International Research Fund (IDRF) Report

Control of Parasites in Ruminant Livestock

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Research Summary (DevNet Research Database)

It is well recognised that access to dietary protein is critical to the future health of South Pacific Islanders. Any current shortfall of animal protein is made up through the importation of meat and dairy products, largely from New Zealand and Australia. There is a want by South Pacific Island nations to be more self sufficient and in the process allow importation substitution. Since their introduction into the Pacific Islands, livestock have been quickly incorporated into the local cultures; particularly chickens and pigs. However, ruminants are particularly successful within integrated farming systems as they can graze and forage grasses found within cash cropping systems, such as coconut plantations and thus do not compete for the production of plant food intended for human consumption.

Parasitism is the most important endemic disease in these grazing animals. Therefore this research project was designed to test the hypothesis that specific supplementation of the diet of Samoan sheep would lead to better control of gut parasites. The sheep used were gifted by the Ministry of Agriculture, Samoa and were part of a rapidly growing national flock originally imported from Fiji ('Fiji Fantastics'). The dietary supplementation was made up of by-products from tuna processing, copra production and the brewing industry. The collaborative research was carried out by academic and technical staff of Lincoln University, University of the South Pacific (Alafua Campus) and the Samoan MAF.

Our results indicate that the gut parasites present in our research animals were resistance to the effects of a (normally) very effective anthelmintic (wormer). Secondly, the effect of feeding the concentrate supplement to our sheep for 17 weeks resulted in a decrease in the number of parasites found in them at slaughter. In addition, the number of parasite eggs that were excreted in the faeces of the supplemented sheep throughout the research trial was less, leading to less pasture contamination. The addition of the concentrate resulted in these supplemented animals suffering less than the pasture-only feed animals as indicated by diminished signs of anaemia. However, the concentrate feed did not improve the growth rates of the sheep. The material costs of the supplementation are in the order of SAT $0.10 per animal per day which may make it cost effective and its adoption is likely to less treatments with chemicals (anthelmintics) which will prolong their usefulness.

In summary, we have shown that a method of supplementing the normal pasture diet of sheep with dietary protein, derived from industrial by-product, enhances the animal's defence against internal parasites and can be beneficially used on Samoan farms.

Background to the research

The South Pacific region has an expanding human population, with the population of Samoa, estimated by the South Pacific Community (2004), to be growing at approximately 0.5-1% per year. This will lead to an increased demand for quality food. A well balanced diet requires the provision of both animal and plant protein and it is likely that these will be limiting factors, ironically, in the South Pacific where there is a bountiful source of food rich in fibre and vitamins. In this region these protein-rich items, if not provided locally, are imported (for example milk products and meat from New Zealand and Australia). There is a desire to become more self-sufficient in order to provide security of supply and also to allow for importation substitution - allowing the procurement of other needed commodities, particularly during the current economic period where returns to the country may diminish. It is unlikely that any increased fish catch will significantly provide for these future dietary needs (Sibert et al, 2006). Livestock could provide this source of protein.

Species	Beef cattle	Buffalo	Chickens	Crocodile	Dairy cattle	Goats	Horses	Pigs	Sheep
A Samoa,	**120**		**34,000**				**12**	**40,000**	
Cook Is	**200**	**0**	**24,300**	**0**	**0**	**5,000**	**70**	**15,900**	**0**
FSM	**50**	**10**	**20,000**	**0**	**0**	**500**	**0**	**10,000**	**0**
Fiji Is.	**170,000**	**0**	**4,500,000**	**0**	**9,000**	**100,000**	**40,000**	**500,000**	**10,000**
F. Polynesia,									
Guam		**60**	**26,332**			**179**		**2,287**	
Kiribati			**61,667**					**47,455**	
Marshall s									
Nauru			**3,000**					**3,000**	
New Caledonia									
Niue	**7**	**0**	**7,000**	**0**	**0**	**0**	**0**	**1,457**	**0**
NMI,	**1,312**		**6,381**		**83**	**276**	**5**	**1,483**	
Palau,	**15**		**6,000**			**300**	**3**	**10,000**	
PNG	**100,000**	**10,000**	**5,000,000**	**10,000**	**1,000**	**50,000**	**1,000**	**3,000,000**	**10,000**
Pitcairn Is.									
Samoa,	**49,000**	**0**	**497,000**	**0**		**2,312**	**2,516**	**258,000**	**309**
Solomon Is	**2,000**	**0**	**230,000**	**0**	**0**	**300**	**30**	**150,000**	**0**
Tokelau			**2,500**					**3,500**	
Tonga,	**10,000**		**100,000**		**400**	**1,000**	**2,000**	**80,000**	**350**
Tuvalu			**14,148**			**5**		**10,202**	
Vanuatu	**214,152**	**0**	**368,251**	**0**	**10**	**8,792**	**200**	**88,694**	**800**
Wallis & Futuna	**20**		**32,000**			**10**	**30**	**30,100**	
Total	**546,876**	**10,070**	**10,932,579**	**10,000**	**10,493**	**168,674**	**45,866**	**4,252,078**	**21,459**

Fig 1. Livestock numbers in South Pacific countries (data from Manueli (2009).
Since their introduction into the South Pacific islands, livestock have quickly been incorporated into the local culture; particularly poultry and pigs but also ruminants (especially cattle and to a lesser extent sheep and goats), depending on the country.

Ruminants have been used particularly in integrated farming systems. For example, cattle graze and forage within coconut plantations as they can efficiently utilise grasses populating these areas. In the process they are very effective at weed control and recycle some of the nutrients back into the soil to be used by the coconut palms. With such a system there is no competition for the production of plant products to be used as human food. A small but rapidly expanding sheep industry exists in Fiji (currently 14,265, *pers comm* MAF Fiji) and some of these animals ('Fiji Fantastics') have been exported to Tonga and Samoa. The nucleus of this flock originates from imported stock (from the West Indies) that have been cross bred with Wiltshire sheep, which are believed to exhibit some tolerance to gastrointestinal parasites. However, this disease remains as the most important endemic disease in these, other breeds of sheep and indeed all ruminants in the region. Control has centred on the use of chemicals (which are imported). These have become less effective with the development of anthelmintic resistance (Baker and Gray, 2004), as has happened worldwide and some farmers have no therapeutic options available at all. Currently, there is a wish to expand the ruminant population and that is likely to depend on the development of rotational grazing strategies to attain optimal grass growth. However, this also is likely to increase the risk of internal parasitism as livestock density is inevitably higher under such systems.

One alternative means of controlling parasites in these grazing animals is by manipulation of their diet. Supplementation of the protein intake of sheep at critical times in their production cycle has been shown to decrease their worm burden. This has been studied in detail at Lincoln University (Kambara et al, 1993) and elsewhere (van Houtert and Sykes, 1996) with diets supplemented with fish meal (van Houtert et al, 1995) and meat and bone meal (Kambara et al, 1993). Another study in Fiji has shown that imported non-protein nitrogen supplements (urea) given with molasses to sheep can also enhance resistance to parasites, presumably by increasing the endogenous production of protein released by ruminal microflora, within the animals ingesting the supplements (Knox and Steel, 1996). Protein sourced from fish material is especially efficacious because it is not totally degraded in the rumen into simple nitrogen-containing substances to be lost in the urine as urea (sometimes termed by-pass protein).

The fishing industry in the South Pacific varies between countries but is substantial. Small amounts of by-products from this industry are currently used for feeding livestock (1600 and 780 metric tonnes in Fiji and the Solomon Islands respectively); fish meal fed to poultry and pigs. However, there is a large Tuna canning operation in Pago Pago (American Samoa) that produces substantial amounts of dried fishmeal (actual tonnage unknown). The coconut industry is a large industry that provides domestic and exportable products and has a highly fibrous material as residue. The annual production of copra meal is 8,800 and 5000 metric tonnes in Fiji and the Solomon Islands respectively (Copra Dev Authority). Copra production in Samoa has not been quantified but it is available at a reasonable price. Both of these products provide high levels of protein (especially fish meal) and carbohydrate and are palatable below certain levels to sheep. Hence the addition of this highly proteinaceous material into the diet should improve immunity against internal parasites in sheep providing a degree of control . In addition, the decreased dependency on chemicals (anthelmintics) used to provide traditional control should

prolong their usefulness, as resistance by the parasites to them will be slower in its development.

