{\text{amount of DM in feed}}$$

The coefficients for each proximate fraction of the DM, eg CP, CF, EE, etc. can be calculated in the same way.

Although the proportion of food not excreted in the faeces is commonly assumed to be equal to that which is absorbed from the digestive tract there are objections to this assumption, particularly in the case of ruminants. For example, in ruminants methane arising from the fermentation of carbohydrate is lost by eructation and thus is not absorbed. This results in an over-estimation of the digestible carbohydrate and digestible energy of the feed. Also because of the presence of metabolic faecal nitrogen in the faeces, i.e. nitrogen arising from non dietary sources such as sloughed epithelial cells, enzymes etc., there is an under-estimation of the proportion of nitrogen absorbed by the animal. Ether extractable substances and minerals of metabolic origin are also found in the faeces, again leading to an under-estimation of their absorption. Consequently, the values obtained in digestibility studies are refered to as 'apparent' digestibility coefficients.

In this thesis, N balance is expressed as g N retained or lost on a daily basis and was calculated using the following equations:-

N balance (g N) = g N feed - (g N faeces + g N urine)

Where useful, N balance data may also be expressed as a percentage of the dietary N intake.

E. Infusion Studies

1. A system for the continuous intravenous infusion of beta-adrenergic agonists into cattle

A method for the accurate continuous infusion of substances over an extended time period was developed in order to assess the effectiveness of long term vascular administration of beta-agonists to growing cattle. During the period of active infusion digestibility and nitrogen balance studies were conducted and these are fully described in Section I. The infusion system was developed to simultaneously control the fluid flow to ten calves accomodated in metabolism stalls. Each calf was fitted with an indwelling jugular vein catheter and in order to prevent interference with this, the conventional headlocking restraint mechanism of the metabolism stalls was removed. Each calf was fitted with a headcollar to either side of which was attached a short chain and ring. The rings encircled the vertical bars at the front of the stalls along which they could slide allowing the calves freedom to lie down, but not to turn round or chew the infusion tubing. Only one bucket support was maintained on each stall, this being attached to the side opposite to that in which the catheter was inserted, thus preventing the calves possibly damaging the catheter by rubbing against the bucket. Water was available ad libitum except when feed was being offered. The restraint method is illustrated by Plate 1.

The arrangement of the infusion tubing was determined on a trial and error basis during a two week pre-experimental period. Catheter design however evolved during the period of active infusion. Various catheter types used are shown in Figure 3. The original design was made from Portex vinyl tubing (PVC) (BSS 52) and had an inserted length of 25 cm. A short, split outer sleeve bearing a single strand of nylon permitted suturing of the catheter to the calf's neck immediately above the insertion site. A locking connection to the infusion tubing was effected by means of a ground 14 G hypodermic needle inserted into the external top part of the catheter. This design was found to be unsatisfactory as the single suture did not provide firm anchorage and the inserted portion of the catheter displayed some tendency to loop out of the calf's neck resulting in expulsion. This was particularly noticeable when the split outer sleeve had also begun to detach itself from the main body of the catheter. A more pertinent problem was the susceptibility of the Portex vinyl material to environmental

a)

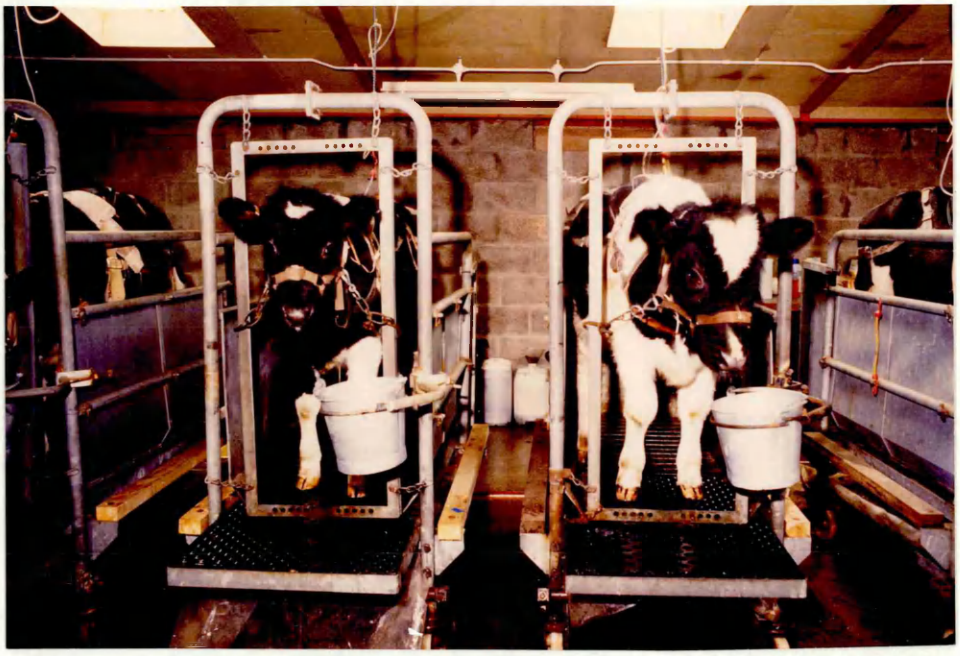
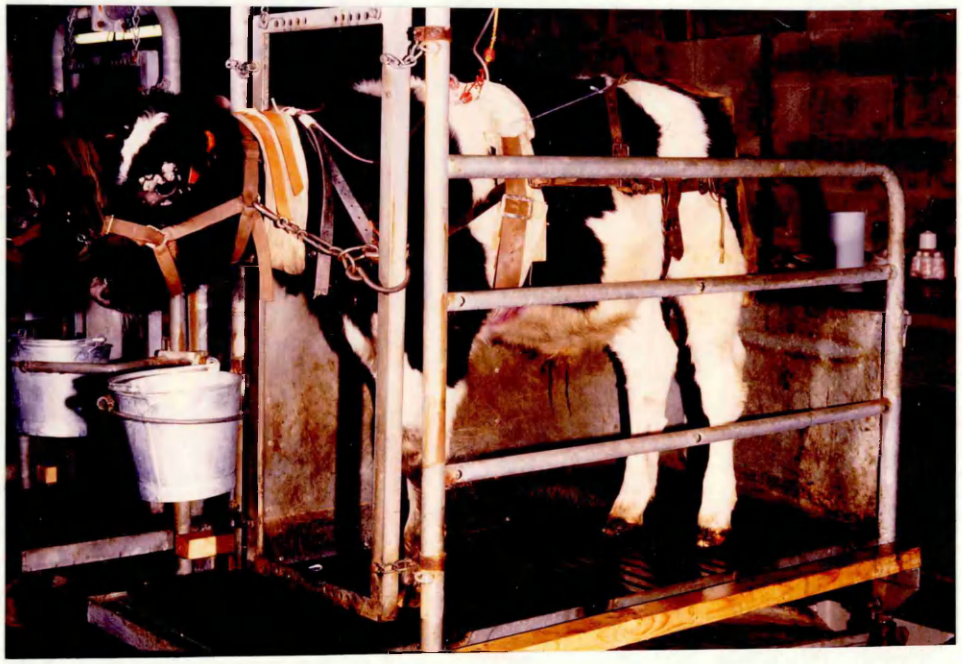


Plate 1. Restraint of calves during infusion studies by means of a headcollar and sliding chains.

b)



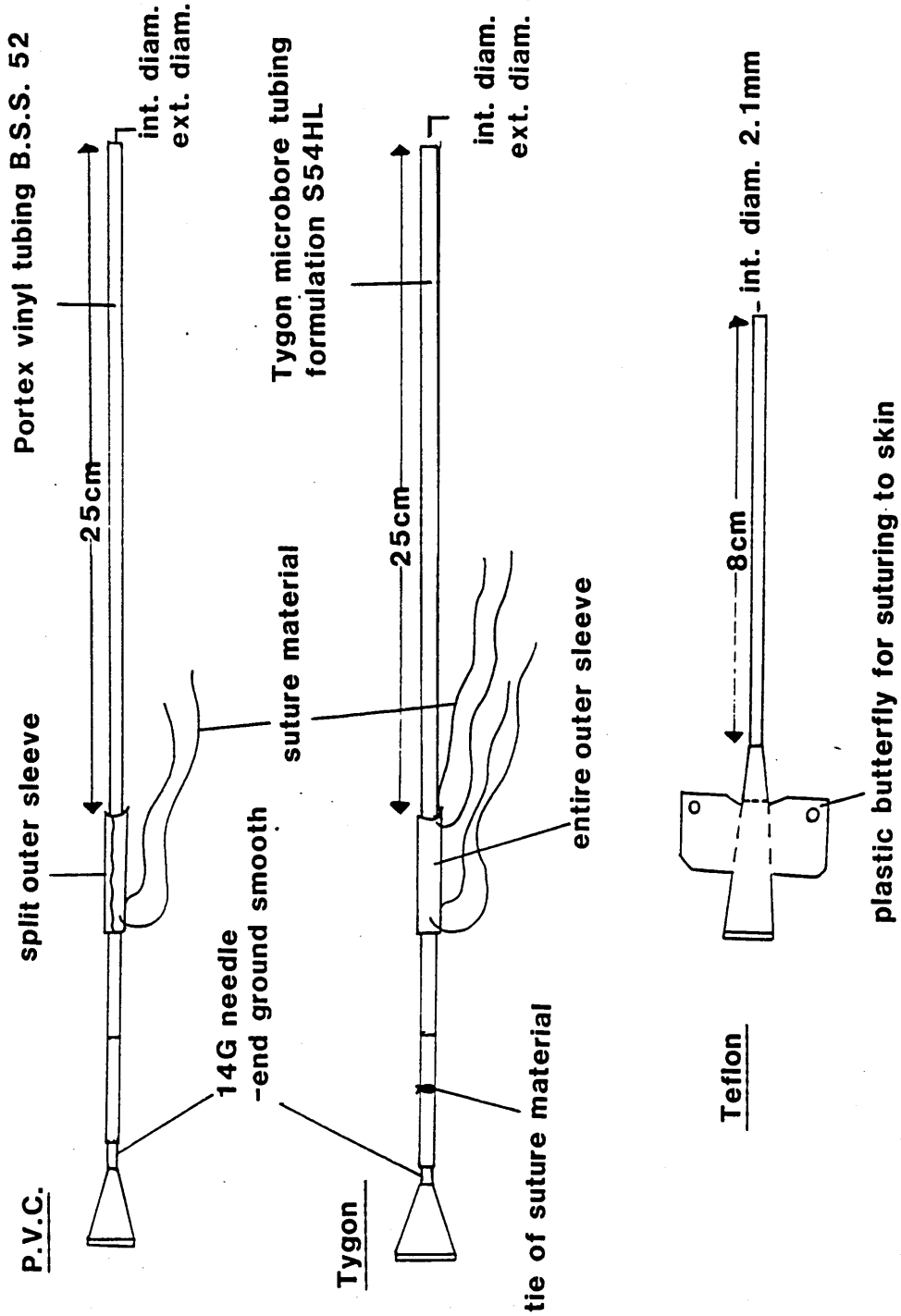


Figure 3. Catheter types used for the continuous intravenous infusion of beta-agonists into cattle.

temperature changes. As environmental temperatures decreased the external top part of the catheter became brittle and fissuring occurred.

The PVC catheter was superseded by a Teflon catheter (Intraflon 2, G.14, i.d. 2.1mm. Vygon, Laboratoires Pharmaceutiques, BP7-95440, Ecouen, France). This came complete with its own internal trochar rendering the insertion procedure relatively simple compared to that previously required (see Appendix 1). The Teflon catheter had no external top part and so was not influenced by environmental temperature changes. Connection to the infusion tubing was via a 'butterfly arrangement' which also permitted suturing to the skin, providing firm anchorage. However the Teflon catheter had an inserted length of only 8 cm and the distal end readily exited from the jugular vein during periods of calf activity resulting in subcutaneous infusion and oedema. For this reason the effective life of the catheter was extremely short.

The ultimate, and extremely successful catheter was of similar design to the PVC catheter but was made from Tygon microbore tubing (formulation S54HL, Norton Industrial Plastics, P.O. Box 350, Akron, Ohio, USA). This material is soft so as not to cause undue damage to the jugular vein wall and also temperature insensitive so as not to become brittle and crack with falling temperatures. An entire outer sleeve bearing two lengths of nylon suture material allowed the catheter to be firmly stitched in position. Protection for all catheters was afforded by a sterile dressing and a canvas neck collar.

The catheter for each calf was connected to one of two peristaltic pumps (Watson-Marlow 5025/501M pumphead), positioned approximately 1.3m above the calves, by means of a single length of Marzinal tubing (1.6mm bore, Watson-Marlow Ltd. Falmouth, Cornwall, England). Support for this tubing was effected by means of a horizontal wire positioned approximately 1 m above the middle of the calves backs, and an elastic strap between the wire and the calf harness. The infusion tubing was tied to the elastic strap in a looped fashion permitting the calves to lie down and stretch the strap without undue tensioning of the tubing. The general arrangement of the infusion tubing is shown in Plate 2, and Figure 4 illustrates in detail how a loop of the infusion tubing was fixed onto the neck strap of the calf harness and another stitched to the calf's neck such that exaggerated calf movement closed the loops rather than tensioning the catheter connection. Marzinal tubing is

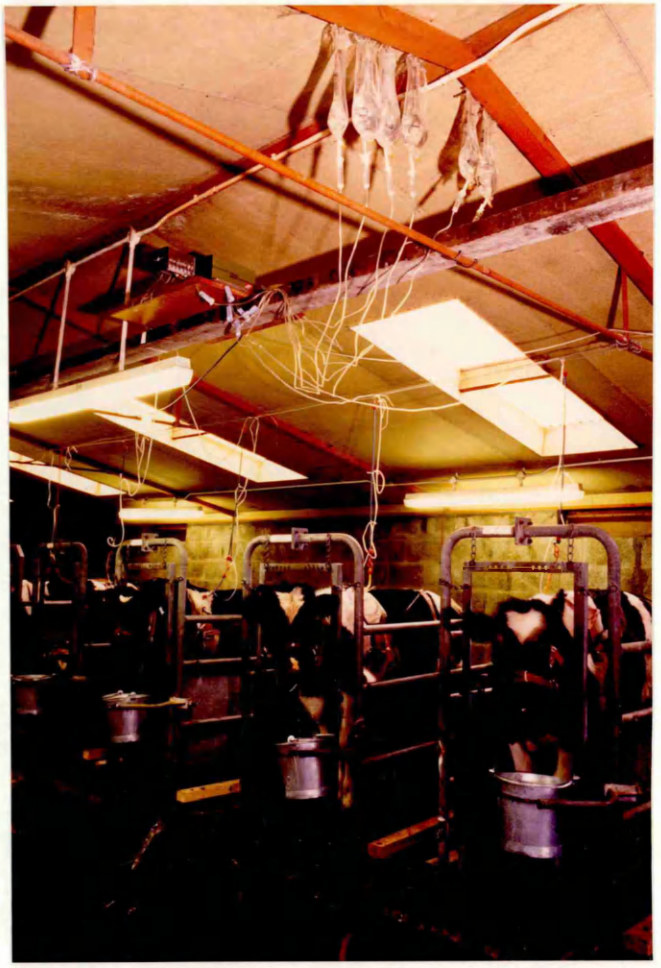


Plate 2. The arrangement of infusion packs, regulatory pumps, tubing and calves during the continuous intravenous infusion of beta-agonists.

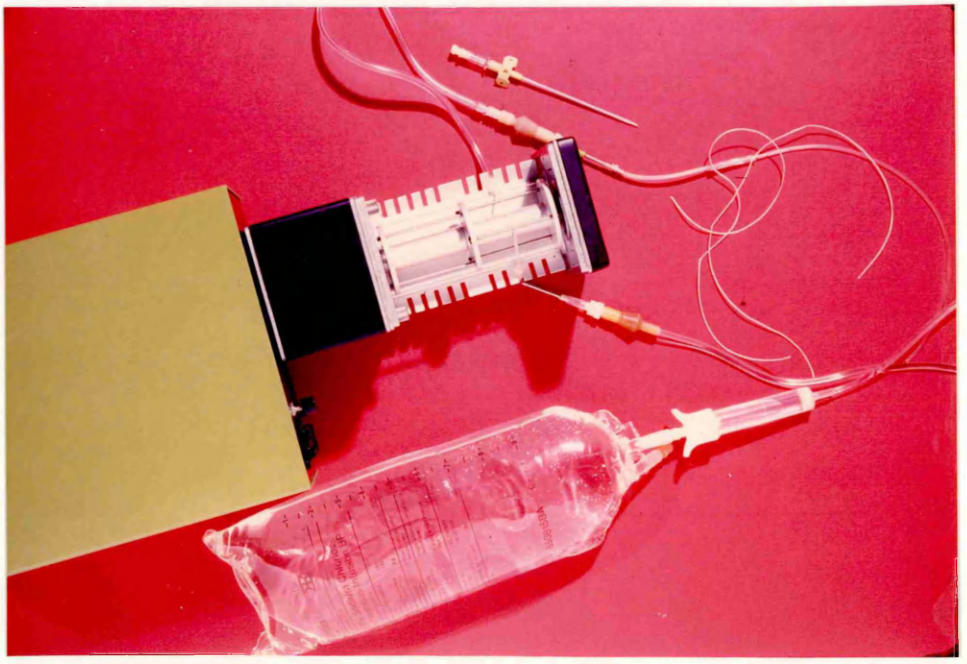


Plate 3. The Watson-Marlow 501M pumphead and associated peristaltic tubing.

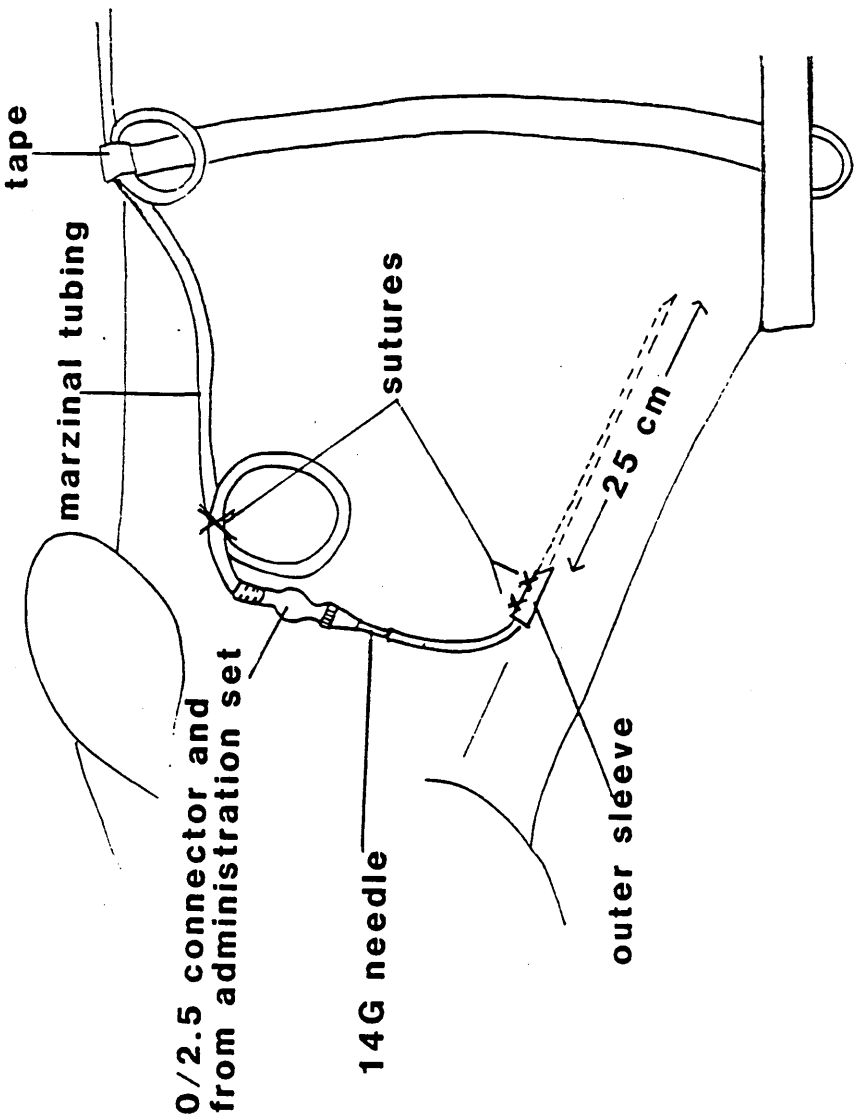


Figure 4. Detail of the inserted Tygon catheter and infusion tubing for the continuous administration of beta-agonists to cattle.

very elastic and formed good airtight connections at both the catheter and pumphead.

Each peristaltic pump simultaneously controlled the fluid flow to five calves and also that in a calibration line which permitted direct measurement, by volume, of flow rate. Flow rates for individual animals were also calculated from the difference in weight of the infusion packs taken at twice daily weighings. Variations in flow rate between individual animals were attributable to changes in delivery pressure at the catheter. Reductions in flow rate occurred if the catheter became kinked, if it became trapped against the vein wall or a valve, or if it was partially blocked by a small blood clot. Such changes could be reversed by examination of the catheter to remove kinks and flushing with heparinized saline. It was found that decreasing temperatures resulted in a general reduction in flow rate possibly through a contraction of the pumphead tubing in both length and diameter, and also a slight increase in the viscosity of the infusion solutions. However the most salient factor responsible for random changes in flow rate was wear on the pumphead tubing. This tubing consisted of 89 mm lengths of 0.1 mm internal diameter silicone rubber which were tensioned across the cylindrical pumphead rotor, as illustrated by Plate 3 and Figure 5. This tubing tended to stretch through time resulting in undesirable random increases in flow rates. In order to maintain accurate control of fluid flow to all calves, the pumphead tubing was changed every six days. Complete sterilised pumphead tubing units, as shown in Figure 6 were exchanged rather than just the tubing itself as it was far easier to form connections to wide bore marzinal tubing and to the administration set from the infusion pack, than to mount relatively narrow bore pumphead tubing onto the pumphead connectors. Thus continued asepsis of the system was promoted. Exchange of a complete tubing unit had the added advantage that all junctions at the pumphead could be sealed by silicone sealant prior to sterilisation and incorporation in the infusion system, thus reducing the potential for air influx.

It was noticed that on removal used pumphead tubing was very distorted and had a mean length of 93 mm, with a range from 90-96 mm. At any given time the infusion rate in lines connected to pump A was greater than that for lines connected to pump B. The arrangement of animals was such that the pumpheads were turning in opposite directions to achieve the same effect and it is possible that direction of

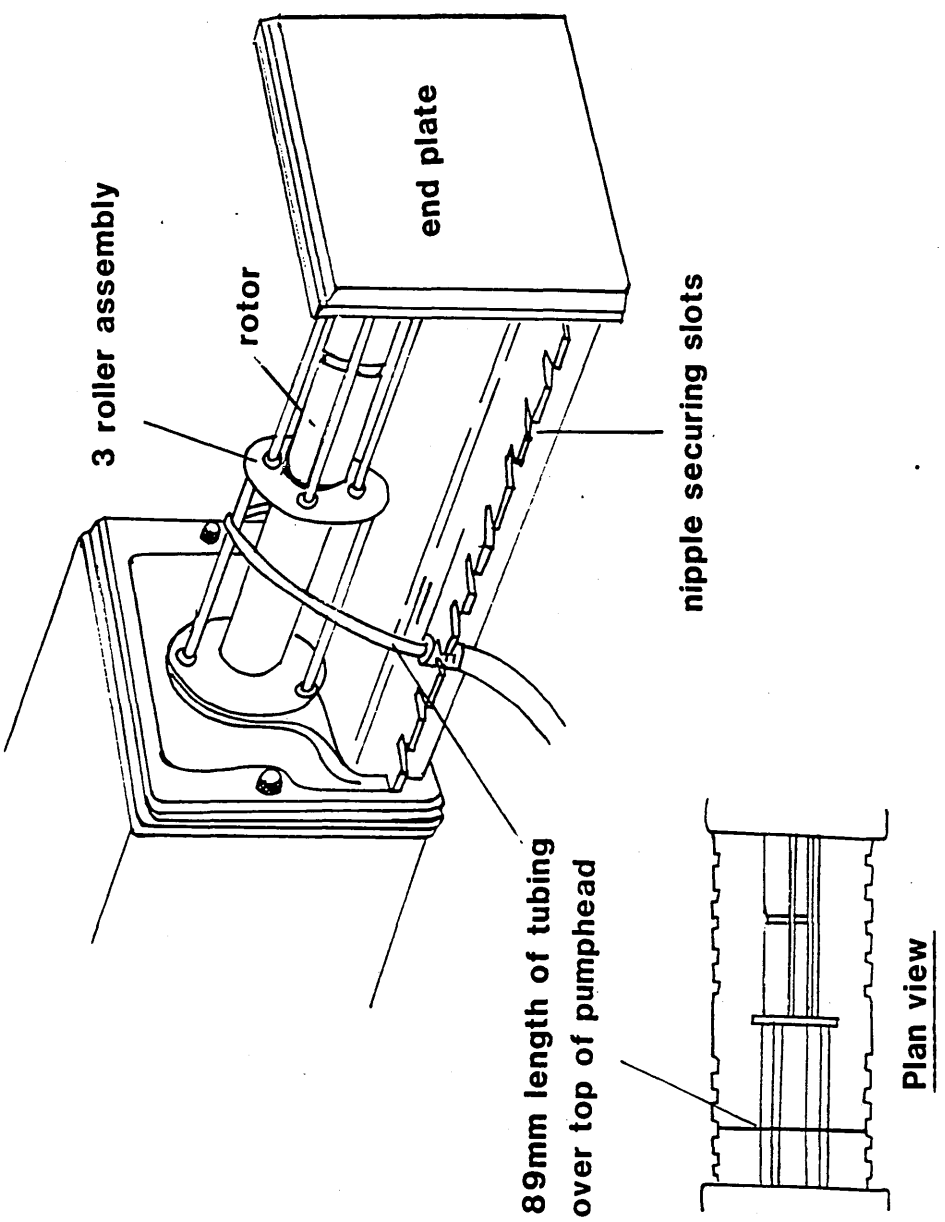


Figure 5. The Watson-Marlow 501M pumphead and associated peristaltic tubing.

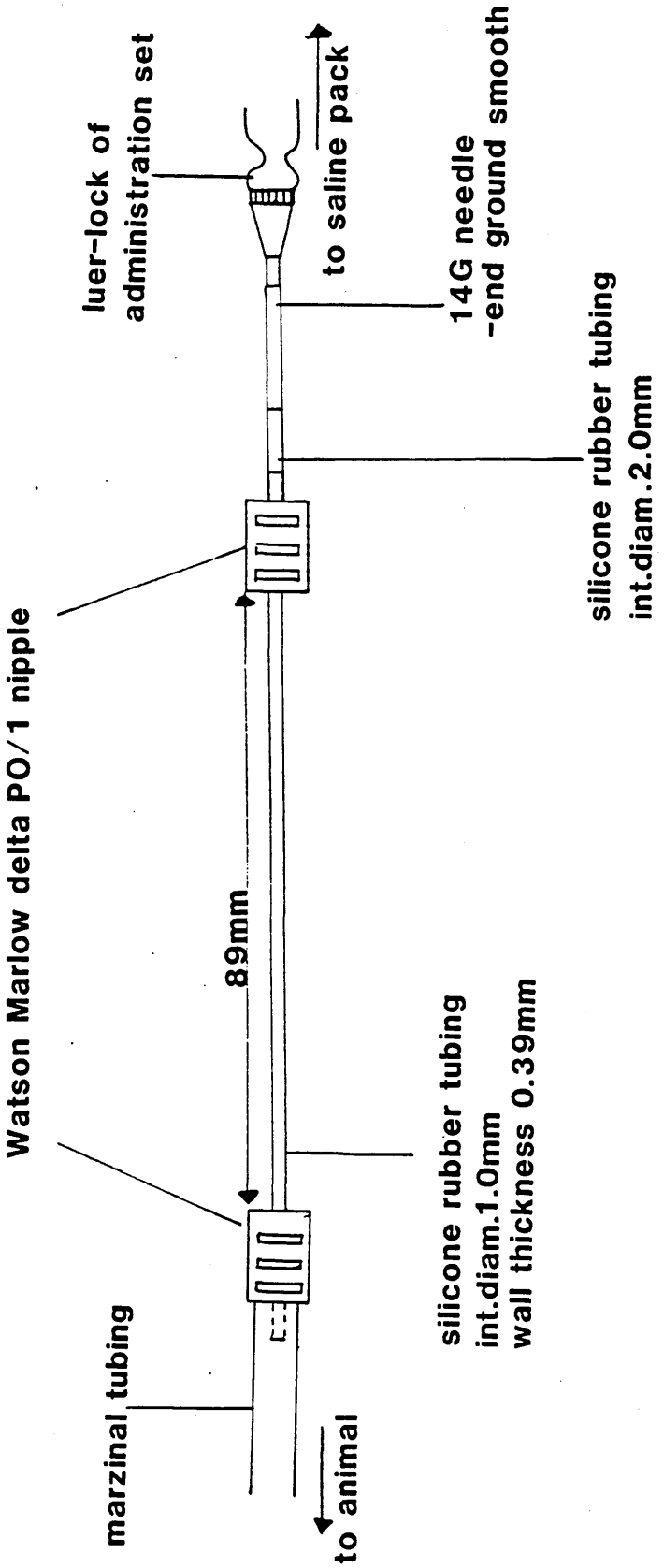


Figure 6. A unit of tubing for the Watson-Marlow 501M pumphead.

pumphead rotation affected the wear on the pumphead tubing. Watson-Marlow have noticed this with other pumphead types.

A detailed description of the procedure involved in changing the pumphead tubing is given in Appendix 1.

The beta-agonists to be infused were held in solution in 1 litre sterile 0.9% saline infusion packs positioned above the pump and connected to the pumphead tubing units by means of infusion fluid administration sets (Travenol, England). Each litre of solution was infused over a four day period, the administration set facilitating rapid replacement of empty infusion packs. Infusion packs were prepared immediately prior to requirement by injection of suitable aliquots of stock solutions of beta-agonists into the saline bags after withdrawal of the same volume of saline. Fluid flow rates were maintained at a level such that each infusion pack lasted slightly longer than four days. Packs were changed at the same time on every fourth day and any residual fluid in the existing pack was rapidly infused before replacement with the new one. The reserve fluid ensured that in the event of slight random increases in flow rate, the infusion packs would not empty before the appointed replacement time. The procedure for preparation and exchange of infusion packs is described in Appendix 1.

2. A modified system for the continuous intraruminal infusion of anthelmintics into growing lambs.

The infusion system developed for the continuous intravenous administration of beta-agonists to cattle was adapted to provide continuous intraruminal infusion of anthelmintic to sheep. The intraruminal infusion system was essentially similar to that for intravenous infusion except that Tygon catheter was replaced by a Foley catheter (22 Fr, Warne Surgical Products, Andover, Hants). The connector from the marzinal tubing was inserted into the top of this catheter and was secured by a small tie. A rubber disc, approximately 5 cm in diameter fitted tightly around the catheter and was positioned against the sheep's skin at the insertion site preventing the catheter from invading the rumen and also minimizing leakage of rumen fluid. The Foley catheter is illustrated in Figure 7 and Plate 4.

During infusion sheep were restrained in standard metabolism stalls. Each sheep was fitted with a dog collar and was chained to the front of the stall. In most cases this prevented animals chewing the

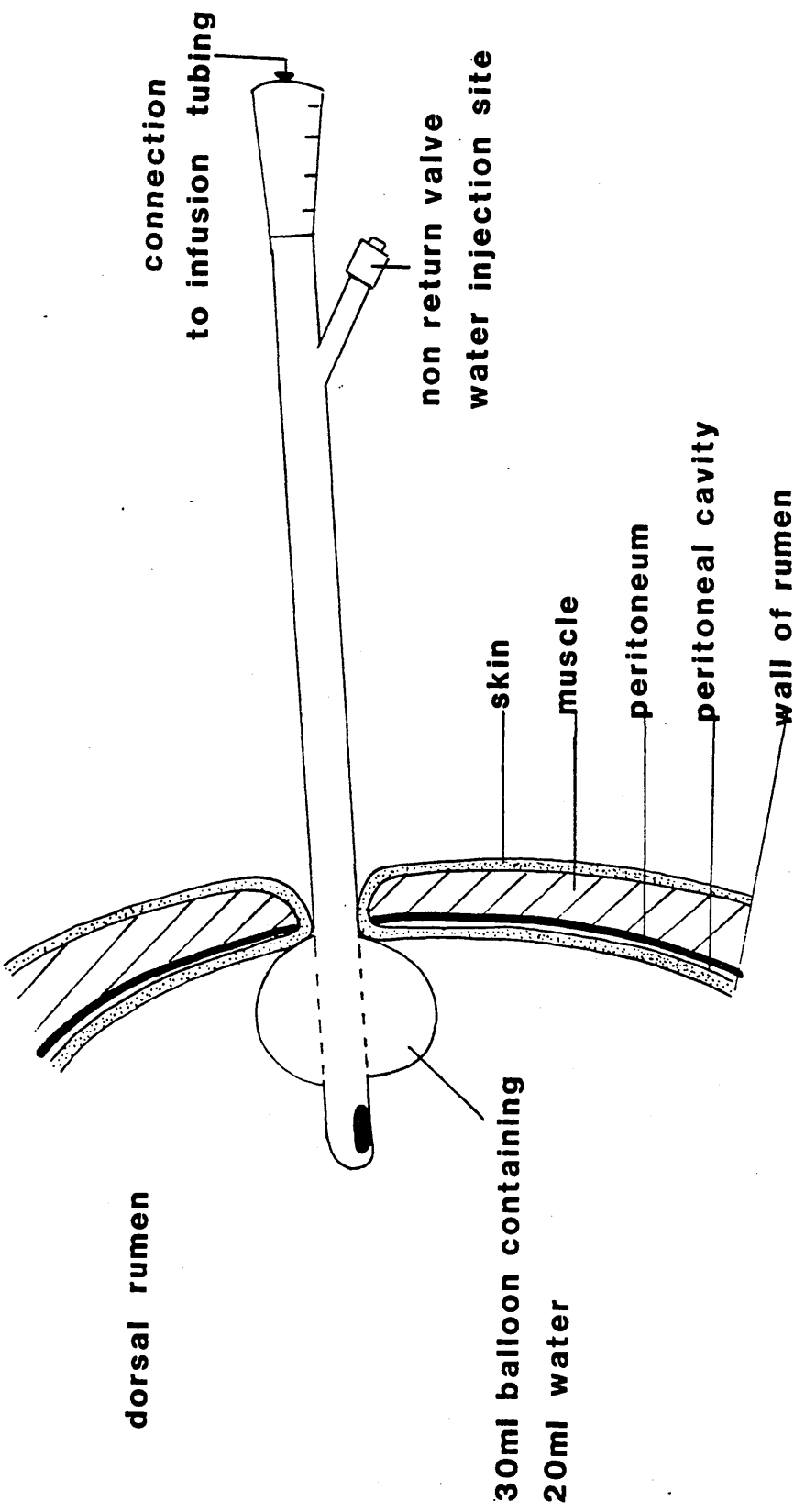


Figure 7. A diagramatic representation of a Foley catheter inserted into the dorsal rumen of a sheep.

Plate 4. The position of the inserted Foley catheter.

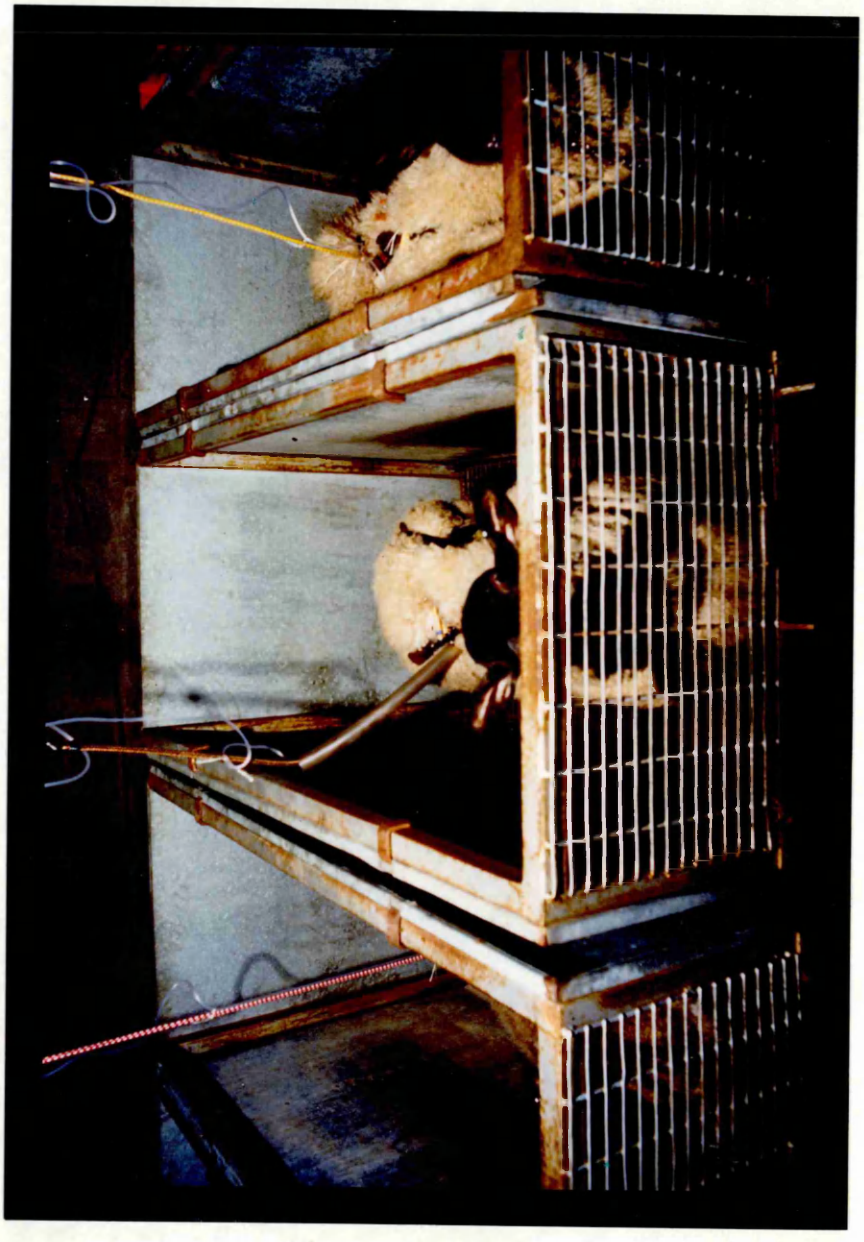


Plate 5. Polythene protector for infusion tubing during the continuous intraruminal infusion of levamisole into lambs.

infusion tubing. Where this was a problem however, the elastic strap and accessible infusion tubing was surrounded by a thick piece of protective polythene tubing, as shown in Plate 5.

3. Daily routine during infusion studies.

Infusion studies involved a fairly intensive daily routine in addition to that required for digestibility and nitrogen balance studies. Greater care and vigilance was required for intravenous compared to intra-ruminal infusion as continued asepsis in the infusion system was essential and the presence of a significant air lock could prove fatal to one of the experimental animals. Problems such as blocked catheters were also more difficult to resolve for the intravenous infusion system.

During intravenous infusion catheters were inspected at least four times per day at 07.30h, 12.00h, 16.00h and 21.00h. It was important to check that the insertion site was dry, dampness being indicative of a cracked and leaking catheter or possible infection. The connection between the catheter and infusion tubing was tested and tightened if found to be loose. The sutures to the skin were checked to ensure that they provided continued firm anchorage of the catheter. These sutures tended to work loose through time with exfoliation and required periodic replacement. Bandaging over the insertion site also required regular replacement.

During intraruminal infusion catheters were inspected three times per day at 07.30h, 16.00h and 21.00h. Both the connection between the catheter and the infusion system and the anchorage of the catheter in the rumen were tested. The water filled balloons of the Foley catheters tended to deflate through time and occasional injection of additional water was required. The rubber of some balloons perished through time, necessitating catheter replacement. At 07.30h and 16.00h the intraruminal insertion site was bathed with antiseptic solution (Hibitane), thoroughly dried and sprayed with antibiotic (Polybactrin).

At times of catheter inspection, infusion tubing was also inspected to ensure that it had not been chewed. During intravenous infusion studies particular attention was also paid to air locks in the infusion tubing and any of significant size were removed as described in Appendix 1.

Following catheter inspection, observation was made of the infusion packs and the peristaltic pumps. It was important to check

DATE	TIME	BAG WT. (gm)	FLUID INFUSED PRE. 24hrs (gm)	OBSERVATIONS	INITS.
21/11	430pm	1070	—	Beg Per IV	LMT
22/11	830am	950	180		LMT
22/11	430pm	880	190		LMT
23/11	8am	760	194		LMT
23/11	430pm	680	200		LMT
24/11	8am	580	180		LMT
24/11	430pm	500	180		LMT
25/11	830am	370	206	PTC	LMT
25/11	430pm	1060	—	New bag	LMT
26/11	830am	940	180		LMT
26/11	430pm	860	200		LMT
27/11	830am	720	220		LMT
27/11	430pm	660	200		LMT
28/11	830am	520	200		LMT
28/11	430pm	440	220		LMT
29/11	830am	330	190		LMT
29/11	430pm	1060	—	New bag	LMT
30/11	830am	930	195		LMT
30/11	430pm	860	200		LMT
1/12	830am	730	200		LMT
1/12	4pm	660	204		LMT

Figure 8. An example of the daily infusion rate record sheets kept separately for each animal during infusion studies.

Date	Observations	Initials
11/12	Pm Nos 2 + 4 ♂ bloated - stomach tube + BIRP	LMT
12/12	calf No 2 eating slowly. several coughs → slight cough.	
12/12	Nothing to report	
13/12	No 4 → ♂ swelling (cold) on back off sides leg	LMT
14/12	No 9 - swelling (hard) around outer part sleeve of catheter + ♂ fluid under skin	
	Temp 103.4 °F.	LMT
15/12	am No 10 not eating v much	
	No 9 - neck more swollen / temp 101.5	
	Given 8 ml depomycin	LMT
16/12	No 3 - thick mucus material at front of cage (from nose/mouth?)	
	Calf 9 - 8 ml DEPOMYCIN	
17/12	Nothing to report	LMT
18/12	calves 1 + 4 - mycophyt 2.	LMT
19/12	calf No 9 - neck more swollen, hard + ♂ hot. Temp = 103.2. - 8 ml Depomycin.	
	8pm - temp = 104. calf eating well + has normal calf otherwise.	
20/12	No 9 - swelling big + hot Temp 103.9 °F am	
	8 ml DEPOMYCIN. Pm No 9 temp = 104.6	
	calf given 14 ml Terramycin @100 IM. +	
	catheter removed. calf → spoken to.	
21/12	calf No 9 - lump gone down below - still hard + ♂ hot. Temp 103.8 °F am.	

Figure 9. An example of the daily log kept during the continuous intravenous infusion of beta-agonists into cattle.

that a drip formed regularly in the reservoir of each administration set connected to each infusion pack. Absence of dripping indicated a blockage somewhere in the particular infusion line, usually at the catheter. The procedure for flushing catheters is described in Appendix 1. The connectors at the pumphead were checked to ensure that they were at the bottom of their supporting slots. Occasionally the action of the pumphead rotor caused them to move to the top of the slots, slightly reducing the contact between the pumphead tubing and rotor resulting in an increase in flow rate. At approximately 08.30h and 16.30h each infusion pack was weighed and the 24 hour flow rate was calculated from the decrease in pack weight compared to that recorded at a similar time on the previous day. The volume of fluid that had passed through the calibration line was also noted. Separate records for the infusion rate to each animal were kept and problems with the catheters or infusion tubing were recorded thereon. A daily record of general observations was also made and examples of these records are given (Figures 8 and 9).

On every fourth day fresh infusion packs were prepared and exchanged for existing packs. Catheters were flushed at this time if residual fluid in existing packs could not be infused at the expected rapid rate. On every sixth day the pumphead tubing was replaced. During intravenous infusion studies units of pumphead tubing which had been prepared and sterilised were supplied in sterile packs. During intraruminal infusion studies, pumphead tubing units were assembled as required.

Detailed time costing of procedures carried out during infusion studies is given in the appropriate sections of this thesis.

F. Radioisotopic Studies

1. Daily routine

Radioisotopic studies were conducted simultaneously with metabolism studies for the experiments described in Section II. The duration of each study period was 16 days during which time calves were accomodated in metabolism stalls. Each calf was dosed daily per os with 10 ml of 0.75 % (w/v) potassium iodide (KI) to prevent uptake of radioactive iodine by the thyroid gland. The dosing commenced four days prior to injection of radioiodine and continued throughout the study period. Blood samples and sub-samples of the total daily faecal and urinary outputs of each animal were collected regularly for the

measurement of radioactivity. Daily intakes of feed and water were recorded.

2. Preparation of isotopes for injection

(i). Tritiated water (TOH) and ^{51}Cr-chromic chloride ($^{51}CrCl_3$)

Stock solutions of TOH and $^{51}CrCl_3$ (Amersham International, Amersham, Buckinghamshire, UK) were diluted with 0.9% (w/v) physiological saline to give solutions with activities of 11.0 and 7.4 MBq/ml respectively.

(ii). ^{125}I-labelled albumin

Bovine serum albumin (Fraction V, 97% pure, Sigma Chemical Company) was trace-labelled with radioiodine by the iodine monochloride method of McFarlane (1958). The protein in a slightly alkaline medium was treated with iodine monochloride to which the radioactive iodine had been added as a carrier-free iodide. A substitution reaction occurred in which ^{125}I replaced hydrogen in the 3 and 5 positions of the benzene rings of the tyrosine groups.

A 2% solution of bovine serum albumin was prepared by dissolving 600 mg of the freeze-dried protein in 30 ml of physiological saline. A stock iodine monochloride solution was prepared by dissolving 5.00 g of potassium iodide and 3.22 g of potassium iodate in 37.50 ml of distilled water. 37.50 ml of concentrated HCl and 5 ml of carbon tetrachloride were added and the solution was shaken for several minutes. 0.1 M potassium iodide was added dropwise until a faint pink colouration was observed. The solution was then diluted 1:350 with physiological saline giving a concentration of 0.42 mg of iodine/ml as iodine monochloride.

Two glycine buffers A and B (pH 8.5 and 9.0) were prepared by adding molar sodium hydroxide (NaOH) to a molar solution of glycine in 0.25 M sodium chloride until the required pH was attained. Buffer A was used to convert iodine monochloride to the hypoiodite and buffer B provided the alkaline medium necessary for the reaction to occur. Procedure:- 15 ml of buffer B was added to 30 ml of 2% bovine serum albumin solution in a 100 ml conical flask. In a separate flask 6 ml of iodine monochloride was added to 15 ml of buffer A. Approximately 37 MBq of carrier-free radioiodine was added to the buffered iodine monochloride solution and then this was transferred to the buffered albumin solution. Iodination of the albumin occurred instantaneously

and the solution was poured into a dialysis sack and dialysed for 48 hours against two changes of physiological saline.

3. Preparation of standards

A known weight (approx. volume 1 ml) of each of the radioisotopically labelled preparations was emptied into a 100 ml volumetric flask. The contents of the flask were made up to the mark with 0.02 N NaOH. 1 ml of this solution was dispensed into counting vials and made up to 10 ml with 0.02 N NaOH. The standards served as corrections against decay, changes in the sensitivity of the counting equipment and for calculation of the injected dose.

4. Injection of isotopes

Known weights of each isotope solution (approx. volume 10 ml) were injected into each calf via a jugular catheter. A double injection of 74 MBq $^{51}CrCl_3$ and 15 MBq of ^{125}I-bovine albumin followed a single injection of TOH four days earlier. Prior to the injection of TOH food and water were withheld from the calves for 18 hours to standardise water in the gut and enhance equilibrium. The jugular catheter was flushed with physiological saline after each injection and prior to its withdrawal from the jugular vein.

5. Collection and preparation of blood, faecal and urine samples for counting

Blood samples (5 ml) were collected from the opposite jugular vein into heparinized vacutainer tubes 15, 30, 60, 120 and 360 minutes after each injection. Twice daily samples were taken thereafter in the intervening period between the TOH and the $^{51}CrCl_3/^{125}I$-albumin injections and for a five day period following the combined injection. For the remainder of the study period blood samples were taken once daily.

A packed cell volume estimation was carried out on all blood samples after which 1 ml aliquots of plasma were pipetted into counting vials and made up to 10 ml with 0.2 N NaOH.

Total daily faecal and urinary outputs were recorded following the combined injection only. The daily faecal collection was thoroughly mixed and eight weighed subsamples of approximately 10 g (approx. volume 10 ml) were packed into counting vials. Four weighed urine subsamples (approx. volume 1 ml) were transferred to counting vials and

made up to 10 ml with 0.2 N NaOH.

Radioactivity measurements for ^{125}I and ^{51}Cr in the plasma, urine and faeces were determined in an automatic well-type scintillation spectrometer (Canberra Packard Minaxi γ Autogamma 5000 series gamma counter). TOH in the plasma was determined using a liquid scintillation spectrometer (Canberra Packard Tricarb Liquid Scintillation spectrometer). The calculation of cross-over factors was based on the relative count rates of the standard solutions at each photo peak. All samples were corrected for background radiation.

6. Calculation and expression of results

(i) Total injected activity (TIA)

This was calculated by determining the net counts/min/ml of several aliquots of each standard solution. The total standard radioactivity (TSR) was calculated as the product of the mean radioactivity in 1 ml of standard solution (cpm) and the volume of the standard (100 ml).

From TSR it follows that the total activity injected into each animal was;

$$\text{Total injected activity} = \frac{\text{wt of labelled preparation injected} \times \text{TSR}}{\text{wt of labelled preparation in standard (g)}}.$$

(ii) Total body water and body water turnover

Gastrointestinal parasitism often produces an alteration in the body composition of the host animal. Commonly there is an increase in the proportion of water in the carcass and thus the full economic implications of parasitism cannot be gauged from changes in body weight alone. Other features of many parasitisms include the occurrence of diarrhoea and inappetence and hence a reduction in water intake by the host animal.

Total body water, its distribution and turnover can be calculated using the dilution principle. Equilibration or extrapolation methods may be employed. In ruminants the contents of the rumen, reticulum and abomasum may constitute up to 25% of the total body water and because of this equilibration of an injected marker takes between three and four hours. Deprivation of food and water for 18 hours prior to injection of the marker slightly reduces the amount of water present in the gut and so enhances equilibration.

Body water measurements were made using TOH as the injected

marker. From the activity in the plasma of blood samples taken at 15, 30, 60, 120 and 360 minutes post injection the point of equilibration of the marker was determined and the total body water was calculated from the equilibration equation thus;

$$\text{Total body water} = \frac{\text{Total radioactivity injected (cpm)}}{\text{Radioactivity of sample removed at equilibration (cpm/ml)}}$$

Total body water was also calculated by the extrapolation method. The plasma activity of each blood sample taken in the four days following the TOH injection was plotted against time using semi-log graph paper. Extrapolation of the negative slope back to zero time gave an estimation of the plasma radioactivity (cpm/ml) if equilibration could occur instantaneously following injection. Using this value in the previous equation permitted calculation of total body water by the extrapolation method. The biological half-life of the TOH (t1/2) was calculated from the slope of this line permitting calculation of body water turnover thus;

$$\text{Turnover (litres/day)} = \frac{0.693 \times \text{total body water (litres)}}{\text{t 1/2 (days)}}$$

(iii) Plasma Volume

This was calculated by the dilution principle using ^{125}I-albumin as the radioisotopic marker. Equilibration of injected ^{125}I-albumin with plasma took approximately 10 minutes and plasma volume was calculated thus;

$$\text{Plasma volume (Vp)} = \frac{\text{Total injected activity (cpm)}}{\text{cpm/ml of plasma at equilibration}}$$

(iv) Faecal Clearance of ^{51}Cr and ^{125}I-albumin

One of the characteristic features of most gastrointestinal parasitic infections is that they lead to changes in the concentration and distribution of the plasma proteins of their host. Usually these take the form of a depression in albumin and elevations in globulin levels. Current thinking suggests that the depression of albumin levels is primarily due to the leakage of this small blood constituent through a damaged mucosa into the digestive tract from which it is subsequently

excreted. Injection of ^{125}I-albumin and subsequent daily measurement of this marker in plasma and faeces permits calculation of the faecal clearance of albumin. However this parameter is underestimated due to reabsorption of iodine from the digestive tract prior to excretion. Injection of $^{51}CrCl_3$ also permits calculation of plasma losses into the gastrointestinal tract and this marker has the advantage that it is not reabsorbed to any significant extent.

Using semi-log graph paper a graph was plotted of plasma activity of ^{125}I-albumin or ^{51}Cr as a percentage of that at equilibration against time. From this the total plasma activity at the beginning of each 24 hour period was determined. The total faecal activity for each 24 hour period was calculated as the product of mean faecal activity of the daily subsamples (counts/gram of faeces) and total faecal output. Faecal clearance for each 24 hour period was then calculated;

$$\text{Faecal clearance ml/day } (^{51}Cr \text{ or } ^{125}I\text{-albumin}) = \frac{\text{total faecal activity (cpm) over 24 hours}}{\text{total plasma activity at start of 24 hour collection period}}$$

(v) Albumin disappearance (t 1/2)

The biological half-life of the ^{125}I-albumin was calculated from the negative slope of the graph of plasma activity of ^{125}I-albumin against time.