Accordingly, we proposed a research trial to assess the importance of dietary protein (from fish and coconut by-products) for the control of internal parasites in young growing lambs. This was be investigated in animals produced within a rotational grazing system.

Preliminary literature review

This review will focus on the evidence showing that dietary protein is critical for development of resistance against internal parasites in sheep.

The observation that the amount and quality of food available to livestock influences resistance to diseases including parasitism is not new to most farmers and veterinarians. However, there is very little scientific data on which to support these observations, be they due to the presence, or absence, of macro or trace elements.

Until recently it was thought that no effective immunity was generated by infection with nematodes; that worms living in the gut were effectively "outside" the body and could neither initiate nor be affected by the immune system. Hence, repeated infections are apparent in livestock throughout life. However, that viewpoint has changed and it is clear that a very complicated immune response occurs against helminths (roundworms, and in general the most problematic parasites) due to the complexity of the life cycle. In order to survive, helminths need to remain within the host for a relatively long period of time to ensure patency (egg production) and in wild sheep populations a balance between worm and host is maintained unless environmental conditions (population density, nutrition or climate) upset it. In general, gastrointestinal parasites (helminths) exist throughout the year in tropical environments as the presence of moisture and high ambient temperatures are necessary for their life cycle to proceed. For this reason parasitism in livestock is regarded as the most important animal health disease.
As one might expect, the immune function against parasites is particularly affected during those phases in an animal's life when their protein requirements are least supplied by their diet; that is, in the young growing lamb or the pregnant or lactating ewe.

In general, sheep need to ingest the infective larvae of intestinal helminths, and therefore be exposed to the developmental stages, for up to 12 weeks to develop resistance. However, this period is dependent on genetics (McEwan, 1998), age (Gibson and Parfitt, 1972) and nutrition (Kambara et al, 1993). In a indoor pen trial at Lincoln University (Kambara et al, 1993), young lambs were sensitised by trickle infection from 2 until 5 months of age, dewormed, and then challenged with Trichostrongylus colubriformis. These young lambs did not show the expected array of immune responses such as: serum antibody increase, mucosal mast cell proliferation, and eosinophilia which contribute to protection in older animals. Despite this, the young lambs were better able to resist the establishment of an artificial parasite infection when their diet had been supplemented with meat and bone, and soya bean meal (20% crude protein [CP] of diet), as compared to 10% CP supplemented with equivalent amounts of energy. They consequently grew faster.

Other data support these findings. In experiments where lambs were infused with casein into the abomasum in order to reduce the variability of protein source, resistance to T. colubriformis (Bown et al, 1991) and Ostertagia circumcincta (Coop et al, 1995) increased. When lambs were fed up to 20% CP as rumen bypass protein (fish meal), in an attempt to increase the amount of utilizable protein, resistance was enhanced (van Houtert, 1995). For this reason protein supplements containing fish products have an inherent advantage over other sources. Recently, researchers have shown that when parasitized lambs on a protein deficient diet are supplemented with methionine or a methionine-lysine mixture, they are more resilient (tolerant) (Coop et al, 1997) and resistant (Yarali, 1997) to infection with T. colubriformis. Thus, supplementation with fish meal may be correcting for the deficiencies of specific amino acids. The addition of urea (non protein nitrogen) to the diet within a molasses block can also reduce parasite infection and pathology and increase productivity (Knox and Steel, 1996), because the non-protein nitrogen is converted into utilisable protein by the ruminal microorganisms. This has been used to good effect in a research trial in Fiji (Knox and Steel, 1996) where molasses is available as a by-product of the sugar industry. However, this is not cheaply available in Samoa and livestock can be poisoned with excess urea.

It is not clear what immune mechanisms are affected by protein supplementation although the function of certain lymphocyte populations may be crucial (Kambara & McFarlane, 1996) in young lambs, and circulating eosinophils and intestinal mast cell protease concentrations may be elevated due to dietary protein in older lambs (van Houtert et al, 1995).

A substantial effort has gone into the selection of genotypes resistant to gastrointestinal parasites (Baker and Gray, 2004). Currently the sheep population in Fiji has increased dramatically and they have been exported to Samoa (in 2004) and Tonga (in 2005). Some of these animals are a result of the cross breeding of Wiltshire sheep with imported genotypes from the West Indies (Barbados Blackbelly). The resultant cross has been shown to be partially resistant to gastrointestinal parasites (Baker and Gray, 2004). It is expected that any dietary advantage due to the proposed trial will be additive to the genetic advantages of this breed, as described by Coop & Holmes (1996).

Pregnant ewes will not be used in this trial but if the results in growing lambs are favourable, then we would recommend a similar regime of dietary supplementation to pregnant and/or lactating ewes.

Source of dietary protein

In the proposal the method of preparing the protein-rich food supplement from available fish by-product had yet to be finalised. In Apia we have access to copra cake /meal, a by-product from two new Copra Oil Mills (who are extracting oil to be used as a fuel substitute), brewers spent grain and dessicated coconut from the coconut cream factory. Using software-based nutritional tables we were to formulate a ration incorporating our heat-treated fish products (largely fish bones and heads) that met the feed requirements of the research animals.

Grazing systems

Efficient use of pasture is fundamental to the high levels of production in the livestock sector in New Zealand and elsewhere. Much of this is based on rotational gazing as it maintains active pasture growth, and avoids senescence, thus enhancing nutritive value. Many of these ideas have been extended to tropical grasslands. Unfortunately, the introduction of such systems has the potential to increase the rate of uptake of infective parasite larvae on the pasture. This is because livestock graze the grass sward to a lower level where the infective parasite larvae inhabit because of their higher density and also because nutritive (including protein) quality diminishes when the animals reach the latter part of their grazing allocation. Hence, it is likely that dietary protein supplementation will be more critical in such a grazing system. Hitherto, the integration of supplementary feeding regimes into a rotational grazing system has been generally only carried out in high producing dairy animals (usually cows) to increase their production at specific times of the year, not for animal health reasons, although these both are inter-related to some degree.

Commercial implications

The proposed trial had been set up to ascertain whether there are significant benefits to animal health by the supplementation of their diet with protein rich products. To that end the emphasis was on rigorous scientific methodology including strict adherence to experimental design and analysis. The findings of such a study are scientifically justified in their own right.
The future commercial value of such an approach was not to be addressed per se but will need to be addressed subsequently if supplementation is successful.

Team member roles

1. Dr Robin McFarlane. Overall Supervisor, who, in conjunction with his Samoan counterpart planned the trials, facilitated communication between research personnel in order to carry out specific duties and will oversee the publication of results in national and international fora.

2. Dr Kenneth Lameta. Academic and veterinarian employed at Alafua Campus, USP. He took on site responsibility for activities surrounding the research trial in Samoa. He communicated with Dr McFarlane and jointly made decisions.

3. Mr Robin McAnulty, took responsibility for supplying parasite larvae for the research trial and giving technical advice to Dr Lameta and a Samoan technician regarding faecal egg counts and worm counts following post mortem.

4. Dr Andy Greer was involved with trial design and setting it up on the ground and giving advice on nutrition and immunity to parasites

Key questions to be explored/addressed in proposal

1. Can resistance to gastrointestinal parasites in sheep be enhanced with the addition of fish by-product material to their diets in a rotational grazing system.

2. Is this 'resistance effect' additive to that already conferred to sheep with a resistant genotype or is this supplementation unnecessary in genetically resistant sheep.

Actual deviations:

The major influence on the project was the tsunami that occured in Samoa on 29th September, 2009. It not surprisingly influenced many aspects. Many of the Samoan personnel had family members and/or friends who had died or at least been affected by it. They decided that they wished the project to proceed but activities were postponed because of it with adjusted timelines.
Despite earlier assurances that 40 animals would be available for the study, in actuality only 22 animals were available at the beginning of the study. Advice obtained from biometricians from Lincoln University was to the effect that this number was adequate to answer the primary question (1 above) presuming a statistical power of 0.8. Hence, Objective 2 relating to the potential additive affects of diet and genetics was not addressed.