(vi) Fractional catabolic rate of albumin (F (CA))

Increases in the faecal clearance of ^{125}I-albumin may be reflected in elevations of the fractional catabolic rate of albumin. The fraction of the intravascular pool of albumin broken down each day was calculated by the method of Campbell et al (1956) based on the excreted activity in urine and faeces. The excreted isotope in the urine and faeces was assumed to be proportional to the amount of labelled albumin in the intravascular pool where degradation occurred. The fraction of the plasma pool broken down each day was determined from the total daily excreted activity (urine and faeces) and the activity present in the plasma at the beginning of the 24 hour collection period.

$$F\ (CA) = \frac{\text{Total excreted activity (U and F) over 24 hours}}{\text{Total plasma activity at beginning of 24h collection period}}$$

The fractional catabolic rate thus calculated provides an index of metabolism and not an absolute value as steady state conditions rarely exist in parasitised animals.

G. Parasitological Techniques

1. Culture and harvesting of infective larvae

Ostertagia ostertagi and Cooperia oncophora larvae were cultured from the faeces of experimentally infected calves which had previously been reared and maintained parasite-free. Ostertagia circumcincta larvae were similarly cultured from the faeces of experimentally infected sheep. Donor animals were fitted with a body harness and faecal collection bag. The complete daily faecal output was mixed with vermiculite to produce a mouldable consistency. The faeces were then incubated in screw top containers for 12-14 days at 26°C. At the end of this period the containers were filled with lukewarm tap water and kept for three hours to allow migration of larvae into the water, after which contents of the containers were poured through a coarse sieve (60 meshes per inch) to remove gross faecal matter. The fluid containing the larvae was then filtered through two layers of eight inch milk filters (Maxa Filters, A. McCaskie, Stirling) by suction using a Buchner apparatus and suction pump. The filter paper was then removed and placed larval side uppermost on a coarse metal sieve (150 micron pores) which was placed on top of a glass funnel. The funnel was closed at the stem with a piece of rubber tubing and a clip and was filled with lukewarm tap water. Motile larvae were left to migrate through the paper and sieve into the water below where they accumulated at the neck of the funnel and were collected 12 hours later.

2. Preparation and administration of larval doses

The concentration of the larval suspension was determined by examining 40 x 0.025 ml aliquots. The suspension was mixed continuously during this procedure in order to evenly disperse the larvae in the fluid. Once the concentration of larvae had been determined appropriate doses were pipetted into universal bottles, also during continuous mixing, and the volume was made up to approximately 15 ml using tap water. With the exception of experiment IIc larval doses were made up in small batches and, where required, short term

storage was at room temperature (16°C). In experiment IIc larger batches of larval doses were prepared and were stored at 4°C until required.

Larval doses were administered per os after thorough mixing. Each bottle was then half-filled with water, shaken and the rinsings also given to each animal.

3. Faecal egg counts

Faecal samples were either collected directly per rectum or from the faecal collection bags. The samples were examined by a modified McMaster technique (Gordon and Whitlock, 1939). In this technique 3 g of faeces were homogenized with 42 ml of water and the resultant suspension was passed through a 250 micron sieve (Endecotts Test Sieves Ltd., Morden, London). After thorough mixing of the filtrate, two 15 ml aliquots were withdrawn into flat bottomed centrifuge tubes (capacity 15 ml) and centrifuged at 2000 rpm for two minutes. The supernatents from both tubes were discarded and the remaining faecal mass broken up by rotary agitation. One tube was then filled with saturated salt solution to its former level, inverted six times and a volume of the resulting suspension quickly transferred by pipette to fill both chambers of a McMaster slide. The number of eggs under the etched area of the slide were counted and the result multiplied by 50 to give an estimation of the number of eggs per gram (epg) of faeces according to the following calculation:-

3 g faeces in 42 ml gives 1 g in 15 ml
Volume under one square equals 0.15 ml
∴ No. of eggs seen in two squares x 50 = No. of eggs/gram

4. Necropsy procedure and estimation of parasite burdens

Sheep were killed by electrical stunning and cattle were euthanased with a captive bolt and pithing. Immediate exsanguination followed by severing each jugular vein. At this time a blood sample was collected. Where Ostertagia sp was the only parasite present the omasum and abomasum were removed intact together with approximately 0.5 m of small intestine. For cattle with a mixed infection of Ostertagia ostertagi and Cooperia oncophora the pyloric sphincter was ligatured on opening the abdominal cavity and the omasum and abomasum were removed together with the entire small intestine. A faecal sample was

obtained from the large intestine.

Abomasa were prepared by firstly removing the omental fat, omasum and remaining duodenum. The abomasum was opened by an incision along its greater curvature and the contents were collected into a graduated bucket. A sample of abomasal fluid (approximately 200 ml) was retained for pH determination using a Radiometer pH meter type PHM 26c (Electric Measuring Instruments Ltd. Copenhagen, Denmark). A pH stick (Gallenkamp) was also used. The abomasal folds were carefully washed under a slow stream of water into the bucket and the volume made up to a standard two litres for sheep and four litres for cattle. After thorough mixing, two 200 ml aliquots of abomasal washings were withdrawn into jars, 10 ml of 40 % formalin was added and the samples were stored for subsequent microscopic examination.

The washed abomasum was laid out on a dissecting board and a nodulation score was ascribed. Cattle abomasa were then cut in half longitudinally and the mucosa from one half was scraped off with a sharp post mortem knife. Entire sheep abomasa were scraped. The mucosal scrapings were digested in three times their own volume of a pepsin-hydrochloric acid mixture (Herlich, 1956) at 42°C for six hours. The digested mixture was then made up to four litres for cattle or two litres for sheep and two 200 ml aliquots were withdrawn, formalized and stored as described previously.

In cattle experimentally infected with Cooperia oncophora the retained small intestine was cut into three sections, each of approximately 6 m which roughly corresponded to the duodenum, jejenum and ileum. Each section was opened along its length using a pair of sharp scissors. The intestinal contents from the separate sections were collected into graduated buckets and the inner surfaces washed under a slow stream of water until a volume of four litres of washings was attained in each case. Two 200 ml aliquots were withdrawn, formalized and stored as for abomasal washings.

To one of each pair of washings and digest samples from each animal 2-3 ml of iodine solution (720 g potassium iodide and 450 g iodine crystals per litre of distilled water) was added and mixed thoroughly. Ten 4 ml aliquots were withdrawn by pipette into scored petri dishes and 2-3 ml of sodium thiosulphite was added to clear the background leaving the parasites stained with iodine. The aliquots were examined under a dissection microscope and parasites present were classified as adult male or female, developing fourth or fifth stage

larvae or early fourth stage larvae on the basis of bursal or vulvular development, the presence of a sheath projection and size respectively. The sum of the parasites present in the ten aliquots from each original subsample was multiplied by the appropriate dilution factor (50 for sheep, 100 for cattle) to give the total number of parasites present in the original two or four litres.

5. Pathology

Tissue samples were excised from the duodenum, jejunum and ileum before opening the gut while those obtained from the abomasum were taken immediately following opening along the greater curvature. Samples for light microscopy were fixed in 10 per cent formalin or Carnoy's fluid. Discs of tissue were also fixed in paraformaldehyde-gluteraldehyde for a minumum of 24 hours for scanning electron microscopy (SEM).

Formalin fixed tissues were post fixed in corrosive formol and processed in a standard 24-hour cycle. Sections (5 um) were stained with haemotoxylin and eosin for general histopathology and to demonstrate globule leucocytes, and Alcain blue (pH 1 and pH 2.5) to demonstrate goblet cells. Carnoy fixed tissue was similarly processed and sections stained with Astra blue and Sofranin to demonstrate mast cells.

For SEM, specimens were coated with gold in an Emscope sputter coater, before viewing in a Phillips 501 B Scaning electron microscope.

6. Morantel sustained release bolus (MSRB)

During the parasitological investigations described in Section II of this thesis the morantel sustained release bolus (MSRB, Paratect, Pfizer Inc., Sandwich, Kent, England) was used to limit parasitic infection. The bolus was first described by Jones (1983) and comprised of a stainless steel tube (9.1 cm in length and 2.7 cm in diameter) fitted at each end with a sintered polythene disc impregnated with cellulose triacetate hydrogel. The tube was encased with a heat shrunk polyolefin band and each end had a circumferential crimped aluminium cap. It was filled with a blend of 22.7 g morantel tartrate (equivalent to 13.5 g morantel base), 14.7 g polyethylene glycol 400 and 4.1 g sodium metaphosphate and was reported by Jones (1983) to have been designed to release approximately 90 mg of base per day for at least 60 days. Experimental results recorded in the same publication

in fact show a declining release rate through time and a residue of 4.69 morantel still present in the bolus after 90 days.

H. Blood biochemical techniques

Blood samples for biochemical analysis were obtained directly from the jugular vein into heparinized vacutainer tubes (Beckton Dickinson Ltd.).

1. Plasma pepsinogen estimation

Plasma pepsinogen was estimated by the method described by Edwards et al (1960). Plasma was incubated with bovine serum albumin (BSA) substrate at pH 2 for 24 hours and the phenolic amino acids liberated (tyrosine) were estimated using Folin-Ciocalteu's reagent. Corrections were made for the normal (non-incubated) content of tyrosine-like substances and also the release of these substances from the BSA substrate by acid-hydrolysis during the incubation period.

Whole blood was centrifuged at 2000 rpm for 20 minutes. Two 0.5 ml aliquots of plasma were withdrawn and added to 2.5 ml of acidified 2% BSA (pH 1.5) in two universal bottles labelled 'Test' and 'Control'. The 'Test' was incubated for 24 hours at 37°C while the 'control' was immediately precipitated by the addition of 5.0 ml of trichloracetic acid (TCA).

BSA blanks were set up (in duplicate) so that corrections could be made for the normal (non-incubated) content of tyrosine-like substances and also for the release of these substances from BSA alone. For each blank 2.5 ml of acidified BSA and 0.5 ml distilled water were dispensed into universals. Two universals (incubated-blanks) were incubated for 24 hours at 37°C and the other two (unincubated-blanks) were immediately precipitated with 5.0 ml 4% TCA.

After mixing precipitated 'controls' and 'unincubated-blanks' were allowed to stand for 10 minutes to ensure efficient floculation of the precipitate. They were then filtered through a Whatman No.44 filter paper. Following incubation 5.0 ml of 4% TCA was added to 'Tests' and 'incubated-blanks' and the resulting suspensions were similarly filtered. 2.0 ml of each filtrate was pipetted into a suitably labelled 50 ml conical flask containing 20 ml of 0.25 N NaOH. A reagent blank was set up containing 2.0 ml distilled water and 20 ml 0.25 N NaOH. Flasks were also set up (in duplicate) containing 2.0 ml of each working tyrosine standard (o.2 µ mols and 0.4 µ mols). Three

ml of diluted Folin-Ciocalteu's reagent was added to all flasks and after standing for 30 minutes the blue colour was read in a spectrometer at 725 mu.

Results were calculated by firstly subtracting the reagent blank from all readings. All readings were converted to u mols tyrosine using a factor calculated from the spectrophotometer readings of the tyrosine standards. The total release of tyrosine on incubation was calculated by subtracting the value of non-incubated BSA and plasma from that from incubated BSA and plasma (A). Similarly the release of tyrosine from BSA substrate due to incubation alone (i.e. no pepsinogen) was calculated by subtracting the value for non-incubated BSA alone from that for incubated BSA alone (B). The subtraction A - B gave the u mols of tyrosine released on incubation of the equivalent of 0.125 ml serum for 24 hours with substrate. (2.5 ml serum originally made up to 15 ml from which 6 ml containing 2.5 x 6/15 = 1.0 ml serum was withdrawn for incubation. Following precipitation, total filtrate volume approximated 16 ml from which 2 ml was withdrawn for addition of Folin-Ciocalteau's reagent. This contained 2/16 x 1.0 = 0.125 ml serum). The amount of tyrosine released per 1000 ml plasma per minute (International Units) was the product of 5.56 and the μ mols released.

$$\text{i.e. } \mu \text{ mols released} \times \underbrace{\frac{1000}{0.125}}_{\text{volume adjustment factor}} \times \underbrace{\frac{1}{24 \times 60}}_{\text{time adjustment factor}}$$

2. Total serum protein, albumin and urea

These were estimated by continuous flow analysis (standard Technicon Auto Analyser II methods).

3. Haematocrit

Haematocrit estimations were performed using an electronic particle counter (Model ZF, Coulter Electronics, Harpendon, Herts.). The machine was calibrated daily using standard blood sample of known value (Coulter Electronics).

I. Statistical analysis

Statistical analysis of the experimental results contained in Sections I and II of this thesis was performed on a microcomputer using an interactive analysis of variance programme and 'MINITAB' (Ryan, Penn

State University) respectively. A formal test (F-test) was applied in order to ascertain which factors significantly contributed to the total variation observed. Where treatment effects were shown to be significant mean values were compared using a multiple comparisons procedure. For the experiment described in Section III an analysis of variance was carried out for each data set. For parasitological parameters this was performed using 'MINITAB' on a microcomputer. Analysis of digestibility and nitrogen balance data was performed using the programme 'RUMMAGE' (PLU, Edinburgh) on an ICL 2988 computer. The model incorporated three main effects and allowed for first and second order interactions.

For some parasitological assessments where population distributions were not normal non-parametric procedures (e.g. Mann-Whitney) were carried out, also using the 'MINITAB' programme.

SECTION I

MANIPULATION OF BOVINE METABOLISM USING EXOGENOUS BETA-ADRENERGIC AGONISTS

Literature Review

Introduction

Physiological Mode of Action

- Lipid Metabolism
- Protein Metabolism
- Indirect Effects

Effects on the Carcass Composition and Performance of Farm Livestock

Meat Quality Effects

Effect of a Withdrawal Period

Side Effects of Beta-Agonists as Repartitioning Agents

Commercial Future for Beta-Agonists

Experiment I

Nitrogen Balance and Digestive Efficiency Studies conducted with Young Steers given Repartitioning Agents

LITERATURE REVIEW

Introduction

In recent years there has been an increasing awareness of health problems which might be associated with high levels of dietary fat. This growing public concern has been fuelled by two reports linking excessive intake of dietary lipid with coronary heart disease (National Advisory Committee on Nutrition Education 1983; Department of Health and Social Security 1984). In the UK approximately thirty three percent of the fat consumed is derived from meat (Jones, 1985). Carcass fat in farm livestock can be limited nutritionally by restrictive feeding but this practice is associated with undesirable reductions in growth rate. Slaughtering at an earlier stage of physiological maturity results in leaner carcasses as fat is a late maturing tissue. However as meat processing costs have risen the tendency has been to produce heavier carcasses which are incompatible with the requirement for lean meat. Control of growth has been achieved chemically using exogenous steroids and/or feed additives but the use of such substances is now limited due to the body of public concern over residue accumulation.

More recently pharmacological agents have been identified which increase the accretion of lean tissue and limit the deposition of fatty tissue in growing animals. These substances, termed 'repartitioning agents', are beta-adrenergic agonists and are similar in structure to epinephrine (adrenalin). Perhaps the best known are clenbuterol (benzyl alcohol, 4-amino-R-(t-butyl-amino) methyl-3,5-dichloro) and cimaterol (anthranilonitrile, 5-(1-hydroxy-2-(isopropylamino)ethyl)-) shown in Figure 10.

Physiological mode of action

The exact mechanism by which synthetic beta-adrenergic agonists exert their repartitioning effect has yet to be fully elucidated. These compounds may exert a direct effect on metabolism and/or an indirect effect by alteration of circulating hormone levels. Receptor systems activated by sympathomimetic amines have been classified as alpha, $beta_1$, or $beta_2$ types dependent on the response elicited by various agonists and antagonists (Lands et al, 1967). It was considered that activation of $beta_1$ receptors was associated with lipolysis and cardiac stimulation, activation of $beta_2$ receptors

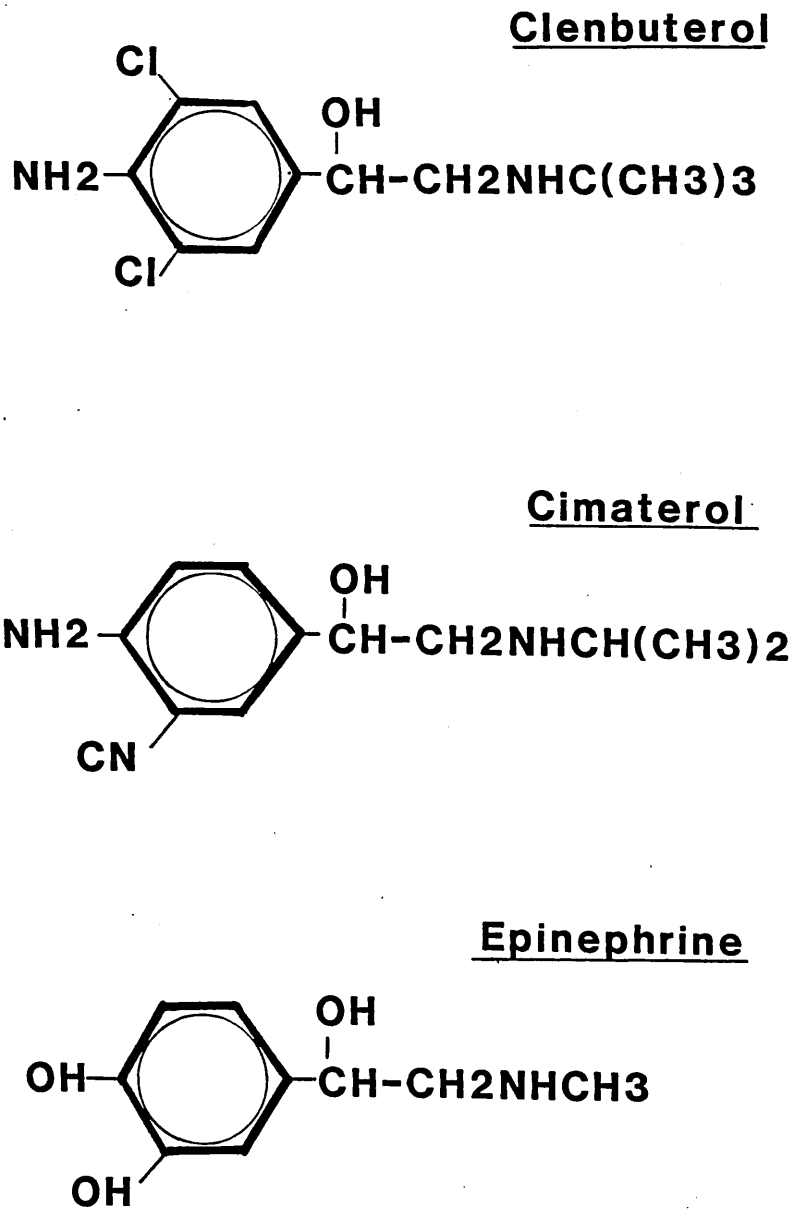


Figure 10. The chemical structures of some beta-adrenergic agonists.

brought about bronchodilation and vasodepression, and that alpha-receptor activity played an important role in vasopressor action, intestinal relaxation, contraction of the vas deferens (rat) and stimulation of the pregnant uterus. More recent evidence however suggests that lipolysis and also brown adipose tissue thermogenesis are mediated by both $beta_1$ and $beta_2$ receptor systems (Belfrage, 1978). The synthetic compounds which stimulate body protein deposition and reduce the proportion of body fat have been loosely classified as $beta_2$ adrenergic agonists.

The proportions of alpha and $beta_2$ receptor types varies between tissues and thus the strength of the response to agonist stimulation also varies. Beta-receptors are present on cell membranes and form one component of the hormone sensitive adenylate cyclase system. The other components of this system are a catalytic moiety which converts adenosine 5'-triphosphate (ATP) into adenosine 3':5'-cyclic monophosphate (cAMP) and a guanine nucleotide regulatory protein (N protein). Activation of beta-receptors by beta-agonists stimulates the membrane bound enzyme adenylate cyclase and this action leads to the intracellular accumulation of cAMP (Stiles et al, 1984). In adipocytes intracellular protein kinase and triacylglycerol lipase are also stimulated (Fain and Garcia-Sainz, 1983). In the presence of cAMP inactive protein kinase disociates into regulatory and catalytic subunits, the latter of which catalyses the ATP dependent phosphorylation of its enzyme substrate. Phosphorylation generally activates enzymes involved in store breakdown and inactivates those involved in store deposition (Vernon and Peaker, 1983). It may be hypothesised that this mechanism promotes lipolysis resulting in liberation of free fatty acids which are then used as an energy source sparing amino acids otherwise destined for gluconeogenesis for protein production.

The direct action of beta-adrenergic agonists on muscle may result in fibre hypertrophy via a decrease in protein degradation and/or an increase in accretion (Ricks et al, 1984a). The evidence in support of this hypothesis is largely equivocal. Detailed investigations of both lipid and protein metabolism have been undertaken with often conflicting results, and the possibility of an indirect effect of beta-agonists via alterations in circulating hormones cannot be discounted.

Lipid metabolism

Lipolysis is the major pathway for the release of free fatty acids and glycerol from adipose tissue. Triglyceride lipase is the rate determining enzyme and it is controlled by an enzyme cascade which involves adenylate cyclase and a protein kinase (Galton and Wallis, 1982). Beerman et al (1985a) demonstrated a 60% increase in plasma free fatty acid concentration over controls in lambs fed the beta-agonist cimaterol for periods of six or twelve weeks. There was a simultaneous decrease in plasma insulin and an increase in thyroxine levels. Intravenous infusions of beta-agonists similarly produced increases in circulating free fatty acid levels in cattle (Blum et al, 1982) and the response was enhanced by temporary starvation. Increased plasma free fatty acids suggest that beta-agonists stimulate lipolysis. Indeed Eadara et al (1987) concluded that 'cimaterol selectively stimulates lipolysis in white adipose tissue and lipoprotein lipase activity in skeletal muscle with no major changes in de novo fatty acid synthesis'.

In contradiction to these findings is a study by Grisdale-Helland et al (1986a) in which no change in plasma fatty acid concentration was observed during infusion of cimaterol into lambs. Plasma insulin concentrations were significantly increased. Duquette and Muir (1985) incubated rat adipose tissue with several beta-agonists including clenbuterol and measured rates of lipolysis and lipogenesis using radiolabelled (^{14}C) acetate and glucose. They concluded that clenbuterol was apparently 5-10 times more potent as an anti-lipogenic than a lipolytic agent. In a similar study involving porcine adipose tissue Mersmann (1987) demonstrated that clenbuterol failed to stimulate lipolysis and concluded from the overall results that the in vivo effect of clenbuterol on adipose tissue was indirect and that there was no interaction with adipose beta-receptors.

The anti-lipogenic activity of clenbuterol is implicated in in vivo studies by Hamby et al (1986). Rambouillet X Finn crossbred wether lambs received 0 and 2 ppm clenbuterol in their diet for approximately 40 days prior to slaughter. Carcass characteristics were compared with those of a similar group of lambs slaughtered at the beginning of the feeding period. It was found that subcutaneous fat thickness had increased by 88% in the control group compared to the initial kill group, but remained unchanged in the clenbuterol treated group. Most investigations into the effects of beta-agonists on

carcass characteristics have not employed an initial slaughter group and so it has been impossible to differentiate between reductions in fatness due to stimulation of lipolysis or depression of lipogenesis. It is interesting that in studies where increased plasma free fatty acids have been observed, the beta-agonist involved has been cimaterol whereas anti-lipogenic activity has generally been attributed to clenbuterol. It is possible that these two beta-agonists act in slightly different ways to achieve the same effects on carcass fatness.

It has been reported that beta-agonists may stimulate lipogenesis and at the same time increase lipolysis. This would result in an increased lipid turnover rate and a consequent reduction in the total energy available for fat deposition. Stimulation of the adrenergic system is known to increase metabolic rate and thereby increase energy expenditure. Williams et al (1987a) estimated that over a 100 day treatment period with clenbuterol the energy expenditure of calves was increased by a mean of 12% (calculated from the total energy intake and the energy retained in the carcass). Increased energy expenditure coupled with increased energy retained as protein is an alternative mechanism for reducing the proportion of total energy available for fat deposition.

Protein metabolism

On the basis of comparative slaughter experiments with calves (Williams et al, 1987a) and similar studies with rats (Reeds et al, 1988) it would appear that the protein anabolic properties of beta-agonists are mainly confined to skeletal muscle. The effect apparently predominates in type II muscle fibres which are characterized as fast contracting mixed glycolytic/oxidative fibres, as opposed to type I fibres which are characterized as slow contracting and oxidative. This was demonstrated by Hamby et al (1986) who noted the tendency for muscle from clenbuterol fed sheep to utilize glucose at faster rates than controls, to have a reduced lipid content and to fail to esterify radiolabelled palmitate at measurable rates. Together with the marked hypertrophy of the muscle these characteristics are indicative of a greater proportion of type II muscle fibres. Following incubation of rat muscle with clenbuterol, Reeds et al (1986) observed an increase in skeletal muscle protein and RNA without any increase in DNA and concluded that the increase in muscle protein mass

arising from beta-agonist treatment was due to muscle hypertrophy rather than hyperplasia. However in certain selected muscles from sheep an approximately equal increase in the cross-sectional area of both type I and type II muscle fibres has been observed (approximately 28%) with an increased percentage of type II fibres present (Beerman et al, 1985b, Beermann et al, 1987). This effect was not apparent in all muscles examined and suggests that the increase in size of some muscles at least results from hypertrophy of both fibre types coupled with selective hyperplasia of type II fibres. The proportion of type I and type II fibres in muscles, together with the degree of maturity attained is likely to influence the magnitude of response to beta-adrenergic stimulation.

The mechanism for adrenergic stimulation of muscle hypertrophy has yet to be identified. In rats given twice daily subcutaneous injections of clenbuterol (1 mg/kg bodyweight) for seven days, the fractional rate of protein synthesis was apparently increased by 34% (Emery et al, 1984) and it was considered unlikely that this was entirely due to an increased intake of metabolizable energy compared to the controls. However the weight of evidence suggests that the major long-term effect of beta-agonists on muscle protein metabolism is a reduction in the rate of protein degradation. MacRae et al (1986) measured plasma leucine turnover and oxidation in sheep at intervals up to 20 days after the start of treatment with clenbuterol. Leucine flux was initially unaltered, was elevated temporarily by day 11 indicating a transient rise in synthesis, whereas leucine oxidation (which can be considered to measure overall amino acid catabolism) was significantly lower on days 4, 11 and 18 as urinary N excretion decreased and N retention increased. The reduction in leucine oxidation may be representative of a reduction in protein degradation. In vitro incubation of muscle cell cultures from the legs of twelve-day old broiler chicken embryos with cimaterol demonstrated no effect on the rate of incorporation of ^{3}H-leucine into the soluble protein fraction and slight but significant increases in the incorporation rate into myosin heavy chains. Thus the apparent effect on protein synthesis was small. However 10^{-7} M cimaterol caused significant increases in the quantity of the myofibrillar fraction, the myosin heavy chain and the total cellular protein in the muscle cultures. Extra protein was deposited in the absence of any marked change in the rate of synthesis and the most probable cause is a reduction in the rate of degradation

(Young et al, 1987).

Similar conclusions may be drawn from studies using sheep (Bohorov et al, 1987) and calves (Williams et al, 1987a). After 37 days of clenbuterol treatment (10 mg/kg diet) fractional rates of protein synthesis in wether lambs were unchanged but protein acretion was increased indicating that degradation rates had fallen. Similarly after 75 days of clenbuterol treatment the fractional degradation rate of protein in calves was only 65% of the control values. Grisdale-Helland et al (1986b) concluded from whole body protein turnover studies in fasted sheep that catecholamine like substances, including cimaterol, exerted their anabolic effect by differential suppression of both endogenous synthesis and degradation of protein.

A distinction must be made between the effects of beta-agonists occurring in skeletal muscle and those occurring in non carcass components. Williams et al (1987a) reported that in clenbuterol treated calves nitrogen retention in the carcass was increased but that retained in the gut, hide and organs was reduced. Jones et al (1985) also noted a reduction in the weights of heart and kidneys of pigs treated with cimaterol. The mechanism for this redistribution of nitrogen within the body has yet to be elucidated.

Indirect effects

Various authors (eg Mersmann 1987, Mirbahar & Eyre, 1985) have suggested that the physiological responses induced by exogenous beta-agonists do not arise from direct stimulation of beta-receptors. It may be summised that the action of these substances is mediated via alterations in circulating hormone levels particularly insulin, growth hormone and thyroxine. Insulin has a known lipogenic effect and is the primary hormone responsible for glucose entry into cells prior to metabolism. Mean plasma insulin levels increase with increasing age and adiposity and marked diurnal fluctuations are observed associated with feeding periods. Ruminant white adipose tissue has a greater sensitivity to insulin than that from non-ruminants. In the fed state insulin plays an important role in maintaining proteolysis at a low level, its mode of action being to reduce the formation of lysosomes by which muscle protein degradation is effected (Lindsay, 1983). Both growth hormone and thyroxine influence protein synthesis and thyroxine is also thought to have a regulatory role in the sensitivity of adipose tissue to beta-agonists (Fain & Garcia-Sainz, 1983).

The evidence for alterations in circulating hormone levels during exogenous beta-adrenergic stimulation is equivocal. Beermann et al, (1985a) reported a 50% reduction of insulin and a 25% increase of thyroxine over controls in lambs receiving 10 ppm cimaterol in their diet. However Grisdale-Helland et al, (1986a) reported increases in the plasma insulin concentration of lambs receiving a cimaterol infusion (69 ng/kg/min).

Chronic treatment of rats with clenbuterol did not affect plasma insulin, growth hormone or triiodothyronine levels (Emery et al, 1984). It has been noted (Stiles et al, 1984) that the symptoms of hyperthyroidism and excessive beta-adrenergic stimulation are similar. Thyroid hormones may increase beta-receptor number or affinity or affect the coupling mechanism within the cell membrane.

More rigorous tests suggest that the involvement of growth hormone in mediating beta-adrenergic stimulation is unlikely. From independent studies examining the cellular aspects and composition of skeletal muscle growth in lambs fed 10 ppm cimaterol for seven or twelve weeks and in swine receiving up to 200 ug/kg/d porcine somatotrophin (growth hormone) from 45 to 100 kg Beermann et al (1987) concluded that, while both agents increased muscle protein deposition, somatotrophin-induced muscle hypertrophy appeared to act through satellite cell proliferation whereas cimaterol induced hypertrophy did not. Thus if an exogenous beta-agonist and growth hormone apparently exert their effect in different ways it is unlikely that the action of the beta-agonist is mediated via endogenous growth hormone. However, Williams et al (1987b) did note a synergistic effect between exogenous bovine growth hormone and clenbuterol in young calves.

Thiel et al (1987) conducted an experiment using normal and hypophysectomized rats given cimaterol at between 0 and 100 ppm in the diet for 14 or 21 days, to determine if the repartitioning effects of cimaterol were dependent on a functional pituitary axis. Muscle weights were increased by approximately 21% in both types of rats indicating that the pituitary axis was not important for the repartitioning effect of this agonist.

The involvement of insulin in mediation of exogenous beta-adrenergic stimulation is more difficult to define. An explanation for the discrepancy between the results of Beermann et al (1985a) and Grisdale-Helland et al (1986a) may lie in the fact that in the former study the sheep were fasted overnight whereas in the

latter blood sampling was carried our for a six hour period immediately following withdrawal of food when background insulin levels would be low and high respectively. If insulin and its antagonist glucogen are involved in the beta adrenergic response they may exert their effect via alterations in membrane fluidity. An increase in membrane fluidity would increase the sensitivity of the membrane bound beta-receptors as these normally exist as separate subunits within the membrane which have to become physically associated before they can be subject to stimulation (Houslay, 1985).

Incidentally beta-receptors can be desensitized by a novel method of 'down regulation' in which receptors are sequested away from the cell surface in small membrane bound compartments which remain physically associated with the cell membrane (Strader et al, 1984). Down regulation may also occur as a result of receptor uncoupling (Stiles et al, 1984).

A reduction in voluntary food intake has occasionally been noted following the use of relatively high levels of beta-agonists in pigs (Moser et al, 1984), cattle (Ricks et al, 1984a) and sheep (Baile et al, 1972). This response is more clearly defined in monogastric than in ruminant animals. Voluntary food intake is thought to be adrenergically controlled and it is possible that a reduction in voluntary food intake mediated by exogenous beta-agonists results in reduced levels of circulating insulin (related to feeding behaviour) and hence reduced lipogenic stimulation by this circulating hormone. This sequence of events is however unlikely as significant reductions in fat accretion have been noted in trials where voluntary food intake has been unaltered.

Another possible mechanism by which beta-agonists may exert their repartitioning effect is by a redirection of blood and hence nutrient flow, altering the pattern of nutrient availability between different tissues. Stimulation of beta-receptors on blood vessels leads to relaxation of the smooth muscle and vasodilation (Vernon & Peaker, 1983). Vatner et al, (1986) demonstrated that there were different patterns of beta-receptor subtypes on different bovine tissues, heart being predominantly $beta_1$, lung predominantly $beta_2$ and the large coronary artery being intermediate with a ratio of $beta_1$: $beta_2$ of 1.5-2.0 : 1.0. It is thus possible that if different blood vessels carry varying proportions of $beta_1$ and $beta_2$ receptors subtypes selective vasodilation may occur during exogenous beta stimulation

resulting in a redirection of nutrient flow.

While the exact mechanisms are still uncertain, the repartitioning of nutrients appears to occur as a result of the direct action of beta-agonists on the receptors of both adipose and muscular tissue with alterations in circulating hormones perhaps having a minor effect. Selective vasodilation resulting a redirection of nutrient supply may also be involved.

Effects on the carcass composition and performance of farm livestock

When included in the normal diet of cattle, sheep, pigs and poultry beta-agonists increase protein deposition in the carcass (+ 15% approximately) and reduce total body fat (- 18% approximately). These effects occur in entire males, castrates and females. Summary tables relating to the published work on the effect of beta-agonists on livestock performance and body composition are given by Williams (1987).

Where different levels of beta-agonists have been given there are indications of dose related changes in carcass composition in pigs up to an inclusion level in the diet of 1.0 ppm. In sheep responses have been achieved with clenbuterol up to 2.0 ppm and cimaterol up to 10.0 ppm in the feed (Williams, 1987). Parenteral administration of cimaterol has been shown to have a similar effect to oral administration in altering the body composition of cattle, sheep and pigs (Hanrahan et al, 1986). By either route of administration, the effects of beta-agonists on body fat and protein have been relatively consistent within each commercial livestock class examined.

Studies in which clenbuterol was orally administered to rats (Berne et al, 1985) demonstrated the existence of a strain X treatment interaction. Whilst the agonist achieved consistent reductions in fatness for all four strains of rat examined, the effect on muscle protein acretion was influenced by the rat strain. No such interaction was observed on feeding cimaterol to a variety of sheep breeds with different propensities to fatten (Hanrahan et al, 1986). However in pigs, investigations using heart muscle and adipose tissue have revealed that the Pietrain breed has between 31 and 38% more beta-receptors than the Large White breed (Bocklen et al, 1986). These receptors have equal affinities for beta-agonists and thus the Pietrain breed may be much more responsive to exogenous beta stimulation.

The majority of trials examining the effects of exogenous

beta-agonists on the body composition of commercial livestock have involved animals at a relatively late stage in their development. Baker et al (1984) suggested that the treatment response is enhanced with increased maturity and liveweight. This was confirmed in an experiment by Mersmann et al (1987) which failed to demonstrate any effect of dietary cimaterol at 0, 0.25 or 0.50 mg/kg diet when fed to growing pigs slaughtered at 60 kg liveweight. Dietary cimaterol has proved extremely effective in altering the carcass composition of finishing pigs. However, Williams et al (1986) was able to demonstrate an increase in nitrogen acretion and a reduction in fat in veal calves given 0.1 or 1.0 mg/kg diet clenbuterol from 21 days of age until a slaughter weight of 150-170 kg was achieved. In this trial it was also noted that the increased nitrogen acretion occurred in the carcass rather than in the hide, gut or viscera yielding a beneficial increase in carcass weight at the expense of non carcass components.

Besides the repartitioning of nitrogen between carcass and non carcass components, animals treated with beta-agonists also undergo conformational changes. When lambs were treated with cimaterol individual muscles responded differently so that the hind leg consistently represented a higher proportion of the carcass (Beermann et al, 1986). This effect may have arisen because the hind limb muscles are relatively late maturing and thus may have been influenced to a greater extent by the beta-agonist. The superior development of the hind quarters of treated animals represents a qualitative improvement in the carcass in addition to any quantative changes which have occurred, increasing carcass value.

In studies with broilers (Dalrymple et al, 1984) the repartitioning effect of dietary clenbuterol was more pronounced in female than in male birds. This effect may be related to the tendency for females of any species to have a greater body fat content than males of the same species at approximately equal age and liveweight. If the repartitioning effect of beta-agonists primarily results from nutrient sparing due to increased rates of lipolysis, then one would expect greater repartitioning effects in initially fatter animals. This hypothesis may also provide the explanation for more pronounced repartitioning effects in more mature or heavier animals where initial levels of fatness would also be greater.

In contrast to the relatively consistent action of beta-agonists on body composition, no concensus has been reached concerning their

effect on performance characteristics. The food conversion efficiency (food intake : liveweight gain) would be expected to improve in an animal utilizing a greater proportion of its nutrient intake for protein deposition as this energetically more efficient than the production of body lipid. However exogenous beta-adrenergic stimulation has been shown to increase metabolic rate resulting in elevated maintenance requirements which may offset any benefits of reduced lipid deposition (Beerman et al, 1986). In addition the maintenance cost of 1 kg protein is greater than that for 1 kg fat once deposited (Moser et al, 1986).

Effects on liveweight gain reflect the relative effects on the rates of protein (and water) and fat deposition. In animals showing rapid liveweight gains or nearing maturity, where the rate of fat deposition is high, there is a large potential for the action of a beta-agonist with respect to fat deposition and liveweight gain may be reduced. Alternatively where the magnitude of the protein anabolic effect is greater than the reduction in fat, weight gain will be increased (Williams, 1987).

Investigations have been made into the effect of beta-agonists under different dietary conditions. Young pigs given adequate as opposed to inadequate dietary protein with or without clenbuterol had improved performance and carcass characteristics but these effects were attributable solely to the protein and not to the beta-agonist (Mersmann et al, 1987). In a separate study with lambs Beermann et al (1986) reported that when fishmeal replaced soya in the diet all measures of skeletal muscle development were increased. When cimaterol was given the response with fishmeal was always less than with the soya, although the observed muscularity was always greater (i.e. the effects of fishmeal and cimaterol were not additive). A differential effect occurred in that the effects of cimaterol and fishmeal on the muscles of the fore-limbs were always smaller than those on the hind limbs. This was attributed to the contrasting growth potential of the different muscle groups since at the start of the experiment the muscles of the hind limbs had attained a smaller proportion of mature size than those of the fore-limbs and so had a greater potential for growth.

Meat quality effects

Meat quality is affected by both the handling of animals prior to slaughter and the procedures adopted immediately after slaughter during the processing stage, in addition to any compositional changes in fat and muscle induced during the life of the animal. Wood and Brown (1987) reported no changes in the firmness of shoulder fat or muscle quality of pigs following dietary administration of a beta-agonist during the finishing period. The pH in the longissimus dorsi at 45 minutes post slaughter and the reflectance of light from this muscle (a measure of paleness) did not differ between treated and control groups. Similar results were obtained in pigs by Moser et al (1986) following cimaterol treatment but a significantly increased marbling score was noted. It has been proposed that cimaterol may increase lipolysis and consequently increase the use of free fatty acids as an energy source in muscle. It is possible that some of these free fatty acids may be esterified by muscle adipocytes increasing the marbling score.

Contrary to these findings are the reports of several authors indicating that treatment with beta-agonists increased the proportion of type II fibres in skeletal muscle (Hamby et al, 1986; Beermann et al, 1985b; Beermann et al, 1987). Type II muscle fibres are associated with a reduced muscle lipid content and increasing the proportion of type II fibres also increases the glycolytic activity of the muscle. Post-mortem glycolysis is important in achieving the correct depression in muscle pH and any factor reducing glycogen levels in muscle prior to slaughter consequently limits post-mortem glycolysis. Muscle pH remains high which facilitates bacterial degradation of the meat and in cattle causes dark cutting; i.e. an undesirable darker meat. Reduced muscle glycogen stores are implicated following the administration of cimaterol to lambs, resulting in a higher ultimate pH (+ 0.31 units) 24 hours after slaughter (Beermann et al, 1985c). Measures designed to limit pre-slaughter stress may be necessary in order to conserve limited glycogen stores following beta-agonist treatment and permit normal post-mortem glycolysis .

Apart from adverse effects on post-mortem glycolysis, treatment of animals with beta-agonists may also increase the tendency for 'cold shortening' to occur. This phenomenon is associated with the rapid chilling of hot carcasses while the muscle is in the early pre-rigor condition and may be a feature of carcasses with reduced fat cover. A muscle temperature drop to between 10 and 15°C produces a greater

degree of inter-digitation of actin and myosin and a greater measure of cross-bonding during the onset of rigor mortis (cold shortening) resulting in increased toughness of the meat. This is measured by changes in shear force values. Beermann et al (1985c) found no evidence of increased meat toughness in lambs following cimaterol treatment. However shear force values were increased for meat from lambs treated with clenbuterol (Hamby et al, 1986) and pigs treated with cimaterol (Jones et al, 1985). Cold shortening is primarily a feature of leaner carcasses rather than a specific effect of beta-agonists.

Effect of withdrawal period

A withdrawal period is usually required for the registration of growth manipulating substances in order to ensure that there is no residual metabolic activity when animals are slaughtered for human consumption. There is a dearth of information about tissue residues resulting from the use of beta-agonists. MacRae et al (1986) found that, although clenbuterol treatment increased mean heat production by 0.5 MJ/day for a 40 kg sheep, in the seven day period immediately after its withdrawal, mean heat production was not significatnly different from pretreatment values. This may be taken as an indication that the metabolic effects of beta-agonists are short-lived and that any residual activity of these compounds arises from their sequestration in organs such as the liver.

During a seven day pre-slaughter withdrawal period cimaterol treated pigs displayed a compensatory increase in feed intake and an accelerated rate of fat deposition compared to untreated controls. However at slaughter levels of carcass fat were still less than in untreated animals, while muscularity remained the same as in treated animals not subject to a withdrawal period (Jones et al, 1985b). A five day withdrawal period in poultry did not negate effects on protein and fat in the carcass compared to treatment up to slaughter (Dalrymple et al, 1984).

Side effects of beta-agonists as repartitioning agents

Apart from its repartitioning activity, clenbuterol hydrochloride displays bronchodilator and tocolytic properties and is marketed in two preparations with the trade names 'Planipart' and 'Ventipulmin'. The former is used in cattle and sheep to relax the uterus and delay the

onset of parturition, while the latter is used as a bronchodilator during pulmonary disease in horses. Contra-indications and warnings state that intravenous dosing can produce transient mild muscular tremor and sweating (Owen, 1981).

Tachycardia has been observed following the initiation of clenbuterol treatment in cattle and sheep. The heart rate of two lambs was raised from 150 to 250 beats per minute within four hours after the commencement of an intragastric infusion containing clenbuterol (1.5 mg/day) and deep rectal temperature was elevated by approximately $1^{o}C$ (Herbert et al, 1985). Within three days one lamb had adapted to the drug and on a second challenge the effect on heart rate was greatly reduced. No such adaptation was made by the other lamb necessitating drug withdrawl.

Brockway et al (1987) reported an increase in heart rate and a decrease in blood pressure during the first three days of oral administration of clenbuterol to sheep. Tachycardia may be a reflex reaction in response to vasodepression ($beta_2$ effect) or it may arise directly from $beta_1$ stimulation. The subsidence of tachycardia indicates that the cardiovascular system can become desensitized to beta-agonists. No such desensitisation is apparent in the muscular system.

In addition to an intial cardio-vascular insult, there is a suggestion that the inclusion of cimaterol in the diet of fnishing pigs precipitates a greater incidence of hoof lesions (Jones et al, 1985). However this apparently does not affect locomotive scores (Jones et al 1985, Moser et al, 1986). It is probable that in repartitioning nutrients towards the carcass, cimaterol promotes the formation of a softer type of horn which is more susceptible to damage.

Commercial future for beta-agonists

The beta-agonists currently available (clenbuterol and cimaterol) exert consistent effects on the body composition of commercial livestock increasing protein acretion (a $beta_2$ effect) and reducing fat deposition (a $beta_1$ effect). These effects are probably mediated by pharmacologically distinct mechanisms. $Beta_1$ stimulation of the cardiovascular system resulting in undesirable side effects (tachycardia and elevated metabolic rate) would limit the use of these current compounds commercially, especially in situations where group feeding results in inaccurate control of administration rate. Even

short term tachycardia represents a severe insult to an animals metabolism and this may necessitate the production of analogues of clenbuterol which selectively stimulate the required traits (Asato et al, 1984).

Effects of beta-agonists on fat deposition are likely to be dimished by the requirement for a pre-slaughter withdrawal period but this may be beneficial in terms of reducing the incidence of cold shortening. Current slaughter practices may have to be altered to take account of reduced muscle glycogen levels in beta-agonist treated animals. Finally further work is required to quantify residues, if any, in meat accruing from the use of repartitioning agents.

EXPERIMENT 1

NITROGEN BALANCE AND DIGESTIVE EFFICIENCY STUDIES CONDUCTED WITH YOUNG STEERS GIVEN REPARTITIONING AGENTS

Introduction

The present experiment describes the nitrogen balance and digestive efficiency results obtained when beta-adrenergic agonists were infused intravenously into young growing steers for a period of 15 continuous weeks. Graded doses of bitolterol (4-Methylbenzoic acid 4-(2-((1,1-dimethylethyl)amino)-1-hydroxyethyl)-1,2-phenylene ester; P-toluic acid 4-(2-(t-butylamino)-1-hydroxyethyl)-o-phenylene ester; α-((tert-butylamino)methyl)-3,4-dihydroxybenzyl alcohol 3,4-di-p-toluate) a beta-agonist of unknown potency were compared with infusions of the established agonist clenbuterol or physiological saline. Administration by continuous intravenous infusion rather than by an oral route negated possible deleterious effects of the digestive system on the beta-agonists weakening their physiological effect and thus promoted accurate assessment of dose response relationships.

Materials and methods

Experimental design

The design was that of two identical 5 x 5 Latin Squares using ten calves (1-5, and 7-11). There were five treatments which were changed after each three week period.

Period	Weeks	1	2	3	4	5	7	8	9	10	11
I	0-3	A	B	C	D	E	A	B	C	D	E
II	3-6	E	A	B	C	D	E	A	B	C	D
III	6-9	D	E	A	B	C	D	E	A	B	C
IV	9-12	C	D	E	A	B	C	D	E	A	B
V	12-15	B	C	D	E	A	B	C	D	E	A

The intended treatment rates were:

A = Physiological Saline (0.9% w/v NaCl)

B = Bitolterol 1 mg/day

C = Bitolterol 5 mg/day

D = Bitolterol 25 mg/day

E = Clenbuterol 1 mg/day

Animals

Castrated male Friesian calves aged four months and weighing approximately 120 kg at the beginning of the trial were used. Ten animals were permanently housed in metabolism stalls following an initial acclimatisation period of at least seven days. In addition, five similar calves were group-housed in a straw-bedded pen and maintained as possible replacements.

Feed

Within each trial period all calves, including the replacement group, received the same daily allowance of a grass/cereal mix/mineral complete cube preparation ('Superstar' cubes, Hamlyn Milling, Balgarvie Mill, Scone, Perthshire; mean analysis (10 samples), crude protein (CP) 147, crude fibre (CF) 143, ether extract (EE) 42 and ash 97 g per kg dry matter (DM). For calves in metabolism stalls this was supplemented with barley husks (siftings) to increase long fibre intake and prevent bloat. The two feed components were offered as a uniform mixture. Replacement calves received additional long fibre from barley straw used as bedding material. Daily feed allowance was increased at the beginning and end of the third treatment period in order to maintain an average daily gain of 0.5 kg. Feed refusals were generally small. Water was available ad libitum.

Infusion treatments

Treatments were administered to calves accommodated in metabolism stalls by the continuous intravenous infusion system described in 'General Materials and Methods' (Section E.1). Preparation of infusion packs containing beta-agonists and maintenance procedures for the infusion system were as described in Appendix 1. Treatments were changed after each 21 day period and this was achieved by simply replacing existing infusion packs with full packs containing the new infusion treatments at a standard time (11.00 h).

Digestibility and nitrogen balance studies

The ten calves were permanently housed in metabolism stalls throughout the 15 week experiment. Separate total collections of urine and faeces were made during days 7-14 and 14-21 of each of the five 21 day treatment periods (I to V). Food intake was also recorded at these times. Subsampling and subsequent chemical analysis of food, faecal

and urine samples were as previously described.

Time cost study

A detailed account was kept of the time required to carry out routine procedures associated with both the metabolism studies and maintenance of the infusion system. An estimation was also made of the total time spent in dealing with unanticipated occurrences such as the loss of a catheter.

Statistical Analyses

The experimental results were assessed in an analysis of variance in which three factors namely period, calf and treatment were included in an array of 10 calves and 5 time periods. A Formal test (F-test) was applied and where treatment effects were significant a multiple comparisons test between mean values was performed. For nitrogen balance data only separate partial analyses of periods I-II and III-V were carried out in order to ascertain whether the increased feed input, and consequent increased nitrogen input, from period III onwards markedly affected the potency of either beta-agonist.

Results

General and clinical observations

The clenbuterol treatment was included as an 'internal marker' in that its effect upon various measured attributes was expected, whereas the effect of the bitolterol titration upon these same attributes was unknown. The design of the experiment was based on treatment periods of three weeks duration. Measurements of digestive capacity and nitrogen balance were not conducted until the second and third weeks of any period in order to facilitate the removal of any potential slight carryover effects and ensure that the animals were properly attuned to the current treatment.

The mean growth rate of the calves over the total experimental period of 15 weeks was 0.54 kg/d. Interestingly, these calves visually gave the impression of a marked increase in muscular development, especially of the hindquarters, when compared to calves maintained on a similar diet as possible replacements. During the total course of the trial it was necessary to replace four of the experimental animals for reasons not related to treatment. Replacement calves were introduced at the beginning of a treatment period to allow acclimatisation. The

calves showed no adverse reactions to treatment except that occasionally a mild muscular tremor together with tachycardia were observed in calves receiving the clenbuterol treatment or bitolterol (25 mg/d) when residual fluid was rapidly infused before replacement with a new infusion pack. The catheter for each calf was replaced on average 3.3 times during the course of the 15 week trial. The principal reasons for replacement were fissuring of the PVC catheters used in the preliminary stages of the trial and calf interference.

Infusion Rates

Stock and working solutions were stored at ambient temperature (12°C) with the exception of the stock bitolterol 25 mg/d which was stored at 4°C. The results of analyses of the treatment samples taken from the saline packs at the end of each treatment period are shown in Table 1. Clenbuterol was administered at the intended target rate of 1.0 mg/d. However, the bitolterol treatments were all lower than target throughout all five periods and over the whole experimental period the dose rates for the 1, 5 and 25 mg/d treatments were 50, 74 and 82% of those intended respectively, namely 0.5, 3.7 and 20.5 mg/d. The stock solutions were known to be stable when stored at ambient temperatures (as above). The observed reductions of bitolterol concentrations is attributed to the adsorption onto the plastic surface of the infusion packs.

Table 1. Concentration and infusion rates, intended and determined for clenbuterol and bitolterol during each of the five treatment periods

	Concentration mg/l saline							Dosage rate mg/d	
	Intended	Actual in Period						Intended	Actual Overall
		I	II	III	IV	V	Overall		
Bitolterol	4	2.1	2.4	2.9	2.1	1.3	2.1	1	0.5
Bitolterol	20	15.6	14.2	15.7	14.4	14.1	14.8	5	3.7
Bitolterol	100	82.9	78.7	86.2	85.1	79.6	82.5	25	20.6
Clenbuterol	4	4.4	4.1	4.3	4.0	4.1	4.2	1	1.0

Digestive efficiency

No carryover was apparent when a comparison of the separate results from the two recording weeks in any period was made. Also a separate examination of the data analysed as two 5 x 5 replicate blocks (ignoring the calf effects, which were small) showed there to be no period X treatment effects.

The mean digestibility coefficients for the various proximate fractions of the complete diet given to the calves for days 7-21 inclusive of each period are presented in Table 2 (individual values are detailed in Appendix 2). There were no significant effects of the infusion treatments on the digestive efficiencies of the calves.

A calculation using the mean energy digestibility coefficient of 0.63 shows that the mean intake of 3.88 kg DM feed containing 68 MJ Gross Energy (GE) yields 43 MJ Digestible Energy (DE) which may be calculated (MAFF, 1984) to supply about 35 MJ Metabolisable Energy (ME). The feed thus had an energy concentration (M/D) of 9 MJ ME/kg DM, and for a calf of mean liveweight 150 kg this ration provides sufficient energy to support a calculated daily liveweight gain (DLWG) of 0.5 kg. The actual mean DLWG observed was 0.54 kg.

Nitrogen balance

Feed allowance was increased during the 15 weeks of the experiment to allow for sustained growth of the calves. Consequently, nitrogen intakes were about 50% greater in periods III-V than in periods I and II (approximately 93 and 67 g nitrogen/day, respectively).

Mean nitrogen balance data for days 7-21 inclusive for periods I-II and periods III-V together with all periods combined revealed a highly significant ($P<0.001$) increase in nitrogen retention for clenbuterol (1 mg/d) compared to saline, with a smaller but also significant ($P<0.05$) increase for bitolterol (25 mg/d). These effects were principally derived through significant ($P<0.01$) reductions in urinary nitrogen excretion, faecal nitrogen excretion being apparently unaffected by treatment.

Separate analyses of periods I-II and periods III-V (where N intakes were about 67 and 93 g/d respectively) revealed no significant treatment effect on nitrogen retention for either level of nitrogen intake. However in periods III-V both clenbuterol (1 mg/d) and bitolterol (25 mg/d) treatments resulted in significant ($P<0.01$, 0.05 respectively) reductions in urinary nitrogen excretion compared to

Table 2. Mean digestibility coefficients and SE mean of the proximate fractions of feed given to calves receiving infusions of physiological saline, 1 mg/d clenbuterol and 1, 5 and 25 mg/d bitolterol.

	DM	CP	CF	EE	ASH	NFE	OM	GE
Saline (A)	0.6258	0.5888	0.3709	0.8511	0.4590	0.7131	0.6433	0.6281
Bitolterol 1 (B)	0.6280	0.6006	0.3632	0.8497	0.4608	0.7204	0.6456	0.6378
Bitolterol 5 (C)	0.6249	0.5860	0.3720	0.8568	0.4497	0.7171	0.6433	0.6281
Bitolterol 25 (D)	0.6191	0.5930	0.3460	0.8573	0.4493	0.7221	0.6371	0.6200
Clenbuterol 1 (E)	0.6196	0.5885	0.3506	0.8542	0.4513	0.7110	0.6367	0.6221
SE Mean	0.0048	0.0057	0.0119	0.0049	0.0097	0.0065	0.0052	0.0060
Significance between treatments	NS	NS	NS	NS	NS	NS	NS	NS

Table 3. Mean nitrogen balance (g/d) with SE mean for days 7 to 21 inclusive of calves receiving infusions of physiological saline, 1 mg/d clenbuterol and 1, 5 and 25 mg/d bitolterol.

Period I and II	Intake	Faeces	Urine	Retention
Saline (A)	67.24	28.55	19.57	18.56
Bitolterol 1 (B)	67.24	29.04	18.36	21.04
Bitolterol 5 (C)	66.20	29.65	17.38	18.59
Bitolterol 25 (D)	66.03	29.13	16.81	20.71
Clenbuterol 1 (E)	67.24	29.00	16.61	21.19
SE Mean	0.71	0.74	0.86	0.65
Significance between treatments	NS	NS	NS	NS
Period III to V				
Saline (A)	93.60	37.75	27.19	28.66
Bitolterol 1 (B)	92.99	35.67	25.77	31.54
Bitolterol 5 (C)	93.08	36.46	23.68	32.95
Bitolterol 25 (D)	92.39	36.31	23.20	32.86
Clenbuterol 1 (E)	93.09	36.96	20.34	35.78
SE Mean	1.19	1.01	1.26	1.56
Significance between treatments	NS	NS	AD* AE,BE**	NS
All periods				
Saline (A)	83.07	34.07	24.38	24.62
Bitolterol 1 (B)	82.71	33.02	22.34	27.34
Bitolterol 5 (C)	82.34	33.72	21.40	27.21
Bitolterol 25 (D)	81.85	33.44	20.44	28.00
Clenbuterol 1 (E)	82.77	33.77	18.97	29.95
SE Mean	0.70	0.62	0.92	1.04
Significance between treatments	NS	NS	AC,BE* AD** AE***	AD* AE***

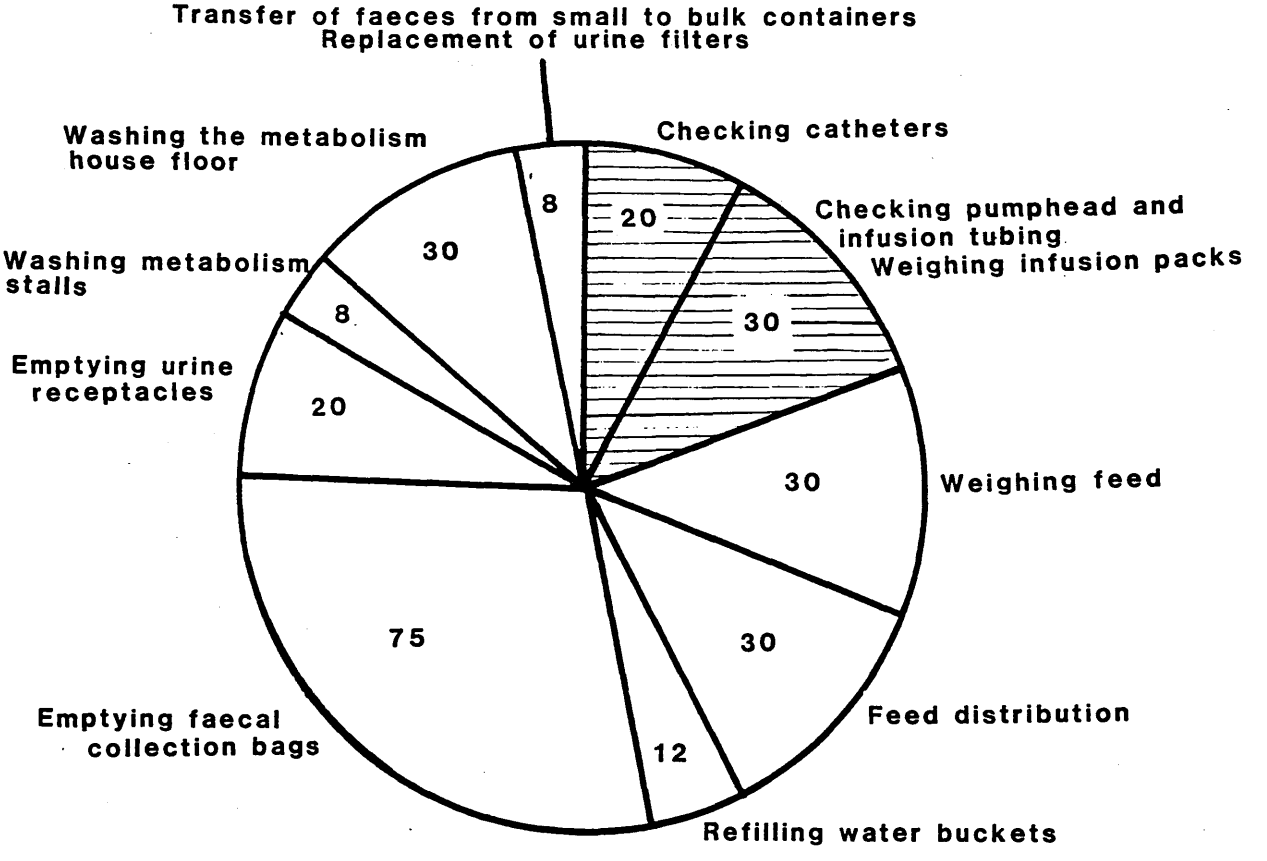
Significance * $P<0.05$, ** $P<0.01$, *** $P<0.001$

saline. Nitrogen balance data expressed in terms of percentage of nitrogen intake and individual calf data are contained in Appendix 2.

Time cost study

The average time required to perform the basic daily routine during this study was approximately four and a half hours per day and a breakdown of the operations involved is given in Figure 11. Of the total routine daily time only 19% was spent in monitoring the infusion system while the remaining 81% of the time was engaged in conducting metabolism studies. In addition to the daily routine, many operations such as changing pumphead tubing and subsampling of urine and faeces were carried out on a regular but not daily basis and the average time cost of these is given in Table 4a. In Table 4b an estimation is made of the considerable time spent in dealing with problems associated with the infusion system.

Of the total time involved in carrying out daily and regular routine procedures during the 15 week experiment, 23.9% was spent on maintenance of the infusion system and the remainder was spent conducting the metabolism studies. The most time consuming operation was emptying the faecal collection bags which accounted for 14.0% of the total time. This was followed by checking the peristaltic pumps and recording flow rates which took 9.7% of the total time. Although checking catheters only took 6.5% of total time actual time spent maintaining the catheters was considerably longer due to the various problems encountered. The time required to deal with such problems was dependent on such factors as environmental temperature (affecting the flexibility of the tubing) and calf cooperation. An unquantifiable but not inconsiderable time was also spent in investigating random decreases in flow rate and straightening kinked catheters. Complete catheter replacement accounted for the majority of non-routine time spent on catheter maintenance as it was the most time consuming operation and was usually eventually necessitated by other catheter problems.



Routine total time cost : 4 hours 23 minutes/day

Values indicate time taken (minutes)
Shading separates metabolism from infusion duties

Figure 11. The time cost of procedures carried out on a daily basis during the continuous intravenous infusion of beta-agonists into cattle.

Table 4. The time cost of additional procedures carried out during metabolism studies with continuous intravenous infusion of beta-agonists into ten steers

a) Regular but not daily procedures

Procedure	Interval (days)	Time Reqd (minutes)
Changing pumphead tubing	6	12
Replacement of empty infusion packs	4	45
Preparation of infusion packs	4	30
Weighing/subsampling bulk faecal and urine collections	7 & 14	360
Washing receptacles for faeces and urine	7 & 14	80
Preparation of faecal slurries	7 & 14	30
Grinding dried faecal and feed samples	7 & 14	70
Washing sample drying trays	7 & 14	20
Labelling sample containers	7 & 14	20
Checking and adjusting calf harness	3	15
Grooming calves	3	45

b) Irregular procedures

Procedure	Occurrence (No. times)	Time Reqd (minutes)
Application of topical ringworm treatment	-	Dependent on infection
*Renewal of bandaging	-	10
Catheter disconnected from infusion tubing	3	15
Chewed tubing - joint with S-type connector	5	20
- complete replacement	2	30
Cracked and leaking catheter	15	30
Needle disconnected from plastic part of catheter	2	20
Catheter looping out of calf's neck with/without stitch detached from skin	12	10
Catheter pulling through outer sleeve	2	12
Teflon catheter lying subcutaneously	8	15
Complete catheter replacement	33	45

*All catheter/tubing problems involved the renewal of bandaging.

Discussion

In previous investigations into the physiological effects of beta-agonists in farm livestock, these substances have either been administered orally or by discrete injections. Both of these routes provide a pulsed dosage of the substance concerned and this may represent a greater insult to the animals metabolism than continuous low level administration. The uptake of beta-agonists from the digestive tract may be influenced by dietary composition and thus the 'metabolic dose rate' is not clearly defined. For this reason results obtained from this trial may not be strictly comparable with those obtained from feeding trials.

Intravenous administration of either clenbuterol or bitolterol did not adversely affect voluntary food intake. Oral administration of clenbuterol to steers at 500 mg/day has been shown to reduce voluntary food intake and also rumen fermentation (Ricks et al, 1984a). Voluntary food intake is thought to be adrenergically controlled and reductions may result from high levels of adrenergic agents crossing the blood/brain barrier and engaging in excessive stimulation. However adrenalin is known to depress rumen motility and it is possible that other agonists may also directly affect the rumen in this way, slowing the on passage of digesta and consequently limiting intake. If rumen fermentation is restricted one would perhaps expect a concomitant reduction in digestive efficiency. In view of the absence of effects on voluntary food intake, the lack of effect of either intravenous clenbuterol or bitolterol on the digestibility of the experimental diet or faecal nitrogen excretion is to be expected.

Both clenbuterol and bitolterol apparently acted at the metabolic level, their principal effect being to reduce urinary nitrogen excretion and thereby increase nitrogen retention as demonstrated at the higher intakes of nitrogen during periods II-V. Higher intakes of nitrogen occurred in the latter periods of the trial because the animals were gaining weight and thus required more food. Reduced urinary nitrogen excretion and consequent increased nitrogen retention was evident, thought not significant, in both the earlier experimental periods (I-II) when nitrogen intake was lower.

From nitrogen balance results it may be concluded that clenbuterol is a much more potent agonist than bitolterol since an infusion of only 1 mg/day induced a greater response than 25 mg/day bitolterol. While the structure of clenbuterol is very similar to that of adrenalin

(Figure 10,) bitolterol has a very different chemical structure (Figure 12, below).

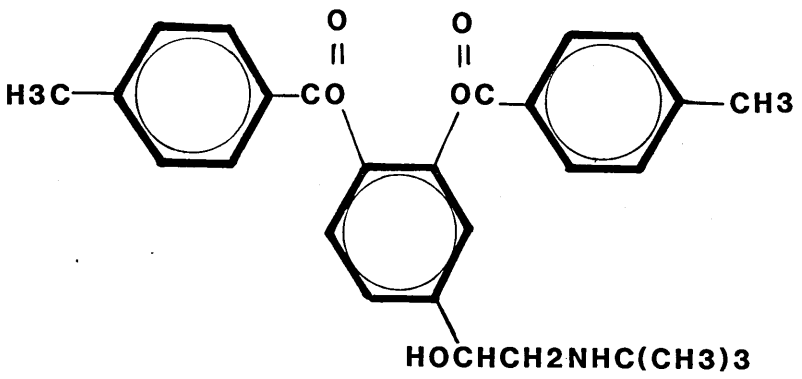


Figure 12. The chemical structure of bitolterol

The repartitioning agents currently used in farm livestock (clenbuterol and cimaterol) have been loosely classified as beta-$_2$ agonists and the exact mechanism by which they exert their effect has yet to elucidated. There is some disagreement as to whether these substances act primarily as lipolytic or antilipogenic agents with regard to fat metabolism, but the consensus regarding protein metabolism appears to be that they reduce degradation. The structure of bitolterol is entirely different from that of clenbuterol and thus it is possible that the observed increases in nitrogen retention may be mediated by an entirely different mechanism. If both agonists act via the same physiological mechanism then it is probable that the strength of the response will be different because the chemical structures are different, resulting in differing intensities of receptor stimulation. Unfortunately no blood analyses were carried out to measure circulating free fatty acid or hormone concentrations in the calves.

The effect of treatment on final carcass composition could not be differentiated as the experimental design was that of a Latin square in

which each animal received each treatment. However the external appearance of the steers was considerably more muscular than that of similar animals maintained on the same diet as possible replacements suggesting that the use of a repartitioning agent in general had improved the lean percentage of the carcass and resulting animal.

With the exception of occasional instances when residual fluid was rapidly infused into animals prior to replacement of infusion packs, no adverse reactions such as tachycardia were observed in any of the calves. Since these reactions represent a severe metabolic insult to the animal, in practice the most efficient way to administer beta-agonists to animals would be by means of a suitably designed implant or slow release device. However the nitrogen balance data from this trial suggests a slightly diminished response to adrenergic stimulation through time and thus long term low level continuous administration may not be practicable.

In view of the relatively high doses of bitolterol required to significantly increase nitrogen retention when administered by intravenous infusion compared to clenbuterol, very high doses indeed would be required if administration was via an oral or subcutaneous route where losses of the drug would inevitably occur. Considering also that excessive doses of this substance produce the same adverse reactions in cattle to excessive doses of clenbuterol, bitolterol apparently has no advantage over existing and more potent beta-adrenergic agonists.

SECTION II

STUDIES ON THE PATHOPHYSIOLOGICAL EFFECTS OF GASTROINTESTINAL NEMATODE INFECTION IN CATTLE

Literature Review

Introduction

Life cycle of Ostertagia ostertagi and Cooperia oncophora

The clinical disease

Pathogenesis

- Structural changes produced by Ostertagia ostertagi
- Structural changes produced by Cooperia oncophora

Effect on Voluntary Food Intake

Feed Utilization Effects

- Gastric pH and serum gastrin
- Pepsinogen and serum proteins
- Protein and energy metabolism
- Body water turnover

Interactions between Ostertagia ostertagi and Cooperia oncophora

Summary

Experiment IIa

Pathophysiological and Parasitological Studies on Type I Ostertagia ostertagi Infection in Calves

Experiment IIb

A Preliminary Study of the Effect of Cold-Conditioning on Infective Larvae of Ostertagia ostertagi

Experiment IIc

Pathophysiological and Parasitological Studies on Pre Type II *Ostertagia ostertagi* Infection in Calves

Experiment IId

Pathophysiological and Parasitological Studies on a Concurrent Infection of *Ostertagia ostertagi* and *Cooperia oncophora* in calves

LITERATURE REVIEW

Introduction

The bovine gastrointestinal tract may be inhabited by a variety of nematodes, the most important genera being Ostertagia, Cooperia, Trichostrongylus and Nematodirus. In North Western European countries the most abundant species are Cooperia oncophora and Ostertagia ostertagi which typically are located in the small intestine and the abomasum respectively (Kloosterman et al, 1984). O. ostertagi is generally regarded as the more pathogenic species (Anderson et al, 1965; Entrocasso et al, 1986a; Frankena, 1987) and has been associated with most recorded clinical outbreaks of bovine parasitic gastroenteritis (PGE) (Armour and Ogbourne, 1982). However the deleterious contribution of C. oncophora to PGE has been difficult to assess as this parasite is expelled rapidly in comparison to O. ostertagi and residual populations are consequently small by the time that clinical symptoms are apparent.

Experimental studies have demonstrated that C. oncophora is a potential pathogen if massive numbers of infective larvae are ingested by susceptible animals (Herlich, 1965; Armour et al, 1987a) and under these conditions its pathogenicity may equal or exceed that of O. ostertagi. Under field conditions Baker (1988) asserts that in cattle Ostertagia does not occur as a causative agent of PGE without the accompaniment of Cooperia and occasionally Nematodirus.

Life cycle

Both O. ostertagi and C. oncophora have a direct life cycle involving distinct free-living and parasitic stages as summarised in Figure 13. In the free-living stage, eggs passed in the faeces develop into first stage larvae (L_1) which hatch, develop and moult to become second stage larvae (L_2). These in turn develop and moult to the third infective stage (L_3). All of this occurs within the faecal pat. Under moist conditions the L_3 larvae then migrate onto the herbage retaining the outer sheath of the L_2 which renders them the most resistant of the free-living stages. Following ingestion, the parasitic cycle involves the development to adults through L_4 and L_5 stages.

The parasitic development of O. ostertagi has been described by several authors (e.g. Clark-Osbourne et al, 1960; Ritchie et al,

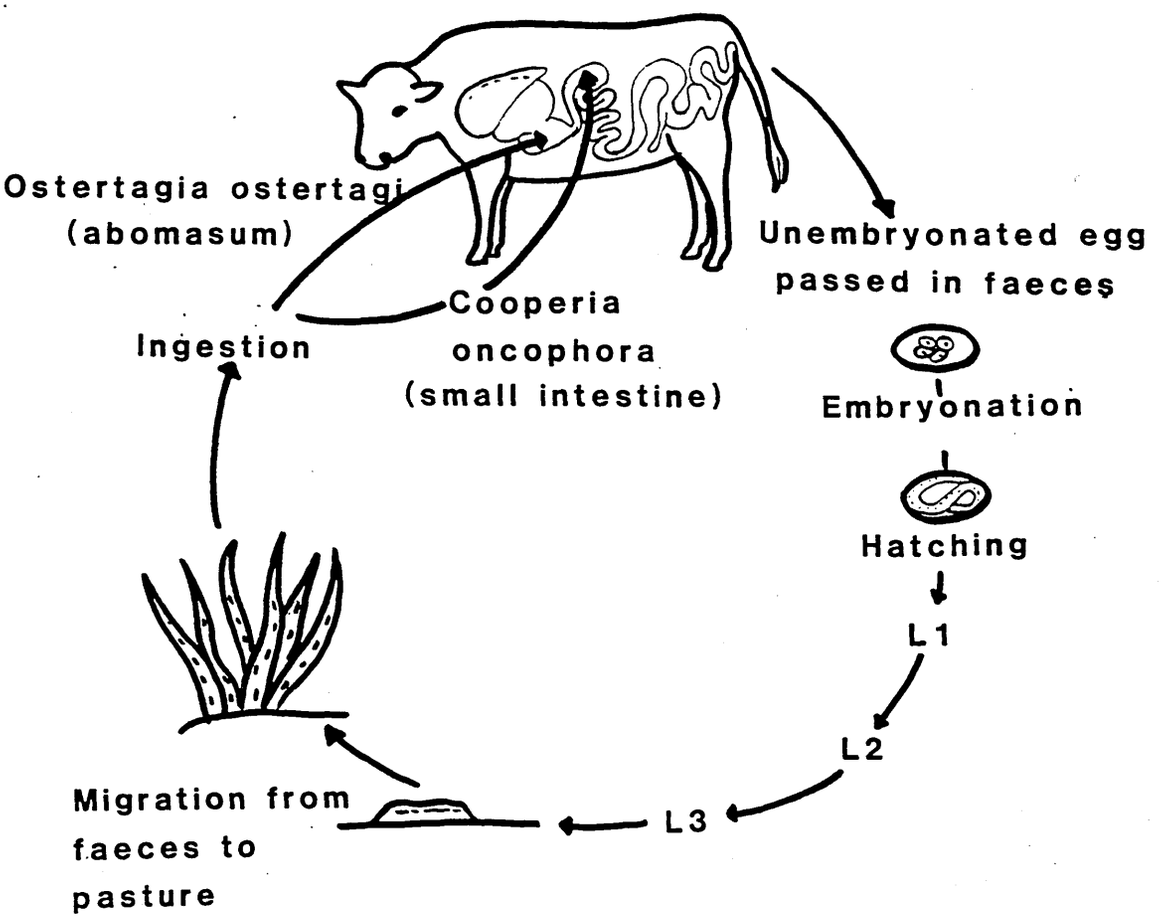


Figure 13. The life cycle of Ostertagia ostertagi and Cooperia oncophora.

1966; Rose, 1969). Maturation from the L_3 to adult stage occurs within the gastric glands of the abomasum and takes between 16 and 21 days. There is much associated tissue damage particularly on emergence of the adult worms into the abomasal lumen.

Development of the parasitic stages of C. oncophora is less well documented. Ingested L_3 exsheath in the abomasum and moult to the fourth larval stage in the small intestine, the final habitat. Isenstein (1963) observed that three days after the oral inoculation of L_3 to calves all of the larvae were located in the proximal small intestine and that the prepatent period varied from 17 to 22 days.

Until recently it was generally accepted that the development of C. oncophora took place in the intestinal lumen and that no mucosal penetration occurs. This was often the reason cited for its apparently low pathogenicity (Frankena, 1987). However Armour et al (1987a) recovered approximately 50% of the larval stages of C. oncophora from the mucosal digests of calves necropsied at 21 and 42 days during a continuous 42 day experimental infection. These authors also noted that in the calf necropsied at 21 days, the worm burden was principally located in the duodenum whereas in the calf necropsied at 42 days the distribution in the intestine and stadial structure had altered such that the majority of worms were present in the ileum. In further calves subsequently necropsied at 82 days (42 days after the cessation of larval inoculation) the majority of infection was again recovered from the duodenum. From these findings the authors speculated that the worm burden may have migrated through the intestine during the course of the infection. However in view of the fact that only one animal was necropsied at 21 and 42 days an equally likely explanation is that differences in digesta, especially with the respect to acidity on exit from abomasum, may have influenced the predilection site in the two animals. Indeed in a separate study (Coop et al, 1979) where nine calves were experimentally infected with C. oncophora the majority of the worm burden was located in the first quarter of the small intestine in all but one animal in which it was located in the second quarter, demonstrating the existence of differences in predilection site in different animals.

The clinical disease

Most recorded field cases of clinical parasitic gastroenteritis have been associated with O. ostertagi infection. Symptoms are

usually apparent in first year grazing cattle towards the end of the grazing season and include ill thrift and failure to gain weight, a 'staring coat', inappetence, lethargy and intermittent profuse diarrhoea which is bright green in colour. In severe infections the eyes may appear sunken and submandibular oedema may be present.

Following reports by Martin et al (1957) of 'atypical parasitic gastritis' in which these symptoms occurred in cattle which had been winter housed and apparently healthy for up to five months a detailed field study of suspected outbreaks of ostertagiasis was carried out in south-west Scotland and northern England (Anderson et al, 1965). These authors classified the disease into three phases, two of which (Type I and II) were clinically obvious. Type I ostertagiasis corresponds to the classical description of parasitic gastritis seen in first season grazing calves between July and October. The majority of the ingested larvae develop to maturity within the expected three week period. In Pre-Type II ostertagiasis large numbers of Ostertagia are present in cattle but the majority are inhibited in the early fourth larval stage. Affected animals show no clnical symptoms and usually have no history of diarrhoea although they have been grazing heavily infected pastures in the autumn. Clinical Type II ostertagiasis arises as a result of the synchronous maturation of these inhibited larvae and may be manifest up to six months after removal of cattle from pasture. The sequential development of Type I and Type II ostertagiasis in young cattle has been described by Entrocasso et al (1986a).

In contrast to O. ostertagi which gives a relatively late and low peak in faecal egg output of infected calves associated with a constant turnover of worms, C. oncophora gives a rapid high peak followed by a dramatic decline as a result of host immunity. The majority of worms are expelled and only a small fraction is retained (Albers, 1981). Using experimental infections and the criterion of faecal egg output Coop et al (1979) concluded that acquired immunity becomes effective within two months after primary inoculation. Thus, although C. oncophora may be a predisposing factor in the occurence of PGE later in the grazing season, by the time clinical symptoms are apparent the numbers of this nematode are relatively small. Under experimental conditions single and multiple inoculations of calves with C. oncophora have resulted in anorexia, depressed weight gain and diarrhoea (Herlich 1965; Armour et al 1987a; Borgsteede and Hendriks, 1979) but these have been of a transient nature in all but extremely

heavy infections and Cooperia alone has never been associated with clinical disease under field conditions.

The propensity for arrested development of C. oncophora in tracer calves has been shown to be greater than for O. ostertagi (Brunsdon, 1971; Bisset and Marshall, 1987) but there is no late season accumulation of early fourth larval stages in resident animals due to host immunity, and no disease syndrome comparable to Type II ostertagiasis in known. However in field cases of Pre-Type II ostertagiasis Anderson et al (1965) did note the presence of low numbers of inhibited Cooperia and it is possible that these may develop synchronously with O. ostertagi and contribute to clinical Type II disease.

Pathogenesis

Structural changes produced by O. ostertagi

The principal lesions of ostertagiasis are white, raised, umbilicated nodules on the surface of the abomasum which may be accompanied by hyperaemia and oedema. These primary lesions, located predominantly in the fundic region, are caused by the enlargement of the larvae within the gastric glands and any cellular changes are initially confined to the parasitised glands.

As larvae develop, the parasitised gland distends and stretches the surrounding glands stimulating multiplication of new cells in order to maintain the continuity of the epithelium. At this time the parietal cells become incorporated into this stretched epithelium and lose their microvillary structure which is necessary for HCl secretion. Parietal cells in the parasitised glands are permanently lost, being replaced by mucous neck cells (Murray, 1968; Murray et al, 1970). Approximately seven days after infection, collagen accompanied by fibroblasts is deposited in the tissue around the parasitised glands and structural fibres abound in the necks of these glands (Stringfellow, 1974). The capillary network around the infected glands is also disrupted (Stringfellow, 1977).

By the time of emergence of adult O. ostertagi from the gastric glands marked cellular changes have occurred and these are associated with clinical symptoms and alterations in the biochemical values of blood and abomasal fluid. Macroscopically there is hyperplasia and loss of cellular differentiation particularly of the parietal cells both in the parasitised and surrounding glands. In heavy infections

where coalescence of these lesions occurs the mucosa assumes a Morocco leather appearance. Ultrastructurally some parietal cells are still distinguishable but are non functional resulting in elevations in the pH of abomasal fluid and permitting the survival of bacteria. The undifferentiated and hyperplastic mucosa is also abnormally permeable to macromolecules due to the breakdown of the junctional complexes between many cells. If the newly emerged adult worms are removed, glands surrounding the parasitised glands are able to resume normal function and re-establish ultrastructural characteristics within 48 hours. However, glands which harboured developing larvae do not readily resume their normal glandular function (Armour et al, 1967a).

Structural changes produced by C. oncophora

A detailed study involving the serial slaughter of calves at regular intervals during infection with C. oncophora has not been undertaken and so consequently little is known about the associated structural damage. It has been generally accepted that the parasitic development of this nematode occurs entirely within the lumen of the small intestine resulting in only limited morphological changes to the tissues immediately in the vicinity of the worms. In calves necropsied two weeks after a 20 week continuous infection with C. oncophora, Coop et al (1979) noted that consistent pathological changes were only present in areas of the small intestine occupied by the worms and that these were comprised of limited compression or distortion of the lateral margins of the intestinal villi in immediate contact with the worm bodies. However, Armour et al (1987a) reported the development of a good immunity after approximately six weeks using a similar infection rate of third stage larvae and thus the limited damage observed after 22 weeks may simply have been that remaining after major repair of the tissues had been effected. Armour et al (1987a) infected calves daily for 42 days with 10,000 C. oncophora infective larvae, sacrificing one animal on day 21, another on day 42 and the remainder 42 days after the last larval inoculation. At 21 days the worm burden, both adults and larvae, was in the duodenum and pathological changes were limited to a flattening of epithelial cells where worms were lying deep between the villi. At 42 days the worm burden was primarily in the jejunum and was associated with an obvious thickening of the mucosa and broadening of the villi. Worms had migrated deep in between the villi and those present in the lumen were

often surrounded by sloughed epithelial cells and mucus. There was obvious destruction and sloughing of the surface epithelium throughout the small intestine and mucosal blood capillaries were congested. In contrast to other reported work, at both 21 and 42 days approximately fifty per cent of larvae had penetrated the intestinal mucosa, particularly in the ileum. By 84 days after the initial larval inoculation only minimal damage to the intestinal mucosa was present although scanning electron microscopy showed the villi to remain flattened and thickened.

Effect on voluntary food intake

A reduction in voluntary food intake is widely recognised as a major factor in the pathogenisis of gastrointestinal parasitic infections. The degree of inappetence varies with the degree and duration of infection and may also be influenced by the quality of the diet, particularly with respect to protein content (Abbott, 1982). There is some evidence to suggest that parasitism of the small intestine has a greater effect on appetite than abomasal parasitism (Steel et al, 1982). The causitive factors have yet to be identified and have recently been reviewed by Symons (1985). Pain may be important but its effect is difficult to quantify. Structural damage to the gastrointestinal tract may also influence the digestion and absorption of nutrients and this in turn may influence voluntary food intake. Leng (1981) proposed that changes in the availability of amino acids for absorption would change the protein : energy ratio of absorbed nutrients and reduce food intake as well as the efficiency of utilization of absorbed amino acids. O. ostertagi infection is responsible for well documented increases in abomasal pH which may inhibit the denaturation of proteins by pepsin and also promote the survival of increased numbers of bacteria resulting in increased deamination. Both of these factors would influence the amino acids available for absorption from the small intestine. C. oncophora may directly limit the uptake of amino acids by reducing absorptive capacity. However as the central portion of the small intestine is primarily responsible for amino acid absorption and this nematode predominantly occupies the proximal portion, extensive malabsorption is unlikely. Indeed Symons (1976) concluded that malabsorption, at least of the major constituents of the diet, is not an important factor exacerbating anorexia.

Reduced absorption of dietary phosphorus has been reported in lambs infected with T. colubriformis (Coop, 1981) and it has been proposed that this is associated with a reduction in salivary secretion resulting in reduced availability to rumen microbes associated with fibre digestion. The consequent reduction in rate of passage of fibre through the rumen may limit intake.

Intake is unlikely to be limited by increases in gut volume resulting from diarrhoea as Grovum and Phillips (1978) could produce no change in voluntary food intake when the wet faecal output of sheep was doubled as a result of abomasal administration of a bulk laxative.

The neuropeptide cholecystokinin has been implicated as a satiety factor but evidence for its involvement in the long term inappetence produced by parasitic infection in largely equivocal.

Feed Utilization Effects

The loss of production arising as a result of gastrointestinal parasitism is primarily associated with reduced voluntary food intake but pair-feeding experiments (e.g. Sykes and Coop, 1976 and 1977) have demonstrated that specific parasite effects also operate to influence the digestion and utilization of dietary nutrients. These effects may be mediated by alterations in the biochemical values of the blood and gastric contents which predispose to changes in the animal's metabolism.

Gastric pH and serum gastrin

O. ostertagi infection is associated with an increase in abomasal pH from the normal 2.2 to 7.0 in heavy infections. Using cannulas to monitor abomasal fluid changes, Jennings et al (1966) reported that the period of maximum change immediately followed the emergence of adult parasites from the gastric glands and was a consequence of the associated parietal cell destruction. Any differentiated parietal cells remaining may show morphological characteristics indicative of reduced activity (Murray et al, 1970). The functional consequences of elevated abomasal fluid pH are a failure to activate pepsinogen (secreted by the zymogen cells) to pepsin resulting in a failure to initiate the denaturation of protein, and a loss of bacteriostatic effect. The increased numbers of bacteria in the abomasum resulting from the latter have often been cited as the cause of diarrhoea which is generally apparent at this time, its onset

and duration closely following the elevated pH (Armour and Ogbourne, 1982).

Normal abomasal pH has been shown to be detrimental to the survival of adult O. ostertagi and the immersion of freshly collected worms in a solution of pH 2.2 resulted in 100% mortality within 60 minutes (Eiler et al, 1981). These authors suggested that the hypochlorhydria observed during ostertagiosis may be partially mediated by a chemical released from the parasite. Local parasite effects on HCl production have also been implicated in studies using sheep infected with Ostertagia circumcincta and surgically prepared with separated fundic pouches (Anderson et al, 1976). During continuous infection of previously worm free animals acid secretion of the parasite free pouches was maintained or increased, whereas that of the abomasum was reduced. However in calves infected by surgical transplantation of adult O. ostertagi (McKellar et al, 1987) no significant alterations in abomasal pH were observed.

Both of these studies reported an increase in plasma gastrin, a hormone concerned with the regulation of digesta flow and abomasal pH, suggesting a physiological mechanism whereby the host is attempting to counteract actual or potential abomasal pH changes. Indeed Anderson et al (1981) reported hypergastrinaemia just eight days after infection of sheep with O. circumcincta implying that in the absence of any change in abomasal pH, the parasites may stimulate the endocrine cells directly to produce gastrin, or indirectly by stimulating the release of a gastrin secretagogue from the gastric mucosa.

Using the gastrin analogue, pentagastrin, Bell et al (1977) concluded that gastric emptying was slowed by gastrin and that the effect was mediated via the smooth muscle of both the body and the antrum rather than a general change in the tone of the gut or inhibition of the antral pump alone. This finding is difficult to reconcile with diarrhoea observed during clinical Ostertagia infection which is suggestive of increased gut motility. However it is possible that differential effects on motility may occur along different portions of the digestive tract.

The effects of C. oncophora on gastric pH and serum gastrin have not been investigated. Gastrin is produced by G-cells which are mainly found in the antrum of the stomach and the duodenum (Olowu-Orokun, 1975). It is possible that the presence of C. oncophora in the duodenum may disrupt the G-cells and gastrin production, particularly

if the mucosa is penetrated by developing larvae. A mixed infection of O. ostertagi and C. oncophora may exacerbate abomasal hypochlorhydria as the presence of Cooperia in the duodenum may limit any gastrin mediated stimulation of abomasal parietal cells, otherwise inhibited by the presence of O. ostertagi.

Pepsinogen and serum proteins

A characteristic feature of O. ostertagi infections is an increase in serum pepsinogen concentrations coincident with emergence of the worms from the gastric mucosa. Abomasal pH is also elevated at this time resulting in partial or complete inactivation of pepsinogen to pepsin. Murray (1969) observed electron opaque material lying between the epithelial cells of an undifferentiated and hyperplastic abomasal mucosa and proposed that the separated cell junctions facilitated the passage of macromolecules, particularly small serum proteins leaking into the digestive tract and inactivated pepsinogen leaking into the circulation. Although this theory of macromolecules flowing in and out of the epithelial sheet has been generally accepted, Stringfellow and Madden (1979) have challenged its validity. In studies involving experimental infection of calves with 250,000 larvae of O. ostertagi there was a significant increase in plasma pepsinogen concentrations in the absence of any detectable leakage across the gastric mucosa. Pepsinogen granules were completely denuded from chief cells suggesting a mechanism whereby pepsinogen was secreted directly into the circulation rather than being taken up from the gastric contents through a damaged vasculature.

Alterations in serum protein concentrations are also characteristic of bovine ostertagiasis where typically there is a depression in serum albumin, a concurrent increase in gamma globulin and little change in total protein (Mahrt et al, 1964; Ross and Todd, 1965). However, in heavy infections the reduction in albumin is eventually accompanied by a drop in globulin resulting in overall hypoproteinaemia. Packed cell volume estimations support the view that haemoconcentration is associated with a fall in albumin levels in early infection but there may be an expansion of plasma volume in the later stages of infection, this being more typical in thin and wasted animals.

Both albumin and immunoglobulin turnover rates are affected by clinical parasitism. Neilsen (1966) demonstrated that these were

greatly accelerated in animals when clinical signs were apparent, but were normal (or even sub-normal) during a quiescent period or convalescence. Subsequent studies have confirmed these findings (Halliday et al, 1968; Holmes and MacLean, 1971). It seems reasonable that increased immunoglobulin turnover is due to the immune response mounted by the host. Increased albumin turnover is probably a consequence of albumin hypercatabolism and it has been suggested that this is primarily due to abnormal leakage of plasma proteins into the gastro-intestinal tract (Mulligan et al, 1968). In support of this suggestion, studies using ^{51}Cr (Holmes and MacLean, 1971) and polyvinyl pyrolidione (PVP) (Jarnum, 1962) have demonstrated increased losses of plasma by this route. However increased endogenous albumin catabolism may also contribute, this being principally associated with the inflammatory changes occurring in the abomasum. There is little doubt that O. ostertagi infection results in increased losses of exfoliated epithelial cells and increased mucus production due to proliferation of goblet cells. Preliminary radioimmunoassay of rat intestine has established that infection with Nippostrongylus brasiliensis results in increased mucin release. This was associated with greater release of DNA which may be indicative of increased epithelial cell shedding and death (Forstner et al, 1982). The role of gastrointestinal mucus in the elimination of parasitic nematodes and protozoa has recently been reviewed by Miller (1987).

Herlich (1965) reported transient hypoproteinaemia in calves given a single inoculum of 350,000 C. oncophora infective larvae. In contrast no significant changes in haematocrit, serum urea, total protein and albumin concentrations were reported when calves were daily infected with 10,000 L_3 for 42 days (Armour et al, 1987a). However these authors did note a slightly increased faecal clearance of plasma in infected animals indicating that some degree of enteric loss was occurring.

Protein and energy metabolism

Nitrogen balance studies have demonstrated that reduced nitrogen retention is a characteristic feature of gastrointestinal parasitism. This generally arises as a result of increased urinary nitrogen loss (Parkins et al, 1973; Armour et al, 1987a) implying a reduction in the efficiency of utilization of absorbed amino acids. Faecal nitrogen may also be increased however as a result of impaired digestion or

increased endogenous losses into the gastrointestinal tract. In view of the apparently large capacity of the bovine intestines to compensate for any parasite-induced alterations in the breakdown or absorption of proteins increased endogenous losses are most likely to be responsible for any decrease in the apparent digestibility of dietary nitrogen, especially at high nitrogen intakes where the capacity for reabsorption of endogenous nitrogen may be exceeded (Coop, 1981). Indeed Bown et al (1988) reported that the inferior nitrogen balances of sheep infected with Trichostrongylus colubriformis were caused by excessive endogenous loss rather than by malabsorption and that this induced protein deficiency was a major factor limiting efficient food utilization. Of the additional endogenous protein excreted in the faeces, only a small proportion is thought to originate as a result of plasma leakage (Steel and Symons, 1982).

Using radiolabelled leucine and tyrosine, the pattern of amino acid utilization in sheep and guinea pigs infected with T. colubriformis, and mice infected with Nematospiroides dubius has been studied (Symons and Jones, 1971; Symons 1985). In general these studies demonstrate that parasitised animals show reduced rates of skeletal muscle protein anabolism which is partially attributable to reduced voluntary food intake. However there is a corresponding increase in protein synthesis by both the liver and the gastric mucosa which is unrelated to intake. In guinea pigs infected with T. colubriformis increased mucosal protein synthesis occurred along the entire length of the small intestine and was probably associated with increased epithelial cell turnover, this being a consequence of mucosal damage at the site of parasitism and compensatory absorption in the distal regions. In sheep infected with T. colubriformis the tyrosine flux, a measure of protein synthesis, was unaltered with respect to bodyweight but increased in relation to intake demonstrating an increased protein turnover. Synthesis of structural liver proteins increased as did amino acid incorporation into membrane bound ribosomes, those responsible for the synthesis of blood proteins (Symons, 1985). It may thus be hypothesized that gastrointestinal parasitisms are responsible for increased endogenous nitrogen losses both in terms of accelerated turnover of the mucosal epithelium and leakage of plasma proteins. Amino acids are consequently diverted from more productive sites to the liver and gastric mucosa to compensate and net synthesis of skeletal muscle is reduced. In severe infections net

catabolism of skeletal muscle may occur, particularly if voluntary food intake is severely restricted.

Reduced intake limits the energy available to an animal at a time when it is faced with increased demands arising from increased protein turnover, but it is induced protein deficiency rather than energy deficiency which limits animal production as demonstrated by Bown et al (1988). Post-ruminal infusions of protein into lambs infected with T. colubriformis replaced endogenous nitrogen losses and restored nitrogen and energy balance whereas isocaloric infusions of energy produced only partial restoration. However these authors did consider that at extremely low food intakes energy availability may be the primary factor limiting growth.

Body water turnover

In view of the inappetence and diarrhoea which typify clinical gastric parasitic gastroenteritis, alterations in body water metabolism are to be expected. In calves infected with O. ostertagi significantly greater amounts of water (approximately 20%) may be excreted in both urine and faeces (Parkins et al, 1982). However diarrhoea may not necessarily be accompanied by an overall increase in fluid loss. Bremner (1982) reported that in calves with heavy infection of Cooperia pectinata urine water losses were considerably below normal and, despite excessive faecal water loss, overall output from infected animals was less than that from worm free controls.

Studies of water metabolism in parasitised ruminants have been conducted using tritiated water (TOH) (Halliday et al, 1965; Baker et al, 1965; Holmes and Bremner, 1971). In general these studies indicate that body water as a percentage of body weight is increased and that water turnover rate is reduced implying a conservation of body water under conditions of reduced intake. Pair feeding experiments using sheep infected with O. circumcincta (Holmes and Bremner, 1971) have demonstrated that anorexia is the primary cause of reduced water turnover and Baker et al (1965) suggested that depletion of serum albumin in bovine ostertagiasis may result in altered osmotic pressure of the serum and physiological conditions promoting generalised oedema. Where increased water retention occurs, the tissue loss attributable to parasite infections cannot be readily determined from changes in bodyweight alone.

Interactions between O. ostertagi and C. oncophora

Under natural field conditions both concurrent and sequential infections of O. ostertagi and C. oncophora occur. During the early grazing season low numbers of both species are ingested with pasture to establish small host parasite populations. Due to the superior fecundity of C. oncophora it is reasonable to suppose that host populations of this nematode initially increase more quickly than those of O. ostertagi. However as a result of the rapid development of host immunity to C. oncophora, O. ostertagi predominates during the later part of the grazing season. Direct interaction between the two nematode species cannot occur because they occupy different portions of the digestive tract. Indirect interaction may be mediated by the hosts physiological or immunological mechanisms.

Physiologically mediated interactions are not inconceivable because O. ostertagi infection usually results in elevations of the pH of abomasal digesta and this may adversely affect the small intestine microclimate. Such an effect has been demonstrated in sheep with infections of Haemonchus contortus and Nematodirus battus in the abomasum and small intestine respectively (Mapes and Coop, 1970). However experimental studies have demonstrated that reciprocal negative interactions between C. oncophora and O. ostertagi only occur in sequential and not in concurrent infections, implicating the involvement of the hosts immune system (Kloosterman et al, 1984). Indeed Keus et al (1981) reported cross-reacting IgG antibodies for O. ostertagi and C. oncophora using the saline extracts of adult worms. More recent work (Frankena, 1987) has shown that primary C. oncophora infection stimulates a degree of acquired immunity which is expressed against challenge infections of C. oncophora and to a lesser extent O. ostertagi in the form of stunted growth and development of the worms. Worm burdens and faecal egg output were not affected and local abomasal responses were independent of those in the small intestine.

Summary

Under experimental conditions infection of calves with O. ostertagi and C. oncophora either singly or in combination may result in clinical parasitic gastroenteritis. Under natural grazing conditions the relative contribution of each species to clinical disease outbreaks is difficult to assess due to differential rates of

development of the host immune response and worm expulsion. O. ostertagi is generally regarded to be the more pathogenic of the two species because of the greater persistency in the host and the well documented penetration of, and damage to, the abomasal mucosa by developing larvae. However substantial damage to the small intestine mucosa has been reported for C. oncophora and experimental studies suggest that the detrimental effects on calf metabolism and production of these two nematode species together far exceed those of either species alone.

GENERAL INTRODUCTION TO EXPERIMENTAL STUDIES

A previous study conducted at the University of Glasgow (Armour et al, 1987a) investigated the deleterious effects of experimental C. oncophora infection in growing calves. Daily infections over a six week period resulted in inappetence, weight loss, impaired nitrogen retention and a loss of plasma proteins into the gastrointestinal tract. During this period infections occurred in the duodenum, jejunum and ileum and many larval stages were present in intestinal mucosa. At necropsy on weeks three, six and ten after the initial inoculation stunting and thickening of the villi were noted with excessive mucus production and even at week twelve there was continued loss of plasma proteins into the gastrointestinal tract despite the expulsion of most of the worms.

The results of this study demonstrated that C. oncophora infections given at a level frequently encountered under natural conditions in the West of Scotland can cause considerable pathophysiological changes in calves which persist beyond the development of a good immunity after approximately six weeks. Despite evidence such as this, C. oncophora has largely been disregarded in favour of O. ostertagi as the primary cause of bovine parasitic gastroenteritis. However it may be that infection with parasites such as C. oncophora is a prerequisite for the development of more severe clinical disease in O. ostertagi infected cattle in the field situation.

Experimental studies in this section follow that of Armour et al (1987a) but use monospecific infections of O. ostertagi and a subsequent mixed infection of O. ostertagi and C. oncophora. Kloosterman et al (1987) concluded that there was no interaction between O. ostertagi and C. oncophora in a concurrent infection and consequently it may be expected that parasitological data for either nematode in the combined infection would resemble that in the respective monospecific infections. However for pathophysiological parameters it is probable that the detrimental effects of a concurrent infection will far exceed those of either monospecific infection because of the greater total area of mucosal damage and consequent reduction in the compensatory digestive capacity of the gut. Such an infection may therefore result in more severe and prolonged clinical disease.

Data from the following series of studies, together with that from Armour et al (1987a) permit a comparison of the relative pathogenicities of O. ostertagi and C. oncophora when given as monospecific infections under similar experimental conditions. More importantly, a comparison can be made between the effects of either monospecific infection and those of the combined infection, this being the sum of the two monospecific infections. C. oncophora was administered to calves at a level frequently encountered under natural grazing conditions (Entrocasso, 1984). Administration of O. ostertagi was at a level calculated to produce mild clinical disease and thus allow maximum expression of any additional detrimental effects attributable to C. oncophora in the combined infection.

In all studies a morantel sustained release bolus (MSRB; Paratect, Pfizer) was given to one group of infected calves to limit larval establishment and the efficacy and mode of action of this anthelmintic device are discussed.

EXPERIMENT IIa

PATHOPHYSIOLOGICAL AND PARASITOLOGICAL STUDIES ON TYPE I OSTERTAGIA OSTERTAGI INFECTIONS IN CALVES

Introduction

The following study investigates the pathophysiological consequences of daily experimental infections of O. ostertagi in calves and the effectiveness of the MSRB in preventing adverse pathophysiological changes.

Materials and Methods

Animals and experimental design

Twenty-five castrated male Friesian calves aged four months, weighing approximately 120 kg, and which had been reared under helminth-free conditions were allocated to three treatment groups.

Group A calves (clean) were maintained free of parasites. Five were used as control animals for digestion and nitrogen economy studies and a further two for radioisotopic investigations.

Group B calves (MSRB) were each given a morantel sustained release bolus (MSRB, Paratect; Pfizer). The calves were subsequently dosed per os with 2,000 O. ostertagi infective larvae daily for six weeks. Five calves were allocated to the digestion studies, two to the radioisotopic investigation and two necropsied 23 and 42 days after initial infection. The bolus was administered in order to limit the establishment of the larvae (Jones 1983).

Group C calves (infected) were treated similarly, but without prior MSRB administration, with five calves allocated for digestion studies, two for radioisotopic work and two for necropsy during the course of the trial (days 23 and 42).

Clean control calves were housed separately from those in infected groups to prevent possible autoinfection from bedding material. All calves were clinically examined and weighed every three weeks and blood and faecal samples were collected weekly for biochemical, haematological and parasitological analyses.

The remaining calves in the metabolism and isotope studies were slaughtered on day 84 of the experiment.

Feed allowance

The calves were individually given a complete ration twice a day (Superstar cubes; Hamlyn Milling). Mean analyses were 0.89 dry matter (DM), with 157 g crude protein (CP), 174 g crude fibre (CF), 43 g ether extract (EE), 102 g ash and 18.0 MJ gross energy (GE) kg DM^{-1}. The daily feed allowance of 3.0 kg/day was calculated to supply sufficient metabolisable energy (ME) to allow for a daily liveweight gain of about 0.35 kg (MAFF et al 1984).

Larval infections

The O. ostertagi larvae were harvested from faecal cultures no more than 21 days prior to oral inoculation of the calves, and were maintained at room temperature (17° C) until required. The daily dose of 2000 larvae was equivalent to that ingested by calves grazing pastures contaminated at a level of 800 infective larvae per kg of dried herbage, assuming a daily intake of 2.5 kg dry matter.

Parasitological techniques

Faecal egg counts were carried out by the modified McMaster method of Gordon and Whitlock (1939). At post-mortem, trichostrongyle parasites were recovered using the technique described by Ritchie et al (1966).

Pathology

Tissue was taken from the fundus and pylorus, fixed and processed as previously described.

Blood analyses

Blood urea, total protein and albumin concentrations were measured using continuous flow analysis (Standard Technicon Auto-Analyser II).

Plasma pepsinogen activity was assayed by the method of Edwards et al (1960) where one international unit (I.U.) is equivalent to the release of one m/mol tyrosine/litre plasma/minute.

Digestibility and nitrogen balance studies

The fifteen calves involved in the metabolism study were allocated to three equal sized treatment groups such that the mean weight and weight range of each group was similar. Of the five calves in each group four were used for the metabolism studies while the remaining

animal was maintained as a potential replacement.

Digestibility and nitrogen (N) balance data were obtained from four animals in each of the three treatment groups on days 14-21, 35-42, 56-63 and 77-84 post infection.

Calves were accommodated in standard metabolism stalls and individually fed twice daily at 0730 and 1630 h for seven days before the following seven day recording period. Individual feed intakes were recorded for the whole 14-day period and faecal and urinary outputs were measured over the seven day collection period. Feed, faecal and urine collection methods and their analyses were as previously described.

Radioisotopic measurements

Radioisotopic studies using $^{51}CrCl_3$, ^{125}I-albumin and tritiated water (TOH) were conducted with two calves from each of the treatment groups clean, MSRB and infected on days 35-47 and 70-82 following the first infection. Each calf received 74 MBq of $^{51}CrCl_3$ and 15 MBq of ^{125}I-bovine albumin by intrajugular catheter at the beginning of each study period. This followed an infusion of 110 MBq of TOH four days earlier. Plasma samples were collected 15, 30, 60, 120 and 360 minutes after injection and then twice daily for five days and then once daily for the remainder of the experimental period. The calves were restrained in metabolism stalls during the investigations and fitted with faecal bags. On each day the total faecal output of each calf was thoroughly mixed before eight 10 g subsamples were taken for ^{51}Cr and ^{125}I radioactivity determination. Total daily urine outputs were also collected and weighed 1 ml subsamples diluted with 0.02 N NaOH before radioactivity measurement. Calves were dosed daily per os with 10 ml 0.75% KI solution for three days prior to the isotope injection and during the subsequent recording period in order to ensure rapid excretion of ^{125}I from catabolised ^{125}I-albumin.

Plasma protein losses in the gastrointestinal tract, plasma volume, and albumin turnover and catabolic rate were calculated as described by Holmes and Maclean (1971). Total body water and water turnover were determined from the plasma disappearance rate of TOH and application of the dilution technique using extrapolated plasma values.

Statistical analysis

Statistical analyses were performed using 'Minitab' (Ryan, Penn State University) on a microcomputer. Metabolism study and blood biochemical data were analysed using a standard analysis of variance and multiple comparisons procedure, while t-tests were performed on parasitological data for infected and MSRB treated calves.

Results

Clinical observations

Infected calves showed marked softening of the faeces and one calf became diarrhoeic between days 49-63 after the start of the infection. The MSRB calves showed only occasional softening of the faeces and the clean calves remained clinically normal throughout. There were several instances of bloat in all groups during the whole course of the trial and one calf (infected) died as a result. There was no depression of appetite due to the parasitic infection.

The mean liveweights of the 15 calves used in the metabolism studies are shown in Figure 14. After 84 days, when the experiment ended, there were no significant differences in the total mean liveweight gains between treatments; these were 27.6, 26.2 and 22.0 kg (0.33, 0.31 and 0.26 kg/d) for the clean, MSRB and infected groups respectively.

Biochemistry

Plasma pepsinogen values are shown for the metabolism study calves in Figure 15. Mean values for the infected calves were significantly ($P<0.001$) greater than both clean and MSRB groups from day 21 and the values for MSRB calves were greater than those of the clean calves between days 14 and 63 of the trial.

Blood urea and total protein concentrations were similar for all treatment groups. However a significant ($P<0.05$) depression in mean albumin concentration was observed for infected calves on day 42 (35.7 mmol/l) compared with the clean and MSRB groups (39.2 and 38.4 mmol/l respectively).