Schedule

1. Set up the research trial (April 2009 – February 2010).

   Much of the preparative work between Drs McFarlane and Lameta took place by e mail, aided by digital photography etc and helped by both of them having first-hand knowledge of the general topographic and land-use features of Samoa.

   The first visit to Apia by Dr McFarlane (2 week) was on February 12-18), to assess availability of industrial by-product to be used as dietary supplement, formulation of the supplement and its standardisation.

   The animal aspects of the trial were organised such as purchase of livestock, grazing management procedures and the setting up of analytical facilities (parasitological and blood analysis) at USP – Alafua campus (see methodology for detail).

   The setting up at USP was expedited by a 10 day visit to Lincoln University during January 2010 by Dr Lameta to learn parasitological techniques necessary for this project (with the oversight of Mr McAnulty). It also allowed him to meet with Drs McFarlane, Greer and colleagues within the Faculty of Agriculture and Life Sciences.

   Milestone 1: Research trial was able to proceed.

2. Start of trial (26 April – 1 May, 2010).

   During the second visit (1 week) to the USP Alafua campus, Dr McFarlane, accompanied by Drs Greer, Lameta and helpers made physical modifications to the animal house and fencing arrangements at the farm at Tanumalala. They set up analytical equipment (taken from Lincoln University) and at USP- Alafua campus and went through some diagnostic procedures with their Samoan counterparts. When satisfied, the trial got under way. Animals were divided into 2 treatment groups, pre-treatment measurements were taken and the various treatments (parasitological and dietary) initiated.

3. Duration of research trial (May 2010 – August 2010)

   The trial proceeded with Samoan technical oversight. Dr McFarlane and Mr McAnulty visited the research site (5 days) at the time of research completion to carry out parasitological analyses and organised the processing of the study samples. This was carried out predominantly on Alafua campus but parallel analyses were carried out at Lincoln University.

4. Analysis of data (September 2010 – September 2011)

   Most of the communication occurred via electronic communication and the statistical analysis was carried out at Lincoln University.

   Milestone 2: Trial and final report completed 4 November, 2011 with Journal publications to follow.

Methodology

1. Animals and diet.

   Proposed use of animals:

   The animals will be housed at the University of the South Pacific livestock facilities. The Principal investigator from Lincoln University will be responsible for attaining Animal Ethics approval from the LU animal ethics committee (this is a routine for academic staff working at a site remote from the campus). The experiments will be carried out on young (4 months old), recently weaned, male lambs. One group will be comprised of the Barbados Blackbelly bred and another group of the Wiltshire (or Suffolk) breed.

   The animals will be grazed using a rotational grazing system – known to more efficient for the production of high quality pasture. The pasture will be 'pre-conditioned' by grazing to a low level with beef cattle. This is necessary as the indigenous grasses growing at this site are stoloniferous and rhizomatous that are poor in quality except for the actively growing tip. In addition, any parasites that the cattle deposit on the pasture are unlikely to be pathogenic for the research sheep.

   All of the research animals (total no, N = 40) will be grazed together as 1 flock so as to eliminate outside variables. The frequency of changing paddocks will be dependent on pasture growth in order to prevent luxuriant growth leading to senescence and poor food quality.

   Half of them (n=10 for BB breed, and n=10 for Wiltshire) will receive extra dietary protein (increasing amounts of 20% crude protein dependant on liveweight); the other half of each breed group will and hence act as control animals.

   Actual use of animals (deviations)

   Following my first visit to Samoa (Appendix 1) the study design was modified. Because of difficulty sourcing the proposed 40 research animals we settled on the use of 22 animals (18 from a Ministry of Agriculture and Fisheries –MAF- farm and 4 privately owned) and the trial to be carried on the Tanumalala research farm. Approval to use these MAF animals, facilities and staff was gained through a personal meeting with the Director of Agriculture and the Minister of Agriculture. This followed an inspection of the 4 MAF farms when a decision was made as the preferred research site, personnel to be involved, assignment of duties etc. The originally proposed site at the Alafua Campus, University of the South Pacific (USP) was dismissed because of the inadequate pasture cover which would not support the animals over the project study period. Similarly, there were various reasons why 3 other MAF farms were not considered such as distance from Alafua, difficulty in subdividing off a suitable grazing area, poor drainage, poor pasture, inadequate supervisory potential and security (see Appendix 1). However, we were unable to subdivide off sufficient paddock plots to allow rotational grazing. Instead we used set stocking starting off with generous

allowances of pasture but by the end of the trial the pasture allocation was minimal (very similar to a NZ farm!).

In addition, the age of animals was wider than we had wished (estimated to be 2-12 months of age), again a reflection on the inability of sourcing enough animals. This was likely to have meant a varying immune status among the trial animals. The research animals were made up of males as females were required for ongoing breeding programmes; that is, 10 wethers (castrated males) and 12 entire males).

Upon the second visit by Drs McFarlane and Greer, the research animals were located, final approval for their use given, and transportation (provided by USP) then followed to the research site. The grazing area was adequately fenced and the animal shed (with slatted floor and thus parasite-free) was modified to house the 2 treatment groups separately overnight, during which time the dietary supplement was offered. That is, the animals in both the supplemented and un-supplemented groups were grazed together during the day but at night were housed separately (at which time the dietary supplementation took place).
The animals were randomly allocated to their 2 treatment groups following stratification with respect to live weight (and indirectly age).

Animal Ethics approval for the project was attained on 10th December, 2009. (Appendix 2).

Proposed diet:

The method of preparing the protein-rich food supplement from fish by-product has yet to be finalised. We have access to copra cake /meal, a by-product from 2 new Copra Oil Mills (who are extracting oil to be used as a fuel substitute), brewers spent grain and dessicated coconut from the coconut cream factory. Using software-based nutritional tables we will formulate a ration that meets our requirements.

Actual diet (with deviations):

Following my first visit to Apia and following regular communications with my collaborator, Dr Ken Lameta at USP it became clear that our pilot attempts to dry the fish by-product material obtained from the Apia fish market was not without its difficulties. Using the USP feed mill prolonged drying times were needed to produce a product able to be stored over a length of time. In addition, it was obvious that the energy required to produce such a product was excessive. Costs were likely to be more than that needed to buy fish meal commercially. After additional advice from Dr Akira Chijiiwa, a nutritionist from Japan (JICA project) we decided to purchase imported dried fish meal, a by-product from the Tuna canneries in Pagopago, American Samoa (Starkist Samoa Company).

2. Parasite treatment and measurements - as per Proposal.

   At the beginning of the research trial all of the animals were to be treated (drenched) with an anthelmintic mixture (containing all 3 families of active ingredients) in order to render them worm-free.

   As well as ingesting infective larvae from the pasture (which we will quantify by counting the numbers of infective larvae per kilogram of pasture dry matter) we will give extra infective doses of parasites to ensure that there is sufficient challenge for the animals. We will use *Haemonchus contortus* and/or *Trichostrongylus colubriformis* - common pathogens in Samoa, at a dose yet to be decided, but in keeping with previous work and likely pasture burdens found in Samoa. We will continue this regime for 12 weeks. Careful note will be taken of the animals and if on veterinary inspection the animals are assessed to be suffering or the levels of parasite eggs in the faeces reach a threshold, then anthelmintic treatment will be carried out under the supervision of the principal investigator from USP. At the same time an assessment of the mucosal colour of the conjunctiva will be assessed using the FAMACHA system colour charts to compare with blood counts (see below).

   Faecal egg counts will be carried out on a weekly basis, starting prior to the anthelmintic treatment at the beginning of the trial.

   A weighing apparatus will be supplied by the USP and each week the live weight of the research animals will be measured.

   Blood sampling will also be carried out weekly and haematocrit (packed cell volume) estimations made as one indication of pathology due to parasites by USP staff. In addition, plasma will be stored for estimations of protein levels.

   Infective larvae for the artificial infections will be generated at Lincoln University and transported to Apia. In this process, an immune suppressed animal will be infected with infective larvae, the faeces collected and incubated. The resulting infective larvae will then be enriched from the faeces and purified. Animal Ethical approval will be necessary for this procedure but as it is of low impact re welfare no problems are anticipated.