Apparent digestibility coefficients

Examination of the apparent digestibility of each of the standard proximate feed fractions for each of the four separate recording periods revealed only a significant depression in the digestive

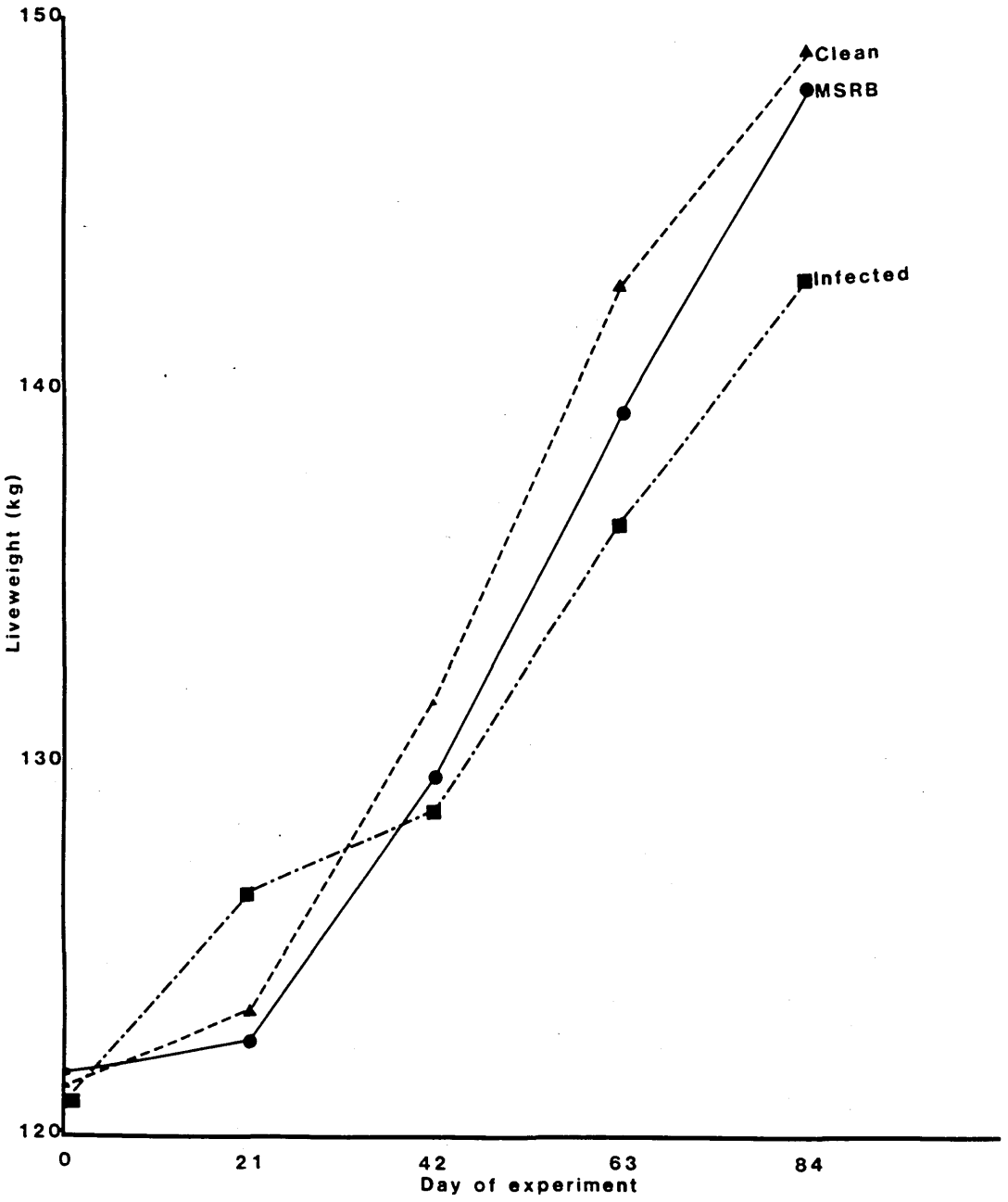


Figure 14. Mean liveweights of metabolism study calves infected with Ostertagia ostertagi, infected after administration of a MSRB or maintained as clean controls.

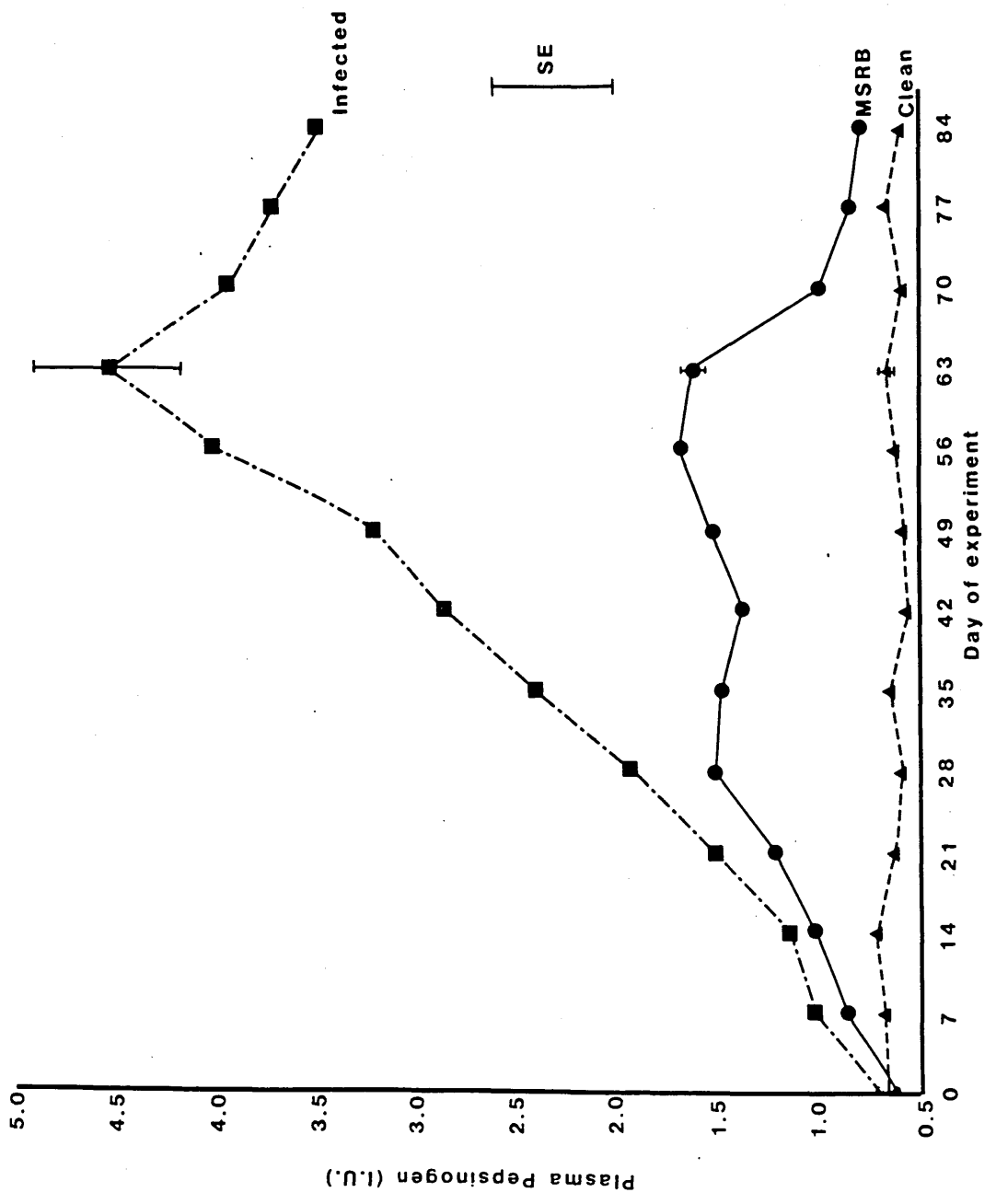


Figure 15. Mean plasma pepsinogen values (I.U.) for metabolism study calves infected with Ostertagia ostertagi, infected after administration of a MSRB or maintained as clean controls.

efficiency of the EE and NFE fractions in infected calves 56-63 days after infection began. No other differences occurred and no trends in the digestibilities with time were noted. Overall values (means of all four recording periods) are presented in Table 5 and individual data is contained within Appendix 3.

Nitrogen balance

Mean results for the daily nitrogen input, output in urine and faeces together with the apparent retentions for the three treatment groups in each of the four recording periods are presented in Table 6. Individual values and data expressed in terms of percentage of nitrogen intake are given in Appendix 3. There were no significant treatment related differences in the nitrogen economy of the calves. Retentions for the clean calves remained relatively constant throughout the trial, but both the infected and MSRB treated animals suffered a reduction in nitrogen retention during days 35-42 as a result of increased urinary nitrogen excretion followed by an apparent recovery during days 56-63.

Radioisotopic measurements

Elevated faecal clearances of both ^{125}I-albumin and $^{51}CrCl_3$ were shown by the infected calves compared to the clean controls and this increased albumin loss to the gastrointestinal tract was reflected in an increase of approximately 30% in the albumin catabolic rate and a reduced ^{125}I-albumin half life (Table 7). MSRB treated calves showed only marginal increases in faecal clearance of ^{125}I-albumin and albumin catabolic rate compared to the clean controls. During days 70-82, one infected calf, O3, had a persistently elevated faecal clearance of $^{51}CrCl_3$ and also an elevated albumin catabolic rate compared to the clean controls. The continued loss of albumin into the gastrointestinal tract was associated with a continued elevation of plasma pepsinogen levels and a high faecal egg output. The other infected calf, O4, was apparently recovering from infection at this time and had a reduced faecal clearance of $^{51}CrCl_3$. In this animal the albumin catabolic rate was particularly low at this time and this is associated with a greatly extended ^{125}I-albumin half life. Plasma pepsinogen had also returned to pre-infection levels.

No adverse pathophysiological differences were observed when comparing the MSRB treated calves to the clean control animals during the second investigation period.

Table 5. Mean overall apparent digestibility coefficients for calves infected with Ostertagia ostertagi, infected after prior administration of a MSRB or maintained as clean controls (±SE)

	Clean	MSRB	Infected
DM	0.633 ±0.006	0.632 ±0.006	0.643 ±0.008
CP	0.606 ±0.009	0.613 ±0.007	0.617 ±0.007
CF	0.298 ±0.018	0.277 ±0.017	0.308 ±0.021
EE	0.884 ±0.004	0.887 ±0.004	0.889 ±0.004
Ash	0.509 ±0.015	0.512 ±0.021	0.516 ±0.015
NFE	0.755 ±0.005	0.755 ±0.007	0.765 ±0.008
OM	0.647 ±0.006	0.644 ±0.006	0.657 ±0.009
GE	0.626 ±0.008	0.631 ±0.007	0.638 ±0.009

n=16, four calves in each of four recording periods

Table 6. Mean nitrogen balance (g/day) ±SE for calves infected with Ostertagia ostertagi, infected after prior administration of a MSRB or maintained as a clean control

	Clean	MSRB	Infected
Days 14-21	n=4	n=4	n=4
Intake	70.67 ±0.23	68.03 ±1.93	69.75 ±0.53
Faeces	27.10 ±1.05	27.12 ±1.15	27.71 ±1.43
Urine	28.20 ±1.60	25.75 ±1.87	24.60 ±1.01
Retention	15.38 ±1.98	15.16 ±0.63	17.45 ±1.74
Days 35-42	n=4	n=4	n=4
Intake	63.72 ±0.00	62.48 ±0.74	63.34 ±0.23
Faeces	25.90 ±0.96	25.91 ±0.90	25.71 ±1.07
Urine	21.98 ±2.31	24.03 ±0.91	25.89 ±1.84
Retention	15.84 ±2.06	12.53 ±1.11	11.74 ±2.55
Days 56-63	n=4	n=4	n=4
Intake	67.09 ±0.00	66.10 ±0.99	66.73 ±0.36
Faeces	25.33 ±0.86	25.48 ±0.94	23.97 ±0.72
Urine	26.98 ±1.34	24.54 ±0.55	25.82 ±1.25
Retention	14.79 ±1.40	16.08 ±1.39	16.94 ±1.20
Days 77-84	n=3	n=4	n=4
Intake	67.66 ±0.00	67.66 ±0.00	67.59 ±0.07
Faeces	27.91 ±1.96	24.49 ±0.82	25.31 ±0.15
Urine	24.60 ±0.94	24.14 ±1.49	26.36 ±1.23
Retention	15.15 ±1.99	19.04 ±1.57	15.93 ±1.20

Missing data during days 77-84 is due to one calf suffering from bloat.

Table 7. Pathophysiological changes in calves infected with Ostertagia ostertagi infected after prior administration of a MSRB or maintained as clean controls

		Clean		MSRB		Infected	
Days 35-47		B1	B2	Y63	Y64	O3	O4
Plasma Volume	Vp (ml/kg)	50.2	47.6	42.9	45.4	46.5	45.3
Faecal Clearance	I-albumin (ml/day)	37.4	39.5	41.5	41.0	46.9	58.9
Faecal Clearance	Cr (ml/day)	272.8	321.1	341.4	307.8	432.3	437.1
Catabolic rate of albumin	%	4.5	5.2	5.2	5.5	6.8	6.1
Albumin Disappearance	I-albumin T1/2 (days)	27.8	20.0	23.0	27.6	18.7	18.4
Total body water	% bodyweight	73.3	74.1	74.0	72.5	84.6	73.4
TOH Disappearance	T1/2 (days)	170	168	144	168	207	227
Days 70-82		B1	B2	Y63	Y64	O3	O4
Plasma Volume	Vp (ml/kg)	46.4	47.1	41.6	47.3	51.5	47.9
Faecal Clearance	I-albumin (ml/day)	46.3	30.6	28.9	23.7	42.6	54.1
Faecal Clearance	Cr (ml/day)	345.6	324.0	222.8	279.4	295.4	292.6
Catabolic rate of albumin	%	5.4	7.2	5.7	4.8	6.7	4.8
Albumin Disappearance	I-albumin T1/2 (days)	12.4	16.0	19.4	20.6	16.3	35.8
Total body water	% bodyweight	65.1	72.4	70.6	62.7	69.0	69.7
TOH Disappearance	T1/2 (days)	252	145	161	129	103	129

During days 35-47 total body water as a percentage of bodyweight was increased in one infected calf and the tritiated water half-life was increased in both infected calves. However during the second period body water measurements were normal for all six calves and no significant differences were recorded.

Plasma volume measurements for both investigation periods, days 35-47 and days 70-82, revealed no significant differences between treatment groups.

Pathology

The gross appearance of the abomasa in the infected and MSRB calves slaughtered 23 days after infection began were similar with nodules due to O. ostertagi being confined to the fundic region. There was no associated congestion or oedema at this time.

At necropsy at 42 days, nodules were apparent on both the fundus and pylorus of the abomasum from the infected calf and had colaesced forming ulcerative lesions. There was obvious congestion and oedema, thickening of the larger folds and enlargement of the lymph nodes. This substantial abomasal damage was associated with the observed symptoms of Type 1 ostertagiasis, notably a softening of faeces and mild diarrhoea. Nodules were still present in the fundic region of the abomasum of the MSRB treated calf necropsied at 42 days, but more widespread abomasal damage was not apparent and no clinical signs were seen.

Few nodules due to O. ostertagi were observed in abomasa from either infected or MSRB treated calves necropsied at 84 days. However, the abomasa from some infected calves still appeared congested with a morocco leather appearace typical of more chronic ostertagiasis and widespread secondary fungal tracts were present as a consequence of the raised abomasal pH (Ritchie et al, 1966). This contrasted with the normal appearance of abomasa from MSRB treated calves.

Parasitology

The weekly trichostrongyle faecal egg counts (Appendix 3) showed that the faeces of the clean controls were negative throughout the trial. In the MSRB group only occasional positive counts were recorded (maximum mean of 70 epg) whereas the infected calves had positive counts by day 28 rising to a mean maximum of over 700 epg on day 49 and declining steadily to values of 250 epg at day 84 when the experiment

Table 8. Mean burdens of Ostertagia ostertagi and abomasal pH for calves slaughtered 23, 42 and 84 days after initial infection

Treatment	Day	L3 given	n	L4	L5	Adult	Total	Abomasal pH
MSRB	23	46000	1	2200	7600	7200	17000	
Infected	23	46000	1	4400	14000	17200	35600	
MSRB	42	84000	1	200	3400	12400	16000	
Infected	42	84000	1	800	2400	30550	33750	
Clean (±SE)	84	0	7	0 ±0	0 ±0	0 ±0	0 ±0	2.93b ±0.16
MSRB (±SE)	84	84000	7	114b ±40	0 ±0	129 ±99	243b ±123	3.58Ab ±0.36
Infected (±SE)		84000	*6	1883a ±677	0 ±0	6233 ±3062	8117a ±2792	4.23A ±0.17

* One infected calf died as a result of bloat
Significance ab $P<0.05$, Ab $P<0.01$

Plate 6. Scanning electron micrograph (x 80) of pylorus from an infected calf necropsied on day 84. Note worms on the mucosal surface and the large amount of mucous plus necrotic debris.

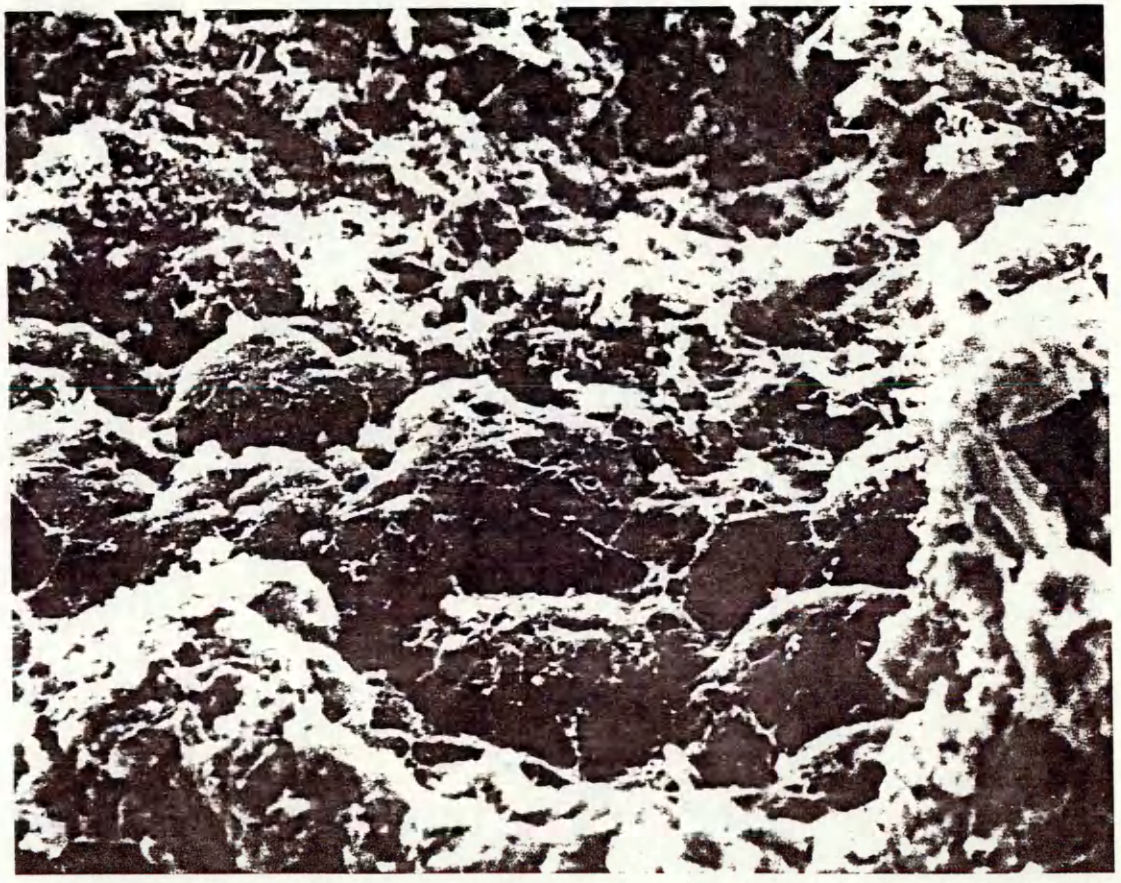


Plate 7. Scanning electron micrograph (x 320) of pylorus from an MSRB calf necropsied on day 84 after infection. The mucosal surface epithelium appeared intact but the cells looked swollen and their

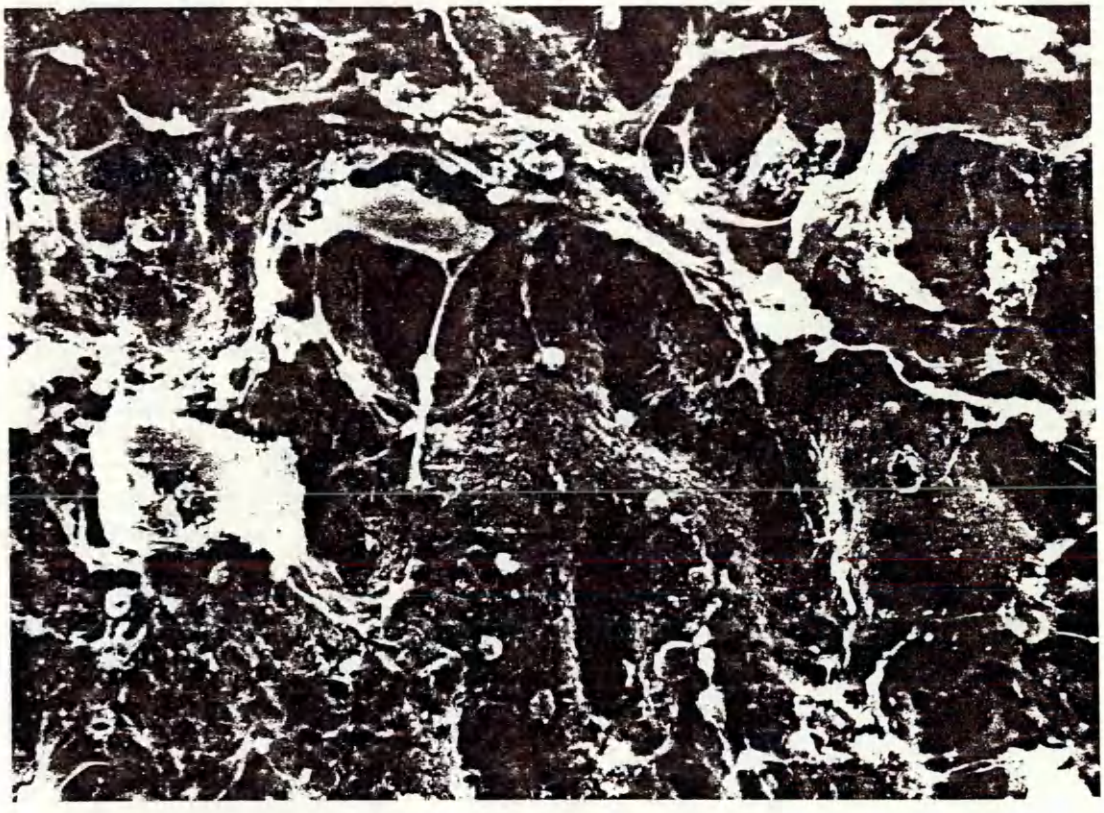


Plate 8. Scanning electron micrograph (x 320) of pylorus from a clean calf necropsied on day 84. The mucosal surface epithelium is intact with a small amount of mucus present. Note the clear delineation between individual cells.

was terminated.

Examination by scanning electron microscopic techniques confirmed that epithelial cellular hyperplasia was still present in the infected calves necropsied on day 84; worms could be seen on the mucosal surface which was covered with a thick layer of mucus and necrotic material (Plate 6). The mucosal surface epithelium of the MSRB calves necropsied on day 84 appeared intact but the cells looked swollen with some excess mucus present and the cell junctions were not fully delineated (Plate 7). In contrast the mucosal surfaces of the clean calves were intact with scant mucus being present (Plate 8).

The mean worm burdens of the calves slaughtered on days 23, 42 and 84 are given in Table 8. (Individual values are presented in Appendix 3). No O. ostertagi were recovered from the clean calves. The establishment of L_3 larvae in the infected calves slaughtered 23 and 42 days after the initial infection was 77% and 40% respectively whereas in the MSRB calves the corresponding values were 37% and 19%. By day 84 means of 8117 and 243 total worms remained in the infected and MSRB groups respectively. Very few developing larvae were present at that time, and the abomasal pH was significantly ($P<0.01$) higher in the infected calves compared to the clean group.

Discussion

The life cycle of O. ostertagi involves several larval stages. Ingested third stage larvae enter the gastric glands of the abomasum and develop through the fourth and fifth larval stages before emerging as adults approximately 21 days later. The principal lesions of ostertagiasis are white raised umbilicated nodules and these, together with associated abomasal damage have been described by Ritchie et al, (1966).

Both the worm burden data and visual appraisal of abomasa in this study demonstrated the efficacy of the MSRB in reducing the establishment of O. ostertagi and preventing associated adverse pathophysiological changes.

The anthelmintic morantel acts on nematodes as a potent agonist against acetyl choline muscle receptors causing contraction and depolarisation (Harrow and Gration, 1985). However there is only a relatively low activity against larval stages found in the gastric mucosa (Cornwell et al, 1973). Plasma pepsinogen values (Fig. 15) for the infected and MSRB treated calves show similar increases up to

day 21 of infection at which time young adult parasites would be emerging from the glands. Thereafter values for the infected calves continued to rise, while those for the MSRB treated calves were maintained at a level slightly higher than that of the clean control calves indicating that the MSRB was limiting infection but that some degree of larval development was occurring. The development of these larval stages may have a beneficial effect in priming the host immune system so that through time this may act in combination with the MSRB limiting larval establishment and apparently increasing the anthelmintic efficacy. It is interesting that Borgsteede (1983) suggested that morantel has a sublethal effect on the larvae allowing them to grow sufficiently to induce an immune response in the host before they were expelled.

Despite evidence of relatively severe and persistent abomasal damage, no depression of appetite or daily liveweight gain of the infected calves was apparent. Possibly increased water retention was responsible for part of the liveweight gain of the infected calves and there was evidence to support this during days 35-47 of the pathophysiological study.

The pathophysiological measurements gave some indication of the adverse effects of O. ostertagi on calf metabolism. Alterations in pathophysiological values were greatest during the expected period of emergence of adult worms from the abomasal mucosa (days 21-63). During this time plasma pepsinogen values for the infected calves became elevated in relation to the values for the clean controls. Parasite development within the gastric glands causes loss of differentiation and stretching of the abomasal mucosa resulting in cell junction breakdown and increased permeability to macromolecules and hence a rise in plasma pepsinogen concentration.

Leakage of blood constituents into the abomasa of infected calves also occurred at this time as was demonstrated by increased faecal clearances of both ^{125}I-albumin and $^{51}CrCl_3$, increased albumin catabolic rates and reduced half life of the radioactive albumin compared to the clean calves. Loss of albumin in this manner leads to increased rates of synthesis, and in severe infections, where this cannot match increased losses, hypoalbuminaemia results (Mulligan et al, 1963).

Pathophysiological measurements made after the completion of worm emergence (days 70-82) revealed differing responses of the two infected

calves. One calf continued to show evidence of leakage of macromolecules between the blood and the abomasum while the other was apparently recovering from infection and was normal for most of the pathophysiological parameters measured. The particularly low value for albumin catabolic rate recorded for this calf implies the occurrence of post-infection hypocatabolism of albumin.

The increased half life of tritiated water and the slight increase in total body water indicates a degree of water retention by the infected calves during the first pathophysiological investigation. This contrasts with work by Halliday et al, (1968) in which there was no evidence of increased water retention in calves suffering from Type I ostertagiasis following administration of large single doses of O. ostertagi larvae. Holmes & Bremner (1971) found that in sheep given a large single infection of O. circumcincta a reduction in body water turnover was principally associated with anorexia induced by parasitic infection. Calves in this experiment did not become inappetent and the cause of the reduced body water turnover rate is unclear.

The alterations to the protein metabolism of the infected and to a lesser extent the MSRB treated calves, as shown by the pathophysiological study, occurred without alterations in apparent protein digestion. The high abomasal pH of the infected calves implies that abomasal protein digestion may have been reduced, and that a possible compensatory increase in digestive efficiency occurred in the lower gut. The increased leakage of albumin into the abomasa of the infected calves did not result in significant increases in faecal nitrogen content and thus this albumin was possibly broken down and recycled as ammonia.

Finally, it is interesting that the level of infection used in this trial, which may be encountered under natural grazing conditions in the U.K., produced pathophysiological changes in infected calves without acute clinical disease. Prior administration of a MSRB prevented these changes by reducing the establishment of infection and possibly promoting the development of the immune response.

EXPERIMENT IIb

A PRELIMINARY STUDY OF THE EFFECT OF COLD CONDITIONING ON INFECTIVE LARVAE OF OSTERTAGIA OSTERTAGI

Introduction

Arrested larval development, or hypobiosis, is a predisposing factor in the development of Type II ostertagiasis. This phenomenon may be defined as the temporary cessation of development of a nematode at a precise point in its early parasitic life and in the case of Ostertagia species this occurs at the early fourth larval stage (EL_4). An increased incidence of hypobiosis has been associated with autumn grazing (Anderson et al, 1965; Armour et al, 1969a) and Martin et al (1957) originally suggested that this resulted as a consequence of immunity acquired by the host in response to larval challenge during the grazing season. However Armour et al (1969a) reported similar levels of EL_4 in both autumn grazing permanent and tracer calves irrespective of number of days grazed and subsequently suggested (Armour et al, 1969b) that arrested development may be a feature of ageing larval populations or may be attributed to the effect of the autumn environment on either the larvae or the host. Other work (Armour and Bruce, 1974; Michel et al, 1974; Michel et al, 1975) demonstrated that autumn temperature changes are primarily responsible for the induction of hypobiosis and that host endocrine changes are not involved. Spontaneous redevelopment of arrested larvae usually occurs 16-18 weeks after entry into the host, 21-23 after the commencement of the conditioning treatment (Michel et al, 1976).

The present trial investigates the effect of storage of O. ostertagi L_3 at $4^{o}C$ prior to oral inoculation of calves on the incidence of arrested larval development and assesses the MSRB as a means of controlling nematode populations in the calf.

Materials and Methods

Animals

Nine castrated male Friesian calves aged eighteen weeks, weighing approximately 140 kg and which had been reared under helminth-free conditions were used. Throughout the trial they were loose housed together in a straw bedded pen and received a daily feed allowance of 3 kg hay and 1 kg of a standard 15% CP (fresh matter) concentrate pellet

(190 Keystart Rearer Pencils, BOCM Silcock Ltd., Basingstoke, Hampshire). The concentrate allowance was reduced slightly towards the end of the trial period to ensure that the ration was completely consumed.

Treatments

Equal numbers of calves were allocated to each of three treatment groups.

Group 1 calves were dosed per os with 2,000 O. ostertagi infective larvae daily for 42 days. The larvae were harvested from faecal culture and were no more than 24 hours old at oral inoculation.

Group 2 calves were similarly dosed but larvae were initially chilled and subsequently maintained at 4°C for 28-35 days prior to oral inoculation.

Group 3 calves were dosed per os with 4,000 freshly harvested O. ostertagi infective larvae every second day for 42 days. One calf in each treatment group additionally received a morantel sustained release bolus (MSRB, Pfizer Ltd.) at the commencement of the trial (control and bolus treated calves).

Faecal samples were obtained per rectum from all calves at weekly intervals and examined for the presence of O. ostertagi eggs by a modified McMaster technique (Gordon and Whitlock, 1939). Blood samples were also taken for plasma pepsinogen determination.

Calves were necropsied 84 days after the initial larval inoculation and the abomasa examined for O. ostertagi as previously described.

Results

Clinical Observations

Administration of freshly harvested infective larvae to calves resulted in reduced voluntary food intake and intermittent profuse diarrhoea, these symptoms being typical of Type I ostertagiasis and necessitating the necropsy in extremis on day 63 of one calf receiving 4,000 L_3 every second day. Prior administration of an MSRB prevented these changes and calves inoculated with cold-conditioned larvae also remained clinically normal throughout the trial.

Table 9. Plasma pepsinogen values (IU) for calves infected with 2000 fresh or chilled O. ostertagi infective larvae daily or 4000 larvae every second day with or without the prior administration of a MSRB.

	2000 fresh L3/day			2000 chilled L3/day			4000 fresh L3/2 days		
Day	*B11	B12	B13	*W21	W22	W23	*R13	R14	R15
7	0.812	0.790	0.864	0.737	0.856	0.948	0.613	0.685	0.748
14	-	-	-	-	-	-	-	-	-
21	0.717	0.876	0.905	0.837	0.716	0.863	1.182	0.519	1.002
28	0.926	1.327	1.140	1.358	0.859	0.907	1.368	0.875	0.965
35	1.048	2.053	1.963	1.712	1.634	1.439	1.587	2.583	2.340
42	1.147	5.119	4.882	1.534	2.644	2.481	1.675	3.339	3.111
49	2.044	5.376	5.543	2.144	3.105	3.200	2.071	2.810	2.769
56	1.612	6.996	6.480	2.265	3.396	2.984	2.089	1.830	2.410
63	1.549	7.460	6.410	2.202	3.519	3.336	1.550	2.290	2.337
70	1.518	6.812	6.399	1.554	3.298	3.544	1.812	-	3.067
77	1.222	6.494	5.788	1.506	3.237	3.002	1.481	-	2.515
84	1.013	4.761	5.043	1.259	2.796	3.178	1.198	-	2.312
		Mean			Mean			Mean	
7		0.827			0.902			0.717	
14		-			-			-	
21		0.891			0.790			0.761	
28		1.234			0.883			0.920	
35		2.008			1.537			2.462	
42		5.001			2.563			3.225	
49		5.460			3.153			2.790	
56		6.738			3.190			2.120	
63		6.935			3.428			2.314	
70		6.606			3.421			3.067	
77		6.141			3.120			2.515	
84		4.902			2.987			3.212	

* MSRB administered

Table 10. Faecal egg counts (epg) for calves infected with 2000 fresh or chilled O.ostertagi infective larvae daily, or 40000 larvae every second day, with or without the prior administration of a MSRB.

	2000 fresh L3/day			2000 chilled L3/day			4000 fresh L3/2 days		
Day	*B11	B12	B13	*W21	W22	W23	*R13	R14	R15
7	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	50	0
35	–	–	–	–	–	–	–	–	–
42	250	400	300	150	100	50	200	50	50
49	50	0	150	300	800	250	50	300	150
56	–	–	–	–	–	–	–	–	–
63	–	150	250	150	450	300	50	200	350
70	1000	400	0	200	400	250	300	–	150
77	200	500	250	–	150	200	50	–	150
84	–	–	–	–	–	–	–	–	–
		Mean			Mean			Mean	
7		0			0			0	
14		0			0			0	
21		0			0			0	
28		0			0			25	
35		–			–			–	
42		350			75			50	
49		75			525			225	
56		–			–			–	
63		200			375			275	
70		200			325			150	
77		375			175			150	
84		–			–			–	

* MSRB administered

Table 11. Worm burden at necropsy and abomasal pH for calves infected with 2000 fresh or chilled O.ostertagi larvae daily or 4000 larvae every second day

Treatment	Calf	L4	Adult	Total	%L3 given	Abo. pH	Lesion score
Group 1 2000 chilled L3/day	*W21	10800	3600	14400	17	2.5	1
	W22	11200	12200	23400	28	2.9	1
	W23	12400	7400	19800	24	-	-
Mean of controls		11800	9800	21600	26		
+SE		+600	+2400	+1800	+2		
Group 2 2000 fresh L3/day	*B11	0	2200	2200	3	2.3	1
	B12	0	12800	12800	15	3.2	2
	B13	0	12400	12400	15	-	-
Mean of controls		0	12600	12600	15		
+SE		+0	+200	+200	+0		
Group 3 4000 fresh L3/2 days	*R13	0	4600	4600	6	2.1	1+
	R14	0	17900	17900	21	3.4	1
	R15	0	15600	15600	19	-	-
Mean of controls		0	16750	16750	20		
+SE		+0	+1150	+1150	+1		

* MSRB administered
Calf slaughtered in extremis on day 63
Lesion score; 0=none, 3=severe

Plasma pepsinogen

Plasma pepsinogen values (Table 9) were increased above initial levels from day 28 for calves in all treatment groups, this being particularly so for calves inoculated with 2,000 fresh larvae per day. Prior administration of an MSRB limited the extent of this elevation.

Parasitology

Positive faecal egg counts were recorded for all calves from day 35 until the end of the trial period (Table 10).

At necropsy 84 days after the initial larval inoculation white raised umbilicated nodules typical of O. ostertagi infection were observed on the abomasa of all calves. Examination of the worm burdens revealed no developing stages present in calves inoculated with fresh larvae. In those inoculated with cold-conditioned larvae however, approximately 14% of the L_3 given had become inhibited at the early fourth larval stage (55% of the total worm burden). Prior administration of an MSRB reduced total worm burdens by approximately 78% in calves receiving fresh larvae but due to its apparent inactivity against inhibited forms, worm burdens were only reduced by 38% in calves receiving cold-conditioned larvae. Worm burdens and associated data are presented in Table 11.

Discussion

From the results of this study it may be concluded that exposure to low temperatures, even for a relatively short period of time, is a stimulus for inhibited parasitic development of O. ostertagi larvae. Variations in the dosing regime using fresh larvae were not effective in inducing hypobiosis thus confirming the results of Anderson et al, (1967). However cold-conditioning per se only induced 54% of the 84 day worm burden to become hypobiotic and it is possible that under natural conditions other factors such as decreasing daylength may also be involved. Alternatively the relatively low percentage of inhibited larvae may reflect the use of a laboratory strain of O. ostertagi for experimental infection. Armour et al (1967b) have previously reported that laboratory strains show a reduced propensity for arrested development compared to field strains, this possibly being attributable to repeated passage through donor animals. Variation between field strains for this characteristic has also been reported however (Borgsteede and Eysker, 1987; Smeal and Donald, 1982).

The MSRB provided good control of infections arising from fresh larval inoculation but, while giving good control of cold-conditioned larvae undergoing normal development, it was apparently without effect against those entering the hypobiotic state. Thus, in contrast to Bliss and Jones (1983) who suggest that the MSRB present and active in the reticulum/rumen of cattle prevents the establishment of ingested larvae, these results may indicate that the MSRB is primarily effective against adult worms. Alternatively, within a single strain of O. ostertagi there may be two morphs with differing propensities for arrested development. Third stage larvae which readily respond to cold-conditioning may be metabolically different from those that do not and this may reduce the efficacy of morantel against them on ingestion. Experimental studies suggest that inhibiting and non-inhibiting morphs of O. ostertagi do exist (Armour, 1970; Smeal and Donald, 1984).

A reduced efficacy of the anthelmintic fenbendazole against larvae in the hypobiotic state has occasionally been reported (Duncan et al, 1977) but this is not attributable to reduced anthelmintic uptake by larvae in a supressed metabolic state (Prichard et al, 1978). Rather, the reduced energy requirements of such larvae dictate that lethal concentrations of this anthelmintic, which acts by disrupting parasite energy metabolism, must be maintained for a greater length of time in order to kill hypobiotic larvae compared to metabolically active developing larvae and adult worms. The presence of the MSRB in the rumen/reticulum of cattle should ensure that high concentrations of morantel are maintained in the host animal for some considerable time. A possible explanation for the apparent lack of effect against hypobiotic larvae is that morantel acts by the induction of a neuromuscular block inducing rapid paralysis (Harrow and Gration, 1985) and thus promotes the expulsion of adult worms with digesta flow. However it is unlikely that this mode of action would effect the destruction and removal of hypobiotic larvae from within the gastric mucosa.

Anderson (1985) considered that currently the main application of controlled release technologies, such as the MSRB, was in reducing pasture contamination prior to and during a period when survival and development of the free-living stages of a parasite was especially favourable. Used in this way, the MSRB would limit pasture larvae exposed to conditions of late autumn and thus be effective in the prophylaxis of Pre-Type II and Type II ostertagiasis.

EXPERIMENT IIc

PATHOPHYSIOLOGICAL AND PARASITOLOGICAL STUDIES ON PRE TYPE II OSTERTAGIA OSTERTAGI INFECTION IN CALVES

Introduction

Pre-Type II ostertagiasis was first described by Anderson et al (1965) and normally occurs during the winter in cattle which have been grazing pastures heavily contaminated with O. ostertagi larvae in late autumn. No clinical symptoms are apparent despite the presence of large numbers of hypobiotic larvae in the abomasae of infected animals and it is the synchronous development of these larvae in the spring which eventually precipitates clinical Type II disease. Gastrointestinal damage and associated physiological changes arising from O. ostertagi infection are primarily attributable to the emergence of adult worms from the gastic mucosa and consequently the Pre-Type II condition would be expected to have minimal impact on animal metabolism. However Anderson et al (1965) did consider that cattle with Pre-Type II ostertagiasis frequently appeared ill-thriven. The following study investigates the pathophysiological consequences of Pre-Type II ostertagiasis in cattle produced by daily infections of cold-conditioned O. ostertagi larvae.

Materials and Methods

Animals and experimental design

The experimental design was essentially that described in Experiment IIa. Twenty-five castrated male Friesian calves aged four months, weighing approximately 120 kg, and which had been reared under helminth-free conditions were allocated to three treatment groups.

Group A calves (clean) were maintained free of parasites. Five were used as control animals for digestion and nitrogen economy studies and a further two for radioisotopic investigations.

Group B calves (MSRB) were each given a morantel sustained release bolus (MSRB, Paratect; Pfizer). The calves were subsequently dosed per os with 2,000 cold-conditioned O. ostertagi infective larvae daily for six weeks. Five calves were allocated to the digestion studies, two to the radioisotopic investigation and two necropsied 21 and 42 days after initial infection.

Group C calves (infected) were treated similarly, but without

prior MSRB administration, with five calves allocated for digestion studies, two for radioisotopic work and two for necropsy during the course of the trial (days 21 and 42).

All calves were housed together and the parasite-free status of the clean control animals was ensured by dosing fortnightly with 10 ml fenbendazole (Panacur, Hoechst). Blood and faecal samples were collected at approximately weekly intervals for biochemical and parasitological analyses and calves were clinically examined and weighed every three weeks.

Feed allowance

The calves were individually given a complete ration twice a day (Superstar cubes; Hamlyn Milling). Mean analyses were 0.89 dry matter (DM), with 143 g crude protein (CP), 147 g crude fibre (CF), 43 g ether extract (EE), 91 g ash and 18.0 MJ gross energy (GE) kg DM^{-1}. This was supplemented with barley husks (siftings) to produce a uniform mixture during periods when calves were accommodated in metabolism stalls in order to increase long fibre intake and prevent bloat. The initial daily feed allowance of 3.0 kg starcubes and 0.3 kg siftings was increased to 4.0 kg starcubes and 0.4 kg siftings on day 42 to allow for sustained growth of the calves.

Larval infections

The O. ostertagi larvae were harvested from faecal cultures less than 21 days prior to oral inoculation of the calves, and were maintained at 4°C following a period of initial chilling in order to induce hypobiosis.

Parasitology and pathology

Parasitological and pathological techniques were as described in Experiment IIa.

Blood analyses

Blood was analysed for plasma pepsinogen by the method of Edwards et al (1960) where one international unit (I.U.)is equivalent to the release of one m mol tyrosine/litre plasma/minute.

Digestibility and nitrogen balance studies

The fifteen calves involved in the metabolism study were allocated to three equal sized treatment groups on the basis of liveweight. Digestibility and nitrogen balance data were obtained from four animals in each group on days 14-21, 35-42, 56-63 and 77-84 as described in Experiment IIa, the lightest animal in each group being maintained as a potential replacement.

Radioisotopic measurements

Radioisotopic studies were conducted with two calves from each of the treatment groups clean, MSRB and infected on days 35-47 and 70-82 following the first infection. Each calf was given an intramuscular injection of 110 MBq of tritiated water (TOH) after overnight withdrawal of food and water to standardize water present in the gut. Total body water and body water turnover were determined by the dilution technique using extrapolated plasma values as previously described.

The TOH injection was followed by a combined intravenous injection of ^{125}I-bovine albumin and $^{51}CrCl_3$ three days later. During the first recording period 59 MBq of $^{51}CrCl_3$ were given, this being increased to 73 MBq during the second recording period. 15 MBq of ^{125}I-albumin was given in both recording periods. Plasma, faecal and urine samples were obtained, analysed and used for calculation of plasma volume, plasma protein losses into the gastrointestinal tract, albumin turnover and albumin catabolic rate as previously described.

Statistical analysis

Analysis of variance and t-tests were performed using 'Minitab' (Ryan, Penn State University) on a microcomputer, as for Experiment IIa.

Results

Clinical observations

All calves remained clinically normal throught the trial and it was not necessary to include the calves maintained as possible replacements in the metabolism studies. Periodic softening of faeces did occur among the calves but this cleared up spontaneously and did not appear to be treatment related.

The mean liveweights for the 15 calves used in the metabolism

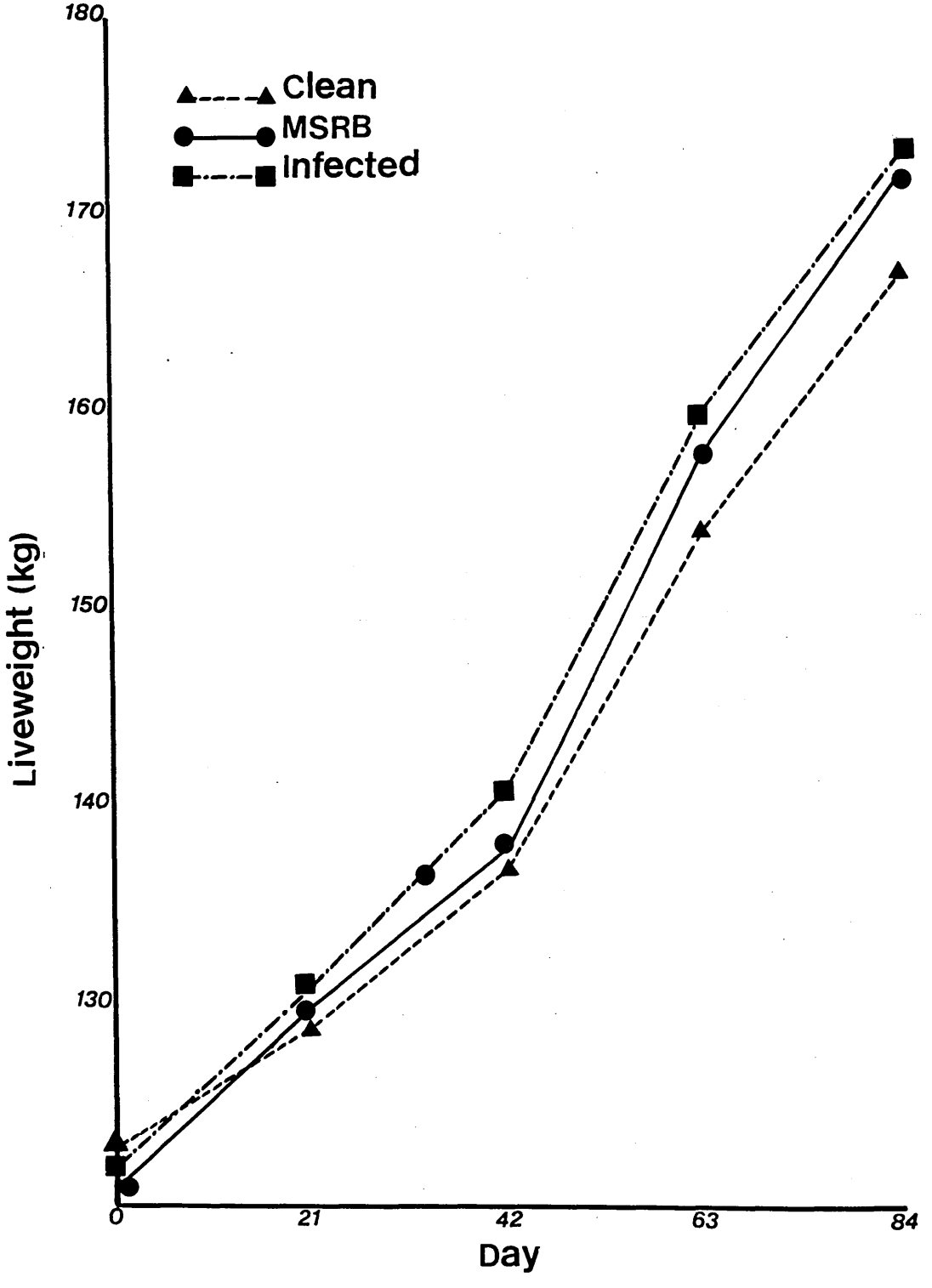


Figure 16. Mean liveweights of metabolism study calves infected with cold-conditioned Ostertagia ostertagi, infected after administration of a MSRB or maintained as clean controls.

Table 12. Mean plasma pepsinogen values for metabolism study calves infected with cold-conditioned O.ostertagi larvae, infected after prior administration of a MSRB or maintained as clean controls

Day (n=5)	7	21	28	42	56	70	84	Overall
Clean	0.575	0.585b	0.710A	0.633B	0.630A	0.618ab	0.602B	0.622
+SE	+0.053	+0.041	+0.038	+0.056	+0.052	+0.041	+0.065	+0.019
MSRB	0.534	0.824a	1.073b	1.483A	1.355b	1.002b	0.771Ab	1.005
+SE	+0.049	+0.071	+0.087	+0.134	+0.132	+0.063	0.044	+0.061
Infected	0.472	0.867a	1.147Bb	1.772A	1.863aB	1.342A	0.949A	1.202
+SE	+0.033	+0.041	+0.074	+0.169	+0.176	+0.136	+0.072	+0.088

Significance ab P<0.05, Ab P<0.01, AB P<0.001

Table 13. Mean faecal egg output (epg) for metabolism study calves infected with cold-conditioned O.ostertagi larvae, infected after prior administration of a MSRB or maintained as clean controls

Day	21	28	35	42	49	56	63	70	77	84
Clean	0	0	-	0	0	0	0	0	0	0
+SE	+0	+0	-	+0	+0	+0	+0	+0	+0	+0
MSRB	10	20	-	20	60	70	20	10	40	40
+SE	+10	+12	-	+12	+60	+34	+20	+10	+40	+10
Infected	0	30	-	230	50	170	160	60	90	10
+SE	+0	+20	-	+68	+39	+25	+37	+29	+33	+10

studies are shown in Figure 16. There were no significant differences in the total mean liveweight gains between treatments, these being 45.4, 51.0 and 50.6 kg (0.54, 0.61 and 0.60 kg/day) for the clean, MSRB and infected groups respectively.

Blood and faecal analyses

Mean values for plasma pepsinogen and faecal egg output for the fifteen metabolism study calves are presented in Tables 12 and 13 respectively. No positive faecal egg counts were recorded for clean control calves and plasma pepsinogen was maintained at normal levels (<1.0 I.U.). Positive faecal egg counts were first recorded on day 21 for MSRB treated and day 28 for infected calves continuing at a low level and reaching mean maxima of 70 and 230 epg respectively. A small but significant increase in plasma pepsinogen relative to the clean controls was noted for both infected and bolused groups from day 21. MSRB treated calves reached a mean maximum value of 1.483 I.U. on day 42 while values for the infected calves ranged from 0.472 to 1.863 I.U.

Apparent digestibility coefficients

Examination of apparent digestibility data for each of the four separate recording periods revealed significant treatment related differences for most of the standard proximate feed fractions during days 56-63 immediately following a feed increase. In general, apparent digestibilities were highest for the MSRB treated calves and lowest for the control group with the infected group being intermediate. Similar trends were apparent during days 14-21 and 35-42 but statistical significance was not attained. Mean values for each recording period, together with the overall mean for all four recording periods are presented in Table 14. (Values for individual calves are given in Appendix 4).

Nitrogen balance

Mean results for the daily nitrogen (N) input, output in urine and faeces and the calculated retentions for the three treatment groups in each of the four recording periods together with that for the overall experiment are presented in Table 15. Individual values and the data expressed in terms of a percentage of nitrogen input are given in Appendix 4. No significant differences in nitrogen balance occurred except in period III when the MSRB group of calves showed a

Table 14. Mean apparent digestibility coefficients for calves infected with cold-conditioned O. ostertagi larvae, infected after prior administration of a MSRB or maintained as clean controls (±SE)

	Clean	MSRB	Infected
Days 14-21			
DM	0.566 ±0.006	0.588 ±0.004	0.580 ±0.019
CP	0.597 ±0.005	0.598 ±0.017	0.605 ±0.019
CF	0.180 ±0.015	0.239 ±0.016	0.215 ±0.033
EE	0.916 ±0.004	0.921 ±0.003	0.916 ±0.007
ASH	0.416 ±0.009	0.465 ±0.016	0.434 ±0.020
NFE	0.663 ±0.006	0.676 ±0.004	0.674 ±0.018
OM	0.581 ±0.005	0.600 ±0.004	0.595 ±0.019
Energy	0.573 ±0.007	0.601 ±0.004	0.599 ±0.017
Days 35-42			
DM	0.572 ±0.006	0.612 ±0.011	0.581 ±0.016
CP	0.609 ±0.010	0.630 ±0.019	0.614 ±0.005
CF	0.219 ±0.018b	0.321 ±0.019A	0.266 ±0.027Ab
EE	0.907 ±0.001	0.914 ±0.003	0.901 ±0.005
ASH	0.399 ±0.018	0.423 ±0.018	0.387 ±0.025
NFE	0.672 ±0.001	0.703 ±0.008	0.675 ±0.015
OM	0.590 ±0.005	0.632 ±0.011	0.601 ±0.015
Energy	0.583 ±0.004	0.620 ±0.013	0.585 ±0.016
Days 56-63			
DM	0.564 ±0.007Bb	0.613 ±0.007A	0.582 ±0.004b
CP	0.610 ±0.012	0.638 ±0.007	0.621 ±0.009
CF	0.162 ±0.022B	0.261 ±0.024Ab	0.224 ±0.007b
EE	0.930 ±0.004A	0.911 ±0.001b	0.902 ±0.003Bb
ASH	0.398 ±0.008a	0.435 ±0.011b	0.383 ±0.014Aa
NFE	0.667 ±0.007Bb	0.710 ±0.007A	0.679 ±0.004b
OM	0.582 ±0.009Aa	0.630 ±0.009b	0.601 ±0.004a
Energy	0.574 ±0.011Aa	0.623 ±0.009b	0.593 ±0.006a
Days 77-84			
DM	0.603 ±0.024	0.587 ±0.008	0.588 ±0.003
CP	0.649 ±0.023	0.628 ±0.014	0.634 ±0.008
CF	0.223 ±0.057	0.195 ±0.012	0.210 ±0.010
EE	0.923 ±0.007	0.910 ±0.003	0.917 ±0.002
ASH	0.424 ±0.037	0.413 ±0.019	0.390 ±0.011
NFE	0.701 ±0.015	0.681 ±0.006	0.686 ±0.006
OM	0.621 ±0.023	0.599 ±0.008	0.607 ±0.003
Energy	0.617 ±0.021	0.595 ±0.006	0.595 ±0.006
Overall			
DM	0.577 ±0.007b	0.599 ±0.005a	0.583 ±0.006ab
CP	0.616 ±0.008	0.623 ±0.008	0.619 ±0.006
CF	0.196 ±0.016b	0.254 ±0.014a	0.229 ±0.011ab
EE	0.919 ±0.003A	0.914 ±0.002Ab	0.909 ±0.003b
ASH	0.409 ±0.010ab	0.434 ±0.009a	0.399 ±0.010b
NFE	0.676 ±0.006	0.693 ±0.005	0.678 ±0.006
OM	0.594 ±0.007b	0.615 ±0.006a	0.601 ±0.006ab
GE	0.587 ±0.007b	0.609 ±0.005A	0.593 ±0.006Ab

Significance ab $P<0.05$, Ab $P<0.01$, AB $P<0.001$

Table 15. Mean nitrogen balance (g/day +SE) for calves infected with cold-conditioned O.ostertagi larvae, infected after prior administration of a MSRB or maintained as clean controls

	Clean	MSRB	Infected
Days 14-21			
Intake	64.59 ±0.61	64.88 ±0.41	65.26 ±0.04
Faeces	26.02 ±0.32	26.10 ±1.06	25.76 ±1.24
Urine	23.34 ±2.09	25.66 ±1.69	24.41 ±0.80
Retention	15.23 ±2.19	13.12 ±2.61	15.09 ±1.64
Days 35-42			
Intake	62.63 ±0.00	62.63 ±0.00	62.63 ±0.00
Faeces	24.48 ±0.61	23.16 ±1.18	24.17 ±0.31
Urine	26.43 ±0.41	25.61 ±1.55	25.40 ±2.57
Retention	11.72 ±0.98	13.86 ±2.68	13.06 ±2.84
Days 56-63			
Intake	83.98 ±0.51	84.86 ±0.00	84.86 ±0.00
Faeces	32.74 ±0.90	30.74 ±0.62	32.15 ±0.73
Urine	32.15 ±1.41	30.24 ±1.05	31.54 ±0.78
Retention	19.10 ±0.98b	23.88 ±0.74a	21.17 ±1.45ab
Days 77-84			
Intake	84.75 ±0.00	80.87 ±3.38	84.75 ±0.00
Faeces	29.77 ±1.91	29.96 ±0.65	31.03 ±0.71
Urine	34.95 ±0.69	32.70 ±1.81	31.61 ±0.70
Retention	20.04 ±2.36	18.21 ±3.70	22.12 ±0.77
Overall			
Intake	73.99 ±2.69	73.35 ±2.63	74.37 ±2.70
Faeces	28.25 ±0.97	27.49 ±0.89	28.28 ±0.95
Urine	29.22 ±1.32	28.55 ±1.05	28.24 ±1.08
Retention	16.52 ±1.16	17.27 ±1.62	17.86 ±1.29

Significance ab $P<0.05$

significantly improved nitrogen retention compared to the clean, but not the infected group of calves. This was apparently the result of reductions in both urinary and faecal nitrogen output.

Radioisotopic measurements

Faecal clearances of both ^{125}I-albumin and $^{51}CrCl_3$ were elevated for infected and to a lesser extent MSRB treated calves relative to clean controls during the first recording period indicating an increased loss of plasma into the gastrointestinal tract. This persisted into the second recording period for one infected and one MSRB treated calf. Values calculated using ^{125}I-albumin were lower than those using $^{51}CrCl_3$ because of enzyme action and reabsorption of a considerable portion of the iodine label which was later excreted in the urine. Increased plasma faecal clearances were partially reflected by a reduced ^{125}I-albumin half-life in one MSRB treated and one infected calf but the catabolic rate of albumin did not differ between treatment groups to any marked extent.

Plasma volumes expressed as ml/kg were increased for infected calves in both recording periods. Relatively lower values were recorded in the second period for all calves, this possibly being an effect of increased body weight. Body water turnover rates were also marginally increased for infected calves during days 35-47.

Pathology and parasitology

Occasional nodules due to O. ostertagi infection were apparent in the fundic region of abomasa from the infected and MSRB treated calves slaughtered 21 days after the initial larval inoculation.

The gross appearance of the abomasa from both the infected and MSRB calves necropsied at 42 and 84 days was similar, few nodules due to O. ostertagi again being confined to the fundic region. There was no evidence of widespread abomasal damage and lymph nodes were only slightly enlarged.

Worm burdens at necropsy for infected and MSRB treated calves are given in Table 16. No O. ostertagi were recovered from clean control calves. Larval establishment in the infected calves necropsied at 21 and 42 days was 32 and 45% of the L_3 given respectively whereas in the MSRB treated calves it was 20 and 22%. By day 84 post infection means of 14343 and 11657 O. ostertagi were recorded for the infected and MSRB treated calves (17 and 14% of the L_3 given respectively).

Table 16. Pathophysological changes in calves infected with cold-conditioned O.ostertagi larvae, infected after prior administration of a MSRB or maintained as a clean control

Days 35-47		Clean B31	Clean B32	MSRB W35	MSRB W36	Infected O37	Infected O40
Plasma Volume	Vp (ml/kg)	43.1	44.3	42.8	41.3	48.4	46.9
Faecal Clearance	I-albumin (ml/day)	54.0	44.2	69.3	79.0	80.7	75.4
Faecal Clearance	Cr (ml/day)	216.9	175.5	268.7	295.7	336.7	317.4
Catabolic rate of albumin	%	5.2	4.3	5.2	5.0	5.3	5.3
Albumin Disappearance	I-albumin T1/2 (days)	20.2	26.0	22.0	14.5	23.0	17.5
Total body water	% bodyweight	73.7	70.4	72.0	68.4	70.1	68.6
Body water turnover	(l/day)	6.4	7.5	6.6	9.3	8.2	8.0
TOH Disappearance	T1/2 (days)	11.7	8.4	11.0	7.0	8.9	7.4

Days70-82		B31	B32	W35	W36	O37	O40
Plasma Volume	Vp (ml/kg)	37.1	39.9	38.5	37.6	41.9	40.9
Faecal Clearance	I-albumin (ml/day)	40.8	42.9	41.8	67.8	98.3	53.2
Faecal Clearance	Cr (ml/day)	308.7	258.1	438.4	248.1	651.3	301.1
Catabolic rate of albumin	%	6.8	5.3	5.3	6.4	5.9	6.0
Albumin Disappearance	I-albumin T1/2 (days)	15.4	24.9	18.4	11.9	18.2	18.4
Total body water	% bodyweight	65.7	65.4	68.5	71.0	70.0	68.3
Body water turnover	(l/day)	10.9	12.5	12.3	14.4	12.1	14.1
TOH Disappearance	T1/2 (days)	4.2	4.7	5.5	4.7	6.4	3.9

Table 17. Worm burdens at necropsy of calves infected with cold-conditioned O. ostertagi larvae, infected after prior administration of a MSRB or maintained as a clean control

Slaughter (Days PI)	L3 given	Group	Calf	L4	L5	Adult	Total	L4 as % Total	Total as % L3
21	42000	MSRB Infected	W34	4200	700	3400	8300	50.6	19.8
			O39	3400	2000	8200	13600	25.0	32.4
42	84000	MSRB Infected	W33	8600	2800	7400	18800	45.7	22.4
			O36	13900	2400	21200	37500	37.1	44.6
84	84000	MSRB	W1	14600	200	800	15600	93.6	18.6
			W2	10800		600	11400	94.7	13.6
			W3	8000		800	8800	90.9	10.5
			W4	5400		2600	8000	67.5	9.5
			W5	8800		2000	10800	81.5	12.9
			W35	10600		2200	12800	82.8	15.2
			W36	13400		800	14200	94.4	16.9
			MEAN	10229	29	1400	11657	86.5	13.9
			±SE	±1195	±29	±315	±1043	±3.8	±1.2
		Infected	O6	8900	200	7800	16900	52.7	20.1
			O7	18400	200	2600	21200	86.8	25.2
			O8	8400		2500	10900	77.1	13.0
			O9	11800		3800	15600	75.6	18.6
			O10	9800		1600	11400	86.0	13.6
			O37	10100		2100	12200	82.8	14.5
			O40	10800		1400	12200	88.5	14.5
			MEAN	11171	57	3114	14343	78.5	17.1
			±SE	±1279	±37	±836	±1421	±4.7	±1.7

No O.ostertagi were found in the clean control calves

Table 18. Abomasal pH at necropsy of calves infected cold-conditioned O. ostertagi larvae, infected after prior administration of a MSRB or maintained as a clean control

Treatment	Calf	Abo.pH	Mean ±SE
Clean	B13	3.48	4.23
	B14	4.32	+0.20
	B15	-	
	B16	4.19	
	B17	-	
	B31	4.66	
	B32	4.50	
MSRB	W1	4.70	4.31
	W2	3.84	+0.23
	W3	3.99	
	W4	-	
	W5	-	
	W35	4.01	(no bolus)
	W36	5.00	
Infected	O6	3.95	4.15
	O7	4.35	+0.17
	O8	4.21	
	O9	3.47	
	O10	-	
	O37	4.13	
	O40	4.76	

Of these 79% in the infected calves and 87% in the bolused calves were in the form of arrested fourth stage larvae.

Larval infections occurred without significant alterations in the pH of the abomasal fluid (Table 18).

Discussion

The results reported here endorse those of Experiment IIb in that relatively short term exposure to low temperatures (4°C) was sufficient to induce arrested development in O. ostertagi third stage larvae. This is at variance however with the findings of Kloosterman et al (1984) who observed only a sporadic occurrence of arrested development following storage of O. ostertagi third stage larvae at 4°C for up to twelve weeks prior to oral inoculation of calves.

Worm burden data again provides evidence that the MSRB is primarily active in promoting the expulsion of adult worms, numbers of inhibited and developing stages of O. ostertagi being similar in both infected and MSRB treated calves particularly at the 84 day necropsy. Removal of adult worms by the action of morantel did not promote the continued development of early fourth larval stages (Michel, 1971) and thus the arrested development induced by limited chilling was truly akin to the hypobiotic state of Pre-Type II ostertagiasis.

The established infections resulted in some abomasal mucosal damage as is evidenced by elevated plasma pepsinogen values for both infected and MSRB treated calves. These occurred from day 21 following the initial larval inoculation and may be attributed to the emergence of adult worms from the gastic mucosa and associated stretching and breakdown of cell junctions resulting in pepsinogen leakage from the abomasum (Armour, 1970). It is possible that higher values were observed in infected compared to MSRB treated calves because the presence of the bolus limited larval infection (Jones and Bliss, 1983). However in view of the similar numbers of developing stages in both groups of calves, a more likely explanation is that the enhanced removal of adult worms in MSRB treated calves promoted repair of abomasal damage. McKellar (1984) has demonstrated that the establishment of an adult population of O. ostertagi by transplantation can result in dramatic increases in plasma pepsinogen in the absence of any developmental damage to the abomasal mucosa. The presence and limited development of the nematodes stimulated the immune systems of both infected and MSRB treated calves as is evidenced by the

slight enlargment of the lymph nodes.

Radioisotopic measurements indicated that the physiological effects of a predominantly arrested O. ostertagi population were small. Increased plasma faecal clearances for infected and MSRB treated calves may be attributed to abomasal damage resulting from the development to and emergence of adult worms. However leakage of plasma into the gastrointestinal tract did not occur to a sufficient extent to affect the catabolic rate of albumin. The absence of body water effects possibly reflects the normal feed intake maintained by all calves.

Significant treatment effects on the apparent digestibilities of the various proximate feed fractions and on nitrogen retention occurred only during days 56-63 immediately following a feed increase. Interestingly it was the clean control calves rather than the infected calves which were poorest for both of these parameters, with the MSRB treated calves being superior in each case. Similar, but non significant trends were noted for days 14-21 and 35-42. An explanation for this may lie in the regular treatment of clean control calves with fenbendazole. Various authors (Entrocasso et al, 1986b; Fellenius et al, 1981) have suggested that benzimidazoles such as fenbendazole may inhibit acid secretion by the abomasum and thus impair protein digestion. There was no significant difference in abomasal pH at necropsy between the three treatment groups but, while slightly elevated values for infected and MSRB treated calves may have been due to O. ostertagi, those for the clean calves may indeed have been attributable to the continued use of fenbendazole. However effects on apparent digestibility are more than effects on acid production alone as the significantly depressed digestibility values for clean control calves occurred at a time when O. ostertagi was likely to be exerting its maximum on the abomasa of calves in the other treatment groups. Jara et al (1984) have demonstrated that benzimidazoles depress the rumen fermentation ability of sheep and that fenbendazole has the greatest effect on rumen volatile fatty acid production. Increased frequency of dosing results in a sustained depression and molar concentrations of volatile fatty acids may also be altered (Hodgson and Jessop, 1987). Thus the use of fenbendazole may have reduced the productive efficiency of the clean control calves by interfering primarily with rumen fermentation, this being particularly apparent at a time when the digestive system was adapting to a feed increase. In

contrast, the continuous administration of morantel from the MSRB was without digestibility effects.

It may be concluded that Pre-Type II ostertagiasis does not produce marked pathophysiological changes in infected calves, that the MSRB is primarily active against adult O. ostertagi and that fenbendazole may interfere with ruminant digestion.

EXPERIMENT IId

PATHOPHYSIOLOGICAL AND PARASITOLOGICAL STUDIES ON A CONCURRENT INFECTION OF OSTERTAGIA OSTERTAGI AND COOPERIA ONCOPHORA IN CALVES

Introduction

In North Western European countries the most abundant species of gastrointestinal nematodes of cattle are Ostertagia ostertagi and Cooperia oncophora (Kloosterman et al, 1984). Most published work indicates that the establishment of significant populations of C. oncophora in first season grazing calves is confined to the spring and summer months, the rapid acquisition of immunity resulting in expulsion of most of the worms by the autumn. In contrast, O. ostertagi has greater persistency in the host and it is the accumulation of this nematode which is generally credited as the cause of late season clinical parasitic gastroenteritis (PGE). However significant numbers of C. oncophora were reported in clinically affected calves which had been grazing infective pastures for four months (Entrocasso et al, 1986b) and it is clearly possible that absolute immunity to this nematode may not develop in all cases. Even where infection is expelled the possible role of early season C. oncophora in precipitating late season disease has not been discounted (Baker 1988). The following study investigates the effects of a concurrent experimental infection of O. ostertagi and C. oncophora on the digestive efficiency, nitrogen economy and pathophysiology of parasite naive calves.

Materials and methods

Animals and experimental design

Twenty-five castrated male Friesian calves aged four months, weighing approximately 120 kg, and which had been reared under helminth-free conditions were allocated to three treatment groups.

Group A calves (clean) were maintained free of parasites. Five were used as control animals for digestion and nitrogen economy studies and a further two for pathophysiological investigations using radioisotopes.

Group B calves (MSRB) were each given a morantel sustained release bolus (MSRB, Paratect; Pfizer). The calves were subsequently dosed per os with 2,000 O. ostertagi and 10,000 C. oncophora infective

larvae daily for six weeks. Five calves were allocated to the digestion studies, two to the radioisotopic investigation and two necropsied 21 and 42 days after initial infection.

Group C calves (infected) were treated similarly, but without prior MSRB administration, with five calves allocated for digestion studies, two for radioisotopic work and two for necropsy during the course of the trial (days 21 and 42).

Clean control calves were housed separately from those in infected groups to prevent possible autoinfection from bedding material. All calves were clinically examined and weighed every three weeks and blood and faecal samples were collected weekly for biochemical, heamatological and parasitological analyses.

The remaining calves in the metabolism and radioisotope studies were slaughtered on day 84 of the experiment.

Feed allowance

The calves were individually given a complete ration twice a day (Superstar cubes; Hamlyn Milling). Mean analyses were 0.88 dry matter (DM), with 170 g crude protein (CP), 130 g crude fibre (CF), 56 g ether extract (EE), 105 g ash and 18 MJ gross energy (GE) kg DM^{-1}. This was supplemented with barley husks (siftings) to produce a uniform mixture during periods when calves were accommodated in metabolism stalls. The daily feed allowance of 3.0 kg starcubes and 300 g siftings was calculated to give a mean average daily gain of approximately 0.4 kg/day. Where calves became inappetent as a result of the experimental infection feed was initially offered four times per day and subsequently continuously in an attempt to stimulate intake.

Larval infections

Infective larvae were harvested from faecal cultures less than 21 days prior to oral inoculation of the calves and were maintained at room temperature (17^{o}C) until required.

Parasitology and Pathology

Faecal egg counts, pathological techniques and the post-mortem recovery of trichostrongylate parasites was as described in General Materials and Methods.

Blood analyses

Blood total protein and albumin concentrations for calves used in the pathophysiological study were determined using continuous flow analysis (Standard Technicon Auto-Analyser II). Plasma pepsinogen activity was assayed by the method of Edwards et al (1960) where one international unit is equivalent to the release of one m mol tyrosine/litre plasma/minute.

Digestibility and nitrogen balance

The fifteen calves involved in the metabolism study were allocated to three equal sized treatment groups such that the mean weight and weight range of each group was similar. Four calves from each group were used for digestibility and nitrogen balance studies during days 14-21, 35-42, 56-63 and 77-84 post infection as described in Experiment IIa, the fifth animal being maintained as a potential replacement.

Radioisotopic measurements

Radioisotopic studies using $^{51}CrCl_3$, ^{125}I-albumin and tritiated water (TOH) were conducted with two calves from each of the treatment groups clean, MSRB and infected on days 35-47 and 70-82 following the first infection. Each calf received 74 MBq of $^{51}CrCl_3$ and 15 MBq of ^{125}I-albumin by intrajugular catheter at the beginning of each study period. This followed an injection of 110 MBq of TOH four days earlier. Calves were dosed daily per os with 10 ml 0.75% KI solution for three days prior to and ten days after the ^{125}I-albumin injection to ensure rapid excretion of ^{125}I from catabolised ^{125}I-albumin. Collection of plasma, faecal and urine samples, and a calculation of pathophysiological parameters was as described in Experiment IIa.

Statistical Analyses

Statistical analyses were performed using 'Minitab' (Ryan, Penn State University) on a microcomputer. Metabolism study and blood biochemical data were analysed using a standard analysis of variance. Where treatment effects were shown to be significant a multiple comparisons procedure was performed. Two sample t-tests were performed on log transformed parasitological data for infected control and MSRB treated calves.

Results

Clinical observations

Clean control calves remained clinically normal throughout the trial. All infected control calves displayed symptoms typical of Type I ostertagiasis, these first becoming noticeable at around day 21 and varying in severity from a reduced rate of eating and intermittent diarrhoea in some individuals to almost complete inappetence, lethargy, profuse watery diarrhoea and a dramatic liveweight loss in others. These symptoms necessitated the premature necropsy of two infected calves on days 58 and 66. One clean control calf also died from bloat on day 74. Symptoms subsided towards the end of the trial but the appearance of the infected control calves was greatly inferior to that of clean control and MSRB treated calves at necropsy on day 84. Prior administration of an MSRB prevented adverse clinical changes other than a slightly reduced rate of eating compared to the clean controls and an occasional softening of the faeces.

The mean liveweights of the 15 calves used in the metabolism study are shown in Figure 17. A statistically significant difference ($P<0.01$) in liveweight between the infected calves and those in the other treatment groups first became apparent on day 42, this being highly significant ($P<0.001$) on day 63. After 84 days when the experiment ended total mean liveweight gains were 39, 41 and 5 kg for the clean, MSRB and infected groups respectively.

Biochemistry

Mean plasma pepsinogen values for infected and MSRB treated metabolism study calves were significantly elevated above clean control levels from day 7. Values for infected calves reached a mean maximum of 5.41 I.U. on day 42 and remained elevated at just below this level. MSRB treated calves similarly displayed peak plasma pepsinogen values on day 42 (3.78 I.U.) but these subsequently returned to clean control levels by day 84. Mean plasma pepsingoen values are presented in Table 19 and complete individual calf data is contained within Appendix 5.

Blood total protein and albumin concentrations were determined for. calves involved in the radioisotopic studies using each of the two recording periods (Table 20). There was no evidence of hypoalbuminaemia and associated hypoproteinaemia for either of the MSRB treated calves compared to the clean controls. During days 35-42 infected calves were hypoalbuminaemic and hypoproteinaemic compared to

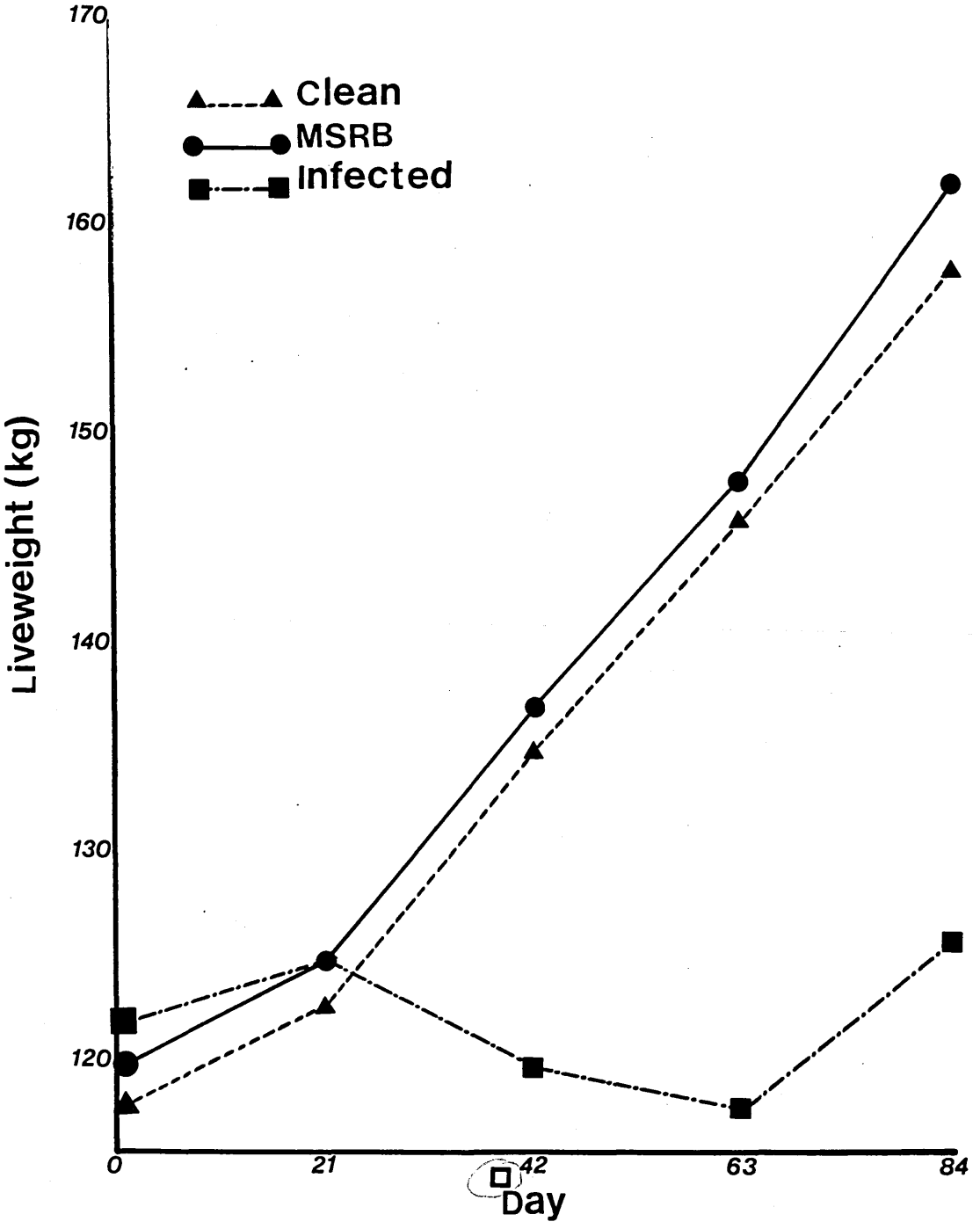


Figure 17. Mean liveweights of metabolism study calves concurrently infected with Ostertagia ostertagi and Cooperia oncophora, infected after administration of a MSRB or maintained as clean controls.

Table 19. Mean plasma pepsinogen values (IU +SE) for metabolism study calves concurrently infected with Ostertagia ostertagi and Cooperia oncophora, infected after prior administration of a MSRB or maintained as clean controls

Day	Clean (A)	MSRB (B)	Infected (C)	Significance
0	0.523 ±0.051	0.537 ±0.032	0.535 ±0.055	
7	0.572 ±0.052	1.028 ±0.090	1.345 ±0.257	AC**
14	0.555 ±0.048	1.443 ±0.167	1.609 ±0.221	AB,AC**
21				
28	0.586 ±0.053	2.335 ±0.316	3.565 ±0.642	AB*,AC***
35	0.618 ±0.021	3.391 ±0.313	4.895 ±0.968	AB**,AC***
42	0.585 +0.024	3.777 ±0.378	5.412 ±1.227	AB*,AC***
49	0.586 ±0.051	3.091 ±0.277	4.442 ±0.868	AB**,AC***
56	0.588 ±0.028	3.422 ±0.462	5.344 ±0.653	BC*,AB,AC***
63	0.612 ±0.030	2.838 ±0.406	5.169 ±0.976	AB*,BC** AC***
70	0.647 ±0.030	1.594 ±0.184	5.225 ±0.687	AC,BC***
77	0.701 ±0.017	1.359 ±0.123	5.176 ±0.736	AC,BC***
84	0.667 ±0.031	1.244 ±0.133	4.619 ±6.624	AC,BC***

Significance * P<0.05, ** P<0.01, ***P<0.001

Table 20. Serum constituents (g/litre) of radioisotope calves infected with Ostertagia ostertagi and Cooperia oncophora, infected after administration of a MSRB or maintained as clean controls

Days 35-47	Clean		MSRB		Infected	
			Total protein			
Day	B17	B18	Y79	Y80	O20	O23
36	72	71	67	63	66	59
39	72	72	73	67	64	60
43	71	72	72	67	67	58
46	70	69	69	66	63	55
Day			Serum albumin			
36	34	35	32	32	29	26
39	35	37	34	33	29	25
43	33	36	32	31	27	22
46	32	34	31	31	25	20

Days 77-84	Clean		MSRB		Infected
			Total protein		
Day	B17	B18	Y79	Y80	O24
71	69	67	67	67	60
74	68	68	69	66	59
78	72	-	69	67	62
81	70	-	68	67	63
Day			Serum albumin		
71	32	34	32	30	22
74	31	33	33	30	21
78	34	-	33	30	21
81	32	-	33	32	20

Severity of clinical symptoms necessitated the premature necropsy of calves O20 and O23 on days 58 and 66 respectively
Calf B18 died from bloat on day 74

both clean and MSRB treated calves. Similar reductions in the respective blood constituents were also apparent for the replacement infected calf used during days 70-84.

Apparent digestibility coefficients

Examination of the apparent digestibility of each of the standard proximate feed fractions for each of the four separate recording periods revealed a consistent depression in the digestive efficency of the crude protein, ether extract and gross energy fractions for infected calves compared to calves in the other treatment groups once a patent infection was established. These effects were most marked during days 35-42 and at this time the apparent digestibility of the ash proximate fraction was also reduced. Over the entire experiment the apparent digestibility of the dry matter was significantly reduced for infected compared to MSRB treated but not clean control calves. Mean data for each recording period is presented in Table 21 and individual calf values are contained within Appendix 5.

Nitrogen balance

Nitrogen retention was significantly reduced for infected compared to clean control and MSRB treated calves during days 35-42 and 56-63 (Table 22) as a result of increases in both faecal and urinary nitrogen output. The increase in faecal nitrogen loss was highly significant during days 35-42. During days 77-84 nitrogen retention was improved for calves in all treatment groups mainly as a result of reduced urinary nitrogen loss compared with preceeding periods. At this time significant differences between treatments did not occur but mean faecal nitrogen loss was increased with a consequent decrease in nitrogen retention for infected compared to MSRB treated and clean control calves. Over the entire experiment nitrogen retention of infected calves was significantly reduced, this principally arising from both a significant reduction in intake and a significantly increased faecal nitrogen loss compared to other treatment groups. Nitrogen balance data for individual calves, and also that expressed as a percentage of nitrogen intake is given in Appendix 5.

Radioisotopic measurements

Pathophysiological changes in the calves, as measured using radioisotopic techniques are summarised in Table 23. During days 35 to

Table 21. Mean digestibility coefficients (+SE) for calves concurrently infected with Ostertagia ostertagi and Cooperia oncophora, infected after prior administration of a MSRB or maintained as clean controls

	Clean	MSRB	Infected
Days 14-21	n=4	n=4	n=4
DM	0.6403 ±0.0115	0.6600 ±0.0094	0.6568 ±0.0153
CP	0.6051 ±0.0112	0.6343 ±0.0077	0.6080 ±0.0122
CF	0.3882 ±0.0247	0.4109 ±0.0215	0.4232 ±0.0321
EE	0.9345 ±0.0027	0.9346 ±0.0017	0.9307 ±0.0028
ASH	0.4784 ±0.0196	0.4630 ±0.0331	0.4576 ±0.0203
NFE	0.7163 ±0.0091	0.7410 ±0.0051	0.7353 ±0.0160
OM	0.6600 ±0.0105	0.6810 ±0.0078	0.6780 ±0.0167
Energy	0.6119 ±0.0156	0.6308 ±0.0104	0.6301 ±0.0173
Days 35-42	n=4	n=4	n=3
DM	0.6294 ±0.0180	0.6352 ±0.0082	0.5958 ±0.0095
CP	0.6258 ±0.0125a	0.6346 ±0.0103A	0.4972 ±0.0319B
CF	0.3327 ±0.0366	0.3571 ±0.0236	0.3580 ±0.0237
EE	0.9026 ±0.0066A	0.9023 ±0.0043A	0.8146 ±0.0231B
ASH	0.4816 ±0.0188A	0.4690 ±0.0269a	0.3260 ±0.0469b
NFE	0.7117 ±0.0175	0.7183 ±0.0038	0.7190 ±0.0054
OM	0.6472 ±0.0182	0.6552 ±0.0069	0.6283 ±0.0074
Energy	0.6630 ±0.0190a	0.6379 ±0.0063a	0.5773 ±0.0076b
Days 56-63	n=4	n=4	n=3
DM	0.6366 ±0.0172	0.6401 ±0.0089	0.6003 ±0.0111
CP	0.6242 ±0.0147A	0.6154 ±0.0170A	0.5280 ±0.0181b
CF	0.3492 ±0.0299	0.3532 ±0.0168	0.3750 ±0.0163
EE	0.9206 ±0.0050a	0.9224 ±0.0013A	0.8898 ±0.0124b
ASH	0.4647 ±0.0268	0.4804 ±0.0186	0.4106 ±0.0780
NFE	0.7207 ±0.0202	0.7256 ±0.0070	0.6872 ±0.0182
OM	0.6561 ±0.0174	0.6654 ±0.0112	0.6214 ±0.0128
Energy	0.6395 ±0.0216	0.6478 ±0.0096	0.5789 ±0.0167
Days 77-84	n=4	n=4	n=4
DM	0.6475 ±0.0168	0.6508 ±0.0098	0.6180 ±0.0126
CP	0.6175 ±0.0169	0.6085 ±0.0075	0.5788 ±0.0119
CF	0.3585 ±0.0302	0.3611 ±0.0237	0.3405 ±0.0239
EE	0.9265 ±0.0025a	0.9285 ±0.0093a	0.8944 ±0.0104B
ASH	0.4604 ±0.0366	0.4879 ±0.0206	0.3962 ±0.0643
NFE	0.7582 ±0.0128	0.7606 ±0.0088	0.7367 ±0.0089
OM	0.6694 ±0.0150	0.6700 ±0.0103	0.6440 ±0.0104
Energy	0.6659 ±0.0165a	0.6592 ±0.0090a	0.6066 ±0.0138B
Overall			
DM	0.6384 ±0.0074ab	0.6465 ±0.0048a	0.6205 ±0.0089b
CP	0.6181 ±0.0066A	0.6232 ±0.0051A	0.5588 ±0.0143B
CF	0.3571 ±0.0147	0.3706 ±0.0114	0.3753 ±0.0146
EE	0.9211 ±0.0036A	0.9219 ±0.0033A	0.8867 ±0.0126b
ASH	0.4713 ±0.0121A	0.4851 ±0.0117A	0.4018 ±0.0272b
NFE	0.7267 ±0.0084	0.7363 ±0.0051	0.7238 ±0.0078
OM	0.6582 ±0.0073	0.6679 ±0.0048	0.6455 ±0.0084
Energy	0.6375 ±0.0096A	0.6439 ±0.0049A	0.6011 ±0.0089B

Significance ab $P<0.5$, Ab aB $P<0.01$, AB $P<0.001$

Table 22. Mean nitrogen balance (g/day) for calves concurrently infected with Ostertagia ostertagi and Cooperia oncophora, infected after prior administration of a MSRB or maintained as a clean control (±SE)

	Clean	MSRB	Infected
Days 14-21	n=4	n=4	n=4
Intake	72.35 ±0.28	72.29 ±0.34	72.22 ±0.25
Faeces	28.74 ±0.85	26.42 ±0.56	28.29 ±0.85
Urine	22.98 ±0.57	21.69 ±1.38	22.19 ±0.91
Retention	20.63 ±1.10	24.18 ±1.88	21.74 ±0.55
Days 35-42	n=4	n=4	n=3
Intake	72.78 ±0.00	72.57 ±0.21	65.22 ±4.63
Faeces	27.23 ±0.91b	26.52 ±0.76b	32.49 ±0.72A
Urine	29.67 ±0.91	27.92 ±1.82	30.57 ±1.06
Retention	15.88 ±0.93A	18.13 ±2.31A	2.15 ±5.00b
Days 56-63	n=4	n=4	n=3
Intake	75.04 ±0.00	75.04 ±0.00	70.90 ±3.14
Faeces	28.22 ±1.10	28.79 ±0.85	33.50 ±2.23
Urine	29.50 ±1.53	26.25 ±1.15	30.48 ±2.55
Retention	17.32 ±0.78A	20.00 ±0.84A	6.92 ±3.59b
Days 77-84	n=4	n=4	n=4
Intake	72.13 ±1.70	73.83 ±0.00	72.35 ±1.48
Faeces	27.56 ±1.06	28.91 ±0.56	30.42 ±0.32
Urine	17.56 ±0.79	21.50 ±1.34	19.60 ±2.97
Retention	27.01 ±1.82	23.43 ±0.85	22.34 ±4.02
Overall	n=16	n=16	n=14
Intake	73.08 ±0.49a	73.43 ±0.30a	70.47 ±1.33b
Faeces	27.94 ±0.47A	27.66 ±0.44A	30.91 ±0.69B
Urine	24.93 ±1.38	24.34 ±0.97	25.02 ±1.65
Retention	20.21 ±1.24a	21.43 ±0.96A	14.54 ±2.88b

Significance ab $P<0.05$, Ab aB $P<0.01$, AB $P<0.001$

Table 23. Pathophysiological changes in calves concurrently infected with Ostertagia ostertagi and Cooperia oncophora, infected after prior administration of a MSRB or maintained as clean controls

		Clean		MSRB		Infected	
Days 35-47		B17	B18	Y79	Y80	O20	O23
Plasma Volume	Vp (ml/kg)	56.8	52.6	54.7	52.3	55.7	47.0
Faecal Clearance	I-albumin (ml/day)	58.8	32.9	87.2	104.2	142.0	208.5
Faecal Clearance	Cr (ml/day)	320.6	378.6	417.0	599.0	714.8	782.0
Catabolic rate of albumin	%	5.1	4.8	4.9	5.7	6.2	9.7
Albumin Disappearance	I-albumin T1/2 (days)	19.3	21.6	19.1	17.8	13.0	10.0
Total body water	% bodyweight	69.5	66.1	73.0	74.5	77.6	69.0
Body water turnover	(l/day)	9.3	12.7	8.1	8.4	9.5	10.1
TOH Disappearance	T1/2 (days)	6.7	4.3	7.9	9.5	7.1	5.9

Days 70-82		B17	B18	Y79	Y80	O24
Plasma Volume	Vp (ml/kg)	52.6	55.7	56.6	55.7	53.8
Faecal Clearance	I-albumin (ml/day)	66.5	21.0	60.7	100.7	94.8
Faecal Clearance	Cr (ml/day)	384.7	248.4	371.4	519.5	903.7
Catabolic rate of albumin	%	4.8	3.2	4.4	4.5	7.8
Albumin Disappearance	I-albumin T1/2 (days)	20.3	-	20.0	17.3	10.4
Total body water	% bodyweight	60.0	59.8	62.0	69.4	71.1
Body water turnover	(l/day)	8.5	9.0	8.5	9.6	10.1
TOH Disappearance	T1/2 (days)	6.5	6.0	7.0	6.5	5.7

Acute clinical ostertagiasis necessitated the premature necropsy of calves O20 and O23 on days 58 and 66 respectively
Calf B18 died of bloat on day 74

47 both infected and to a lesser extent MSRB treated calves showed increased faecal clearances of ^{125}I-albumin and ^{51}Cr compared to clean controls with consequent increases in the catabolic rate of albumin and decreases in the ^{125}I-albumin half-life. In infected calves these changes were associated with hypoalbuminaemia and hypoproteinaemia and were particularly marked in one calf (023) in which plasma volume was also reduced.

Measurements using TOH provided evidence of increased water retention in both MSRB treated and one infected control calf but values for infected calves must be regarded with some caution as inappetence and diarrhoea prevented steady state conditions from operating.

During days 70-84 one clean control calf (B18) died as a result of non-treatment related causes and the physiological values obtained may be atypical as they are based on incomplete data. A direct comparison between values obtained for infected calves during days 35-47 and 70-84 cannot be made because of the premature necropsy of both calves used during the first part of the study. However values obtained for the similarly infected replacement calf indicate a continued increase in the faecal clearance of both ^{125}I-albumin and ^{51}Cr accompanied by an increase and decrease of approximately 100% in the catabolic rate of albumin and the ^{125}I-albumin half-life respectively compared to clean controls.

Responses of the two MSRB treated calves during days 70-84 differed in that one calf (Y80) displayed evidence of continued physiological disturbances (increased faecal clearances of ^{125}I-albumin and ^{51}Cr and reduced half-life of ^{125}I-albumin) compared to clean controls, while physiological values for the other calf (Y79) had returned to control levels. Examination of worm burdens at the 84 day necropsy reveals that this may have been attributable to the greater residual infection in Y80 rather than any differential effects on repair of the gastric mucosa.

Plasma volume for the infected calf was again slightly reduced compared to clean and MSRB treated calves and there was evidence of hypoalbuminaemia resulting in slight hypoproteineamia. There were no major differences in body water measurements between treatment groups.

Pathology

Oedema was apparent in the abomasum of the infected calf necropsied 21 days after initial infection. Microscopic examination of

abomasa from both the infected and MSRB treated calves revealed small areas of necrosis, cellular infiltration of the lamina propria and replacement of zymogen and parietal cells with hyperplastic undifferentiated cells, particularly in the pylorus. Developing O. ostertagi were present in the dilated gastric glands and on the mucosal surface.

The entire small intestines from both infected and MSRB treated calves displayed evidence of mucosal damage attributable to the development of C. oncophora, this being particularly marked in the jejunum. Here, in addition to stunting and thickening, some areas of villi were completely flattened and villous adhesion was apparent. In the MSRB treated calf sloughed epithelial cells formed a sheet around lumen dwelling worms. No worms were detected in the ileum of either calf.

At the 42 day necropsy the gross appearance of the abomasa from the infected and MSRB treated calf was similar to that at day 21 but microscopic examination revealed a strong cellular reaction, particularly in the pyloric region. In the infected calf there was destruction of the surface epithelium, congestion of mucosal blood vessels and developing O. ostertagi in dilated gastric glands. Dilated gastric glands for both calves contained macrophages but for the MSRB treated calf worms were only present on the mucosal surface.

For the infected calf necropsied at this time there was extensive damage to the entire small intestinal mucosa with severe destruction and removal of epithelial cells, especially the tips of the villi, mucosal oedema and congestion of mucosal and submucosal blood vessels. Mucosal damage in the MSRB treated calf was limited to some stunting and thickening of the villi in the duodenum and jejunum. A strong cellular reaction was apparent in both calves, there being an infiltration of the lamina propria by eosinophils and lymphocytes. Worm penetration of the lamina propria was also apparent.

There was no obvious mucosal damage to the abomasum of the MSRB treated calf examined on day 84 but a cellular reaction was still apparent. In contrast, the mucosa from the infected calf showed extensive ulceration, oedema and necrosis particularly in the fundic region where there was replacement of most of the zymogen and parietal cells by non-functional, undifferentiated hyperplastic cells. Hyperplasia of the mucus cells was apparent in the pyloric region.

Persistent damage to the mucosa of the small intestine was

apparent in both calves, this being more severe and extensive in the infected calf. Large numbers of sloughed epithelial cells were present in the proximal lumen and there was cellular infiltration of the lamina propria.

Parasitology

The weekly trichostrongyle faecal egg counts (Table 24, Appendix 6) showed that the faeces of clean control calves were negative throughout the trial. A positive faecal egg count was recorded on day 14 for both MSRB treated and infected calves, with positive counts for all calves in each of these treatment groups being first recorded on days 35 and 21 respectively. Values for MSRB treated calves reached a mean maximum of 150 epg on day 35 while a mean maximum of 4980 epg was recorded for infected calves on day 49 followed by a subsequent decline to 430 epg on day 84 when the experiment was terminated.

Similar total burdens of O. ostertagi were recorded for MSRB treated and infected calves at the 21 day necropsy but the establishment of C. oncophora in the MSRB treated calf was only 19% of that in the infected calf. At the 42 day necropsy this percentage was reduced to 5%, prior administration of the MSRB also promoting a 25% reduction in O. ostertagi burdens. The infectivity of the O. ostertagi L_3 in the mixed infection was particularly high, there being a greater than 100% establishment (possibly attributable to natural variation during preparation of infective larval doses) in both MSRB and infected calves necropsied on day 21, and establishments of 65 and 85% respectively of the larval dose administered at day 42.

By day 84 a marked loss of worms had occurred mean burdens of O. ostertagi and C. oncophora being reduced from 71800 and 128200 on day 42 to 19983 and 16017 respectively on day 84 for infected calves. For MSRB treated calves reductions from 54200 and 6800 to 1457 and 386 O. ostertagi and C. oncophora respectively were recorded.

C. oncophora was mainly located in the duodenum of calves necropsied on day 21, but by day 42 appreciable numbers of worms were additionally recovered from the jejunum and ileum, particularly in the infected calf where the total population was considerably greater. By day 84 the low numbers of C. oncophora recovered from MSRB treated calves were principally located in the duodenum, while the larger numbers recovered from infected calves were more evenly distributed along the entire small intestine. Mean total worm burdens are

Table 24. Mean faecal egg counts (epg +SE) for calves concurrently infected with O.ostertagi and C.oncophora or infected after prior administration of a MSRB

Day	MSRB	Infected
0	0 ± 0	0 ± 0
7	0 ± 0	0 ± 0
14	10 ± 0	10 ± 10
21	0 ± 0	1030 ± 108
28	50 ± 22b	1270 ± 270a
35	150 ± 42B	2220 ± 626A
42	50 ± 39b	3290 ± 868A
49	50 ± 7b	4980 ±3458a
56	90 ± 40b	3270 ±2012a
63	90 ± 46	980 ± 523
70	30 ± 20b	860 ± 566a
77	50 ±101b	1770 ±1258a
84	50 ± 16	430 ± 162

Significance ab P<0.05, Ab P<0.01, AB P<0.001
No positive counts were recorded for clean controls

Table 25. Mean worm burdens (+SE) at necropsy of calves concurrently infected with Ostertagia ostertagi and Cooperia oncophora, infected after prior administration of a MSRB or maintained a a clean control

			Ostertagia ostertagi			Cooperia oncophora		
Treatment	Day	n	Devel	Adult	Total	Devel	Adult	Total
MSRB	21	1	36800	25400	62200	3200	4800	8000
Infected	21	1	42200	22800	65000	18400	24600	43000
MSRB	42	1	19200	35000	54200	1600	5200	6800
Infected	42	1	17000	54800	71800	30800	97400	128200
Infected	58	1	0	108400	108400	6200	151100	157300
Infected	66	1	400	96800	97200	3500	104500	108000
MSRB	84	7	514	943b	1457b	57	329	386
+SE			+168	+784	+796	+57	+136	+124
Infected	84	6	663	19350A	19983a	200	15817	16017
+SE			+445	+9263	+9075	+163	+10154	+11213

Significance ab P<0.05, Ab P<0.01

presented in Table 25 while individual calf values are detailed in Appendix 5.

Discussion

The results of this study demonstrated that a combined infection of O. ostertagi and C. oncophora produced sustained severe metabolic and pathophysiological changes in young growing calves which were far in excess of those produced by either monospecific infection given in an entirely comparable manner (Armour et al, 1987a; Taylor et al, 1988). Experimental infections were given at levels which may be encountered under natural grazing conditions in the west of Scotland (Entrocasso 1984) but it is debatable how closely oral dosing can mimic the intake of infective larvae from pasture. Epidemiological studies (Rose, 1962 and 1963) have demonstrated that moisture availability has an effect upon the migration of infective larvae from the faecal pat to the pasture and that during conditions of low moisture availability highly contaminated pastures are not necessarily highly infective. Thus the true pattern of natural calf infection may well not be a continuous low level intake but rather a saw-tooth pattern where each peak of infection represents the liberation of L_3 from the faecal reservoir following rainfall. The relative availability and infectivity of O. ostertagi and C. oncophora L_3 must also be considered in that, while pasture levels of both species are maximal during the latter part of the grazing season (Ibarra-Silva, 1988; Rose, 1963), early season exposure of susceptible calves to low levels of C. oncophora infective larvae may have stimulated the immune response so that animals are refractory to maximal levels of challenge. However Entrocasso et al (1986b) reported considerable numbers of C. oncophora in calves clinically affected with parasitic gastroenteritis which had been grazing for four months and it is possible that absolute immunity to this nematode may not develop in situations where the increase in pasture infectivity is delayed, as was the case in this study. Thus where climatic conditions are such, simultaneous intake of infective O. ostertagi and C. oncophora larvae by completely susceptible animals may predispose to severe clinical disease as reported in the current work.

Several interesting findings emerge from the parasitological data obtained during this study, both from the infected control and MSRB treated groups of calves. Firstly establishment of O. ostertagi was

extremely high with over one hundred percent of administered infective larvae establishing in both infected and MSRB treated calves at three weeks. This compares with 77 and 33% respectively in calves similarly infected with O. ostertagi alone (Experiment IIa) and an expected 20% where larvae are given as a single inoculum (Ibarra-Silva, 1988). In contrast the establishment of C. oncophora at three weeks in the infected calf was only 58% of that recorded for a comparable monospecific infection (Armour et al 1987a) but necropsy at subsequent dates revealed that the establishment of this nematode was also relatively increased. Previous experimental studies have reported increased establishment rates for infections of mixed species or genera compared to pure infections (Al-Saqur, 1982; Ross et al, 1968) and it has been postulated that this may be due to an immunosuppressive effect. However, Kloosterman et al, (1984) failed to demonstrate any interactions between C. oncophora and O. ostertagi when given as a concurrent infection and the identification of cross reacting antibodies (Keus et al, 1981) suggests that the presence of one species may actually be detrimental to the other. Prior administration of a MSRB was apparently less effective in controlling C. oncophora when given concurrently with O. ostertagi than when given alone, but worm burdens were maintained at low levels with a mean of only 386 worms being recorded on day 84.

The failure of the MSRB to bring about notable reductions in O. ostertagi burdens at 21 days again suggests that morantel is primarily active against adult worms of this species. This suggestion is further supported by examination of the 42 and 84 day worm burdens, the observed reduction in O. ostertagi in the MSRB treated calves being principally due to a reduction in adults, numbers of developing stages being similar for both treatment groups. Conversely prior MSRB treatment brought about apparent reductions in both developing and adult C. oncophora. This species has been previously regarded to be lumen dwelling but both Armour et al (1987a) and the current study have demonstrated some larval penetration of the small intestinal mucosa. The length of time which larvae spend within the mucosa is not known, nor indeed is it known whether mucosal penetration is a prerequisite for parasitic development. It is likely however that developing larval stages of C. oncophora are more greatly exposed to morantel than those of O. ostertagi which develop deep within the gastric glands. Morantel acts by the induction of a neuromuscular

block and paralysis (Harrow and Gration, 1985) and this mode of action can only be effective if paralysed worms are subsequently removed with digesta. Any developing C. oncophora larvae lying deep between the villi in close association with the intestinal mucosa may evade anthelmintic action and complete their parasitic development.

Armour et al (1987a) reported the migration of C. oncophora populations through the small intestine. In contrast evidence from the current study suggests that the duodenum is the predilection site. However this may be attributable to the induction of a more favourable duodenal microclimate by the presence of O. ostertagi in the abomasum. Duodenal overpopulation apparently resulted in colonisation of the more distal jejunum and ileum. Data pertaining to the 84 day necropsy suggested that adult worms remained in that region of the small intestine where their parasitic development took place and that no anterior migration occurred to more preferred sites following loss of worms from these regions.

Despite burdens of 54200 O. ostertagi and 6800 C. oncophora recorded for the MSRB treated calf at the 42 day necropsy, a peak mean faecal egg count of only 150 epg was recorded for remaining MSRB treated calves at approximately this time. This compares with 4980 epg recorded for infected calves and may be indicative of greatly reduced populations of the more prolific C. oncophora. Additionally anthelmintic action may have resulted in suppression of egg laying or an almost absolute effect against emerging prepatent adult females. It was noted that adult C. oncophora from MSRB treated calves appeared stunted and that gravid females contained fewer eggs compared to those from infected calves. The reduction in faecal egg output is extremely pertinent in that the main application of controlled release technology has been considered to be a reduction in pasture contamination (Anderson, 1985).

There was a considerable variation in the severity of clinical symptoms among infected calves and necropsy data suggests that this was attributable to variations in the ability to resist parasite establishment rather than variations in the ability to withstand the effects of an established infection. The combined stresses of larval challenge, confinement in metabolism stalls and injection of radioisotopes may have predisposed to the large infections and acute clinical symptoms which necessitated the premature necropsy of two calves.

Examination of plasma pepsinogen data reveals increased values for both infected and MSRB treated calves compared to clean controls from as early as seven days post initial infection. Such increases are usually attributed to the emergence of young adult O. ostertagi from the gastric glands and associated epithelial stretching and breakdown of cell junctional complexes resulting in increased mucosal permeability. In view of the high numbers of establishing larvae in this study, a possible explanation for the observed pre-emergence increases in plasma pepsinogen concentrations may be the causation of a hypersensitivity type reaction at the gastric mucosa. Such a reaction has been reported in older immune animals (Armour et al, 1979) and in rats infected with Nippostronglyus brasiliensis worm expulsion is inititated by an increase in mucosal permeability to facilitate antibody passage (Barth et al, 1966; Jones and Ogilvie, 1971). However calves in this study were parasite-naive and it is unlikely that an effective antibody response would develop in such a short time. An alternative explanation is that the presence of C. oncophora in the duodenum may have damaged G-cells resulting in an indirect suppression of abomasal acid secretion. It may be speculated that this reduced the conversion of pepsinogen to pepsin, increasing the concentration gradient for pepsinogen between the abomasum and plasma, and resulting in elevated plasma levels. Elevated plasma pepsinogen levels have been observed in monospecific infections of C. oncophora (J. Armour, personal communication). Fox et al (1987) have reported increased plasma pepsinogen values by day seven in calves orally inoculated with 100,000 O. ostertagi larvae. Given an expected 20% establishment (Ibarra-Silva, 1988), numbers of larvae invading the gastric mucosa would actually be less than the current study and increased plasma pepsinogen levels may thus be a consequence of the purely physical effects of mucosal invasion on a massive scale.