   Actual parasite treatments and measurements (with deviations):

   All research animals were treated with a triple action anthelmintic (*Matrix*) at 1ml/3.5Kg liveweight, at the commencement of the study. During the second visit infective larvae (L3) of *H Contortus* were imported into Samoa. These were anthelmintic-susceptible strains cultured at Lincoln University. These parasites were administered orally, weekly, to all of the sheep in the trial, at a dose of 1000 L3 per animal. In addition, weighing, blood and faecal collection was carried out weekly as proposed.

   In retrospect we were told by our Samoan collaborators that a power outage had occurred in mid-July. Following analysis of these larval cultures during our third visit, it confirmed our belief that a proportion of the larval cultures had died

because of no refrigeration during that time. This was consistent with the subsequent parasitological findings.

3. Food and blood analysis

Analysis of the food supplements offered to the animals was to be carried out at Lincoln University. The transport of such products - blood (see above) and food supplements (restricted biological products) from Samoa will be covered under the existing Lincoln University Importation permit of restricted biological products (#2008034621), as long as the product remains within the existing containment and transitional facility (Transitional facility, NZ MAF Ref # 489). This has been approved by the Lincoln University Institutional Biosafety Committee (IBSC).

Actual food analyses (with deviations)

The logistical difficulty of bringing rations back into NZ was alleviated by doing the blood and ration analyses in Apia.
Initial blood analyses were carried out in a modified centrifuge that didn't prove satisfactory until replaced by another analytical technique. This was not finalised until appropriate laboratory glassware had been sourced from Australia via NZ to Samoa. In all, it caused a delay in commencing the measurement of packed cell volume (measurement of anaemia) of 6 weeks. A centrifuge and 2 microscopes from NZ were gifted to USP in the process.

The diet that was decided on was calculated from computerised tables (AFRC and Australia feed standards for livestock) and was as follows:

Supplemented animals:
1 Kg pasture (predominantly Signal and Batiki swards)
20g dry fishmeal
100g dry copra meal
880g wet brewers grain
This was calculated to supply a diet of 19.8% crude protein

Un-supplemented animals:
Pasture (predominantly Signal and Batiki awards) *ad libitum*
This was calculated to supply a diet of 12% crude protein

4. Post mortem.

A small subset of the research animals will be slaughtered humanely (methods to be used have already been approved by the Lincoln University Animal Ethics Committee) by the Principal investigators at the end of the trial. A post mortem examination will be carried out and the gastrointestinal contents will be taken to estimate worm burdens.

Actual necropsies (with deviations):

Of the 22 study animals, 10 were euthanased and a detailed necropsy was performed during the third visit by Dr McFarlane and Mr McAnulty. There was a particular focus on the parasite burden throughout the gastrointestinal tract. Samples of gut contents were transported back to Lincoln University and duplicates were analysed in the lab of Dr Lameta at Alafua campus, USP.

Considerable time was spent up-skilling personnel in parasite analysis (parasite egg in faeces and total worm counts at necropsy). This was carried out for Dr Lameta at Lincoln University during his visit to NZ in 2009 and to Dean (a technician at Alafua, USP) on the job in Apia.

5. Statistical analysis and presentation

Data from liveweight measurements, faecal egg counts and worm burdens will be assessed by parametric analysis (eg analysis of variance followed by pairwise comparisons of the treatment means) following transformation of date if normalisation is necessary. Alternatively, data may be analysed by non-parametric analysis if data is heavily skewed (which is typical with parasitological data).

Actual data analysis (with deviations):

Following communication with Mr David Hunter, Alafua USP, it was decided that it was unnecessary to gain further training in statistical analysis for Dr Lameta, as it was available 'next door' and thus his second proposed trip to NZ was cancelled. Measurements made repeatedly on the same animal were subjected to repeated measurements analysis.

Additional biometrical advice was and graphical assistance was obtained from Dr Richard Sedcole and Dr Feng Piao, respectively, at Lincoln University.

Results and discussion:

1. Parasite burdens in experimental sheep at slaughter.

   Five of each treatment group (N=10) were slaughtered and the developing larval and adult stages of the resident parasites were counted both in the abomasums (4^{th} stomach) and the proximal 6m of the small intestine. Counts were taken of the luminal washings and also the same area of digestive tract was digested to liberate developing parasites in the mucosal membrane.
   The results are displayed in Figures 2, 3 and 4 - showing means +/- SEM.

   The parasite burden in the abomasum was a product of those infective larvae administered to the research animals each week plus those ingested infective larvae that were resident on grazed pasture. In general, the worm burdens were relatively low which supported the belief that some of the larvae administered to the animals were non-viable (Fig 2). There was no significant difference in abomasal worm burden due to administration of the concentrate food (p=0.356).

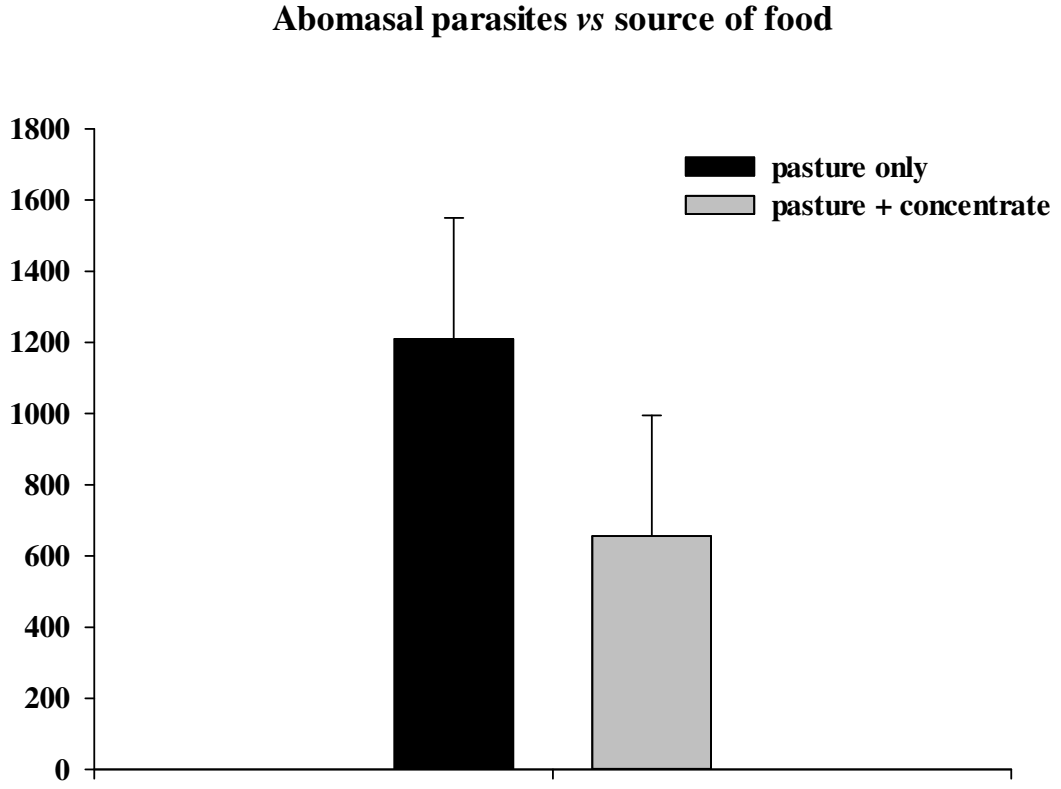

Fig 2.

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Source_of_food	0.96	1	0.96	8.0	0.356

Kruskal-Wallis Test : H = 0.88 DF = 1 P = 0.347

These results are in conflict with a range of other research findings where pathogenic burdens were established with oral doses of *H contortus* infective larvae of 1000 L3 (Mugambi et al 1996) in Red Maasai and Dorper lambs or 600 L3 in Dorset Horn lambs (Knox and Steel, 1996). This strongly suggests that the loss in viability due to refrigeration failure during the trial meant that inadequate parasite establishment occurred with little re-infection following grazing of infective pasture.