For MSRB treated calves the duration of elevated plasma pepsinogen levels approximated to the time required for larvae within the mucosa to complete their development and thereafter levels returned to those of control calves indicating repair of mucosal damage. In contrast levels for infected calves remained elevated until the trial was terminated demonstrating the severe and sustained mucosal damage in these calves. Both the magnitude and duration of the increase was greater than that recorded for a monospecific infection (Experiment IIa) and reflects the greater establishment achieved.

In the interests of animal welfare, only the least clinically affected animals were used for metabolism studies and consequently the data obtained cannot indicate the full detrimental effects of infection. In comparison to clean and MSRB treated calves, apparent digestive efficiencies for gross energy and the crude protein and ether extract proximate feed fractions were consistently and significantly reduced for infected calves once a patent infection had established. This contrasts with data obtained for either monospecific infection in which no treatment related differences in apparent digestive efficiency were reported. Protein digestion is initiated in the abomasum and completed in the proximal small intestine, liberated amino acids being principally absorbed from the mid third of the small intestine (Steel and Symons, 1982). Abomasal O. ostertagi infection is thought to produce a pH mediated limitation on abomasal protein digestion but in a monospecific infection this is nullified by a compensatory increase in protein digestion in the small intestine. In the present concurrent infection, colonisation of the entire small intestine by C. oncophora, and associated mucosal damage, evidently prevented any compensatory response resulting in a reduction in the apparent digestibility of crude protein in infected calves. No such reduction was apparent in MSRB treated calves as the bolus restricted nematode populations minimising abomasal pH changes and confining the damage due to C. oncophora infection to the anterior small intestine allowing a possible compensatory response in the posterior portion.

The reduced apparent digestibility of dietary protein was undoubtedly one source of the significantly increased faecal nitrogen losses recorded for infected calves. Perhaps the major contributor to this additional faecal nitrogen however was the greatly increased losses of plasma into the gastrointestinal tract. Studies using ^{125}I-albumin and $^{51}CrCl_3$ revealed that plasma losses in infected calves were double those in clean controls with losses for MSRB treated calves being increased by 50%. Sloughed epithelial cells arising as a result of mucosal damage represent a third, and possibly very significant source of increased faecal nitrogen but methods for quantification of these losses are as yet unavailable. Bacterial degradation of some of this additional nitrogen entering the caecum to ammonia may have been responsible for increased urinary nitrogen losses in infected calves. It is probable that increased turnover of epithelial cells and plasma albumin were primarily responsible for the

observed reduction in the apparent digestibility of gross energy.

Radioisotopic measurements revealed marked deviations from control values in the physiological parameters measured for infected calves. Although direct comparison between the two recording periods could not be made due to the premature necropsy of the calves used during days 35-47, disturbances in albumin metabolism apparently persisted until the end of the trial despite the expulsion of a large proportion of the worm burden. Studies using $^{51}CrCl_3$ and ^{125}I-albumin revealed a substantial loss of plasma into the digestive tract, presumably through a severely disrupted mucosa while blood analysis revealed that the reverse loss of pepsinogen was also occurring. This leakage of albumin was the cause of the observed reduction in the half-life of ^{125}I-albumin, the increase in catabolic rate of albumin and the resultant hypoalbuminaemia. Previous studies (Mahrt et al, 1964) have reported a concommitant increase in serum globulin to maintain a constant level of serum proteins but in this study the extent of albumin leakage was such that hypoproteinaemia also occurred. During both recording periods there was evidence of declining serum albumin concentrations but serum proteins were maintained at a slightly subnormal level giving indication of some degree of globulin compensation.

Comparison of radioisotopic data for the present concurrent infection with similar data for a monospecific O. ostertagi infection (Experiment IIa) demonstrates the greatly increased pathogenicity of the two nematodes in combination compared to O. ostertagi alone. C. oncophora infection was associated with considerable damage to the mucosa of the small intestine which would significantly contribute to increased gastrointestinal plasma loss. Moreover, the apparent increase in establishment of O. ostertagi in the presence of C. oncophora would increase abomasal damage over that for a monospecific infection and also contribute to the relative increase in plasma loss and detrimental changes in albumin metabolism.

Clinical symptoms and adverse changes in digestive efficiency and nitrogen economy were prevented by prior administration of a MSRB but radioisotopic studies revealed slightly increased gastrointestinal plasma loss, and small deviations from normal albumin metabolism, particularly during the first recording period. Together with increased plasma pepsinogen levels these provide evidence of limited mucosal damage attributable to developing nematode larvae. However

there was no apparent insult to the animals metabolism and liveweight gains for MSRB treated animals were actually superior to those for clean controls. By the second recording period plasma pepsinogen values had returned to control levels as had physiological values for one MSRB treated calf. The sustained elevated gastrointestinal plasma loss of the other MSRB calf was not associated with alterations in albumin metabolism.

Studies using TOH provided slight evidence for water retention by both infected and MSRB treated calves during the first recording period. While accurate values for MSRB treated calves were obtained, those recorded for infected calves must be regarded with some caution. Body water determination by either extrapolation or equilibration methods assumes that water intake and output by an animal are approximately equal so that steady state conditions apply. In animals clinically affected by ostertagiasis, diarrhoea and inappetence dictate that water output exceeds input and under these conditions extrapolated plasma values based on the dilution principle may give an overestimation of body water. During body water measurements all calves showed some liveweight loss associated with the stress of confinement in metabolism stalls and changes in gut fill. Liveweight changes for infected calves were greater than for the other treatment groups as they were additionally attributable to metabolic effects of nematode activity and this would again lead to an overestimation of total body water on a ml/kg basis. Examination of total body water volumes does however still indicate some degree of water retention by infected calves during the first recording period.

The results of this study demonstrated that in combination O. ostertagi and C. oncophora produce severe adverse alterations in calf metabolism which far exceed those produced by either species alone. A synergistic effect apparently operates in that the reciprocal establishment of either species is increased in the presence of the other, thereby increasing the relative pathogenicity. Additionally the presence of C. oncophora in the small intestine limits the extent of compensatory digestive responses to O. ostertagi infection of the abomasum and thus limits the ability of the metabolic response to replace plasma constituents lost to the gastrointestinal tract through a damaged mucosa. Prior administration of a MSRB limited metabolic and physiological changes by limiting nematode populations, although it was apparently ineffective against developing stages of O. ostertagi.

The limited larval development which did occur resulted in non-consequential alterations in plasma pepsinogen concentrations and some physiological parameters measured while permitting stimulation of the immune response as was evidenced by enlarged abomasal lymph nodes at necropsy.

SECTION III

EPIDEMIOLOGY AND CONTROL OF RUMINANT OSTERTAGIASIS

Literature Review

Introduction

Epidemiology of Ostertagia ostertagi and Cooperia oncophora

Epidemiology of Ostertagia circumcincta

Control

Experiment III

Parasitological and Metabolism Studies Conducted with Growing Lambs Infected with Ostertagia circumcincta and given Graded Doses of Levamisole

LITERATURE REVIEW

Introduction

Epidemiology may be defined as a study of the factors affecting the occurrence of disease in a population (Armour and Ogbourne, 1982). The main features governing the epidemiology of parasitic gastroenteritis of cattle and sheep in Britain are now well recognised (Michel 1969a; Pott et al, 1978; Armour 1980; Thomas et al, 1986). Regular seasonal patterns of pasture contamination and host animal infection are known to occur, these being modified to some extent by weather conditions and grazing control. Clinical disease is generally only manifest towards the end of the grazing season and may be controlled by the use of modern anthelmintic drugs. However, it is known that subclinical disease, and even the continual intake of infective larvae from pasture are associated with production losses (Jacobs et al, 1981; Sykes and Coop, 1977) and, from an understanding of the population dynamics of the free-living nematode larvae, prophylactic control programmes have been devised which limit the exposure of susceptible stock.

Epidemiology of *Ostertagia ostertagi* and *Cooperia oncophora*

Observations on the ecology of the free-living stages of *O. ostertagi* and *C. oncophora* revealed that their development requirements were similar (Rose, 1961 and 1963). Infective larvae of both species can survive on herbage over winter until the spring of the following year when the rate of mortality increases sharply and pasture larval numbers fall to a low level. Ingestion of residual larvae by susceptible stock facilitates the completion of development and adult worms produce eggs which are passed with the faeces to recontaminate the pasture. *C. oncophora* is more fecund than *O. ostertagi* and is primarily responsible for early season increases in pasture contamination. Infective larvae ingested by grazing cattle stimulate immunity within approximately two months of exposure and consequently the cycling of *C. oncophora* infection through the host animal falls to a low level in the latter part of the grazing season. However, residual pasture infectivity from early season contamination may be maintained at high levels and experimental studies have demonstrated the accumulation of considerable worm burdens in parasite-naive tracer calves (Ibarra-Silva, 1988). Large numbers of *C. oncophora* have also

been reported in calves clinically affected by ostertagiasis and which had been grazing infective pastures for four months (Entrocasso et al, 1986b). However in this study pasture numbers of C. oncophora infective larvae did not increase significantly until the end of July, and this atypically late increase may have been responsible for the apparent lack of immunity (K. Bairden, personal communication).

Field experiments concerned with the epidemiology of O. ostertagi (Michel, 1966 and 1969b) demonstrated that calves turned out in late April became infected and were excreting eggs in their faeces by the third week of May. However infective larvae resulting from this infection were not recovered from the herbage until July when the pasture larval population rapidly increased to a high level which was maintained until the following spring. Larvae ingested after July either caused clinical disease in the autumn or were accumulated as inhibited fourth stages in the host. Diseased calves excreted large numbers of eggs during September and October but these did not appreciably increase pasture larval contamination.

O. ostertagi usually only completes one generation a year and the cycling between pasture and the host animal comprises of two phases. The 'contamination phase' occurs during the early part of the grazing season during which infection of susceptible stock acts to multiply herbage infection. This results in a massive increase in pasture contamination during the latter part of the grazing season, the 'autoinfection phase', where eggs excreted during the contamination phase have developed into infective larvae reinfecting stock and precipitating clinical disease.

The exact timing of the two phases is primarily dependent on weather conditions. Environmental temperature controls the rate of development of eggs to infective larvae and moisture influences survival of the preinfective stages and migration of L_3 onto herbage. Provided that environmental temperature is sufficiently high and faeces remain in a compact pat, eggs will complete their development during both rainy and dry periods. The rate of development increases with increasing temperature and a minimum of 2-3 weeks is required for completion during July. Differential rates of development of eggs excreted before July predisposes to the synchronous appearance of large numbers of infective larvae on the pasture during July when rates of development are maximal. Eggs passed in faeces during September and

October may fail to develop to the resiliant infective stage before the faeces disintegrates. Mortality of the preinfective stages is greatest when faecal pats are subject to rapid drying. Dry weather may also restrict the movement of L_3 onto herbage if the disintegration rate of faecal pats is reduced by surface crusting. Under these conditions the faecal pat acts as a reservoir of infective larvae giving rise to widespread herbage infestation when wetting facilitates disintegration (Rose, 1962 and 1970). As a consequence of the effects of moisture a highly contaminated pasture may not be highly infective to stock until rain and/or heavy dews permit the free movement of infective larvae. At such time a massive intake of these larvae by host animals may precipitate clinical disease and herein lies an explanation for clinical symptoms occurring typically during the autumn months when moisture is more freely available.

Epidemiology of Ostertagia circumcincta

Closely allied to O. ostertagi, O. circumcincta has been considered to be the predominant sheep abomasal nematode in Northern Britain (Boag and Thomas, 1971 and 1977). Serial slaughter trials (Armour et al, 1966) have demonstrated that the parasitic stages of O. circumcincta in sheep produce similar pathophysiological and biochemical changes to O. ostertagi in cattle but that the rate of development to the young adult stage is more rapid, requiring approximately eight days. Patent infections are established within 21 days of larval ingestion by which time a loss of some adult worms has occurred. Like O. ostertagi infective larvae of O. circumcincta can successfully overwinter on pasture persisting until May and providing an immediate source of infection for susceptible grazing animals. However the epidemiological picture is complicated as post-parturient ewes may provide an additional source of infection due to the temporary breakdown of immunity during lactation. The increased faecal egg output ('spring-rise') of ewes observed at this time has been attributed to the redevelopment of inhibited early fourth larval stages within the ewes (Thomas and Boag, 1973) but in the same publication these authors also suggest that where inhibited larvae are effectively controlled a characteristic post-parturient rise in ewe faecal egg output can arise solely as a result of their ingestion of overwintering infective larvae while in an immunosuppressed state. The relative importance of the two sources of infection may vary with

climatic conditions. Boag and Thomas (1971) could detect no residual overwintered infection on pastures contaminated in the previous autumn by grazing ewes and suggested that this was attributable to low ewe faecal egg output and a newly established sward which afforded little protection. Gibson and Everett (1967) recognised the existence of overwintered infection but considered it to be small and unimportant compared with the egg output of the ewes. However they later concluded that both were significant factors in lamb infection (Gibson and Everett, 1971). The population dynamics of infective larvae on initially clean pastures and those harbouring overwintering larvae form two distinct patterns. On initially contaminated pasture, availability of infective larvae increases to a peak during April/May this being attributable to overwintering infection. Following a decline, pasture larval numbers reach a second and more substantial peak in August/September, the original generation of larvae being replaced by one derived from ewe egg excretion in the springtime. On initially clean pasture only the second peak of infection is apparent. The magnitude of the increases is dependent on the initial contamination and the patterns indicate the probable occurrence of one major generation of parasites in the clean pasture lambs and two generations in those on contaminated pasture (Boag and Thomas, 1977). On clean pasture lambs do not appear to be appreciably involved in the cycling of pasture infection until the larval population explosion attributable to ewe contamination and thus may safely graze with the ewes until such time as this occurs (Boag and Thomas, 1971).

Control

Measures aimed at preventing clinical parasitic gastroenteritis generally attempt to avoid exposure of susceptible stock to heavily contaminated pasture during the latter part of the grazing season. This may be achieved by the strategic use of anthelmintics and/or grazing control. Mixed or alternate grazing by cattle and sheep where pasture nematodes are mutually exclusive promotes the removal of L_3 by the non-receptive host without harmful effect, thus reducing contamination for the receptive host. Mixed or alternate grazing by animals of the same species but different ages may be used to similar effect, the older immune animals reducing the contamination to which the younger susceptible animals are exposed. Theoretically rotational grazing systems should promote the control of parasitic gastroenteritis

by breaking the cycle of infectivity between the herbage and the host during periods when pastures are rested. However, Morley and Donald (1980), stated that practicable rotational grazing systems do not control trichostrongyles as resting periods are relatively short compared to the longevity of the infective larvae. Michel (1969c) has shown that a straightforward rotational system involving only cattle is less effective than set-stocking, possibly because the latter denudes free-living larvae of grass cover so that they succumb more readily to adverse environmental conditions.

Perhaps the most successful grazing control measure was that advocated by Michel (1966, 1968) in which susceptible stock are treated with anthelmintic and moved to safe pasture, usually a hay or silage aftermath, just prior to the infective larval population explosion on the original pasture. However, due to vagaries in weather conditions, the exact timing of the dramatic increase in pasture larvae is difficult to predict and may not always coincide with the availability of alternative clean grazing.

Where clean grazing is available at turnout the safest control strategy for both cattle and sheep enterprises is to limit pasture contamination by grazing stock in early season thus preventing a massive build up by late season. For sheep enterprises, removal of the existing ewe worm burden prior to turnout onto clean pastures effectively controls lamb infection, but no such control is achieved for ewes and lambs grazing contaminated pastures in the spring due to autoinfection from overwintering larvae (Boag and Thomas 1973, Thomas and Boag 1973). Alternative anthelmintic treatment of lambs at weaning may be equally effective where this is accompanied by a move to clean pasture, but treated lambs remaining on contaminated pasture may actually harbour greater worm burdens than untreated animals due to interference with the development of immunity. The size of the post-parturient rise in ewes, and the extent of the contamination phase in cattle determine the magnitude, but not the timing of late season increases in pasture infectivity and hence indirectly influence the occurrence of clinical disease.

A variety of modern anthelmintic drugs are available with both broad and narrow spectrums of activity and these have been subject to recent reviews (Marriner 1986; Arundel 1985; Behm and Bryant 1985). Control strategies may be prophylactic employing regular use of anthelmintics throughout the grazing season to remove existing worm

burdens and prevent pasture contamination (Taylor et al, 1985) or may be instigated once clinical symptoms become apparent. However severe production losses may result from both clinical and subclinical ostertagiasis and experimental studies suggest that in the latter case these may arise primarily from a reduction in voluntary food intake attributable to the continual presence of developing larvae in the abomasal mucosa (Sykes and Coop, 1977).

Anthelmintic efficacy and persistency are governed by the dose rate administered and the pharmacokinetic behaviour of the drug within the host animal. Conventional anthelmintic formulations are administered by oral, dermal or parenteral routes and, with the exception of ivermectin, peak blood concentrations are maintained for only relatively short periods facilitating the removal of an existing infection and concurrently ingested larvae but failing to prevent the establishment of infective larvae subsequently ingested. As a consequence, treatment is required at intervals of two to three weeks to prevent patent infections. More recently systems for the continuous administration of anthelmintics have been developed and have the advantages of a reduced need to handle stock and possibly more complete control of incoming infection achieved at a daily dose rate which is a fraction of the therapeutic dose.

Systems include medication of feed or drinking water (Jones et al 1978; Downey et al 1974; Downey and O'Shea 1977; Downey 1987) and intraruminal devices providing either pulsed or continuous medication e.g. the oxfenbendazole pulse release bolus designed to provide five therapeutic doses of oxfenbendazole at approximately three weekly intervals, the first of which is released three weeks after administration (Morgan and Rowlands 1986; Jacobs et al 1987a; Mitchell 1987), the morantel sustained release bolus designed to release morantel tartrate continuously for 90 days (Jones 1981; Borgsteede et al 1985; Armour et al 1981) and the prototype ivermectin sustained release bolus (Pope et al, 1985, Egerton et al 1986, Soll et al, 1987). Anderson (1985) considered that the main application of controlled release technology was for the prolonged reduction in the levels of pasture contamination prior to and during a period when development and survival of free-living nematode larvae was especially favourable. Delaying administration until after turnout has produced reduced benefits with respect to acquired worm burdens and daily liveweight gains (Jacobs et al 1987b; Armour et al 1981).

A sudden increase in numbers of larvae on pasture late in the grazing season has been noted in several experiments in which contamination rates early in the season have been reduced by either conventional treatments or the use of a morantel bolus. Thus early season anthelmintic treatment may delay rather than prevent the rise in herbage contamination and thus predispose cattle to Type II rather than Type I ostertagiasis.

There is some evidence to suggest that the use of controlled release devices interferes with the development of immunity to gastrointestinal nematodes (Jacobs et al, 1987c; Borgsteede et al 1985) but not to such an extent as to influence clinical disease.

Concern has been expressed that controlled release devices may increase the selection pressure on nematodes for anthelmintic resistance. This is especially pertinent for diffusion devices such as the morantel sustained release bolus where the release rate is considerably below the effective level during the terminal stages. However Tornquist and Tolling (1987) presented no evidence for reduced efficacy when the MSRB was used in six successive years. Such devices, applied only once in the early grazing season for a relatively short time are in fact likely to produce a lower selection pressure than frequent anthelmintic dosing throughout the grazing season as the period of drug release will be small in comparison to the total survival time of the infective stages.

A wide choice of control strategies for gastrointestinal nematodes exists and the choice of system is dependent on cost effectiveness and integration with other farm enterprises. Management decisions aimed at helminth control cannot be considered in isolation from other parts of the farm enterprise as they compete for labour, finance, land, skills and perhaps other resources.

EXPERIMENT III

PARASITOLOGICAL AND METABOLISM STUDIES CONDUCTED WITH GROWING LAMBS EXPERIMENTALLY INFECTED WITH OSTERTAGIA CIRCUMCINCTA AND GIVEN GRADED DOSES OF LEVAMISOLE

Introduction

Parasite control programmes which employ regular use of anthelmintics to maintain a low infection status in stock provide the greatest independence from other farm enterprises. However they are costly with respect to both time and labour and prolonged use of anthelminitics in this manner may increase selection pressure for nematode resistance. Administration of an intraruminal device to cattle at turnout prevents early season pasture contamination and consequently late season disease (Jones, 1981). Similar administration to lambs at weaning may control both lamb and pasture infection where animals are moved to 'safe' pastures and weaning occurs within 12 weeks of birth. For lambs maintained with their dams throught the grazing season, administration of a controlled release device at approximately 10 weeks of age may control lamb infection in the face of increasing pasture contamination although constant exposure to infective larvae may give reduced benefits compared with weaning to safe pasture.

At time of writing the anthelmintic levamisole hydrochloride is available for use in sheep as an oral drench, in-feed granules, an injectable solution or a topical application and all of these routes require regular administration to achieve effective parasite control. The present work assesses the effectiveness of continuous delivery into the rumen of graded doses of levamisole hydrochloride in controlling a low level infection of Ostertagia circumcincta in growing lambs and thus gives an indication of the payload which would be required should this anthelmintic be incorporated into a sustained release device.

Materials and Methods

Animals and experimental design

Thirty six parasite naive cross-bred lambs, of average liveweight 35 kg were allocated to six treatment groups. Lambs in groups 1-5 were dosed daily per os with 4,000 infective O. circumcincta larvae for 21 days, while those in group 6 were maintained as a parasite-free control. In addition, lambs in groups 2-5 received continuous

infusions, directly into the rumen, of levamisole hydrochloride in solution in 0.9% w/v saline at rates of 1.0, 1.5, 2.0 and 3.0 mg/kg/liveweight/day for 24 days commencing on the first day of experimental infection. The animals were restrained in standard metabolism stalls and the levamisole solutions were administered via surgically fitted indwelling ruminal catheters. The infusion rates were controlled by constant running peristaltic pumps (Watson-Marlow Ltd.). A full description of the infusion system is given in General Materials and Methods. Group 1 lambs remained untreated and acted as an infected control.

Allocation to treatment was on the basis of sex and liveweight, there being three female and three castrated male lambs in each of treatment groups 1-4 and in group 6. Effects of 3.0 mg/kg/day levamisole (group 5) were examined after preliminary analysis of data from the other treatments and consequently all lambs in treatment group 5 were female.

Three female lambs from each treatment group were necropsied on day 24 post initial infection; necropsy of the remaining lambs being undertaken on day 28.

Feed allowance

Lambs were individually given a daily allowance of 1 kg grassnuts in two approximately equal feeds at 07.30 and 16.30h. Mean analysis was 0.92 dry matter (DM) with 123 g crude protein (CP), 286 g crude fibre (CF), 30 g ether extract (EE) and 56 g ash per kg DM.

Larval infections

The O. circumcincta larvae were harvested from faecal cultures no more than four days prior to oral inoculation of the lambs and were maintained at room temperature (17°C) until required.

Parasitological techniques

Faecal samples were obtained from all lambs per rectum on day 0, 7, 14, 21 and for remaining lambs on day 28 and were examined for the presence of strongylate eggs by the modified McMaster technique of Gordon and Whitlock (1939). At necropsy examination of the abomasa and recovery of adult and developing Ostertagia cirumcincta was as previously described.

Digestibility and nitrogen balance studies

Digestive efficiency studies were carried out separately during days 0-7, 7-14, 14-21 and 21-28. Concurrent nitrogen balance studies were conducted for male lambs in each treatment group except the parasite-free control during days 0-7, 7-14 and 14-21, and for all male lambs during days 21-28. Collection and analyses of urine and faeces was as described in General Materials and Methods.

Statistical analysis

A analysis of variance was carried out for each data set. For parasitological parameters this was performed using 'Minitab' (Ryan, Penn State University) on a microcomputer. Analysis of digestibility and nitrogen balance data was performed using the programme 'Rummage' (PLU Edinburgh) on an ICL 2988 computer. The model incorporated treatment, time and sex (in the case of digestibility coefficents) as main effects and allowed for first and second order interactions.

Results

General observations

During the trial none of the lambs became inappetent or showed overt signs of clinical ostertagiasis. This contrasts with Sykes and Coop (1977) where a similar rate of infection in similar animals resulted in a 20% reduction in voluntary food intake within two to three weeks. The catheter insertion procedure was highly successful in all cases and there was no evidence of infection or inflammation. The extent to which the skin contracted around the inserted catheter varied between individual sheep but generally leakage of rumen fluid at the insertion site was small, and was minimized by the presence of the rubber disc.

Apparent digestibility coefficients

The apparent digestibilities of several proximate feed fractions were significantly affected by treatment over the whole 28 day period. Dry matter, crude protein, crude fibre and ash digestibilities were reduced with a consequent increase in nitrogen-free-extract digestibility for lambs receiving 3.0 mg/kg/day levamisole (Table 26). Treatment did not affect the digestibility of the gross energy. Sex influenced the apparent digestibility of the crude protein and ash proximate fractions during the whole 28 day period, these being

Table 26. Mean digestibility coefficients (0-28 days) for lambs infected with Ostertagia circumcincta and given graded doses of levamisole

Levamisole mg/kg/day	0	1	1.5	2	3	Clean control	Pool SE
DM	0.502b	0.548a	0.525	0.520	0.510b	0.524	0.009
CP	0.476	0.482	0.473	0.472	0.438b	0.491a	0.015
CF	0.401c	0.442ac	0.422ac	0.434ac	0.386b	0.448a	0.011
ASH	0.380	0.408a	0.396a	0.388a	0.329b	0.372	0.015
NFE	0.573b	0.598	0.595	0.589	0.613a	0.589	0.008
GE	0.486	0.513	0.510	0.507	0.498	0.508	0.010

Values with different superscripts are significantly different using a multiple comparisons procedure with overall significance level of 0.05

Table 27. Mean nitrogen balance (g/day +SE) for lambs infected with Ostertagia circumcincta and given graded doses of levamisole

n=3	Infected control	Levamisole 1.0 mg/kg/day	Levamisole 1.5 mg/kg/day	Levamisole 2.0 mg/kg/day	Clean control
Days 0-7					
Intake		18.09 ±0.00	18.09 ±0.00	18.09 ±0.00	
Faeces		8.85 ±0.39	8.92 ±0.38	8.93 ±0.23	
Urine		7.60 ±0.12	7.90 ±0.36	7.31 ±0.22	
Retention		1.64 ±0.43	1.26 ±0.70	1.85 ±0.20	
Days 7-14					
Intake	18.61 ±0.00	18.61 ±0.00	18.61 ±0.00	18.61 ±0.00	
Faeces	9.19 ±0.42	9.02 ±0.31	9.22 ±0.71	9.10 ±0.21	
Urine	7.67 ±0.29	7.13 ±0.43	8.22 ±0.45	7.43 ±0.49	
Retention	1.75 ±0.55	2.46 ±0.74	1.17 ±0.96	2.08 ±0.62	
Days 14-21					
Intake	17.90 ±0.00	17.90 ±0.00	17.9 ±0.00	17.90 ±0.00	
Faeces	9.27 ±0.15	8.40 ±0.76	10.14 ±0.36	9.30 ±0.26	
Urine	7.02 ±0.43	7.85 ±0.14	8.61 ±0.46	7.70 ±0.22	
Retention	1.61 ±0.34	1.66 ±0.76	-0.85 ±0.14	0.89 ±0.47	
Days 21-28					
Intake	17.90 ±0.00	17.90 ±0.00	17.90 ±0.00	17.90 ±0.00	17.90 ±0.00
Faeces	8.97 ±0.26	9.83 ±0.72	8.83 ±0.33	8.74 ±0.19	8.88 ±0.31
Urine	7.55 ±0.07	7.59 ±0.06	7.75 ±0.28	7.63 ±0.18	7.47 ±0.17
Retention	1.38 ±0.31	0.49 ±0.75	1.31 ±0.33	1.53 ±0.24	1.55 ±0.42

Missing data is due to a shortage of metabolism accomodation

Table 28. Mean faecal egg output (epg) at days 21 and 28 and mean worm burdens at necropsy of lambs infected with Ostertagia circumcincta and continuously infused intraruminally with graded doses of levamisole hydrochloride

Levamisole mg/kg/day	0	1	1.5	2	3	Pool SE
epg						
Day 21	617a	394	113b	354	75b	120
Day 28	358	192	333	542	50	185
Worms						
L4	1767	2967	1083	983	1717	908
L5	7000a	8150a	4483b	3342b	850c	1264
Adult	22492a	19392a	12925a	9058b	4575b	3249
Total	31258a	30508a	18492b	13383b	7142c	4098

Values with different superscripts are significantly different $P<0.05$

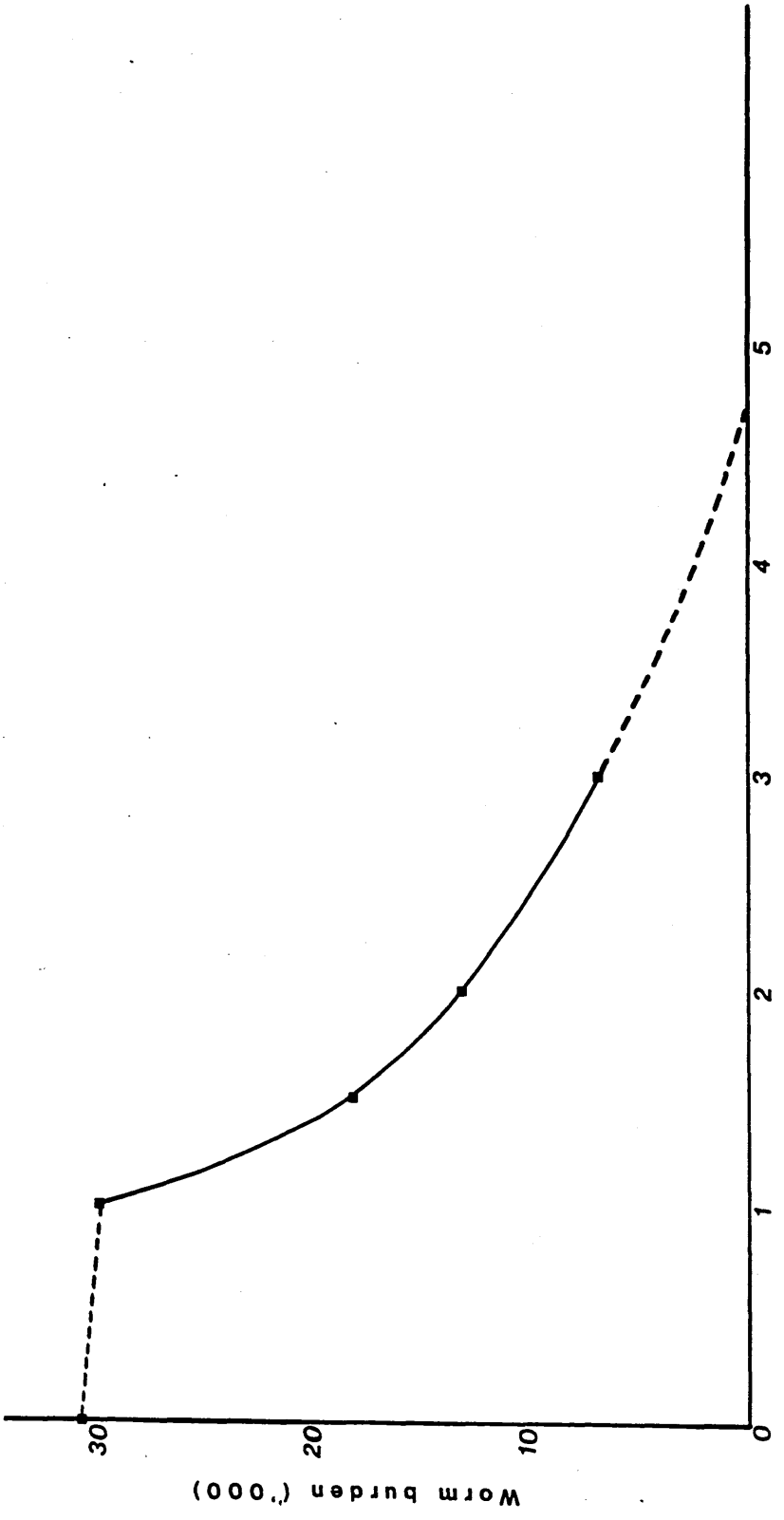


Figure 18. Mean worm burdens at necropsy of lambs infected with Ostertagia circumcincta and given graded doses of levamisole by continuous intraruminal infusion.

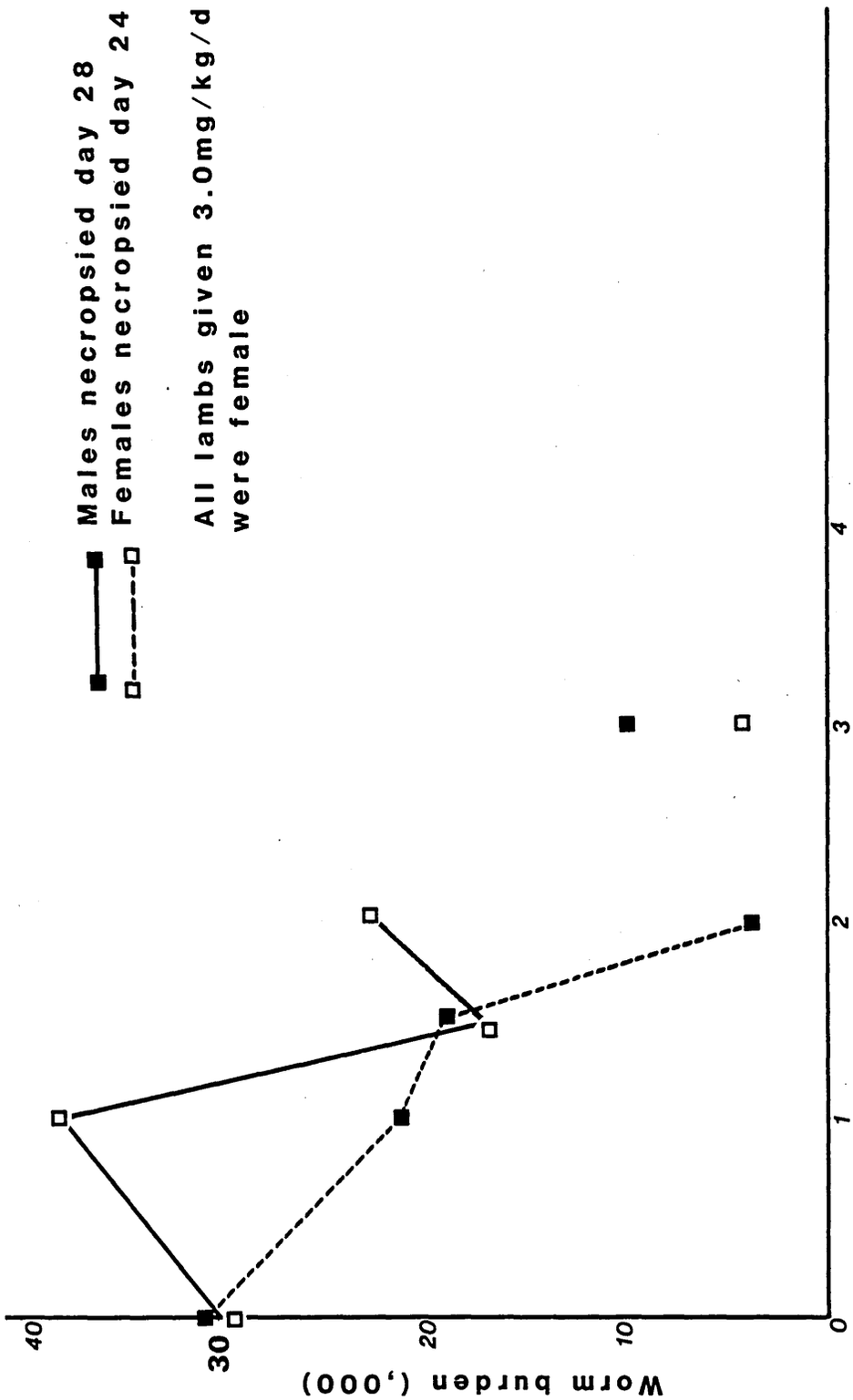


Figure 19. Mean worm burdens at necropsy of male and female lambs infected with Ostertagia circumcincta and given graded doses of levamisole.

significantly ($P<0.05$) reduced for female compared to male lambs.

Recording period had a significant ($P<0.05$) effect on the apparent digestibility of each proximate feed fraction and a significant interaction also occurred between recording period and treatment. Mean digestibility data for the various proximate feed fractions in each recording period and values for individual animals are contained within Appendix 6.

Nitrogen balance

Mean results for the daily nitrogen input, output in urine and faeces together with the calculated retentions for each of the four recording periods are given in Table 27. Individual values and the data expressed in terms of percentage of nitrogen input are given in Appendix 6. There were no significant differences in nitrogen economy between treatments. Urinary nitrogen loss did differ significantly between recording periods and was generally greatest during 14-21 days post initial infection.

Parasitology

Infusions of 1.5 and 3.0 mg/kg/day levamisole resulted in a significant reduction in faecal egg output on day 21 compared to infected controls (Table 28).

Increasing daily doses of levamisole resulted in increasing reductions in O. circumcincta worm burdens at necropsy compared to the infected control. These reductions were significant for the 1.5, 2.0 and 3.0 mg/kg/day treatments (Figure 18, Table 28, Appendix 6). Comparison of data for male lambs necropsied on day 24 and female lambs necropsied on day 28 reveals deviations from the general overall pattern (Figure 19) which may be attributable to differences in slaughter date and/or sex.

The pH of the abomasal contents at necropsy (Appendix 6) was not significantly affected by treatment but for lambs on all treatments values were greater than the accepted norm (<pH 2.5).

Discussion

The system of intraruminal infusion used in this study provided an extremely accurate means of administering titrated doses of anthelmintic to individual animals. It did not completely mimic a sustained release device in that effects of rumen environment on

diffusion or dissolution rates of such a device were not taken into account, but it did provide a precise method of assessing the effect of different drug levels on the parasite population.

Sykes and Coop (1977) reported a 20% reduction in voluntary food intake for lambs infected with 4000 O. circumcincta L_3/day but no such effect was apparent in this study possibly because lambs were offered a standard amount of food per day which was below appetite and so consequently differences in voluntary food intake were not expressed. The same authors also reported reductions in the apparent digestibility of nitrogen and energy for infected lambs compared to controls fed ad libitum and in contrast apparent digestibility data here reveals only a significant reduction in crude fibre digestibility for infected compared to clean control lambs.

O. circumcincta completes its parasitic development in the gastric glands of the abomasum and the emergence of young adult parasites 8-12 days after ingestion is associated with mucosal damage. It may be expected therefore that lambs harbouring the highest numbers of O. circumcincta, i.e. those on the lower levamisole treatments, would have the poorest apparent digestive efficiencies. However examination of the digestibility data for the whole 28 day period reveals the reverse to be true and suggests that levamisole, like the substituted benzimidazoles, may adversely affect rumen fermentation. Reduced apparent digestibility of crude fibre may be attributable to reduced volatile fatty acid production as a result of suppression of both cellulolytic and carbohydrate dependent microorganisms (Jara et al, 1984; Hodgeson and Jessop, 1987). Concommitant inhibition of acid secretion in the abomasum (Felenius et al, 1981) may have been responsible for the significant reduction in crude protein digestibility. At necropsy abomasal pH of all lambs was elevated above normal values and this may be evidence of inhibition of acid secretion in lambs receiving the highest levels of levamisole, while reflecting mucosal damage in lambs on the low/zero levamisole treatments. Despite alterations in crude protein digestibility and abomasal pH, nitrogen economy did not differ between treatment groups.

The significant treatment-time interaction for digestive efficiencies possibly occurred because the suppressant effect of levamisole operated throughout the trial while that due to parasitic mucosal damage only became apparent following maturation and emergence of the adult worms.

Increasing daily doses of levamisole hydrochloride resulted in increased reductions in total worm burden at necropsy, this response being curvilinear in nature. The data predicts that a daily dose rate of 4.7 mg/kg is required for complete control. This compares with 3.5 mg/kg/day required for the control of O. ostertagi in grazing calves when administered via the drinking water (Downey and O'Shea, 1977) and may not be an economic proposition as a single oral dose of only 7.5 mg/kg administered at 21 day intervals provides effective control at present. Continual administration at a dose rate which provides only partial parasite control may accelerate selection for resistance to this anthelmintic. Resistance of Ostertagia to levamisole has already been reported in sheep, goats and cattle (Le Jambre 1979; Gillham and Obendorf, 1985; Geerts et al, 1987).

Separate examination of the worm burden data for male and female lambs reveals deviations from the overall pattern. These may be attributable to differences in sex and/or slaughter date. For rat and mice host-parasite systems infections are relatively more persistent in male hosts and this has been attributed to differences in steroid hormones (Waddell et al, 1971; Dobson 1961a and 1961b). A similar effect has been reported in sheep for parasites with extensive somatic migrations (Dobson, 1965). The intimate contact between developing O. circumcincta and the abomasal mucosa would ensure exposure to sex hormones but effects are likely to be minimal in this study as male sheep were castrated and female sheep were physiologically immature.

The results of this study demonstrate that continuous administration of levamisole hydrochloride directly into the rumen can limit the establishment of the abomasal parasite O. circumcincta but a daily dose in excess of 3.0 mg/kg/day is required which must be compared with the recommended therapeutic treatment dose of 7.5 mg/kg . Continuous administration at a dose effective for parasite control may interfere with ruminant digestion.

GENERAL CONCLUSIONS AND DISCUSSION

The experimental work described in this thesis used similar techniques to explore two separate aspects of nitrogen metabolism in the ruminant. One study investigated the effect of exogenous beta-adrenergic agonists on the digestive efficiency and nitrogen economy of young growing cattle. Similar nitrogen balance and digestive efficiency techniques were used to investigate the detrimental effects of gastrointestinal parasitism in both cattle and sheep. Additional studies utilising radioisotopes provided more detailed information on the metabolism of nitrogen in infected cattle. In persuit of each of the study objectives a continuous infusion system was developed for the accurate long-term administration of exogenous substances by either intravenous or intraruminal routes.

Beta-agonists, also termed repartitioning agents, have been shown to increase lean tissue accretion in farm livestock at the expense of fat but the precise mode of action has yet to be elucidated. Increased lean tissue deposition may result from reduced protein degradation or increased accretion, while converse changes in lipid metabolism may result in decreased fatness. Initial experimental work investigated the effects of clenbuterol or graded doses of bitolterol (beta-agonists) on the digestive efficiency and nitrogen economy of growing Friesian calves. The experimental design was that of two identical 5 X 5 Latin squares using ten calves and five treatments which were changed after each three week period. Administration of 1 mg/day clenbuterol or 25 mg/day bitolterol resulted in a significant increase ($P<0.01$) in nitrogen retention as a result of a significant ($P<0.001$) reduction in urinary nitrogen excretion. Apparent digestive efficiency and faecal nitrogen excretion were unaffected by treatment indicating that nutrients were being repartitioned within the animal.

Improvements in nitrogen retention accruing from the use of beta-agonists were enhanced during the latter part of the experiment following an increase in daily feed allowance to allow for the sustained growth of the calves. It is possible that relative increases in nitrogen and energy availability from the ration at this time permitted a greater beta-agonist mediated stimulation of nitrogen accretion. However, it is equally possible that use of these agents resulted in a reduction in protein degradation which was magnified by

increased protein turnover attributable to increased nutrient intake. Effective dose rates (on the basis of weight of agent per kg liveweight) of the beta-agonists were reduced throughout the trial as a result of increased calf liveweight and the enhanced responses, coincident with increased nutrient intake at lower effective dose rates emphasises the importance of nutrient supply when administering these substances.

Further studies, possibly involving radioisotopes, are required to fully elucidate the mechanism by which beta-agonists exert their repartitioning effect. Studies are also necessary to investigate the effect of these agents in animals of different age, sex and physiological condition. Finally, investigations into possible residue accumulation are required. The leaner more muscular animals resulting from use of beta-agonists produce carcasses which are in line with current consumer requirements but exogenous manipulation to achieve this end is perhaps unlikely to find widespread consumer acceptance at the present time.

In the second and third sections of this thesis the detrimental effects of gastrointestinal parasitism in both cattle and sheep were investigated. In all studies animals were orally inoculated with infective larvae over an extended period of time in order to simulate intakes in a field situation. However in the field, larval availability and animal grazing behaviour are very much influenced by climatic vagaries and thus exact simulation is not really possible. Declining pasture quality towards the end of the grazing season, as opposed to a consistent experimental diet, may also influence animal response to infection. However importantly, experimental infection does permit a comparison of the pathogenicity of nematode species under standardised conditions.

In section II the detrimental effect of a monospecific infection of Ostertagia ostertagi in cattle and that of a similar concurrent infection with Cooperia oncophora were examined. Previous work (Armour et al, 1987a) permitted further comparison with a monospecfic C. oncophora infection given in an indentical manner to the C. oncophora in the combined infection. Results of these studies demonstrated that the pathogenicity of concurrently administered O. ostertagi and C. oncophora was far greater than that of either species alone. Enhanced establishment of both species in a combined

infection was partially responsible for this effect but the cause of this enhancement is unclear and contradicts Kloosterman et al (1984) who failed to demonstrate any interactions in a concurrent infection of these two nematodes. Increased establishment of O. ostertagi gave rise to more severe abomasal damage as was evidenced by greater and more persistent increases in plasma pepsinogen levels, this persistency being attributable to the increased time required for repair of the abomasal mucosa. Severe damage to the intestinal mucosa resulting from C. oncophora infection was apparent even after expulsion of the majority of the worms and in some animals extended for the entire length of the small intestine. This contrasts with most published work which suggests that C. oncophora is a relatively non-pathogenic lumen dwelling species, and confirms the results of Armour et al (1987a).

Mucosal damage resulting from both monospecfic and the combined infections permitted the leakage of pepsinogen from the abomasum and the reverse leakage of small plasma constituents (albumin) into the gastrointestinal tract. In concurrently infected calves the considerable loss of plasma by this route greatly increased the catabolic rate of albumin such that increased rates of synthesis couldnot compensate and hypoalbuminaemia associated with hypoproteinaemia occurred. Together with inappetence, reduced apparent digestibilities of both dietary crude protein and energy, and an additional energy requirement for repair of a damaged mucosa, this placed severe limitations on nutrients available for growth and represented a severe insult to the animals metabolism. In addition increased bacterial degradation of undegraded protein reaching the caecum, its absorption as ammonia, and its subsequent conversion to urea prior to excretion in the urine also represented an extra drain on available energy.

Prior administration of a morantel sustained release bolus limited larval infection and prevented clinical symptoms. The anthelmintic morantel tartrate was apparently primarily active against adult parasites, development of immature stages resulting in minor alterations in pathophysiological values and the stimulation of immunity in the absence of disease. The study on Pre-Type II ostertagiasis demonstrated that morantel is inactive against inhibited fourth stage larvae of O. ostertagi.

Both the combined infection described in this thesis and the study of Armour et al (1987a) have demonstrated the potential pathogenicity

of C. oncophora infection in parasite naive calves. Further studies are required to more clearly define the parasitic development of this nematode and in particular to identify the predeliction site and the proportion of time spent by developing stages within, or in close association with, the intestinal mucosa. Thus underlying metabolic causes of the disease processes attributable to this nematode could be more clearly identified. Further studies are also required into possible interactions between O. ostertagi and C. oncophora and the pathogenic consequences of infections established using different proportions of these two species. Finally, detailed studies on the morphological and reproductive characteristics of nematodes from morantel treated cattle may help to elucidate the precise mode of action of this anthelmintic.

In section III nitrogen balance and digestive efficiency studies were used to investigate the adverse effects of Ostertagia circumcincta infection in growing lambs. The effectiveness of continuously administered levamisole in controlling the infection was also investigated using intraruminal infusion to mimic a controlled release device. Daily infections of 4000 O. circumcincta to sheep for 21 days did not cause any clinical signs. There were no significant differences in nitrogen economy between treatments but the apparent digestibilities of the dry matter and crude fibre fractions of the diet were significantly ($P<0.05$) reduced for infected compared to clean control lambs. An infusion of 3.0 mg/kg/day levamisole resulted in a 77% reduction in worm burden at necropsy compared to the infected control but, compared to the clean control, it also resulted in significant ($P<0.05$) reductions in the apparent digestibilities of all of the major feed fractions. Abomasal pH at necropsy was similar for infected lambs and those receiving 3.0 mg/kg/day levamisole indicating that the inhibitory effect of levamisole on acid secretion was similar to that accruing to parietal cell destruction by developing parasites. It is interesting that the detrimental effect of low level anthelmintic administration on the digestive efficiency of the lambs used in this study was apparently more severe than that of parasitic infection and this could act as a constraint on production where parasite challenge is at a low level. However this effect is likely to be small in comparison to that of parasite infection on a massive scale.

The study predicted that a daily dosage in excess of 3.0 mg/kg/day levamisole would be required should this anthelmintic be incorporated

into an intraruminal device and possible future work could include the construction of, and experimentation with such a device for control of gastrointestinal nematodes in both cattle and sheep. However the therapeutic dose of levamisole is 7.5 mg/kg and such a device may not be economic.

In conclusion studies for this thesis have demonstrated that the use of exogenous pharmacological agents in healthy livestock can alter the normal partitioning of nutrients to effect greater retention of nitrogen and thus possibly increase the efficiency of lean meat production. Subsequent studies very clearly demonstrated the detrimental effect of gastrointestinal parasitism on nitrogen metabolism in cattle. Mucosal damage resulting from parasite activity resulted in a reduced apparent digestibility of dietary crude protein and increased losses of plasma proteins to the gastrointestinal tract. Together with increased turnover of mucosal cells this caused a diversion of nitrogen from productive to less productive sites in order to maintain homeostasis. Studies with sheep demonstrated that the use of modern anthelmintic drugs at concentrations and dosing frequencies required for complete parasite control may adversely affect ruminant digestion.

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APPENDIX 1

INFUSION STUDIES PROCEDURES

Catheter Insertion Procedures

- The insertion of an indwelling jugular vein catheter
- The insertion of a ruminal catheter

Procedure for changing Pumphead Tubing

Procedure for Preparing Infusion Packs

Procedure for Exchange of Infusion Packs

Problems Encountered during Infusion Studies

- Immediate action
- Problems with infusion tubing
- Problems with catheters

Catheter insertion procedures

The insertion of an indwelling jugular vein catheter

The calf was anaesthetized by giving 0.5 to 1.0 ml of Rompun (xylazine, Bayer UK Ltd, Suffolk) as an intramuscular injection in the rump region. An area of the calf's neck immediately over the jugular vein midway between the jaw and shoulder was shaved and cleaned with sterilising solution (1:500 Marinol blue : 70% alcohol with a few crystals of sodium nitrate). Approximately 2 ml of Locaine 2% local anaesthetic (lignocaine hydrochloride BP 2% with adrenaline acid tartrate BP, Veterinary Drug Co. PLC, York, UK) was injected under the skin in front of and above the proposed insertion site, and a cut approximately 1 cm long was made through the skin above the raised jugular vein.

The teflon catheter together with its internal trochar were inserted into the jugular vein through the dermal cut, the trochar being removed when the catheter was in position. For PVC and Tygon catheters a 14 G x 2 inch hypodermic needle was inserted into the jugular vein through the dermal cut and a metal stylet was fed down through the needle until it was approximately 20 cm inside the vein. The needle was then removed and the catheter was fed into the jugular vein over the stylet, the stylet being removed when the catheter was correctly positioned.

Once in position, all catheter types were flushed with heparinized saline solution (2 ml 5000 IU heparin in 1000 ml 0.9% saline), drawing back also to obtain blood and thus verify the correct position of the catheter in the vein. Flushed catheters were filled with 0.75 ml of 5000 IU heparin and stoppered before being stitched to the skin and finally connected to an air free infusion line.

The insertion of a ruminal catheter

The insertion of an indwelling catheter into the dorsal ruminal pouch of a sheep involved a two stage surgical procedure. Each sheep was sedated using 0.25 ml Rompun and prepared by shaving an area approximately 15 cm x 15 cm in the region betwen the last rib and the tuber coxae directly below the transverse processes. This area was cleaned using sterilising solution (Hibitane) and a vertical incision, approximately 4 cm long, was made through the skin, muscle and peritoneum to expose the surface of the rumen. The skin was stitched to the surface of the rumen using eight sutures of fine nylon, one at

either end of the incision and three along either side. The suturing needle did not penetrate the rumen itself.

For the following 10 days sheep were maintained in individual peat bedded pens. The area of surgery on each sheep was thoroughly cleaned twice daily with sterilising soltution (Hibitane) and sprayed with antibiotic (Polybactrin). If infection was detected by the presence of pus an intramuscular injection of long acting antibiotic (Terramycin L.A., Pfizer) was given. By day 10, sheep were beginning to shed stitches and any remaining stitches were removed allowing relaxation of the skin around the incision and exposure of any pockets of infection. In all cases the skin had completely knitted on to the surface of the rumen. During the subsequent four days any slight infections were rigorously treated with antibiotic (Polybactrin). On day 14 a scalpel and artery forceps were used to puncture the surface of the rumen through the original incision and a Foley catheter (size 22 Fr, Warne surgical products) was inserted. The 30 ml balloon was inflated using 20 ml of warm water, thus anchoring the catheter in the rumen. Prior to insertion, a tightly fitting rubber washer, approximately 5 cm in diameter, was mounted onto each catheter. This was maintained against the animals skin preventing the catheter from falling into the rumen and also minimising leakage of ruminal fluid from around the insertion site.

Once inserted, catheters were immediately connected to the infusion system and approximately 30 ml of fluid was rapidly infused to ensure that the catheter was free of ruminal contents.