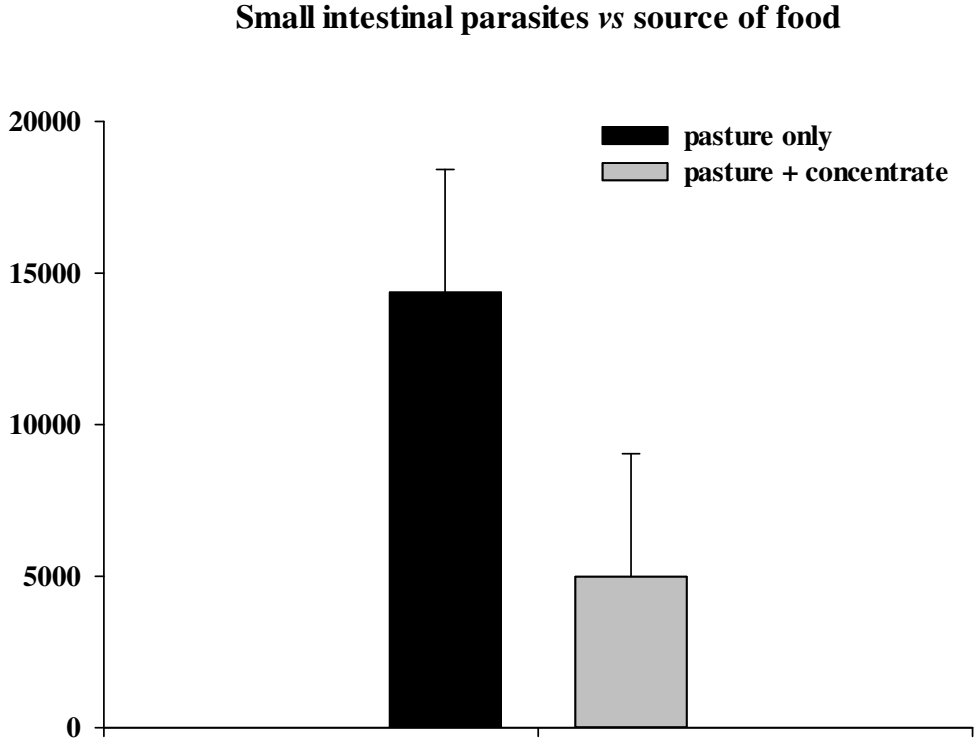

Fig 3

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Source_of_food	2.67	1	2.67	8.0	0.141

Kruskal-Wallis Test : H = 3.94 DF = 1 P = 0.047

However, the parasite burdens of the small intestine in the experimental animals were substantial ranging from 860 to 35,420 made up of *Trichostronglylus* (predominantly) with less *Cooperia spp* and a few *Nematodirus spp*. This indicated a high degree of re-infection from the pasture as no additional infective small intestinal parasite larvae were administered by the experimenters. This was not surprising as this pasture had been grazed by sheep previously. However, we had anticipated *H contortus*, an abomasal worm, to be the predominant gastrointestinal parasite for sheep grazing the mixed Batiki and Signal pasture. Given the constraints of the experiment we were unable to carry out pasture parasite larvae estimations to confirm the pasture contamination by these parasites.

Supplementation of the diet with concentrate resulted in a significant decrease in small intestinal worm numbers (p=0.047), (Fig 3).

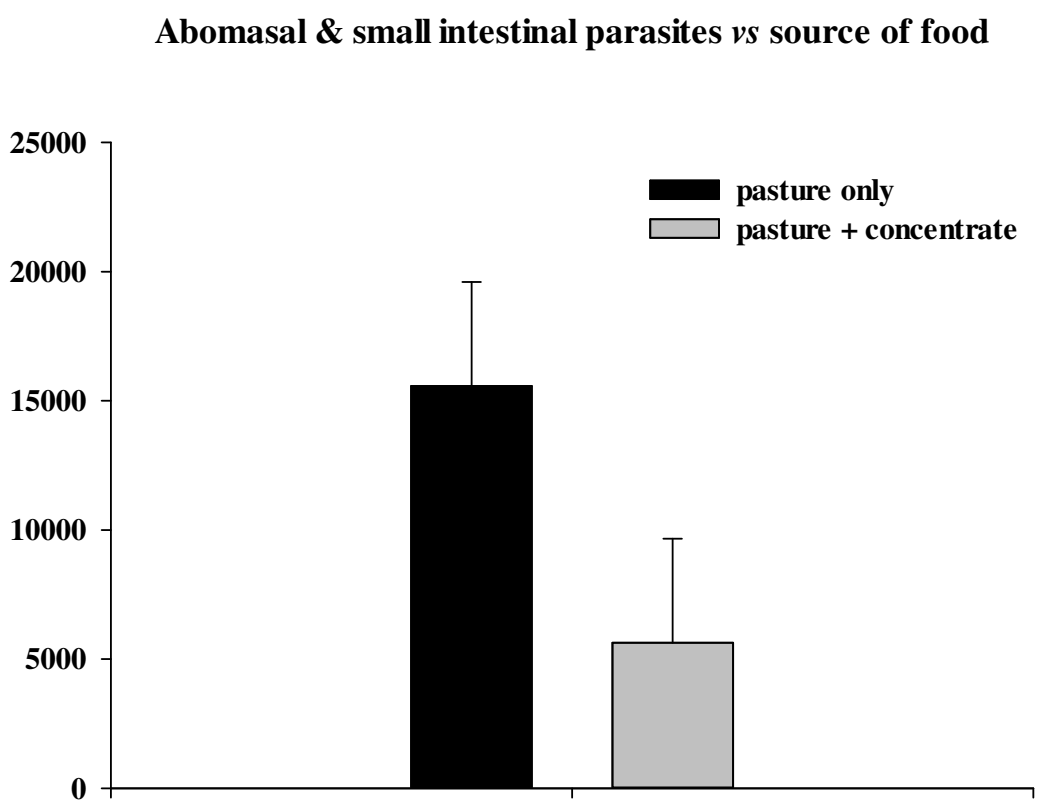

Fig 4.

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Source_of_food	3.04	1	3.04	8.0	0.119

Kruskal-Wallis Test : H = 3.94 DF = 1 P = 0.047

When the worm numbers recovered from the abomasum and small intestine were added together (that is, the likely total gastrointestinal parasite burden) there was a significant effect due to the eating of the concentrate ($p<0.047$), (Fig 4). Clearly the majority of the worm burden was contributed by those in the small intestine and hence it is not surprising that total worm is affected by dietary supplementation. One confounding factor was the inability of the anthemintic to remove all of the existing parasite infection at the beginning of the trial (see section 2). However, previous work has indicated that any adult *Trichostrongylus spp* surviving the anthelmintic treatment were unlikely to have survived the duration of the trial period and show up in the worm count (Dobson et al, 1990). Hence the worm burdens are likely to be the result of establishment from ingested infective larvae.

These results are consistent with previous findings (Kambara et al, 1993, van Houtert et al, 1995) in Coopworth and Merinos, respectively where dietary supplements rich in protein enhanced the animals' resistance to worm establishment.

As the gold standard for resistance to parasites is the ability of the animal to resist/reject the adult parasite form, this finding is crucial for the question that prompted this research which was, 'c*an resistance to gastrointestinal parasites in sheep be enhanced with the addition of fish by-product material to their diets in a rotational grazing system*'. The answer is yes when sheep are set-stocked.

2. Faecal egg counts (FEC) as eggs per gram faeces (EPG).

   At the beginning of the trial all of the animals were treated with Matrix which is a product containing representatives of 3 anthelmintic action families (abamectin, levamisole and oxfendazole). We expected this formulation to be highly efficacious against any parasites harboured in our study animals. We were surprised to find the product to be very inefficient at removing the worm burden as reflected in the faecal egg counts (48% efficacy 14 days after treatment), shown in Fig 5 (2 time points removed because of incomplete sampling). As Matrix was given at a high dosage (1ml/3.5Kg LW), this is strong evidence that drench resistance to all 3 families is present in this sheep parasite population on this research farm. Historically, anthelmintic use on the MAF sheep farms had been frequent – and based on treating any animals exceeding the threshold of 300 eggs per gram of faeces, when checked on a monthly basis. Clearly, there is some cause for concern for future worm control based on the use of chemicals from these 3 action families. This also helps justify our project whose long term goal was to minimise the use of chemical controls in the future by using alternative strategies (see *Background to the research, P.4).*

   There was a significant effect of dietary supplementation on faecal egg output ($P<0.001$). In general, there was little change in the parasite burdens over time ($p=0.157$), but in certain individual animals particularly the younger ones (#113, 121, 125, 126) there were moderate numbers of eggs that fluctuated over time. This is justification of our apportioning study animals into the 2 treatment groups based on liveweight (age). However, the worm burdens assessed by FEC were different (pasture+concentrate greater than pasture only) between the 2 treatment groups prior to the trial starting (30/4/2010) and the combined anthelmintic treatment at the beginning of the trial was ineffective. Thus it is difficult to deduce what faecal eggs are contributed by L3 larvae that had been ingested and matured into egg-laying adults in the course of the trial and those eggs produced by worms resident from the beginning of the trial. The decrease in egg counts shown at 3-4 weeks into the trial most likely reflects partial worm kill off and the increase in FEC from that point on in likely to be from established adult worm ingested as L3 larvae.

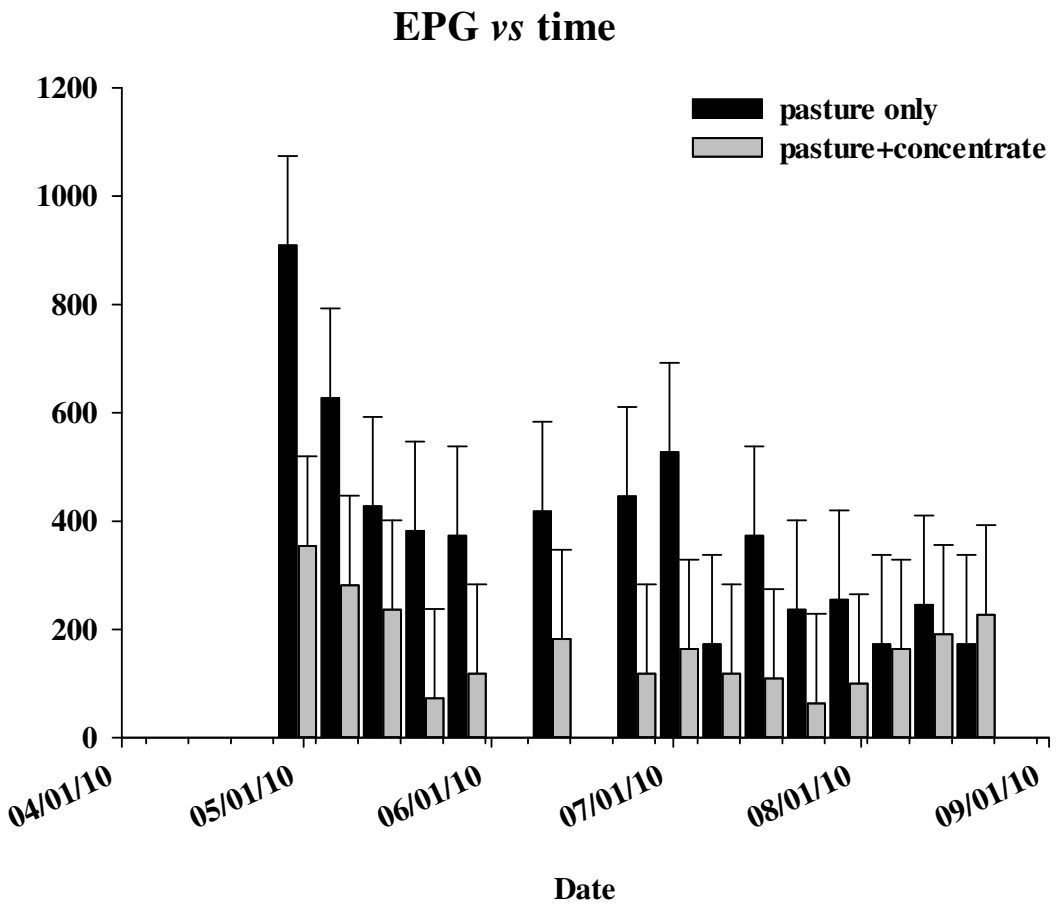

Fig 5.

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Supplementation	12.20	1	12.20	199.9	<0.001
Day	19.58	14	1.39	260.3	0.157
supplementation.day	7.90	1	0.56	287.70	0.892

Taken together with the worm data these results indicate that this supplement was beneficial in decreasing worm burdens and are supported by other research conducted in young sheep where dietary supplementation lowered the level of parasitism due to rejection or decreased establishment (reviewed by Coop and Holmes, 1996, van Houtert and Sykes, 1996).

The mechanisms that are believed to be involved with increasing the supplemented lamb's resistance to small intestinal parasitism are varied but may include innate immunity – particularly in the very young lamb (Kambara et al, 1993) with gamma lymphocyte population change (Kambara and McFarlane, 1995), circulating eosinophils and intestinal mast cell proteases (van Houtert et al, 1995) and antibody (Stear et al, 2007).

3. Packed cell volume (PCV).

Measurements of PCV were delayed whilst analytical equipment was imported into Samoa and commenced on the 18 June, 6 weeks after the beginning of the trial and one time point was deleted because of technical error (Fig 6).

The PCV changed significantly over time ($p<0.01$), initially decreasing in both treatment groups when the faecal egg counts (FEC) were maximal and then increasing later in the trial when there was diminished FECs, presumably indicating lessoning worm burdens.

Dietary supplementation lead to an increased PCV ($p=0.004$) overall, indicating less anaemia, presumably as a result of parasitism, in this group.

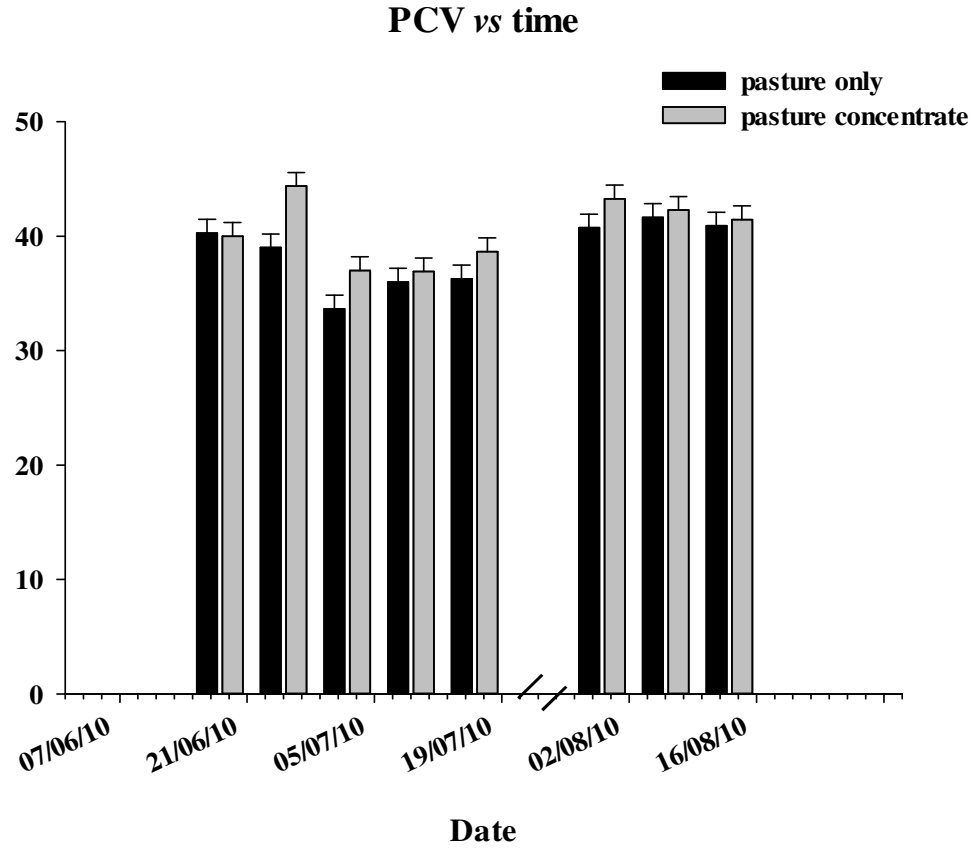

Fig 6.