Procedure for changing pumphead tubing

The pumphead tubing associated with each pump was changed sequentially. For each infusion line the squeeze valve on the administration set was closed, effectively immobilizing the infusion fluid. A pair of artery clamps were attached to the marzinal tubing of the same line close to the connection at the pumphead. The pumphead connectors were lifted out of their supporting slots and a disconnection was made between one connector and the marzinal tubing. The ground 14 G hypodermic needle of the pumphead tubing unit was then unscrewed from the administration set connector permitting removal of the complete pumphead tubing unit. A new unit was attached to the administration set and the squeeze valve was partially opened to allow the unit to fill with fluid and thus prevent an air lock. Finally

reconnection was made to the marzinal tubing and the pumphead connectors were placed in their supporting slots, tensioning the new pumphead tubing across the rotor. The artery clamps were removed and the squeeze valve fully opened.

Procedure for preparing infusion packs

Infusion packs were prepared directly prior to requirement by replacing aliquots of 0.9% saline taken from one litre packs (Viaflex) with the same quantity of the drug to be infused, taken from a concentrated stock solution. Each pack was infused over four days.

The stock solution of levamisole hydrochloride was freshly prepared by dissolving 1.40 g levamisole hydrochloride in 100 ml of glass distilled water. The required infusion rates were 1.0, 1.5, 2.0 and 3.0 mg/kg/day (35, 52, 70 and 105 mg/day for sheep of mean liveweight 35 kg). Thus infusion packs administered over four days required 0.14, 0.21, 0.28 and 0.42 g of levamisole and hence 10, 15, 20 and 30 ml of the stock solution respectively. Packs were prepared simply by replacing either 10, 15, 20 or 30 ml of saline with the same quantity of stock solution using a sterile 30 ml syringe. The intended infusion rate was marked on each pack prior to its preparation and once injected levamisole and saline were mixed by inverting the pack several times. Any levamisole hydrochloride stock solution remaining after preparation of each batch of infusion packs was discarded.

Stock solutions of beta-agonists were supplied in sealed bottles with rubber caps. The concentration of the clenbuterol stock solution was 5 mg/15 ml and the three stock solutions of bitolterol were at concentrations of 4, 20 and 100 mg/15 ml. Three ml of the clenbuterol stock solution was required for a four day infusion at a rate of 1 ml/day, and 15 ml of each bitolterol solution was required for infusion rates of 1, 5 and 25 mg/day respectively. There was evidence to suggest that the 100 mg/15 ml stock solution of bitolterol was deteriorating at a rate of 5% per 4 weeks and in an attempt to retard this, the solution was stored at 4°C. Prior to injection the solution was gently warmed to reverse floculation. The other stock solutions were stored at room temperature.

Greater care was required when preparing packs for intravenous rather than intraruminal infusion as complete asepsis within the infusion system was paramount. Beta-agonist stock solutions were injected in a similar way to levamisole stock solution but separate

syringes were maintained for each separate stock solution and also for the withdrawal of saline. Both the rubber caps of the stock solution bottles and the injection sites on the saline bags were sprayed with sterilising solution (1:500 Marinol blue : 70% alcohol with a few crystals of sodium nitrate). A bacterial filter (Millex-GS, Millipone) was also placed between the injection needle and syringe during the injection process. It was important to ensure that this filter was full of stock solution and that the syringe contained the required amount before injection commenced as some solution was always retained by the filter. Injected stock solution was thoroughly mixed by inversion of the infusion pack.

Procedure for exchange of infusion packs

Infusion packs were exchanged on every fourth day at the same time (11.00 h). Any residual fluid in existing packs was rapidly infused by lifting the connectors out of their supporting slots on the infusion pack side of the pumphead, effectively removing the influence of the pump on each infusion line and allowing gravity assisted flow of infusion fluid. As each existing infusion pack emptied, the squeeze valve on the associated administration set was closed and the connector was replaced in its slot at the pumphead. The empty infusion pack was taken down from it supporting nail, disconnected from the administration set and discarded. The reservoir and connector for the administration set were then inverted and inserted into the replacement infusion pack, its connecting tube being uppermost. Once connected, the infusion pack and reservoir were turned the right way up and the infusion pack was placed on its supporting nail. The inversion of the reservoir and replacement pack reduced the occurrence of air locks during the exchange procedure. Generally, when the squeeze valve on the administration set was opened, a drip formed immediately in the reservoir and no air locks were present.

Problems encountered during infusion studies

Immediate action

On discovery of any abnormal occurrence during infusion studies, the flow of fluid in the particular infusion line was temporarily terminated by closing the squeeze valve on the administration set until the problem could be resolved. In most cases it was also necessary to flush the catheter and establish its patency. Jugular vein catheters

were flushed with heparinized saline. A pair of artery forceps were applied to the catheter prior to and during its disconnection from the infusion tubing and reconnection to a sterile syringe of heparinized saline. The forceps were then removed to permit flushing. If the catheter was patent the forceps were reapplied and the syringe was removed and replaced with a stopper. Catheters flushed with heparinized saline were thus stored over short periods while any problems with the infusion system were resolved. If long term catheter storage was required, catheters were filled with 0.75 ml 5000 IU heparin. Artery forceps were always applied to a jugular catheter before disconnection from the infusion system or from a syringe, or before stopper removal as this prevented blood backflow into the catheter possibly resulting in occlusion. If, on flushing, a jugular vein catheter was found to be non-patent it was removed and surgically replaced.

Intraruminal catheters were flushed with warm water using a 60 ml syringe and fitted with a luer-lock connector from an administration set. The connector permitted a tight junction to be formed at the top of the catheter. If a catheter was found to be non-patent, the water was withdrawn from the balloon into a syringe, permitting removal from the sheep. The catheter was reinserted following thorough washing under running water to remove ruminal contents, the usual cause of the obstruction.

Problems with infusion tubing

Occasionally animals would chew through the marzinal tubing. The section of tubing below the chewed portion would rapidly empty of infusion fluid resulting in a back flow of blood or rumen contents into the catheter causing an obstruction. If this occurred the patency of the catheter was established by flushing, and patent catheters were temporarily 'stored' while a repair was effected. Small sections of chewed tubing were cut out using a sterile scalpel blade and the two cut ends were joined using a double ended connector (Watson-Marlow, delta S). If a large section of tubing was damaged, the whole of the marzinal tubing was renewed, commencing at the pumphead. Before connection to the catheter, it was essential to ensure that the whole of the marzinal tubing was filled with fluid and this was most readily achieved by temporarily releasing the infusion line from the influence of the peristaltic pump allowing gravity assisted fluid flow.

If a significant air-lock was detected in the marzinal tubing during intravenous infusion studies it was removed by disconnecting the catheter and instigating gravity assisted fluid flow.

During either of these procedures, the amount of fluid, and drug, lost from the system was estimated and the drug loss was rectified by injection of an additional quantity of stock solution into the infusion pack.

Problems with catheters

Very few problems were encountered with the intraruminal catheters or the indwelling jugular catheters made from Tygon microbore tubing. The major problem with PVC jugular catheters was fissuring during periods of cold weather. The fissure was located by flushing with heparinized saline. Artery forceps were applied immediately below the damaged portion and the top and damaged part of the catheter were removed using sterile scissors. The 14 G hypodermic needle was then inserted into the remaining part of the catheter and held in place with suture material. A second flushing with heparinized saline established the patency of the catheter and, if patent, it was reconnected to the infusion tubing.

If a PVC jugular catheter began to loop out of a calf's neck it was generally rapidly expelled from the jugular vein. Early detection permitted slight prolongation of the life of the catheter by immobilisation with an additional suture or bandaging and medical adhesive. If the outer sleeve of a PVC catheter was becoming detached from the main catheter body, it was rejoined using 'super glue' or tightly bound in place using suture material.

Teflon jugular catheters had no external portion and so artery forceps could not be applied if disconnection was required. If a Teflon catheter was observed to be lying subcutaneously it was removed immediately.

Each time a jugular catheter was replaced a slight thickening of the jugular vein wall was detected and thus there was a finite number of times that replacement could occur. For this reason PVC and Teflon catheters were superceded by the longer life Tygon catheter.

APPENDIX 2

ADDITIONAL DATA FOR EXPERIMENT 1

2a. Individual calf apparent digestibility coefficients.

2b. Individual calf nitrogen balance data (g/day).

2c. Individual calf nitrogen balance data (% intake).

2d. Overall mean nitrogen balance data (% intake).

Table 2a. Individual digestibility coefficients for calves receiving infusions of physiological saline, 1 mg/d clenbuterol (clen) or 1, 5 and 25 mg/d bitolterol (Bi)

CALF	1	2	3	4	5	7	8	9	10	11
Period IA	Saline	Bil	Bi5	Bi25	Clen	Saline	Bil	Bi5	Bi25	Clen
DM	0.6310	0.6293	0.6109	0.6002	0.6328	0.5980	0.5912	0.6020	0.5968	0.5765
CP	0.5622	0.5677	0.5293	0.5349	0.5436	0.5297	0.5114	0.5430	0.5307	0.5036
CF	0.4530	0.4572	0.3956	0.3874	0.4365	0.3768	0.3570	0.3856	0.4151	0.3600
EE	0.8356	0.8598	0.8388	0.8193	0.8287	0.8328	0.8318	0.8337	0.8263	0.8306
Ash	0.3731	0.4159	0.3910	0.3441	0.3887	0.3341	0.3248	0.3601	0.3518	0.3105
NFE	0.7324	0.7187	0.7181	0.7104	0.7429	0.7111	0.7110	0.7076	0.6946	0.6882
OM	0.6594	0.6528	0.6351	0.6284	0.6596	0.6271	0.6206	0.6286	0.6238	0.6054
E	0.6406	0.6431	0.6174	0.5998	0.6440	0.6064	0.6659	0.6161	0.5967	0.5841
Period IB										
DM	0.6500	0.6099	0.5999	0.5899	0.5910	0.6498	0.6100	0.5997	0.5899	0.6002
CP	0.6123	0.5586	0.5476	0.5308	0.5239	0.5764	0.5493	0.5184	0.5170	0.5749
CF	0.4380	0.3978	0.3459	0.3046	0.3603	0.4209	0.3779	0.3555	0.3413	0.3299
EE	0.8640	0.8409	0.8491	0.8410	0.8481	0.8534	0.8287	0.8320	0.8507	0.8592
Ash	0.4005	0.3422	0.3840	0.3763	0.3105	0.4630	0.3831	0.4068	0.3148	0.3926
NFE	0.6856	0.7137	0.7051	0.7051	0.7203	0.7512	0.7154	0.7065	0.7085	0.7008
OM	0.6780	0.6399	0.6241	0.6139	0.6324	0.6707	0.6354	0.6214	0.6208	0.6234
E	0.6550	0.6240	0.6042	0.5506	0.6093	0.6575	0.5913	0.5987	0.6019	0.6093
Period IIA	Clen	Saline	Bil	Bi5	Bi25	Clen	Sal	Bil	Bi5	Bi25
DM	0.6452	0.6455	0.6202	0.6191	0.6284	0.6160	0.6016	0.6812	0.6148	0.5962
CP	0.6107	0.5944	0.5734	0.5555	0.5739	0.5502	0.5453	0.6013	0.5529	0.5507
CF	0.4563	0.4717	0.4030	0.3930	0.3974	0.3945	0.3761	0.5429	0.3505	0.3025
EE	0.8599	0.8536	0.8807	0.8333	0.8533	0.8195	0.8140	0.7650	0.8296	0.8303
Ash	0.3786	0.3937	0.4333	0.3729	0.3873	0.4282	0.3848	0.4430	0.3943	0.4249
NFE	0.7369	0.7345	0.7056	0.7243	0.7311	0.7109	0.6998	0.7734	0.7283	0.7010
OM	0.6746	0.6732	0.6408	0.6463	0.6550	0.6367	0.6255	0.7074	0.6413	0.6150
E	0.6484	0.6593	0.6306	0.6217	0.6439	0.6214	0.6059	0.6832	0.6246	0.6042
Period IIB										
DM	0.6317	0.6108	0.6416	0.6126	0.6960	0.6323	0.6114	0.6420	0.6440	0.6008
CP	0.6038	0.5942	0.6154	0.6039	0.6777	0.6246	0.6034	0.5914	0.5848	0.5947
CF	0.3999	0.4131	0.3454	0.3658	0.5411	0.3781	0.3392	0.4387	0.4956	0.2257
EE	0.8953	0.8705	0.8591	0.8631	0.9077	0.8723	0.8712	0.7955	0.8592	0.8620
Ash	0.4119	0.3181	0.4527	0.4315	0.5006	0.4628	0.4756	0.4034	0.3693	0.4510
NFE	0.7166	0.6956	0.7410	0.6918	0.7638	0.7120	0.6894	0.7348	0.7330	0.7078
OM	0.6534	0.6397	0.6602	0.6305	0.7174	0.6490	0.6247	0.6656	0.6739	0.6156
E	0.6337	0.6255	0.6471	0.6020	0.7103	0.6373	0.6124	0.6427	0.6474	0.6027
Period IIIA	Bi25	Clen	Saline	Bil	Bi5	Bi25	Clen	Saline	Bil	Bi5
DM	0.6598	0.6496	0.6289	0.6511	0.6116	0.6253	0.6446	0.6387	0.6153	0.6130
CP	0.6314	0.6445	0.6307	0.6551	0.6260	0.6263	0.6603	0.6089	0.6144	0.6804
CF	0.4149	0.4007	0.3401	0.3806	0.3163	0.2828	0.3606	0.3669	0.2911	0.3521
EE	0.8782	0.8590	0.8452	0.8810	0.8648	0.8783	0.8874	0.8780	0.8922	0.8831
Ash	0.5044	0.5060	0.4968	0.5403	0.5601	0.5037	0.5317	0.4838	0.5315	0.4101
NFE	0.7430	0.7272	0.7135	0.7260	0.6782	0.7182	0.7199	0.7281	0.6970	0.6981
OM	0.6752	0.6639	0.6420	0.6621	0.6168	0.6373	0.6558	0.6540	0.6237	0.6332
E	0.6576	0.6550	0.6209	0.6442	0.6081	0.6218	0.6401	0.6380	0.6101	0.6166

Table 2a. continued

Period IIIB										
DM	0.6563	0.6845	0.6603	0.6903	0.6352	0.6607	0.6468	0.6576	0.6463	0.6515
CP	0.6522	0.6609	0.6359	0.6935	0.6344	0.6644	0.6416	0.5972	0.6456	0.6233
CF	0.4200	0.4610	0.3828	0.4329	0.3483	0.3666	0.3518	0.4252	0.3515	0.3445
EE	0.8805	0.8762	0.8339	0.8734	0.8396	0.8747	0.8584	0.8446	0.8500	0.8663
Ash	0.5166	0.5605	0.5697	0.6087	0.5312	0.5676	0.5322	0.4749	0.4831	0.5907
NFE	0.7271	0.7561	0.7424	0.7589	0.7142	0.7387	0.7305	0.7491	0.7363	0.7353
OM	0.6698	0.6965	0.6690	0.6982	0.6452	0.6697	0.6579	0.6752	0.6622	0.6574
E	0.6550	0.6848	0.6552	0.6897	0.6432	0.6610	0.6508	0.6597	0.6461	0.6532

Period IVA	Bi5	Bi25	Clen	Saline	Bil	Bi5	Bi25	Clen	Saline	Bil
DM	0.6593	0.6234	0.5948	0.6049	0.6238	0.6487	0.6115	0.5963	0.6410	0.6409
CP	0.6575	0.5942	0.5799	0.5660	0.6232	0.6393	0.6039	0.6171	0.6295	0.6478
CF	0.4165	0.3364	0.2459	0.3060	0.2690	0.3497	0.2804	0.2284	0.3430	0.3341
EE	0.8658	0.8562	0.8465	0.8317	0.8612	0.8678	0.8554	0.8607	0.8552	0.8370
Ash	0.5130	0.4380	0.4887	0.4635	0.5446	0.5496	0.5226	0.5180	0.5171	0.5169
NFE	0.7317	0.9058	0.6894	0.6987	0.7136	0.7300	0.6976	0.6782	0.7252	0.7257
OM	0.6731	0.6410	0.6048	0.6182	0.6313	0.6581	0.6199	0.6035	0.6527	0.6526
E	0.6597	0.6281	0.6035	0.6031	0.6285	0.6531	0.6126	0.5960	0.6409	0.6434

Period IVB										
DM	0.6317	0.6055	0.5794	0.6105	0.6004	0.6123	0.6138	0.5823	0.6290	0.5507
CP	0.6329	0.5817	0.5762	0.5715	0.6098	0.5805	0.5953	0.5602	0.6275	0.5633
CF	0.3152	0.2779	0.2380	0.3372	0.2710	0.3742	0.2785	0.2306	0.2967	0.1344
EE	0.8965	0.8847	0.8310	0.8414	0.8674	0.8311	0.8524	0.8546	0.8654	0.8490
Ash	0.4607	0.4197	0.4143	0.4557	0.4682	0.4497	0.4771	0.4667	0.5275	0.4127
NFE	0.7185	0.7020	0.6736	0.6955	0.6829	0.7008	0.7066	0.6781	0.7119	0.6531
OM	0.6475	0.6228	0.5947	0.6248	0.6127	0.6318	0.6264	0.5936	0.6384	0.5635
E	0.6356	0.6118	0.5815	0.6032	0.6033	0.6077	0.6144	0.5863	0.6294	0.5466

Period VA	Bil	Bi5	Bi25	Clen	Saline	Bil	Bi5	Bi25	Clen	Saline
DM	0.6578	0.6579	0.5974	0.6395	0.6229	0.6607	0.6022	0.6148	0.6164	0.6066
CP	0.6282	0.6066	0.5796	0.6195	0.6107	0.6485	0.5538	0.5555	0.6007	0.5818
CF	0.4218	0.4017	0.2900	0.3798	0.3027	0.3741	0.2842	0.3645	0.3119	0.2935
EE	0.8811	0.8671	0.8616	0.8465	0.8742	0.8875	0.8535	0.8276	0.8828	0.8769
Ash	0.5144	0.5749	0.5211	0.5206	0.5469	0.5600	0.4834	0.4379	0.5251	0.5561
NFE	0.7403	0.7415	0.6825	0.7232	0.7111	0.7430	0.7057	0.7144	0.7010	0.6900
OM	0.6736	0.6670	0.6058	0.6526	0.6313	0.6719	0.6153	0.6361	0.6266	0.6121
E	0.6563	0.6527	0.5949	0.6284	0.6178	0.6580	0.6002	0.6072	0.6125	0.6055

Period VB										
DM	0.6329	0.6426	0.5937	0.6103	0.5985	0.5639	0.6526	0.6215	0.6006	0.6196
CP	0.5797	0.5342	0.5400	0.5367	0.5381	0.4993	0.5827	0.6552	0.5371	0.5594
CF	0.3969	0.4184	0.3372	0.3705	0.3567	0.2857	0.4397	0.3549	0.3179	0.3789
EE	0.8661	0.8690	0.8288	0.8521	0.8377	0.7863	0.8500	0.8774	0.8149	0.8419
Ash	0.4377	0.4657	0.4731	0.4392	0.4518	0.3994	0.4932	0.4536	0.4391	0.4934
NFE	0.7295	0.7454	0.6825	0.7077	0.6890	0.6696	0.7424	0.7015	0.7056	0.7072
OM	0.6553	0.6629	0.6076	0.6300	0.6154	0.5828	0.6709	0.6398	0.6193	0.6341
E	0.6384	0.6460	0.5972	0.6105	0.6005	0.5641	0.6569	0.6282	0.6054	0.6247

Table 2b. Individual nitrogen balance data (g/day) for calves receiving infusions of physiological saline, 1 mg/d clenbuterol (clen) or 1, 5 and 25 mg/d bitolterol (Bi)

CALF No.	1	2	3	4	5	7	8	9	10	11
N (g/d)										
PERIOD IA	Saline	Bil	Bi5	Bi25	Clen	Saline	Bil	Bi5	Bi25	Clen
Food	68.24	68.24	68.24	68.24	68.24	68.24	68.24	68.24	68.24	68.24
Faeces	29.87	29.87	32.11	31.92	31.14	32.08	33.34	31.19	32.03	33.87
Urine	18.52	14.99	16.53	11.64	15.58	18.71	14.71	18.45	16.46	12.48
Balance	19.85	23.38	19.60	24.68	21.52	17.45	20.19	18.60	19.75	21.89
PERIOD IB										
Food	68.24	68.24	68.34	68.24	68.24	68.24	68.24	68.24	68.24	68.24
Faeces	26.83	30.55	31.30	32.46	32.94	29.31	31.19	33.33	33.43	29.41
Urine	20.58	19.67	14.69	11.34	14.75	21.67	15.62	16.77	17.68	16.81
Balance	20.83	18.02	22.25	24.44	20.55	17.26	21.43	18.14	17.13	22.02
PERIOD IIA	Clen	Saline	Bil	Bi5	Bi25	Clen	Saline	Bil	Bi5	Bi25
Food	66.49	66.49	66.49	66.49	66.49	66.49	66.49	66.49	61.75	66.49
Faeces	23.71	26.97	28.36	29.55	28.33	29.90	30.23	26.51	27.61	29.87
Urine	22.62	26.07	17.66	14.93	14.53	17.53	19.81	18.95	22.62	19.97
Balance	20.16	13.45	20.47	22.01	23.63	19.06	16.45	21.03	11.53	16.65
PERIOD IIB										
Food	66.15	66.15	66.15	66.15	56.33	66.15	66.15	66.15	62.44	66.15
Faeces	26.21	26.85	25.44	26.21	18.16	24.83	26.23	27.03	25.93	26.81
Urine	17.90	18.09	19.15	19.19	19.18	18.99	17.95	16.87	20.65	18.96
Balance	22.04	21.21	21.56	20.75	18.99	22.33	21.97	22.25	15.86	20.38
PERIOD IIIA	Bi25	Clen	Saline	Bil	Bi5	Bi25	Clen	Saline	Bil	Bi5
Food	89.15	89.15	89.15	89.15	80.71	89.15	89.15	89.15	86.96	89.15
Faeces	32.86	31.69	31.39	30.75	30.19	32.42	30.29	34.87	33.53	32.95
Urine	29.12	29.92	23.10	23.81	15.90	23.92	17.68	24.93	23.63	26.30
Balance	27.17	27.54	34.66	34.59	34.62	32.81	41.18	29.35	29.80	29.90
PERIOD IIIB										
Food	89.39	89.39	89.39	89.39	87.82	89.39	89.39	89.39	88.84	89.39
Faeces	31.09	30.31	32.55	27.39	32.10	30.00	32.04	36.01	31.48	33.68
Urine	32.83	30.28	21.40	23.07	22.76	21.43	15.94	19.45	23.78	21.70
Balance	25.47	28.80	35.44	38.93	32.96	37.96	41.41	33.93	33.58	34.01
PERIOD IVA	Bi5	Bi25	Clen	Saline	Bil	Bi5	Bi25	Clen	Saline	Bil
Food	98.48	98.48	98.48	98.48	98.48	98.48	98.48	98.48	97.57	98.48
Faeces	33.73	39.96	41.36	42.72	37.10	35.52	39.01	38.34	36.15	34.69
Urine	34.77	24.48	17.92	29.02	26.89	18.88	18.66	15.55	26.75	28.96
Balance	29.98	34.04	39.19	26.74	34.49	44.08	40.81	44.59	34.67	34.83
PERIOD IVB										
Food	100.13	95.85	100.02	100.13	100.13	92.63	100.13	100.13	96.40	100.04
Faeces	36.75	40.10	42.39	42.91	39.07	38.86	40.52	42.27	36.97	43.69
Urine	27.54	22.16	19.59	33.80	26.04	24.54	22.04	20.46	29.62	23.18
Balance	35.84	33.60	38.04	23.42	35.02	29.23	37.57	37.40	29.81	33.17
PERIOD VA	Bil	Bi5	Bi25	Clen	Saline	Bil	Bi5	Bi25	Clen	Saline
Food	95.84	95.84	95.84	95.84	95.84	80.31	95.84	81.25	83.45	95.84
Faeces	35.63	37.70	40.29	36.46	37.31	28.10	42.76	36.11	33.32	40.08
Urine	29.84	23.71	25.04	22.52	29.76	27.38	24.46	17.20	17.52	37.92
Balance	30.37	34.43	30.51	36.86	28.77	24.83	28.62	27.94	32.61	23.84
PERIOD VB										
Food	94.28	94.28	94.28	94.28	91.50	93.99	94.28	87.18	89.31	90.36
Faeces	39.62	43.91	43.37	43.68	42.26	47.06	39.34	30.06	41.34	39.81
Urine	23.66	20.67	19.23	18.85	29.66	29.03	22.90	22.34	17.89	26.89
Balance	31.00	29.70	31.68	31.75	19.58	17.90	32.04	34.78	30.08	23.66

Table 2c. Individual nitrogen balance data (% intake) for calves receiving infusions of physiological saline, 1 mg/d clenbuterol (clen) or 1, 5 and 25 mg/d bitolterol (Bi)

Calf No.	1	2	3	4	5	7	8	9	10	11
PERIOD 1A	Saline	Bi1	Bi5	Bi25	Clen	Saline	Bi1	Bi5	Bi25	Clen
Feed N g/d	68.24	68.24	68.24	68.24	68.24	68.24	68.24	68.24	68.24	68.24
N% Faeces	43.77	43.77	47.05	46.78	45.63	47.01	48.86	45.71	46.94	49.63
N% Urine	27.14	29.97	24.22	17.06	22.70	27.42	21.56	27.04	24.12	18.29
N% Retained	29.09	34.26	28.72	36.17	31.54	25.57	29.59	27.26	28.94	32.08
PERIOD 1B										
Feed N g/d	68.24	68.24	68.24	68.24	68.24	68.24	68.24	68.24	68.24	68.24
N% Feed	39.32	44.77	45.87	47.57	48.27	42.95	45.71	48.84	48.99	43.10
N% Urine	30.16	28.82	21.52	16.62	21.61	31.76	22.89	24.57	25.91	24.63
N% Retained	30.52	26.41	32.60	35.81	30.11	25.29	31.40	26.58	25.10	32.27
PERIOD IIA	Clen	Saline	Bi1	Bi5	Bi25	Clen	Saline	Bi1	Bi5	Bi25
Feed N g/d	66.49	66.49	66.49	66.49	66.49	66.49	66.49	66.49	61.57	66.49
N% Feed	35.66	40.56	42.65	44.44	42.61	44.97	45.46	39.87	44.71	44.92
N% Urine	34.02	39.21	26.56	22.45	21.85	26.36	29.79	28.50	36.63	30.03
N% Retained	30.32	20.23	30.97	33.10	35.54	28.67	24.74	31.63	18.67	25.04
PERIOD IIB										
Feed N g/d	66.15	66.15	66.15	66.15	56.33	66.15	66.15	66.15	62.44	66.15
N% Feed	39.62	40.59	38.46	39.62	32.24	37.54	39.65	40.86	41.53	40.53
N% Urine	27.06	27.35	28.95	29.01	34.05	28.71	27.13	25.50	33.07	28.66
N% Retained	33.32	32.06	32.59	31.37	33.71	33.76	33.21	33.64	25.40	30.81
PERIOD IIIA	Bi25	Clen	Saline	Bi1	Bi5	Bi25	Clen	Saline	Bi1	Bi5
Feed N g/d	89.15	89.15	89.15	89.15	80.71	89.15	89.15	89.15	86.96	89.15
N% Feed	36.86	35.55	35.21	34.49	37.41	36.37	33.98	39.11	38.56	36.96
N% Urine	32.66	33.56	25.91	26.71	19.70	26.83	19.83	27.96	27.17	29.50
N% Retained	30.48	30.89	38.89	38.80	42.89	36.80	46.19	32.92	34.27	33.54
PERIOD IIIB										
Feed N g/d	89.39	89.39	89.39	89.39	87.82	89.39	89.39	89.39	88.84	89.39
N% Feed	34.78	33.91	36.41	30.64	36.55	33.56	35.84	40.28	35.43	37.68
N% Urine	36.73	33.87	23.94	25.81	25.92	23.97	17.83	21.76	26.77	24.28
N% Retained	28.49	32.22	39.65	43.55	37.53	42.47	46.32	37.96	37.80	38.05
PERIOD IVA	Bi5	Bi25	Clen	Saline	Bi1	Bi5	Bi25	Clen	Saline	Bi1
Feed N g/d	98.48	98.48	98.48	98.48	98.48	98.49	98.48	98.48	97.57	98.48
N% Feed	34.25	40.58	42.00	43.38	37.67	36.07	39.61	38.93	37.05	35.22
N% Urine	35.31	24.86	18.20	29.47	27.30	19.17	18.95	15.79	27.42	29.41
N% Retained	30.44	34.56	39.79	27.15	35.02	44.76	41.44	45.28	35.53	35.37
PERIOD IVB										
Feed N g/d	100.13	95.85	100.02	100.13	100.13	92.63	100.13	100.13	96.40	100.04
N% Feed	36.70	41.84	42.39	42.88	39.02	41.95	40.47	42.21	38.35	43.67
N% Urine	27.50	23.11	19.57	33.76	26.01	26.49	22.01	20.43	30.73	23.17
N% Retained	35.80	35.05	38.04	23.39	34.97	31.56	37.52	37.36	30.92	33.16
PERIOD VA	Bi1	Bi5	Bi25	Clen	Saline	Bi1	Bi5	Bi25	Clen	Saline
Feed N g/d	95.84	95.84	95.84	95.84	95.84	80.31	95.84	81.25	83.45	95.84
N% Feed	37.18	39.34	42.04	38.04	38.93	34.99	44.62	44.44	39.93	41.82
N% Urine	31.13	24.74	26.13	23.50	31.05	34.09	25.52	21.17	20.99	33.31
N% Retained	31.69	35.92	31.83	38.46	30.02	30.92	29.86	34.39	39.08	24.87
PERIOD VB										
Feed N g/d	94.28	94.28	94.28	94.28	91.50	93.99	94.28	87.18	89.31	90.36
N% Feed	42.03	46.57	46.00	46.33	46.19	50.07	41.73	34.48	46.29	44.06
N% Urine	25.09	21.93	20.40	19.99	32.41	30.89	24.29	25.63	20.03	29.76
N% Retained	32.88	31.50	33.60	33.68	21.40	19.04	33.98	39.89	33.68	26.18

Table 2d. Mean nitrogen balance data (% intake) for days 7-21 inclusive for calves receiving infusions of physiological saline, 1 mg/d clenbuterol or 1, 5 and 25 mg/d bitolterol

Period I and II	Faeces	Urine	Retention
Saline (A)	42.42	29.08	27.58
Bitolterol 1 (B)	43.12	27.34	31.28
Bitolterol 5 (C)	44.03	25.86	27.62
Bitolterol 25 (D)	43.93	25.61	31.41
Clenbuterol 1 (E)	43.05	25.24	31.50
SE Mean	0.68	2.25	1.00
Significance between treatments	NS	NS	NS
Period III to V			
Saline (A)	40.28	28.96	30.75
Bitolterol 1 (B)	38.34	27.77	33.88
Bitolterol 5 (C)	39.24	25.42	35.55
Bitolterol 25 (D)	39.22	25.22	35.56
Clenbuterol 1 (E)	39.63	21.97	38.39
SE Mean	0.88	1.39	1.69
Significance between treatments	NS	BE* AE**	NS
All periods			
Saline (A)	41.13	29.38	29.49
Bitolterol 1 (B)	40.26	26.90	32.84
Bitolterol 5 (C)	41.37	26.14	32.49
Bitolterol 25 (D)	41.10	25.05	33.90
Clenbuterol 1 (E)	41.00	23.36	35.64
SE Mean	0.56	1.01	1.21
Significance between treatments	NS	AC,BE* AD** AE***	AD* AE***

Significance * $P<0.05$, ** $P<0.01$, *** $P<0.001$

APPENDIX 3

ADDITIONAL DATA FOR EXPERIMENT IIa

3a. Individual calf apparent digestibility coefficients.

3b. Individual calf nitrogen balance data (g/day).

3c. Individual calf nitrogen balance data (% intake).

3d. Mean nitrogen balance data (% intake).

3e. Mean faecal egg counts (epg).

3f. Individual calf worm burdens at necropsy.

Table 3a. Individual digestibility data for calves infected with Ostertagia ostertagi, infected after prior administration of a MSRB or maintained as a clean control

	Clean				MSRB				Infected			
Days 14-21	B12	B13	B14	B15	Y67	Y69	Y70	Y71	O8	O9	O10	O11
DM	0.6796	0.6076	0.6702	0.6237	0.6683	0.6400	0.6433	0.6054	0.6813	0.6970	0.6618	0.6331
CP	0.6214	0.5892	0.6532	0.6028	0.6252	0.6164	0.5889	0.5756	0.5583	0.6450	0.6231	0.5849
CF	0.4139	0.2333	0.4231	0.3138	0.3856	0.3520	0.3617	0.2539	0.3862	0.4920	0.3681	0.3213
EE	0.8920	0.8534	0.8914	0.8513	0.8893	0.9049	0.8947	0.8586	0.8934	0.8855	0.8535	0.8885
ASH	0.6515	0.5438	0.5279	0.5068	0.6531	0.5552	0.5736	0.5478	0.5826	0.5806	0.6456	0.5798
NFE	0.7646	0.7170	0.7592	0.7170	0.7504	0.7279	0.7369	0.7095	0.8097	0.7820	0.7484	0.7302
OM	0.6830	0.6152	0.6872	0.6377	0.6702	0.6502	0.6516	0.6123	0.6931	0.7109	0.6637	0.6395
Energy	0.6630	0.5452	0.6665	0.6144	0.6582	0.6324	0.6353	0.5840	0.6647	0.6861	0.6530	0.6262
Days 35-42												
DM	0.6177	0.6268	0.6268	0.6168	0.6070	0.6000	0.6145	0.6167	0.6313	0.5505	0.6216	0.6300
CP	0.5699	0.6246	0.6322	0.5893	0.5671	0.6006	0.6023	0.6150	0.6404	0.5619	0.6050	0.6109
CF	0.2757	0.2358	0.2334	0.2174	0.2793	0.1705	0.1923	0.2291	0.2205	0.1477	0.2594	0.2387
EE	0.8840	0.8848	0.8952	0.8733	0.8984	0.8791	0.8778	0.8604	0.8922	0.8749	0.8646	0.8647
ASH	0.4912	0.5277	0.5046	0.5456	0.3820	0.5119	0.5246	0.5204	0.5422	0.4732	0.4865	0.5152
NFE	0.7467	0.7539	0.7562	0.7490	0.7457	0.7353	0.7526	0.7432	0.7596	0.6680	0.7516	0.7671
OM	0.6320	0.6379	0.6406	0.6249	0.6323	0.6100	0.6246	0.6275	0.6413	0.5592	0.6368	0.6429
Energy	0.6126	0.6206	0.6275	0.6040	0.6104	0.5854	0.6117	0.6067	0.6200	0.5357	0.6133	0.6231
Days 56-63												
DM	0.6153	0.6022	0.6671	0.6144	0.6269	0.6101	0.6403	0.6150	0.6430	0.6772	0.6601	0.6151
CP	0.5905	0.6278	0.6524	0.6190	0.6000	0.5925	0.6430	0.6324	0.6193	0.6344	0.6625	0.6190
CF	0.2620	0.1939	0.3537	0.2323	0.2531	0.2194	0.2534	0.1957	0.2887	0.3924	0.2775	0.2002
EE	0.8974	0.8921	0.9014	0.8893	0.8965	0.8914	0.8769	0.8728	0.9054	0.8986	0.9084	0.9112
ASH	0.4857	0.4485	0.5936	0.4443	0.4440	0.5100	0.5002	0.5201	0.5079	0.5485	0.5000	0.4145
NFE	0.7409	0.7349	0.7694	0.7493	0.7714	0.7398	0.7539	0.7472	0.7711	0.7904	0.7959	0.7651
OM	0.6305	0.6202	0.6757	0.6343	0.6483	0.6217	0.6446	0.6260	0.6587	0.6921	0.6787	0.6385
Energy	0.6108	0.6006	0.6683	0.5987	0.6252	0.6225	0.6377	0.6307	0.6325	0.6713	0.6543	0.6229
Days 77-84												
DM	0.6376	0.6508	0.6382	0.6335	0.6567	0.6383	0.6796	0.6424	0.6443	0.6439	0.6534	0.6366
CP	0.5897	0.6368	0.5534	0.5359	0.6446	0.6112	0.6681	0.6282	0.6319	0.6212	0.6248	0.6247
CF	0.3399	0.3375	0.3771	0.3221	0.3249	0.3150	0.3680	0.2746	0.3144	0.3457	0.3364	0.3364
EE	0.8937	0.8988	0.8588	0.8870	0.9041	0.8892	0.9030	0.8877	0.8928	0.8967	0.9020	0.8905
ASH	0.3823	0.5103	0.4930	0.4803	0.4346	0.3308	0.5935	0.5962	0.4987	0.4616	0.4934	0.4234
NFE	0.7869	0.7734	0.7710	0.7830	0.8016	0.8012	0.7922	0.7661	0.7733	0.7717	0.7857	0.7677
OM	0.6640	0.6666	0.6569	0.6508	0.6818	0.6730	0.6893	0.6476	0.6607	0.6645	0.6715	0.6606
Energy	0.6529	0.6640	0.6276	0.6348	0.6713	0.6573	0.6866	0.6384	0.6396	0.6514	0.6651	0.6509

From day 21 calf 09 was replaced by 07
Calf B14 was bloated from day 77-84

Table 3b. Individual nitrogen balance data (g/day) for calves infected with Ostertagia ostertagi, infected after prior administration of a MSRB or maintained as clean controls

	Clean				MSRB				Infected			
Calf	B12	B13	B14	B15	Y67	Y69	Y70	Y71	O8	O9	O10	O11
Days 14-21												
Intake	70.89	70.90	69.99	70.90	70.90	62.76	70.90	67.54	70.83	70.41	68.52	69.24
Faeces	26.84	29.11	24.28	28.16	26.56	24.09	29.13	28.68	31.28	25.00	25.82	28.73
Urine	28.16	32.14	28.21	24.29	31.05	22.98	25.73	23.24	24.19	22.82	27.48	23.90
Retention	15.90	9.65	17.50	18.45	13.29	15.69	16.04	15.62	15.36	22.59	15.22	16.61
Days 35-42												
Intake	63.72	63.72	63.72	63.72	63.72	61.64	63.72	60.82	63.72	63.69	62.76	63.17
Faeces	28.14	24.56	24.06	26.85	28.33	25.26	26.02	24.03	23.51	28.64	25.45	25.23
Urine	19.96	28.89	19.66	19.39	23.72	26.58	23.60	22.23	26.37	30.89	23.61	22.67
Retention	15.62	10.27	20.00	17.48	11.67	9.80	14.10	14.56	13.84	4.16	13.70	15.27
Days 56-63												
Intake	67.09	67.09	67.09	67.09	67.09	67.09	67.09	63.12	67.09	67.09	65.66	67.09
Faeces	27.46	24.97	23.31	25.56	26.85	27.32	23.97	23.76	23.64	24.52	22.17	25.56
Urine	25.41	30.96	26.10	25.46	24.97	25.35	22.92	24.92	29.19	26.13	23.41	24.55
Retention	14.23	11.16	17.68	16.07	15.27	14.42	20.20	14.44	14.26	16.44	20.08	16.98
Days 77-84												
Intake	67.66	67.66	47.56	67.66	67.66	67.66	67.66	67.66	67.66	67.66	67.38	67.66
Faeces	27.77	24.58	21.23	31.38	24.03	26.29	22.45	25.17	24.90	25.64	25.28	25.41
Urine	26.46	23.96	26.75	23.39	28.59	22.87	22.68	22.42	28.21	28.74	24.52	23.95
Retention	13.43	19.12	-0.42	12.89	15.04	18.50	22.53	20.07	14.55	13.28	17.58	18.30

From day 21 calf 09 was replaced by 07
Calf B14 was bloated from day 77-84

Table 3c. Individual nitrogen balance data (% intake) for calves infected with Ostertagia ostertagi, infected after prior administration of a MSRB or maintained as a clean control

	Clean				MSRB				Infected			
Calf	B12	B13	B14	B15	Y67	Y69	Y70	Y71	O8	O9	O10	O11
Days 14-21												
Faeces	37.86	41.06	34.69	39.72	37.46	38.38	41.09	42.46	44.16	35.51	37.68	41.49
Urine	39.72	45.33	40.31	34.26	43.79	36.62	36.29	34.41	34.15	32.41	40.11	34.52
Retention	22.42	13.61	25.00	26.02	18.74	25.00	22.62	23.13	21.69	32.08	22.21	23.99
Days 35-42												
Faeces	44.16	38.54	37.76	42.14	44.46	40.98	40.83	39.51	36.90	44.97	40.55	39.94
Urine	31.32	45.34	30.85	30.43	37.23	43.12	37.04	36.55	41.38	48.50	37.62	35.89
Retention	24.51	16.12	31.39	27.43	18.31	15.90	22.13	23.94	21.72	6.53	21.83	24.17
Days 56-63												
Faeces	40.93	37.22	34.74	38.10	40.02	40.72	35.73	37.64	35.24	36.55	33.76	38.10
Urine	37.87	46.15	38.90	37.95	37.22	37.79	34.16	39.48	43.51	38.95	35.65	36.59
Retention	21.21	16.63	26.35	23.95	22.76	21.49	30.11	22.88	21.26	24.50	30.58	25.31
Days 77-84												
Faeces	41.04	36.33	44.64	46.39	35.52	38.86	33.18	37.20	36.80	37.90	37.52	37.56
Urine	39.11	35.41	56.24	34.57	42.26	33.80	33.52	33.14	41.69	42.48	36.39	35.40
Retention	19.85	28.26	-0.88	19.05	22.22	27.34	33.30	29.66	21.50	19.62	26.09	27.05

From day 21 calf 09 was replaced by 07
Calf B14 was bloated from day 77-84

Table 3d. Mean nitrogen balance (% intake +SE) for calves infected with Ostertagia ostertagi, infected after prior administration of a MSRB or maintained as clean controls

	Clean	MSRB	Infected
Days 14-21	n=4	n=4	n=4
Faeces	38.33 ±1.38	39.85 ±1.16	39.71 ±1.93
Urine	39.91 ±2.26	37.78 ±2.06	35.30 ±1.67
Retention	21.76 ±2.82	22.37 ±1.31	24.99 ±2.41
Days 35-42	n=4	n=4	n=4
Faeces	40.65 ±1.51	41.45 ±1.06	40.59 ±1.66
Urine	34.49 ±3.62	38.49 ±1.55	40.85 ±2.80
Retention	24.86 ±3.24	20.07 ±1.82	18.56 ±4.05
Days 56-63	n=4	n=4	n=4
Faeces	37.75 ±1.28	38.53 ±1.14	35.91 ±0.93
Urine	40.22 ±1.99	37.16 ±1.11	38.68 ±2.08
Retention	22.04 ±2.09	24.31 ±1.96	25.41 ±1.93
Days 77-84	n=3	n=4	n=4
Faeces	41.25 ±2.91	36.19 ±1.21	37.45 ±0.23
Urine	36.36 ±1.39	35.68 ±2.20	38.99 ±1.81
Retention	22.39 ±2.95	28.13 ±2.32	23.57 ±1.79

Missing data during days 77-84 is due to one calf suffering from bloat.

Table 3e. Mean faecal egg counts (eggs/gram faeces) +SE for calves infected with Ostertagia ostertagi, infected after prior administration of a MSRB or maintained as clean controls.

	Metabolism study				Pathophysiology study		
Day	Clean	MSRB	Infected	n	Clean	MSRB	Infected
0	0 ± 0	0 ± 0	0 ± 0	4	0 ± 0	0 ± 0	0 ± 0
7	0 ± 0	0 ± 0	0 ± 0	4	0 ± 0	0 ± 0	0 ± 0
14	0 ± 0	0 ± 0	0 ± 0	4	0 ± 0	0 ± 0	0 ± 0
21	0 ± 0	0 ± 0	0 ± 0	4	0 ± 0	0 ± 0	50 ± 20
28	0 ± 0	20 ± 20	20 ± 12	3	0 ± 0	13 ± 13	367 ±130
35	0 ± 0	40 ± 24	240 ± 93	3	0 ± 0	25 ± 21	617 ±174
42	0 ± 0	10 ± 10	310 ±143	3	0 ± 0	0 ± 0	783 ±437
49	0 ± 0	0 ± 0	*725 ±344	2	0 ± 0	0 ± 0	400 ±250
56	0 ± 0	20 ± 20	313 ± 38	2	0 ± 0	0 ± 0	375 ±225
63	0 ± 0	40 ± 40	588 ±218	2	0 ± 0	0 ± 0	150 ± 50
70	0 ± 0	70 ± 46	538 ±165	2	0 ± 0	0 ± 0	475 ±225
77	0 ± 0	10 ± 10	313 ±202	2	0 ± 0	0 ± 0	425 ±275
84	0 ± 0	30 ± 20	263 ± 99	2	0 ± 0	0 ± 0	275 ± 25

For the metabolism study n=5. After * for the infected treatment n=4 due to the death of a calf.

Table 3f. Worm burdens at necropsy for calves infected with Ostertagia ostertagi or infected after prior administration of a MSRB

Slaughter (days P.I.)	Treatment	Calf	L3 given	L4	L5	Adult	Total	Abo. pH
21	MSRB	Y66		2200	7600	7200	17000	-
21	Infected	O6	42000	4400	14000	17200	35600	-
*35	Infected	O9		600	0	16800	17400	-
42	MSRB	Y65		200	3400	12400	16000	-
42	Infected	O5	84000	800	2400	30550	33750	-
84	MSRB	Y63		200	0	0	200	2.30
		Y64		200	0	0	200	2.60
		Y67		0	0	0	0	3.68
		Y68		200	0	700	900	3.18
		Y69		200	0	200	400	3.79
		Y70		0	0	0	0	4.80
		Y71		0	0	0	0	4.70
84	Infected	O3		1000	0	1500	2500	3.70
		O4		200	0	6600	6800	4.20
		O7		1200	0	19400	20600	4.78
		O8		1300	0	9600	10900	4.45
		O10		4800	0	200	5000	3.80
		O11		2800	0	100	2900	4.47

*O9 died from bloat on day 35. Clean calves were worm free.

APPENDIX 4

ADDITIONAL DATA FOR EXPERIMENT IIc

4a. Individual calf apparent digestibility coefficients.

4b. Individual calf nitrogen balance data (g/day).

4c. Individual calf nitrogen balance data (% intake).

4d. Mean nitrogen balance data (% intake).

Table 4a. Individual digestibility coefficients for calves infected with cold-conditioned Ostertagia ostertagi larvae, infected after prior administration of a MSRB or maintained as a clean control

	Clean				MSRB				Infected			
Days 14-21	B13	B14	B15	B17	W1	W2	W3	W4	06	08	09	010
DM	0.5572	0.5754	0.5758	0.5552	0.5814	0.5873	0.5822	0.5996	0.5371	0.6152	0.6071	0.5615
CP	0.5914	0.5913	0.6118	0.5941	0.5851	0.5701	0.5865	0.6486	0.5607	0.6492	0.6192	0.5920
CF	0.1659	0.2240	0.1685	0.1611	0.2798	0.2079	0.2195	0.2502	0.1317	0.2148	0.2949	0.2194
EE	0.9071	0.9219	0.9208	0.9126	0.9248	0.9121	0.9196	0.9255	0.9098	0.9242	0.9297	0.8988
ASH	0.3936	0.4212	0.4381	0.4095	0.4208	0.5003	0.4710	0.4677	0.3907	0.4869	0.4346	0.4252
NFE	0.6558	0.6677	0.6758	0.6516	0.6644	0.6855	0.6742	0.6815	0.6391	0.7149	0.6942	0.6457
OM	0.5734	0.5907	0.5894	0.5697	0.5973	0.5960	0.5932	0.6127	0.5516	0.6279	0.6242	0.5750
Energy	0.5597	0.5801	0.5876	0.5626	0.6038	0.5975	0.5920	0.6087	0.5646	0.6267	0.6292	0.5760
Days 35-42												
DM	0.5896	0.5648	0.5691	0.5653	0.5850	0.6210	0.6058	0.6372	0.5907	0.5344	0.6030	0.5965
CP	0.6369	0.5923	0.6049	0.6021	0.6039	0.6476	0.5941	0.6750	0.6083	0.6034	0.6209	0.6237
CF	0.2714	0.2091	0.1902	0.2060	0.2731	0.3131	0.3319	0.3656	0.2901	0.1892	0.3100	0.2735
EE	0.9102	0.9045	0.9060	0.9061	0.9115	0.9213	0.9076	0.9138	0.8998	0.8887	0.9131	0.9030
ASH	0.4363	0.3844	0.4075	0.3684	0.3692	0.4448	0.4397	0.4388	0.3841	0.3192	0.4202	0.4257
NFE	0.6739	0.6680	0.6740	0.6700	0.6843	0.7125	0.6948	0.7208	0.6866	0.6293	0.6927	0.6910
OM	0.6055	0.5836	0.5859	0.5858	0.6074	0.6393	0.6231	0.6578	0.6121	0.5567	0.6220	0.6143
Energy	0.5948	0.5747	0.5821	0.5818	0.5903	0.6292	0.6085	0.6517	0.6004	0.5397	0.6096	0.5912
Days 56-63												
DM	0.5500	0.5540	0.5721	0.5811	0.6192	0.5921	0.6294	0.6098	0.5769	0.5748	0.5897	0.5862
CP	0.5931	0.5924	0.6092	0.6455	0.6397	0.6164	0.6476	0.6473	0.6026	0.6114	0.6298	0.6406
CF	0.1121	0.1471	0.1721	0.2147	0.2789	0.2006	0.3165	0.2496	0.2418	0.2059	0.2278	0.2215
EE	0.9353	0.9228	0.9233	0.9384	0.9132	0.9075	0.9135	0.9098	0.9059	0.8932	0.9011	0.9066
ASH	0.4119	0.3841	0.3846	0.4131	0.4031	0.4474	0.4391	0.4511	0.3433	0.4072	0.3801	0.3998
NFE	0.6543	0.6569	0.6775	0.6794	0.7205	0.6942	0.7203	0.7033	0.6755	0.6711	0.6900	0.6806
OM	0.5633	0.5705	0.5902	0.6050	0.6401	0.6061	0.6477	0.6251	0.5994	0.5910	0.6099	0.6048
Energy	0.5527	0.5619	0.5815	0.6014	0.6286	0.6000	0.6417	0.6164	0.5940	0.5764	0.6019	0.5993
Days 77-84												
DM	0.6017	0.6702	0.5761	0.5657	0.5799	0.6036	0.5805	0.5673	0.5937	0.5822	0.5883	0.5865
CP	0.6402	0.7148	0.6164	0.6235	0.6282	0.6601	0.6331	0.5904	0.6297	0.6341	0.6560	0.6155
CF	0.2344	0.3776	0.1574	0.1209	0.1953	0.2294	0.1701	0.1866	0.2197	0.2336	0.1953	0.1914
EE	0.9137	0.9252	0.9109	0.9428	0.9004	0.9166	0.9071	0.9130	0.9146	0.9109	0.9215	0.9204
ASH	0.4581	0.5095	0.3494	0.3781	0.3628	0.4517	0.4036	0.4322	0.3777	0.4117	0.3663	0.4043
NFE	0.6925	0.7460	0.6914	0.6752	0.6836	0.6933	0.6831	0.6636	0.6973	0.6678	0.6897	0.6892
OM	0.6155	0.6857	0.5979	0.5837	0.6008	0.6182	0.5975	0.5803	0.6145	0.5986	0.6097	0.6040
Energy	0.6182	0.6770	0.5926	0.5828	0.5902	0.6126	0.5927	0.5850	0.6093	0.5832	0.5985	0.5889

Table 4b. Individual nitrogen balance data (g/day) for calves infected with cold-conditioned Ostertagia ostertagi larvae, infected after prior administration of a MSRB or maintained as a clean control

	Clean				MSRB				Infected			
Days 14-21	B13	B14	B15	B17	W1	W2	W3	W4	O6	O8	O9	O10
Intake	62.77	65.29	65.23	65.07	65.29	63.64	65.29	65.29	65.29	65.29	65.15	65.29
Faeces	25.65	26.69	25.33	26.41	27.10	27.35	27.00	22.94	28.68	22.90	24.81	26.64
Urine	25.67	17.09	25.38	25.21	22.94	27.10	29.76	22.85	26.33	25.07	23.36	22.86
Retention	11.45	21.51	14.52	13.45	15.25	9.19	8.53	19.50	10.28	17.32	16.98	15.79
Days35-42												
Intake	62.63	62.63	62.63	62.63	62.63	62.63	62.63	62.63	62.63	62.63	62.63	62.63
Faeces	22.74	25.53	24.74	24.92	24.80	22.07	25.42	20.36	24.53	24.84	23.74	23.57
Urine	25.50	27.11	26.00	27.11	27.91	22.63	28.63	23.27	24.61	32.89	21.83	22.26
Retention	14.39	9.99	11.89	10.60	9.92	17.93	8.58	19.00	13.49	4.90	17.06	16.80
Days56-63												
Intake	83.28	82.93	84.86	84.86	84.86	84.86	84.86	84.86	84.86	84.86	84.86	84.86
Faeces	33.89	33.81	33.17	30.08	30.58	32.55	29.91	29.93	33.72	32.98	31.41	30.50
Urine	28.34	32.74	32.35	35.16	31.62	27.77	32.29	29.29	32.08	33.41	30.85	29.80
Retention	21.05	16.38	19.34	19.62	22.66	24.54	22.66	25.64	19.06	18.47	22.60	24.56
Days 77-84												
Intake	84.75	84.75	84.75	84.75	84.75	84.75	83.18	70.78	84.75	84.75	84.75	84.75
Faeces	30.48	24.17	32.51	31.90	31.51	28.80	30.52	28.99	31.38	31.00	29.15	32.58
Urine	33.81	34.07	35.06	36.86	37.74	30.73	29.56	32.76	32.75	32.50	31.49	29.68
Retention	20.46	26.51	17.18	15.99	15.50	25.22	23.10	9.03	20.62	21.25	24.11	22.49

Table 4c. Individual nitrogen balance data (% intake) for calves infected with cold-conditioned Ostertagia ostertagi larvae infected after prior administration of a MSRB or maintained as a clean control

	Clean				MSRB				Infected			
Day 14-21	B13	B14	B15	B17	W1	W2	W3	W4	O6	O8	O9	O10
Faeces	40.86	40.88	38.83	40.59	41.51	42.98	41.35	35.13	43.93	35.07	38.08	40.80
Urine	40.90	26.19	38.91	38.74	35.13	42.58	45.59	35.00	40.32	38.40	35.86	35.02
Retention	18.24	32.95	22.26	20.67	23.36	14.44	13.06	29.87	15.75	26.53	26.06	24.18
Day 35-42												
Faeces	36.31	40.76	39.50	39.79	39.60	35.24	40.59	32.51	39.17	39.66	37.91	37.63
Urine	40.71	43.28	41.52	43.28	44.57	36.14	45.71	37.15	39.30	52.52	34.86	35.54
Retention	22.98	15.95	18.98	16.92	15.84	28.63	13.70	30.34	21.54	7.82	27.24	26.82
Day 56-63												
Faeces	40.69	40.77	39.09	35.45	36.03	38.36	35.25	35.27	39.74	38.86	37.01	35.94
Urine	34.03	39.48	38.12	41.43	37.26	32.73	38.05	34.51	37.80	39.37	36.36	35.11
Retention	25.28	19.75	22.79	23.12	26.70	28.92	26.70	30.21	22.46	21.77	26.63	28.94
Day 77-84												
Faeces	35.96	28.52	38.36	37.64	37.18	33.98	36.69	40.96	37.03	36.58	34.40	38.44
Urine	39.89	40.20	41.37	43.49	44.53	36.26	35.53	46.29	38.64	38.35	37.16	35.02
Retention	24.14	31.28	20.27	18.87	18.29	29.76	27.77	12.76	24.33	25.07	28.44	26.54

Table 4d. Mean nitrogen balance (% intake +SE) for calves infected with cold-conditioned *Ostertagia ostertagi* larvae, infected after prior administration of a MSRB or maintained as a clean control

	Clean	MSRB	Infected
Days 14-21			
Faeces	40.29 +0.49	40.24 +1.74	39.47 +1.89
Urine	36.19 +3.37	39.58 +2.68	37.40 +1.21
Retention	23.53 +3.25	20.18 +3.95	23.13 +2.51
Days 35-42			
Faeces	39.09 +0.97	36.99 +1.89	38.59 +0.49
Urine	42.20 +0.65	40.89 +2.47	40.56 +4.11
Retention	18.71 +1.56	22.13 +4.28	20.86 +4.20
Days 56-63			
Faeces	39.00 +1.25	36.23 +0.73	37.89 +0.86
Urine	38.27 +1.57	35.64 +1.23	37.16 +0.92
Retention	22.74 +1.14	28.13 +0.87	24.95 +1.71
Days 77-84			
Faeces	35.12 +2.26	37.20 +1.44	36.61 +0.84
Urine	41.24 +0.19	40.65 +2.77	37.29 +0.82
Retention	23.64 +2.78	22.15 +4.00	26.10 +0.91
Overall			
Faeces	38.38 +0.80	37.66 +0.79	38.14 +0.58
Urine	39.47 +1.06	39.19 +1.19	38.10 +1.06
Retention	22.15 +1.17	23.15 +1.77	23.76 +1.34

Significance ab $P<0.05$

APPENDIX 5

ADDITIONAL DATA FOR EXPERIMENT II

5a. Individual calf plasma pepsinogen values (I.U.).

5b. Individual calf apparent digestibility coefficients.

5c. Individual calf nitrogen balance data (g/day).

5d. Individual calf nitrogen balance data (% intake).

5e. Mean nitrogen balance data (% intake).

5f. Individual calf faecal egg output (epg).

5g. Individual calf worm burdens at necropsy.

Table 5a. Plasma pepsinogen values (IU) for calves concurrently infected with Ostertagia ostertagi and Cooperia oncophora, infected after prior administration of a MSRB or maintained as a clean control

Metabolism study

Treatment	Day / Calf	0	7	14	21	28	35	42	49	56	63	70	77	84
Clean	B26	0.440	0.509	0.481		0.497	0.577	0.530	0.529	0.544	0.613	0.646	0.689	0.624
	B27	0.640	0.637	0.576		0.650	0.563	0.649	0.539	0.532	0.559	0.656	0.674	0.602
	B28	0.421	0.463	0.444		0.510	0.628	0.596	0.516	0.635	0.692	0.572	0.661	0.753
	B29	0.651	0.743	0.722		0.765	0.654	0.529	0.786	0.673	0.665	0.749	0.736	0.728
	B30	0.462	0.510	0.552		0.510	0.667	0.622	0.559	0.556	0.533	0.611	0.748	0.626
MSRB	Y96	0.580	0.859	1.411		1.710	3.158	3.645	2.304	2.422	2.494	1.603	1.440	1.480
	Y97	0.642	1.071	1.802		3.241	4.410	5.143	3.988	5.081	4.166	2.280	1.781	1.553
	Y98	0.498	0.790	0.831		1.659	2.513	2.863	3.101	3.449	2.994	1.369	1.065	0.922
	Y99	0.488	1.139	1.520		2.182	3.223	3.406	2.806	3.450	2.875	1.516	1.316	1.325
	Y100	0.477	1.279	1.653		2.884	3.649	3.830	3.254	2.707	1.662	1.203	1.189	0.939
Infected	O46	0.404	0.871	1.181		2.628	3.470	4.004	3.092	4.655	4.418	4.363	5.866	5.853
	O47	0.661	1.920	2.124		4.007	4.928	6.775	5.369	6.071	4.722	4.032	3.677	4.876
	O48	0.582	1.082	1.569		3.726	4.928	5.196	4.687	5.602	6.180	5.715	4.670	3.481
	O49	0.406	0.836	1.073		1.850	2.540	1.923	2.049	3.269	2.326	4.303	3.966	2.887
	O50	0.623	2.014	2.099		5.615	8.337	9.161	7.014	7.121	8.201	7.710	7.703	5.998

Pathophysiological study

Clean	B17	0.470	0.589	0.650		0.600	0.705	0.678	0.554	0.557	0.559	0.712	0.711	
	B18	0.710	0.778	0.686		0.842	0.692	0.650	0.670	0.690	0.692	0.640		
MSRB	Y79	0.500	0.613	0.914		2.706	3.009	2.913	2.467	2.268	1.520	1.184	1.267	
	Y80	0.501	0.812	1.495		3.088	3.557	3.160	3.355	4.916	3.350	2.271	2.463	
	Y81	0.623	0.788	1.228										
	Y82	0.473	1.186	2.111		4.250	5.198	7.145						
Infected	O19	0.485	0.486											
	O20	0.485	0.801	1.085		3.893	6.245	4.723	3.866					
	O21	0.434	0.767	1.773										
	O22	0.541	1.104	1.543		3.535	5.921	4.441						
	O23	0.552	0.872	1.083		4.225	6.039	4.791	3.580	2.907	2.704			
	O24	0.607	0.754	1.036		4.199	5.637	5.886		5.875	4.587	5.152	5.087	

Table 5b. Individual digestibility coefficients for calves concurrently infected with Ostertagia ostertagi and Cooperia oncophora, infected after prior administration of a MSRB or maintained as a clean control

	Clean			MSRB					Infected		
Days 14-21	B26	B27	B28	B29	Y96	Y97	Y98	Y99	O46	O47	O48	O50
DM	0.6671	0.6334	0.6480	0.6126	0.6690	0.6323	0.6652	0.6734	0.6303	0.6996	0.6400	0.6573
CP	0.6222	0.5721	0.6153	0.6106	0.6261	0.6226	0.6319	0.6566	0.5877	0.6427	0.5956	0.6062
CF	0.4357	0.3881	0.4091	0.3200	0.4390	0.3471	0.4255	0.4321	0.3791	0.5183	0.3927	0.4027
EE	0.9402	0.9367	0.9338	0.9273	0.9323	0.9382	0.9366	0.9311	0.9228	0.9363	0.9309	0.9326
ASH	0.4911	0.4202	0.5040	0.4984	0.4985	0.3935	0.4228	0.5370	0.4806	0.4660	0.3980	0.4857
NFE	0.7386	0.7115	0.7203	0.6947	0.7415	0.7273	0.7518	0.7433	0.7006	0.7779	0.7294	0.7330
OM	0.6858	0.6560	0.6633	0.6347	0.6872	0.6577	0.6910	0.6880	0.6462	0.7245	0.6657	0.6756
Energy	0.6470	0.6142	0.6155	0.5709	0.6403	0.6026	0.6513	0.6289	0.5965	0.6777	0.6167	0.6294
Days 35-42	B26	B27	B28	B29	Y96	Y97	Y98	Y99	O46		O48	O50
DM	0.6801	0.6055	0.6302	0.6019	0.6306	0.6155	0.6401	0.6546	0.6132		0.5934	0.5807
CP	0.6456	0.6009	0.6490	0.6075	0.6199	0.6359	0.6191	0.6633	0.5235		0.5344	0.4336
CF	0.4354	0.2648	0.3273	0.3032	0.3187	0.3188	0.3750	0.4157	0.3953		0.3141	0.3647
EE	0.9153	0.9034	0.9074	0.8843	0.9090	0.8959	0.9104	0.8938	0.8252		0.8483	0.7703
ASH	0.5350	0.4779	0.4649	0.4486	0.5011	0.3891	0.4860	0.4999	0.4146		0.2551	0.3082
NFE	0.7631	0.6961	0.7030	0.6847	0.7175	0.7080	0.7229	0.7247	0.7180		0.7198	0.7193
OM	0.6976	0.6209	0.6500	0.6204	0.6462	0.6428	0.6586	0.6733	0.6371		0.6341	0.6136
Energy	0.6838	0.6142	0.6376	0.5962	0.6395	0.6199	0.6436	0.6485	0.5695		0.5924	0.5699
Days 56-63	B26	B27	B28	B29	Y96	Y97	Y98	Y99	O46		O48	O24
DM	0.6761	0.5970	0.6219	0.6515	0.6523	0.6448	0.6137	0.6497	0.5893		0.6224	0.5891
CP	0.6319	0.6176	0.5884	0.6590	0.6321	0.6196	0.5842	0.6258	0.4918		0.5461	0.5460
CF	0.4237	0.3069	0.2951	0.3709	0.3738	0.3690	0.3029	0.3671	0.3856		0.3964	0.3430
EE	0.9303	0.9076	0.9203	0.9242	0.9243	0.9248	0.9207	0.9196	0.9046		0.8996	0.8653
ASH	0.4684	0.4139	0.4387	0.5379	0.4724	0.5232	0.4343	0.4916	0.2567		0.4653	0.5099
NFE	0.7700	0.6713	0.7234	0.7180	0.7390	0.7205	0.7081	0.7346	0.7306		0.7071	0.6509
OM	0.6997	0.6177	0.6427	0.6644	0.6727	0.6871	0.6340	0.6676	0.6271		0.6402	0.5970
Energy	0.6886	0.5866	0.6278	0.6550	0.6625	0.6540	0.6197	0.6551	0.5843		0.6048	0.5476
Days 77-84	B26	B27	B28	B29	Y96	Y97	Y98	Y99	O46	O47	O48	O49
DM	0.6924	0.6540	0.6191	0.6243	0.6239	0.6661	0.6490	0.6643	0.6489	0.5929	0.6270	0.6030
CP	0.6522	0.6405	0.5907	0.5864	0.5914	0.6210	0.6002	0.6213	0.6001	0.5449	0.5878	0.5823
CF	0.4409	0.3260	0.3032	0.3637	0.2923	0.3809	0.3708	0.4002	0.4037	0.3265	0.3425	0.2892
EE	0.9277	0.9317	0.9268	0.9199	0.9276	0.9312	0.9271	0.9280	0.9034	0.8962	0.9130	0.8649
ASH	0.5298	0.5160	0.3846	0.4111	0.4837	0.4859	0.4408	0.5413	0.5179	0.2263	0.3710	0.4694
NFE	0.7932	0.7605	0.7443	0.7349	0.7365	0.7787	0.7659	0.7611	0.7459	0.7367	0.7523	0.7118
OM	0.7115	0.6702	0.6466	0.6493	0.6404	0.6872	0.6734	0.6788	0.6643	0.6359	0.6571	0.6187
Energy	0.7137	0.6617	0.6442	0.6438	0.6359	0.6661	0.6562	0.6784	0.6314	0.5882	0.6288	0.5778

Table 5c. Individual nitrogen balance data (g/day) for calves concurrently infected with Ostertagia ostertagi and Cooperia oncophora, infected after prior administration of a MSRB or maintained as a clean control

	Clean				MSRB				Infected			
Days 14-21												
Calf	B26	B27	B28	B29	Y96	Y97	Y98	Y99	O46	O47	O48	O50
Intake	72.63	72.63	71.52	72.63	72.63	72.63	71.26	72.63	72.63	72.63	71.62	71.98
Faeces	27.43	31.08	27.52	28.94	27.13	27.40	26.22	24.92	29.93	25.93	28.96	28.33
Urine	23.32	23.81	21.29	23.50	24.80	20.52	22.96	18.49	21.37	24.06	20.06	23.28
Retention	21.88	17.74	22.71	20.19	20.70	24.71	22.08	29.22	21.33	22.64	22.60	20.37
Days 35-42												
Calf	B26	B27	B28	B29	Y96	Y97	Y98	Y99	O46		O48	O50
Intake	72.78	72.78	72.78	72.78	72.78	71.95	72.78	72.78	66.05		72.78	56.82
Faeces	25.79	29.04	25.55	28.55	27.66	26.21	27.71	24.50	31.45		33.87	32.16
Urine	30.66	27.03	29.92	31.07	31.97	23.85	29.80	26.07	28.72		30.59	32.41
Retention	16.33	16.71	17.31	13.16	13.15	21.89	15.27	22.21	5.88		8.32	-7.75
Days 56-63												
Calf	B26	B27	B28	B29	Y96	Y97	Y98	Y99	O46		O48	O24
Intake	75.04	75.04	75.04	75.04	75.04	75.04	75.04	75.04	72.91		75.04	64.75
Faeces	27.64	28.71	30.90	25.61	27.34	28.54	31.22	28.07	37.05		34.07	29.38
Urine	27.99	29.45	26.78	33.79	27.96	24.10	24.44	28.50	26.95		29.07	35.43
Retention	19.41	16.88	17.36	15.64	19.74	22.40	19.38	18.47	8.91		11.90	-0.06
Days 77-84												
Calf	B26	B27	B28	B29	Y96	Y97	Y98	Y99	O46	O47	O48	O49
Intake	73.83	73.83	67.03	73.83	73.83	73.83	73.83	73.83	73.83	67.92	73.83	73.83
Faeces	25.68	26.56	27.45	30.53	30.18	27.97	29.52	27.97	29.52	30.91	30.41	30.83
Urine	17.55	19.45	17.65	15.60	19.20	24.83	19.48	22.47	23.82	24.95	17.40	12.22
Retention	30.60	27.82	21.93	27.70	24.45	21.03	24.83	23.39	20.49	12.06	26.02	30.78

Table 5d. Individual nitrogen balance data (% intake) for calves concurrently infected with Ostertagia ostertagi and Cooperia oncophora, infected after prior administration of a MSRB or maintained as a clean control

	Clean				MSRB				Infected			
Days 14-21												
Calf	B26	B27	B28	B29	Y96	Y97	Y98	Y99	O46	O47	O48	O50
Faeces	37.77	42.79	38.48	39.85	37.35	37.73	36.79	34.31	41.21	35.70	40.44	39.36
Urine	32.11	32.78	29.77	32.36	34.15	28.25	32.22	25.46	29.42	33.13	28.01	32.34
Retention	30.13	24.43	31.75	27.80	28.50	34.02	30.99	40.23	29.37	31.17	31.56	28.30
Days 35-42												
Calf	B26	B27	B28	B29	Y96	Y97	Y98	Y99	O46		O48	O50
Faeces	35.44	39.90	35.11	39.23	38.00	36.43	38.07	33.66	47.62		46.54	56.60
Urine	42.13	37.14	41.11	42.69	43.93	33.15	40.94	35.82	43.48		42.03	57.04
Retention	22.44	22.96	23.78	18.08	18.07	30.42	20.98	30.52	8.90		11.43	-13.64
Days 56-63												
Calf	B26	B27	B28	B29	Y96	Y97	Y98	Y99	O46		O48	O24
Faeces	36.83	38.26	41.18	34.13	36.43	38.03	41.60	37.41	50.82		45.40	45.37
Urine	37.30	39.25	35.69	45.03	37.26	32.12	32.57	37.98	36.96		38.74	54.72
Retention	25.87	22.49	23.13	20.84	26.31	29.85	25.83	24.61	12.22		15.86	-0.09
Days 77-84												
Calf	B26	B27	B28	B29	Y96	Y97	Y98	Y99	O46	O47	O48	O49
Faeces	34.78	35.97	40.95	41.35	40.88	37.88	39.98	37.88	39.98	45.51	41.19	41.76
Urine	23.77	26.34	26.33	21.13	26.01	33.63	26.38	30.43	32.26	36.73	23.57	16.55
Retention	41.45	37.68	32.72	37.52	33.12	28.48	33.63	31.68	27.75	17.75	35.24	41.69

Table 5e. Mean nitrogen balance (% intake +SE) for calves concurrently infected with Ostertagia ostertagi and Cooperia oncophora, infected after administration of a MSRB or maintained as clean controls

	Clean	MSRB	Infected
Days 14-21	n=4	n=4	n=4
Faeces	39.72 ±1.11	36.55 ±0.77	39.18 ±1.22
Urine	31.76 ±0.68	30.02 ±1.95	30.73 ±1.21
Retention	28.53 ±1.59	33.44 ±2.53	30.10 ±0.77
Days 35-42	n=4	n=4	n=3
Faeces	37.42 ±1.25b	36.54 ±1.03B	50.25 ±3.19A
Urine	40.77 ±1.25	38.46 ±2.44	47.52 ±4.78
Retention	21.82 ±1.28a	25.00 ±3.21A	2.23 ±7.97b
Days 56-63	n=4	n=4	n=3
Faeces	37.60 ±1.47b	38.37 ±1.13b	47.20 ±1.81A
Urine	39.32 ±2.04	34.98 ±1.53	43.47 ±5.65
Retention	23.08 ±1.05A	26.65 ±1.13A	9.33 ±4.83b
Days 77-84	n=4	n=4	n=4
Faeces	38.26 ±1.69	39.16 ±0.76	42.11 ±1.19
Urine	24.39 ±1.24	29.11 ±1.81	27.28 ±4.50
Retention	37.34 ±1.79	31.73 ±1.56	30.59 ±5.16
Overall	n=16	n=16	n=14
Faeces	38.25 ±0.67A	37.65 ±0.51A	44.11 ±1.43B
Urine	34.06 ±1.80	33.14 ±1.32	36.07 ±2.94
Retention	27.69 ±1.71a	29.20 ±1.33a	19.82 ±4.05b

Significance ab $P<0.05$, Ab aB $P<0.01$, AB $P<0.001$

Table 5f. Individual faecal egg counts for calves concurrently infected with Ostertagia ostertagi and Cooperia oncophora infected after prior administration of a MSRB or maintained as a clean control

Treatment	Day / Calf	0	7	14	21	28	35	42	49	56	63	70	77	84
MSRB	Y96	0	0	50	0	50	100	200	0	0	50	50	50	0
	Y97	0	0	0	0	100	100	0	50	100	0	100	50	50
	Y98	0	0	0	0	100	250	50	100	150	200	0	0	100
	Y99	0	0	0	0	0	250	0	100	200	200	0	100	50
	Y100	0	0	0	0	0	50	0	0	0	0	0	50	50
Infected	O46	0	0	50	900	1200	1600	5100	350	1300	300	300	600	700
	O47	0	0	0	1350	950	4100	4800	18600	11100	3050	3100	6800	900
	O48	0	0	0	750	1150	500	250	100	250	300	50	450	0
	O49	0	0	0	950	2300	3100	2800	2850	3000	550	300	400	300
	O50	0	0	0	1200	750	1800	3500	3000	700	700	550	600	250

No positive faecal egg counts were recorded for clean control calves

Table 5g. Individual worm burdens at necropsy for calves concurrently infected with Ostertagia ostertagi and Cooperia oncophora, infected after administration of a MSRB or maintained as a clean control

			Ostertagia ostertagi			Cooperia oncophora						
						Duodenum		Jejunum		Ileum		
Treatment	Day	Calf	Devel	Adult	Total	Devel	Adult	Devel	Adult	Devel	Adult	Total
MSRB	21	Y81	36800	25400	62200	2800	4400	400	0	0	400	8000
Infected	21	O21	42200	22800	65000	13600	16200	1200	1800	3600	6600	43000
MSRB	42	Y82	19200	35000	54200	400	4600	400	600	800	0	6800
Infected	42	O22	17000	54800	71800	1800	47400	20800	30000	8200	20000	128200
Infected	58	O20	0	108400	108400	400	18700	5400	81200	400	51200	157300
Infected	66	O23	400	96800	97200	0	2700	100	21800	3400	80000	108000
MSRB	84	Y79	800	0	800	0	400	0	0	0	400	800
	84	Y80	400	5600	6000	0	600	0	100	0	0	700
	84	Y96	400	0	400	0	0	0	0	0	0	0
	84	Y97	800	0	800	0	0	0	0	0	0	0
	84	Y98	0	0	0	0	600	0	0	0	0	600
	84	Y99	1200	800	2000	0	200	0	0	0	0	200
	84	Y100	0	200	200	400	0	0	0	0	0	400
		Mean	514	943	1457	57	257	0	14	0	57	386
		±SE	±168	±784	±796	±57	±104	±0	±14	±0	±57	±124
Infected	84	O24	0	58600	58600	0	59400	1000	4000	0	7200	71600
	84	O46	600	1200	1800	0	6400	0	200	0	0	6600
	84	O47	400	30800	31200	200	5700	0	0	0	800	6700
	84	O48	2800	2000	4800	0	0	0	0	0	0	0
	84	O49	0	2900	2900	0	1100	0	400	0	0	1500
	84	O50	0	20600	20600	0	900	0	700	0	8100	9700
		Mean	663	19350	19983	33	12250	167	883	0	2683	16017
		±SE	±445	±9263	±9075	±33	±9493	±167	±633	±0	±1580	±11213

APPENDIX 6

ADDITIONAL DATA FOR EXPERIMENT III

6a. Individual sheep digestibility data, days 0-7.

6b. Individual sheep digestibility data, days 7-14.

6c. Individual sheep digestibility data, days 14-21.

6d. Individual sheep digestibility data, days 21-28.

6e. Mean digestibility data for each collection period.

6f. Individual sheep nitrogen balance data (g/day).

6g. Individual sheep nitrogen balance data (% intake).

6h. Mean nitrogen balance data (% intake).

6i. Individual sheep worm burdens at necropsy.

6j. Individual sheep abomasal pH at necropsy.

Table 6a. Individual digestibility coefficients for lambs infected with ostertagia circumcincta and given graded doses of levamisole. Days 0-7

Treatment	Sheep	DM	CP	CF	EE	ASH	NFE	OM	GE
Infected Control	205	0.3918	0.3936	0.2638	0.5161	0.2704	0.4678	0.3992	0.3756
	198	0.4592	0.3988	0.3799	0.5622	0.2927	0.5298	0.4693	0.4454
	285	0.5522	0.5203	0.4826	0.6410	0.4147	0.6081	0.5605	0.5347
	B13	0.4822	0.4900	0.3882	0.5686	0.4125	0.5345	0.4864	0.4583
	B5	0.4694	0.4634	0.3686	0.5611	0.3228	0.5372	0.4784	0.4628
	B4	0.3976	0.4184	0.2437	0.5181	0.3330	0.4771	0.4015	0.3806
Levamisole 1.0 mg/kg/day	204	0.4612	0.4140	0.3614	0.5633	0.3503	0.5337	0.4680	0.4533
	202	0.5285	0.4674	0.4285	0.5788	0.3989	0.6096	0.5364	0.5198
	291	0.5157	0.5225	0.4094	0.5836	0.4328	0.5776	0.5207	0.5059
	B3	0.5367	0.5461	0.4293	0.6023	0.4688	0.5971	0.5409	0.5136
	B16	0.5059	0.5155	0.4361	0.5596	0.4199	0.5484	0.5112	0.4909
	266	0.4865	0.4710	0.3957	0.5377	0.4640	0.5396	0.4879	0.4642
Levamisole 1.5 mg/kg/day	276	0.5309	0.4701	0.4395	0.5223	0.4786	0.6020	0.5341	0.5144
	243	0.5471	0.4497	0.4708	0.5768	0.4014	0.6269	0.5560	0.5314
	269	0.5207	0.4919	0.4135	0.6375	0.4116	0.5917	0.5273	0.5129
	B6	0.5192	0.5302	0.4150	0.5503	0.4477	0.5799	0.5235	0.5009
	B14	0.5078	0.5259	0.3910	0.5800	0.4840	0.5660	0.5093	0.4907
	257	0.4549	0.4646	0.3383	0.4900	0.3810	0.5227	0.4594	0.4415
Levamisole 2.0 mg/kg/day	295	0.5126	0.4833	0.4130	0.5816	0.4017	0.5825	0.5193	0.4999
	219	0.4843	0.3792	0.3672	0.5897	0.3824	0.5789	0.4905	0.4743
	212	0.5281	0.4705	0.4612	0.5845	0.3989	0.5897	0.5359	0.5137
	B7	0.5204	0.5162	0.4331	0.5700	0.3816	0.5819	0.5288	0.5043
	B10	0.5149	0.5216	0.4200	0.5523	0.4231	0.5735	0.5205	0.4981
	B11	0.4488	0.4817	0.3154	0.5517	0.3895	0.5146	0.4524	0.4377
Levamisole 3.0 mg/kg/day	185	0.4536	0.3702	0.3357	0.5346	0.2649	0.5582	0.4650	0.4385
	244	0.4971	0.4491	0.3549	0.6082	0.4014	0.5944	0.5030	0.4902
	251	0.4796	0.4073	0.3653	0.5990	0.2792	0.5785	0.4981	0.4705
	270	0.4780	0.4289	0.3492	0.5927	0.3184	0.5749	0.4877	0.4624
	271	0.4732	0.275i	0.3781	0.5707	0.2218	0.6000	0.4885	0.4651
	317	0.4926	0.3584	0.3812	0.5823	0.3015	0.6060	0.5042	0.4802
Clean Control	309	0.5434	0.4742	0.4826	0.6270	0.3289	0.6125	0.5565	0.5315
	232	0.5275	0.4773	0.4270	0.5845	0.4448	0.6005	0.5325	0.5098
	261	0.5499	0.4741	0.4988	0.5798	0.4442	0.6062	0.5563	0.5308
	B12	0.4417	0.4450	0.3173	0.5315	0.4060	0.5077	0.4438	0.4150
	B8	0.4837	0.4987	0.3894	0.5228	0.2136	0.5598	0.5001	0.4624
	B15	0.5132	0.4992	0.4513	0.5935	0.3294	0.5665	0.5244	0.4887

Table 6b. Individual digestibility coefficients for lambs infected with Ostertagia circumcincta and given graded doses of levamisole. Days 7-14

Treatment	Sheep	DM	CP	CF	EE	ASH	NFE	OM	GE
Infected Control	205	0.3788	0.3267	0.2635	0.5294	0.2396	0.4632	0.3870	0.3642
	198	0.4607	0.3035	0.3557	0.6099	0.2356	0.5751	0.4740	0.4337
	285	0.5042	0.3787	0.4522	0.6123	0.3150	0.5791	0.5154	0.4861
	B13	0.5405	0.5178	0.4339	0.6639	0.4813	0.6055	0.5440	0.5172
	B5	0.5621	0.5304	0.4354	0.7080	0.4347	0.6470	0.5688	0.5602
	B4	0.4790	0.4611	0.3217	0.6468	0.4246	0.5683	0.4822	0.4727
Levamisole 1.0 mg/kg/day	204	0.5023	0.4496	0.3774	0.6052	0.3534	0.5964	0.5111	0.4944
	202	0.5372	0.4708	0.4327	0.6290	0.3934	0.6232	0.5457	0.5377
	291	0.5270	0.4706	0.4434	0.6235	0.3478	0.6024	0.5376	0.5227
	B3	0.5584	0.5013	0.4664	0.6224	0.4566	0.6321	0.5644	0.5423
	B16	0.5438	0.5487	0.4490	0.4356	0.4197	0.6167	0.5511	0.5226
	266	0.5285	0.4960	0.4368	0.6085	0.4756	0.5894	0.5316	0.4966
Levamisole 1.5 mg/kg/day	276	0.5531	0.4858	0.4406	0.6753	0.4289	0.6397	0.5604	0.5416
	243	0.5516	0.3870	0.5115	0.6551	0.3132	0.6352	0.5657	0.5399
	269	0.5437	0.5449	0.4568	0.6535	0.3417	0.6082	0.5556	0.5310
	B6	0.5606	0.5475	0.4874	0.6154	0.4420	0.6151	0.5676	0.5546
	B14	0.5355	0.5381	0.4306	0.6419	0.4583	0.5963	0.5401	0.5223
	257	0.5424	0.4280	0.4673	0.6539	0.4771	0.6139	0.5463	0.5165
Levamisole 2.0 mg/kg/day	295	0.5553	0.5109	0.4522	0.6401	0.4415	0.6324	0.5621	0.5442
	219	0.5744	0.4646	0.5027	0.6700	0.4383	0.6515	0.5825	0.5679
	212	0.5572	0.4316	0.4853	0.6393	0.3887	0.6425	0.5671	0.5410
	B7	0.5430	0.4923	0.4652	0.6332	0.4133	0.6085	0.5506	0.5176
	B10	0.5155	0.5105	0.4383	0.6497	0.4096	0.5640	0.5218	0.5118
	B11	0.5279	0.5308	0.4162	0.6813	0.4415	0.5906	0.5330	0.5243
Levamisole 3.0 mg/kg/day	185	0.4933	0.4656	0.3680	0.5501	0.3580	0.5830	0.5014	0.4808
	244	0.5016	0.4620	0.3287	0.5414	0.4022	0.6181	0.5076	0.4833
	251	0.4979	0.4247	0.3800	0.5779	0.3170	0.5987	0.5088	0.4869
	270	0.5200	0.4771	0.4092	0.5868	0.3194	0.6121	0.5321	0.5086
	271	0.5209	0.4294	0.3827	0.5551	0.2830	0.6468	0.5353	0.5032
	317	0.5203	0.4784	0.4041	0.5675	0.3448	0.6135	0.5309	0.5075
Clean Control	309	0.4821	0.4154	0.3748	0.6090	0.2776	0.5744	0.4942	0.4500
	232	0.4588	0.3842	0.3324	0.5901	0.2922	0.5594	0.4687	0.4376
	261	0.4686	0.3365	0.3754	0.6190	0.3034	0.5632	0.4783	0.4498
	B12	0.3754	0.3771	0.2390	0.5876	0.3062	0.4469	0.3975	0.3529
	B8	0.4479	0.4375	0.3160	0.5763	0.3438	0.5288	0.4540	0.4190
	B15	0.4516	0.4353	0.3841	0.6097	0.3067	0.5003	0.4602	0.4215

Table 6c. Individual digestibility coefficients for lambs infected with Ostertagia circumcincta and given graded doses of levamisole. Days 14-21.

Treatment	Sheep	DM	CP	CF	EE	ASH	NFE	OM	GE
Infected Control	205	0.4007	0.3575	0.3134	0.5224	0.2727	0.4685	0.4079	0.3904
	198	0.4822	0.3975	0.4180	0.4903	0.2809	0.5608	0.4936	0.4627
	285	0.4926	0.3456	0.4623	0.5507	0.2712	0.5659	0.5051	0.4811
	B13	0.5351	0.4778	0.4911	0.5715	0.3872	0.5880	0.5434	0.5216
	B5	0.5386	0.4985	0.4728	0.6377	0.3543	0.6005	0.5490	0.5220
	B4	0.4857	0.4703	0.3654	0.6011	0.3972	0.5620	0.4907	0.4854
Levamisole 1.0 mg/kg/day	204	0.4762	0.3967	0.3818	0.5064	0.3592	0.5609	0.4828	0.4669
	202	0.5400	0.4607	0.4745	0.5788	0.3708	0.6130	0.5496	0.5242
	291	0.5071	0.4379	0.4358	0.5410	0.3110	0.5842	0.5182	0.5053
	B3	0.5135	0.4725	0.4457	0.6117	0.3842	0.5909	0.5208	0.4970
	B16	0.6622	0.6142	0.6232	0.7152	0.5699	0.7032	0.6674	0.6542
	266	0.5553	0.5061	0.4878	0.6805	0.4676	0.6086	0.5602	0.5362
Levamisole 1.5 mg/kg/day	276	0.5355	0.4878	0.4670	0.5949	0.3678	0.6013	0.5450	0.5330
	243	0.5254	0.3552	0.4920	0.6293	0.2759	0.6066	0.5395	0.5124
	269	0.5065	0.4068	0.4508	0.5931	0.2705	0.5830	0.5198	0.5011
	B6	0.4992	0.4603	0.4184	0.5958	0.3682	0.5639	0.5066	0.4793
	B14	0.4896	0.3941	0.4451	0.5396	0.3195	0.5537	0.4992	0.4729
	257	0.5096	0.4459	0.4457	0.6189	0.3677	0.5710	0.5176	0.4888
Levamisole 2.0 mg/kg/day	295	0.5054	0.4283	0.4381	0.5320	0.3148	0.5818	0.5162	0.4964
	219	0.4839	0.3456	0.4180	0.5317	0.2916	0.5733	0.4948	0.4839
	212	0.5198	0.3596	0.4798	0.5088	0.3199	0.6036	0.5311	0.5010
	B7	0.5076	0.4580	0.4460	0.6121	0.3630	0.5648	0.5158	0.4888
	B10	0.5174	0.4752	0.4440	0.5848	0.3849	0.5808	0.5249	0.5011
	B11	0.5155	0.5073	0.4163	0.5905	0.4098	0.5819	0.5214	0.5181
Levamisole 3.0 mg/kg/day	185	0.5176	0.4669	0.3728	0.5931	0.3512	0.6222	0.5278	0.5094
	244	0.5436	0.4788	0.4138	0.6131	0.4289	0.6380	0.5506	0.5310
	251	0.5267	0.4304	0.4186	0.6126	0.3180	0.6266	0.5394	0.5121
	270	0.5641	0.5282	0.4475	0.6504	0.4052	0.6484	0.5738	0.5473
	271	0.5327	0.4244	0.4198	0.6050	0.3394	0.6372	0.5445	0.5225
	317	0.5272	0.4756	0.3961	0.5996	0.3559	0.6254	0.5377	0.5134
Clean Control	309	0.4507	0.3558	0.3830	0.5493	0.2700	0.5266	0.4609	0.4366
	232	0.4685	0.3872	0.4057	0.6328	0.2372	0.5399	0.4815	0.4542
	261	0.3925	0.2427	0.3299	0.5165	0.2275	0.4756	0.4018	0.3716
	B12	0.3703	0.3607	0.2741	0.4692	0.2295	0.4378	0.3783	0.3622
	B8	0.3710	0.3521	0.2867	0.4899	0.1923	0.4367	0.3811	0.3548
	B15	0.4153	0.3626	0.3407	0.4344	0.2549	0.4871	0.4243	0.3949

Table 6d. Individual digestibility coefficients for lambs infected with Ostertagia circumcincta and given graded doses of levamisole. Days 21-28

Treatment	Sheep	DM	CP	CF	EE	ASH	NFE	OM	GE
Infected Control	B13	0.5488	0.4808	0.4858	0.5953	0.3814	0.6170	0.5583	0.5147
	B5	0.5461	0.4886	0.4673	0.6033	0.3712	0.6211	0.5560	0.5203
	B4	0.5425	0.5276	0.4165	0.5805	0.4166	0.6304	0.5497	0.5212
Levamisole 1.0 mg/kg/day	B3	0.5484	0.4898	0.4605	0.5948	0.4168	0.6249	0.5559	0.5288
	B16	0.5088	0.3701	0.4573	0.5650	0.3043	0.5909	0.5204	0.4830
	266	0.5292	0.4926	0.4328	0.5498	0.3969	0.6068	0.5367	0.5039
Levamisole 1.5 mg/kg/day	B6	0.5408	0.5416	0.4527	0.5992	0.4329	0.5999	0.5470	0.5204
	B14	0.5180	0.4983	0.4029	0.5345	0.4304	0.5978	0.5230	0.4906
	257	0.5409	0.4799	0.4491	0.5727	0.4150	0.6205	0.5481	0.5170
Levamisole 2.0 mg/kg/day	B7	0.5341	0.4935	0.4428	0.5925	0.3894	0.6090	0.5423	0.5076
	B10	0.5371	0.5299	0.4629	0.6041	0.3850	0.5943	0.5458	0.5205
	B11	0.5062	0.5123	0.3940	0.6066	0.3832	0.5772	0.5132	0.4932
Levamisole 3.0 mg/kg/day	185	0.5212	0.4867	0.3717	0.5374	0.3674	0.6272	0.5303	0.5086
	251	0.5139	0.4230	0.3978	0.5155	0.2548	0.6278	0.5291	0.5047
	271	0.5331	0.4576	0.4264	0.5961	0.2798	0.6342	0.5480	0.5189
Clean Control	B12	0.5146	0.4729	0.4304	0.5429	0.3738	0.5868	0.5226	0.5013
	B8	0.5387	0.5324	0.4829	0.5697	0.3807	0.5875	0.5476	0.5288
	B15	0.5608	0.5065	0.4924	0.5581	0.3966	0.6311	0.5701	0.5481

Table 6e. Mean digestibility coefficients (+SE) during each collection period for lambs infected with Ostertagia circumcincta and given graded doses of levamisole

	Infected control	Levamisole 1.0 mg/Kg/d	Levamisole 1.5 mg/Kg/d	Levamisole 2.0 mg/Kg/d	Levamisole 3.0 mg/kg/d	Clean control
Days 0-7	n=6	n=6	n=6	n=6	n=6	n=6
DM	0.4587 +0.0242	0.5805 +0.1145	0.5134 +0.0129	0.5015 +0.0122	0.4796 +0.0058	0.5099 +0.0167
CP	0.4473 +0.0213	0.4894 +0.0196	0.4887 +0.0136	0.4754 +0.0210	0.3822 +0.2540	0.4781 +0.0082
CF	0.3545 +0.0360	0.4101 +0.0115	0.4114 +0.0183	0.4017 +0.0213	0.3615 +0.0070	0.4277 +0.0273
EE	0.5612 +0.0185	0.5709 +0.0091	0.5595 +0.0210	0.5716 +0.0067	0.5818 +0.0103	0.5732 +0.0161
ASH	0.3410 +0.0247	0.4225 +0.0180	0.4341 +0.0174	0.3962 +0.0063	0.2987 +0.0245	0.3612 +0.0364
NFE	0.5258 +0.0206	0.5677 +0.0130	0.5815 +0.0145	0.5702 +0.0113	0.5858 +0.0070	0.5755 +0.0162
OM	0.4659 +0.0246	0.5109 +0.0116	0.5318 +0.0176	0.5079 +0.0128	0.4906 +0.0053	0.5189 +0.0173
GE	0.4429 +0.0241	0.4913 +0.0111	0.4986 +0.0127	0.4880 +0.0114	0.4684 +0.0067	0.4897 +0.0184
Days 7-14	n=3	n=6	n=6	n=6	n=6	
DM	0.5272 +0.0249	0.5329 +0.0007	0.5478 +0.0037	0.5456 +0.0087	0.5090 +0.0052	
CP	0.5031 +0.0213	0.4895 +0.0141	0.4886 +0.0277	0.4901 +0.0148	0.4562 +0.0096	
CF	0.3970 +0.0337	0.4343 +0.0124	0.4657 +0.0123	0.4600 +0.0128	0.3788 +0.0118	
EE	0.6729 +0.0182	0.5874 +0.0306	0.6492 +0.0081	0.6523 +0.0078	0.5631 +0.0071	
ASH	0.4469 +0.0175	0.4078 +0.0215	0.4102 +0.0272	0.4222 +0.0089	0.3374 +0.0167	
NFE	0.6069 +0.0227	0.6100 +0.0068	0.6181 +0.0067	0.6149 +0.0137	0.6120 +0.0087	
OM	0.5317 +0.0257	0.5403 +0.0074	0.5560 +0.0045	0.5529 +0.0092	0.5194 +0.0061	
GE	0.5167 +0.0253	0.5194 +0.0082	0.5343 +0.0057	0.5345 +0.0085	0.4951 +0.0052	
Days 14-21	n=3	n=6	n=6	n=6	n=6	
DM	0.5198 +0.0171	0.5424 +0.0264	0.5110 +0.0069	0.5083 +0.0054	0.5353 +0.0067	
CP	0.4822 +0.0084	0.4814 +0.0305	0.4250 +0.0198	0.4290 +0.0264	0.4674 +0.0154	
CF	0.4431 +0.0392	0.4748 +0.0333	0.4532 +0.0101	0.4404 +0.0095	0.4114 +0.0103	
EE	0.6034 +0.0191	0.6056 +0.0329	0.5953 +0.0127	0.5600 +0.0168	0.6123 +0.0082	
ASH	0.3796 +0.0130	0.4105 +0.0381	0.3283 +0.0190	0.3473 +0.0187	0.3664 +0.0172	
NFE	0.5835 +0.0113	0.6101 +0.0201	0.5799 +0.0086	0.5810 +0.0053	0.6330 +0.0041	
OM	0.5277 +0.0186	0.5498 +0.0260	0.5213 +0.0073	0.5174 +0.0051	0.5456 +0.0064	
GE	0.5097 +0.0121	0.5306 +0.0266	0.4979 +0.0091	0.4982 +0.0044	0.5226 +0.0059	
Days 21-28	n=3	n=3	n=3	n=3	n=3	n=3
DM	0.5458 +0.0018	0.5288 +0.0114	0.5332 +0.0076	0.5258 +0.0098	0.5227 +0.0056	0.5380 +0.0133
CP	0.4000 +0.0145	0.4508 +0.0404	0.5066 +0.0183	0.5119 +0.0105	0.4558 +0.0184	0.5039 +0.0172
CF	0.4565 +0.0207	0.4502 +0.0087	0.4349 +0.0160	0.4332 +0.0205	0.3986 +0.0160	0.4686 +0.0193
EE	0.5930 +0.0067	0.5699 +0.0132	0.5688 +0.0188	0.6011 +0.0043	0.5497 +0.0241	0.5569 +0.0078
ASH	0.3897 +0.0138	0.3727 +0.0347	0.4261 +0.0056	0.3859 +0.0018	0.3007 +0.0341	0.3837 +0.0068
NFE	0.6228 +0.0040	0.6075 +0.0098	0.6061 +0.0072	0.5935 +0.0092	0.6297 +0.0022	0.6018 +0.0147
OM	0.5547 +0.0026	0.5377 +0.0103	0.5394 +0.0082	0.5338 +0.0103	0.5358 +0.0061	0.5468 +0.0137
GE	0.5187 +0.0020	0.5052 +0.0132	0.5093 +0.0094	0.5071 +0.0079	0.5107 +0.0042	0.5261 +0.0136

Data has been omitted for clean and infected control lambs during days 7-14 and 14-21 as the calculated apparent digestibility coefficients were uncharacteristically low. It is speculated that the lambs concerned, which were accomodated in floor pens, ingested peat bedding material in addition to the experimental diet

Table 6f. Individual nitrogen balance data (g/day) for lambs infected with Ostertagia circumcincta and given graded doses of levamisole

	Infected Control			Levamisole 1.0 mg/kg/day			Levamisole 1.5 mg/kg/day			Levamisole 2.0 mg/kg/day			Clean Control		
Sheep	B13	B5	B4	B3	B16	266	B6	B14	257	B7	B10	B11	B12	B8	B15
Days 0-7															
Intake				18.09	18.09	18.09	18.09	18.09	18.09	18.09	18.09	18.09			
Faeces				8.21	8.77	9.57	8.50	8.58	9.69	8.75	8.66	9.38			
Urine				7.43	7.82	7.55	8.00	7.23	8.48	7.73	7.19	7.00			
Retention				2.45	1.50	0.97	1.59	2.28	-0.08	1.61	2.24	1.71			
Days 7-14															
Intake	18.61	18.61	18.61	18.61	18.61	18.61	18.61	18.61	18.61	18.61	18.61	18.61			
Faeces	8.79	8.74	10.03	9.28	8.40	9.38	8.42	8.59	10.64	9.45	9.11	8.73			
Urine	8.12	7.14	7.75	7.48	6.28	7.63	8.76	7.33	8.58	8.29	6.60	7.41			
Retention	1.70	2.73	0.83	1.85	3.93	1.60	1.43	2.69	-0.61	0.87	2.90	2.47			
Days 14-21															
Intake	17.90	17.90	17.90	17.90	17.90	17.90	17.90	17.90	17.90	17.90	17.90	17.90			
Faeces	9.35	8.98	9.48	9.44	6.91	8.84	9.66	10.85	9.92	9.70	9.39	8.82			
Urine	7.40	7.51	6.16	7.63	7.81	8.10	9.37	7.78	8.68	8.06	7.74	7.31			
Retention	1.15	1.41	2.26	0.83	3.18	0.96	-1.13	-0.73	-0.70	0.14	0.77	1.77			
Days 21-28															
Intake	17.90	17.90	17.90	17.90	17.90	17.90	17.90	17.90	17.90	17.90	17.90	17.90	17.90	17.90	17.90
Faeces	9.29	9.16	8.46	9.13	11.27	9.08	8.21	8.98	9.31	9.07	8.42	8.73	9.44	8.37	8.83
Urine	7.70	7.49	7.47	7.46	7.65	7.65	8.11	7.21	7.94	7.75	7.85	7.28	7.75	7.49	7.17
Retention	0.91	1.25	1.97	1.31	-1.02	1.17	1.58	1.71	0.65	1.08	1.63	1.89	0.71	2.04	1.90

Missing data is due to shortage of metabloism accomodation

Table 6g. Individual nitrogen balance data (% intake) for lambs infected with Ostertagia circumcincta and given graded doses of levamisole

	Infected Control			Levamisole 1.0 mg/kg/day			Levamisole 1.5 mg/kg/day			Levamisole 2.0 mg/kg/day			Clean Control		
Sheep	B13	B5	B4	B3	B16	266	B6	B14	257	B7	B10	B11	B12	B8	B15
Days 0-7															
Faeces				45.38	48.48	52.90	46.99	47.43	53.57	48.37	47.87	51.85			
Urine				41.07	43.23	41.74	44.22	39.97	46.88	42.73	39.75	38.70			
Retention				13.54	8.29	5.36	8.79	12.60	-0.45	8.90	12.38	9.45			
Days 7-14															
Faeces	47.23	46.96	53.90	49.87	45.14	50.40	45.24	46.16	57.17	50.78	48.95	46.91			
Urine	43.63	38.37	41.64	40.19	33.75	41.00	47.07	39.39	46.10	44.55	35.46	39.82			
Retention	9.13	14.67	4.46	9.94	21.12	8.60	7.68	14.45	-3.28	4.67	15.58	13.27			
Days 14-21															
Faeces	52.23	50.16	52.96	52.74	38.60	49.39	53.97	60.61	55.42	54.19	52.46	49.27			
Urine	41.34	41.96	34.41	42.63	43.63	45.25	52.35	43.46	48.49	45.03	43.24	40.84			
Retention	6.42	7.88	12.63	4.64	17.77	5.36	-6.31	-4.08	-3.91	0.78	4.30	9.89			
Days 21-28															
Faeces	51.90	51.17	47.26	51.01	62.96	50.73	45.87	50.17	52.01	50.67	47.04	48.77	52.74	46.76	49.33
Urine	43.02	41.84	41.73	41.68	42.74	42.74	45.31	40.28	44.36	43.30	43.85	40.67	43.30	41.84	40.06
Retention	5.08	6.98	11.01	7.31	-5.70	6.54	8.83	9.55	3.63	6.03	9.11	10.56	3.97	11.40	10.61

Missing data is due shortage of metabolism accomodation

Table 6h. Mean nitrogen balance (% intake +SE) for lambs infected with *Ostertagia circumcincta* and given graded doses of levamisole

n=3	Infected control	Levamisole 1.0 mg/kg/day	Levamisole 1.5 mg/kg/day	Levamisole 2.0 mg/kg/day	Clean control
Days 0-7					
Faeces		48.92 +2.18	49.33 +2.12	49.36 +1.25	
Urine		42.01 +0.64	43.69 +2.01	40.39 +1.21	
Retention		9.06 +2.39	6.98 +3.87	10.24 +1.08	
Days 7-14					
Faeces	49.36 +2.27	48.47 +1.67	49.52 +3.83	48.88 +1.12	
Urine	41.21 +1.53	38.31 +2.29	44.19 +2.41	39.94 +2.62	
Retention	9.42 +2.95	13.22 +3.97	6.28 +5.17	11.17 +3.32	
Days 14-21					
Faeces	51.78 +0.84	46.91 +4.27	56.67 +2.02	51.97 +1.44	
Urine	39.24 +2.42	43.84 +0.76	48.10 +2.57	43.04 +1.21	
Retention	8.98 +1.87	9.26 +4.26	-4.76 +0.77	4.99 +2.65	
Days 21-28					
Faeces	50.11 +1.44	54.90 +4.03	49.35 +1.82	48.83 +1.05	49.61 +1.73
Urine	42.20 +0.41	42.39 +0.35	43.32 +1.54	42.61 +0.98	41.73 +0.94
Retention	7.69 +1.75	2.72 +4.21	7.34 +1.86	8.57 +1.34	8.66 +2.36

Missing data is due to shortage of metabolism accomodation

Table 6j. The pH of the abomasal contents of lambs at necropsy following infection with Ostertagia circumcincta and treatment with graded doses of levamisole

Treatment	Sheep	Abomasal pH	Lesion Score
Infected Control	B13	5.90	+
	B5	4.40	0
	B4	4.80	+
	205	4.59	+
	198	5.73	+
	285	4.65	+
	MEAN	5.01	
	±SE	±0.26	
Levamisole 1.0 mg/kg/day	B3	3.80	0
	B16	6.47	+
	266	4.30	+
	204	4.28	+
	202	4.09	+
	291	4.51	1
	MEAN	4.58	
	±SE	±0.39	
Levamisole 1.5 mg/kg/day	B6	3.50	0
	B14	4.60	0
	257	5.20	0
	276	4.24	1
	243	3.35	+
	269	6.58	+
	MEAN	4.58	
	±SE	±0.49	
Levamisole 2.0 mg/kg/day	B7	5.10	0
	B10	4.80	0
	B11	3.90	+
	295	5.30	+
	219	5.98	+
	212	4.26	+
	MEAN	4.89	
	±SE	±0.30	
Levamisole 3.0 mg/kg/day	185	5.30	+
	244	5.53	0
	251	5.09	+
	270	6.09	0
	271	5.94	0
	317	5.27	1
	MEAN	5.54	
	±SE	±0.16	

Lesion Score: 0,+,1, indicates no, occasional, or few lesions
Treatment means are not significantly different.

Table 6i. Worm burdens at necropsy for lambs infected with Ostertagia circumcincta and given graded doses of levamisole

Treatment	Sheep	Sex	L4	L5	Adult	Total
Infected control	205	F	650	6100	27600	34350
	198	F	3400	5600	24000	33000
	285	F	150	7150	19900	27200
	B13	M	1600	6000	19600	27200
	B5	M	4550	3800	14950	23300
	B4	M	250	13350	28900	42500
	MEAN		1767	7000	22492	31258
	SE		±744	±1346	±2171	±2800
Levamisole 1.0 mg/kg/day	204	F	4600	3500	14000	22100
	202	F	4400	800	11250	16450
	291	F	8350	8950	10050	27350
	B3	M	50	11800	36000	47850
	B16	M	250	12500	16950	29700
	266	M	150	11350	28100	39600
	MEAN		2967	8150	19392	30508
	SE		±1385	±1990	±4244	±4699
Levamisole 1.5 mg/kg/day	276	F	550	4550	9800	14900
	243	F	500	4900	4350	9750
	269	F	4700	3300	26300	34300
	B6	M	400	2700	5100	8200
	B14	M	150	5100	15350	20600
	257	M	200	6350	16650	23200
	MEAN		1083	4483	12925	18492
	SE		±726	±536	±3382	±3965
Levamisole 2.0 mg/kg/day	295	F	850	1850	1500	4200
	219	F	50	550	2900	3500
	212	F	0	1000	2450	3450
	B7	M	1000	9500	22800	33300
	B10	M	4000	3300	16100	23400
	B11	M	0	3850	8600	12450
	MEAN		983	3342	9058	13383
	SE		±630	±1338	±3547	±5094
Levamisole 3.0 mg/kg/day	185	F	600	100	300	1000
	244	F	5900	1750	16350	24000
	251	F	1350	1000	750	3100
	270	F	300	0	2800	3100
	271	F	1150	2050	5150	8350
	317	F	1000	200	2100	3300
	MEAN		1717	850	4575	7142
	SE		±851	±364	±2457	±3516

Female lambs were slaughtered on day 24, and male lambs were slaughtered on day 28 after the initial infection.