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
supplementation	8.52	1	8.52	179.9	0.004
day	181.30	8	22.51	147.8	<0.001
supplementation.day	12.91	8	1.61	175.4	0.124

4. Measurement of conjunctival mucosal colour – FAMACHA score

Subjective assessments of conjunctival colour were assessed weekly (Fig 7). In general there was no significant change over time ($p=0.39$) but the supplemented animals had a significantly lower Famacha reading than did the unsupplemented

group (p<0.007). This finding (the reverse of the PCV) is consistent with greater 'anaemia' caused by parasitism in the unsupplemented group.

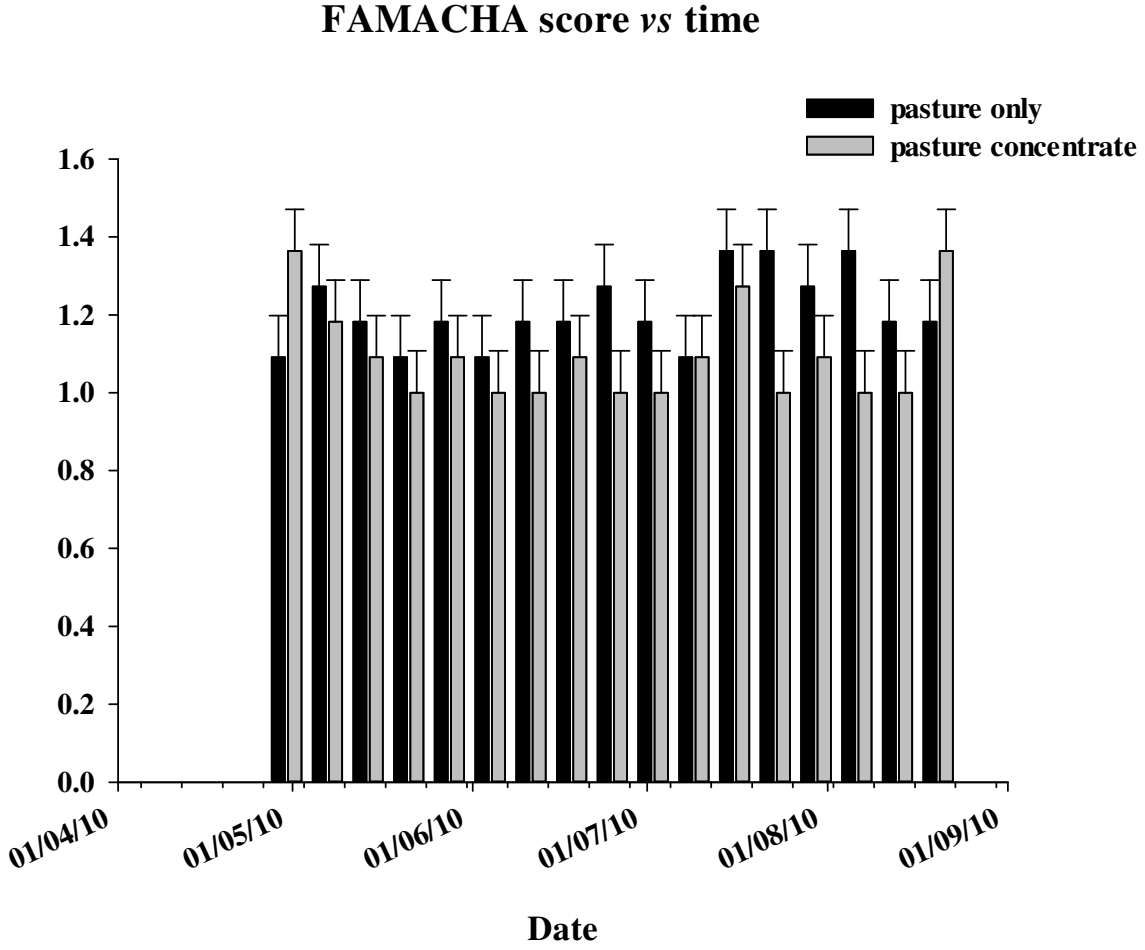

Fig 7.

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
supplementation	7.46	1	7.46	193.8	0.007
day	17.07	16	1.06	295.4	0.393
supplementation.day	18.59	16	1.16	314.8	0.300

There was an expectation of anaemia in all animals as they were experimentally infected with *H contortus* - as these parasites are voracious bloodsuckers. However, as indicated previously the establishment of this species of parasite given experimentally was minimal because of parasite larval death due to refrigeration failure. Therefore, it was re-infection from pasture that caused the decreased PCV and elevated FAMACHA score in the un-supplemented group. The parasites involved were the 'mucosal browsers' of the small intestine (*Trichostronglus and Cooperia spp*) that were detected only upon necropsy (as the faecal eggs were unable to be differentiated from the eggs of the abomasal worms)

5. Changes in liveweight (L.W.) over time.

At the commencement of the trial the 2 study groups were approximately equal in liveweight (23.9kg and 24.1Kg, for the supplemented and un-supplemented groups respectively). Three weeks later 2 extra sheep per treatment group were added to the study which caused a noticeable increase in group-mean liveweight (Fig 8).
Overall there was a significant increase in liveweight over time ($p<0.001$) but there was no effect due to the feeding of concentrate.

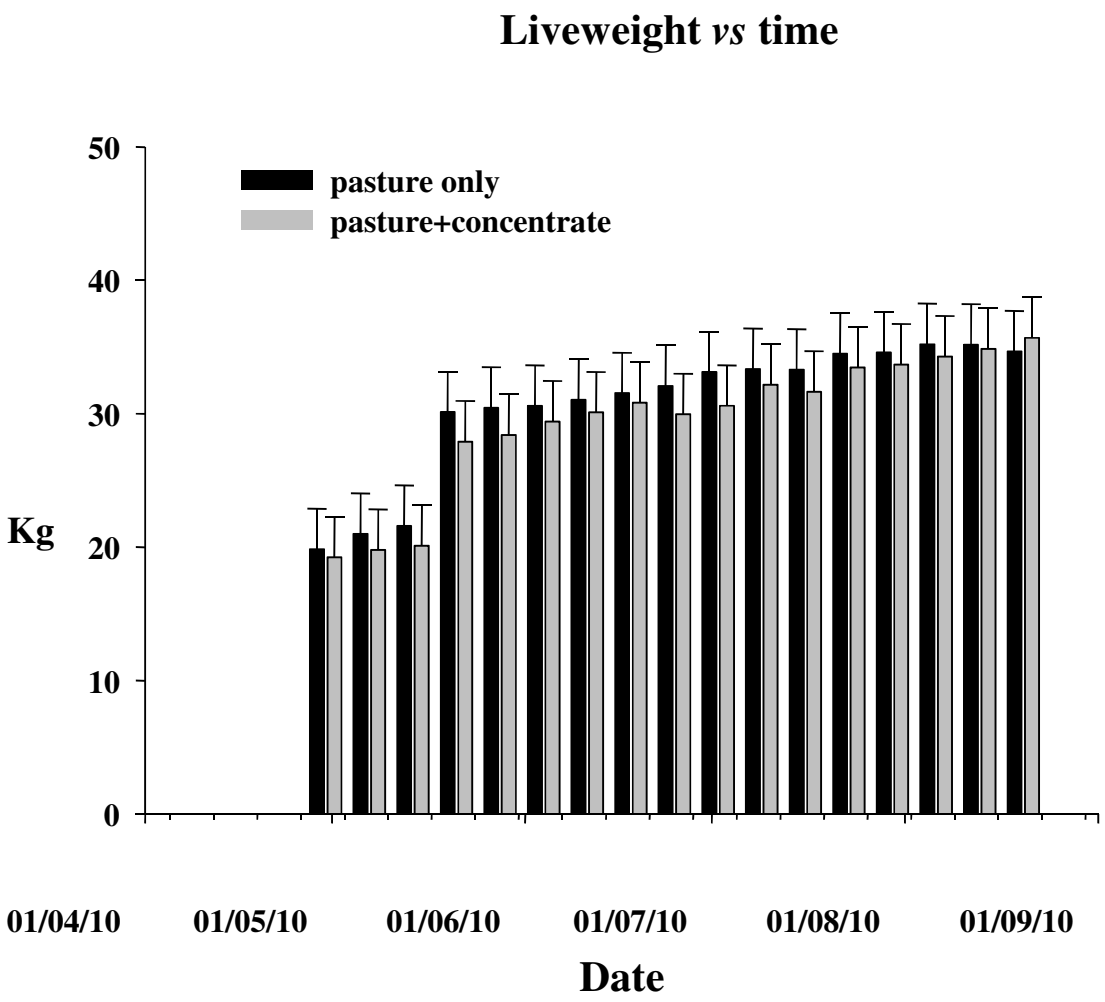

Fig 8.

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
supplementation	0.02	1	0.02	0.885
week	62.29	16	3.89	<0.001
supplementation.week	0.87	16	0.05	1.000

6. Carcass bodyweight at slaughter

At the conclusion of the trial, 10 lambs were slaughtered. They were chosen non-randomly, on the basis of long term breeding objectives. Hence they are not representative of the treatment groups (Fig 10).
There was no significant influence of supplementary feeding on carcass weight (p=0.158).

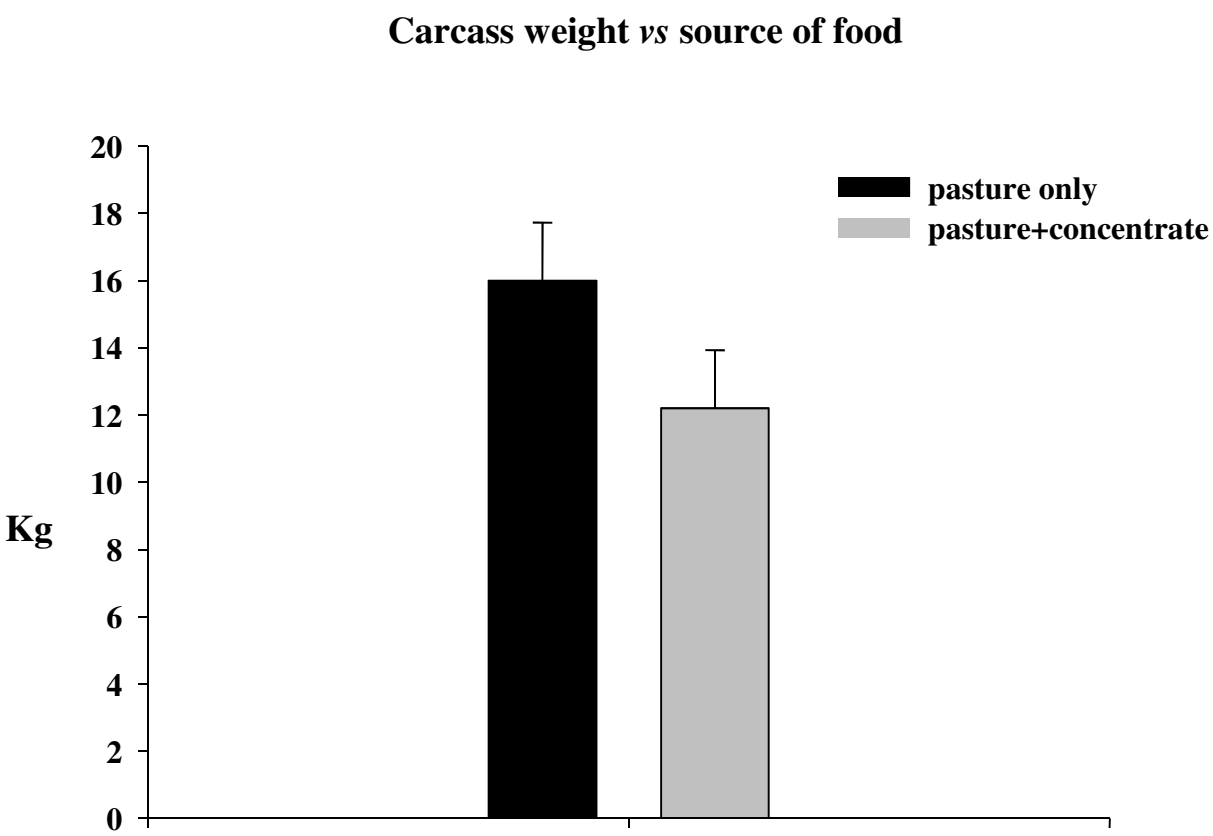

Fig 9.

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Source_of_food	2.43	1	2.43	8.0	0.158

Taken together it is clear that the resilience or tolerance (the ability to grow in the presence of parasites) of the animals was not affected by dietary supplementation in this experiment. This is contrary to other findings and is most likely explained by the varied ages of the lambs in the trial. That is, while the younger lambs would be expected to show improved resilience with supplementation, not so the older ones (Kambara et al, 1993).

Practical use of these findings.

Whilst the practical application of this finding to farmers was not part of the research, the implications are clear. Labour costs aside, the cost of such a diet is approximately$0.10 SAT per sheep per day, where the cost of Brewers grains was $20/tonne, dried copra meal was $10/40kg bag and fish meal was $1/Kg. The proposition to supplement sheep is attractive when lambs can be sold at prices of SAT $6.60/Kg LW.

Conclusions.

- Anthelmintic resistance to three action families exists in the worm populations found in sheep in Samoa. Hence the ability to control these parasites using chemicals is limited.

- Resistance of sheep to parasites can be increased by daily supplementation of a pasture diet with fish meal, copra meal and spent Brewer's grains.

Acknowledgements.

Funding for the project was provided by NZAID to whom we give thanks.
We wish to acknowledge Lincoln University, the University of the South Pacific (Alafua Campus) and the Ministry of Agriculture and Fisheries, Govt of Samoa for their support and provision of facilities and personnel for this project.
The Kiwi contingent would like to give thanks for the kind Samoan hospitality extended to us during our stay.

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APPENDIX 1.

1. Visit MAF farms and Lavomano (15/2/2010).

   'Fiji fantastic' sheep found mostly on MAF farms (see below) and now spread out to commercial farms.

   - Lemafa: Very wet, especially in lower areas. Some sheep (17?) but largely a cattle operation. Didn't see sheep, >40 mins from Apia. Manager not available. Subdivision could be difficult, v lumpy pasture with Batiki, and Sedge pasture predominating.
   - Togitogiga: Big farm with lotsa cattle. Batiki and Sedge predominate. Dryer than Lemafa but still at high altitude. Sheep not doing quite so well; management? Not rotating pasture. Alice and Famusa drenched 12/37 sheep where FEC >300epg (all ages; where 3 less than 4 week, 8 less than 1y).
   - Tanumalala: No manager there but neighbour helps out. Sheep look better. Lotsa of Sedge over Batiki. Easiest to subdivide paddocks? 9km from Apia. Some young animals (8?) scattered grazing. ? Preferred trial site.
   - Avele: Met Sina (CVO) - good value, could help organise importation of *H contortus* if needed. Her group doing the FEC after preparation with A and F. A and F both paravets and graduates from Alafua. FEC assays have been done about every 2-3 months (had hoped every 1 month) and not all animals done each time. Visited the APS clinic and 2 Vets (Sky and Hana). Haematocrit available without tubes.

2. Diet design (with Dr Akira; Japanese Vet going back to Yokohoma next week; JICA volunteer).

   Diet Possibility:

Brewers grain	88% wet weight
Copra meal	10% dry weight
Fish meal	2% dry weight

   Result: 24.9% crude protein (DM basis)
   (protein supply from 57% Brewers grain; 27% Copra M; 16% Fish M)

APPENDIX 2.

Lincoln University
Te Whare Wānaka o Aoraki
CHRISTCHURCH-NEW ZEALAND

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10 December 2009

Dr Robin McFarlane
Faculty of Agriculture & Life Sciences

Dear Robin

Re: Application to Animal Ethics Committee - #336

The Lincoln University Animal Ethics Committee (AEC) recently considered an application from you. It has been approved and a copy (electronic) of the version you generate following consideration of points raised at the meeting will be held on the University files.

> **Application for Animal Ethics Approval No. 336:** Parasite control in sheep with diet enhanced with fish by-product
>
> **Approved**

This approval is subject to some modification of the application to provide some threshold level of faecal egg counts for action and more information about efforts to ensure that management of possible rumen overload (with carbohydrate) will be adequate.

Yours sincerely

Jessie Teo
Secretary
Animal Ethics Committee