Evaluation of serum pepsinogen as a diagnostic marker for parasitism in farmed red deer.

Research internship of H.G. van der Heide, 0149268

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Deer Health and Production

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Contents

Abstract

Aim: This study presents data of pepsinogen analyses from samples collected during a prior investigation of the species of internal parasites, production effects and diagnostic markers for internal parasitism in farmed deer in New Zealand.

Methods: Two groups (n=20) of weaned red deer were naturally infected with *Dictyocaulus* spp. and mixed GI nematodes. The first group (suppressive treated control), was treated every four weeks with anthelmintic. The second group (trigger treated) received only treatment when trigger criteria were met. Individual body weight, faecal egg and larvae counts were measured every two weeks and every four weeks the animals were sampled for serum which was stored frozen.

Results: Serum pepsinogen concentration were measured but were low, with levels not reaching above 225 mU. No difference in pepsinogen mean concentration was seen between the two groups on each sample date. Faecal egg and larvae counts ranged from 0-500 epg and 0-120 lpg in the trigger treated group and few animals in the suppressive treated group were egg or larvae count positive. No correlation was found between mean egg or larvae count and mean serum pepsinogen at any sampling day in either group. A significant inverse correlation was observed between liveweight gain from January to December and mean log pepsinogen for each animal in the trigger treated group. ($r = -0.5177$, $p<0.001$). There was a positive correlation between mean log pepsinogen and liveweight gain for the suppressive treated group ($r = 0.2192$, $p=0.019$), but there was no significant correlation between mean log pepsinogen and sampling date, suggesting this was not a seasonal efffect. There was no difference between groups in the proportion of animals positive for pepsinogen at any sampling date.

Conclusion: Serum pepsinogen concentrations could not be used as a marker of internal parasitism in deer with the apparently low worm burdens and faecal egg and larval counts observed in this study. Further research is necessary in deer with greater worm burdens and faecal egg and larvae counts to evaluate the diagnostic potential of this marker at higher pepsinogen concentrations.

Clinical relevance: Data suggests that in animals with low faecal egg and larvae counts, liveweight gain is a better indicator for subclinical parasite infection then serum pepsinogen.

1. Introduction

The deer industry in New Zealand is the major world supplier of venison. Forty percent of the venison is exported to Germany. The export value of the deer industry accounts for more than $282 million New Zealand dollar in 2008 and venison accounts for about 80% of this amount (Wilson 2008). Production losses result from diseases with no clinical symptoms such as sub-clinical parasite infections. They lead to reduced live weight gain in young deer resulting in reduced venison production (Hoskin 1999). Among the most important parasites are the gastrointestinal (GI) nematodes and the lungworm *Dictyocaulus eckerti* (Swanson 2007).

The deer industry in New Zealand is interested in a more sustainable production system, in which reduction of the use of chemical anthelmintics is one of the points of interest. Reduction of anthelmintic use may result in an increased risk of parasitism so there is need for adequate means of diagnosing and monitoring parasite burdens to determine when intervention is wanted. Until now the diagnosis of GI nematode infections is based on faecal egg count. A diagnostic parameter that could be of interest for potential use in routine herd-health monitoring system for future on-farm parasite control programs and research is serum pepsinogen level. Serum levels of pepsinogen are elevated due to mucosal damage caused by the larval stages of abomasal nematode parasites (Berghen 1993). This damage firstly causes pepsinogen to leak into the blood between broken cell junction complexes (Lawton 1996) and secondly leads to the expulsion of pepsinogen direct from the parietal cells into the bloodstream.(Berghen 1993).

It has been demonstrated that blood pepsinogen levels correlate with abomasal worm burden in natural infected sheep (Stear 1999).There is also a significant linear relationship between serum pepsinogen concentration and gastrointestinal nematode counts in deer which were artificially parasitized (Hoskin 2000). In cattle, blood pepsinogen has been used as a diagnostic tool for ostertagiosis (Anderson 1965). Further, it was suggested that pepsinogen levels in first-year calves can be used for prognostic diagnosis (Ploeger 1994). If this applies for deer, the pepsinogen assay could be very useful as a diagnostic/monitoring technique to assist determination of the need or otherwise for anthelmintic treatment or alternative control strategies.

This report investigates whether there is a significant correlation between subclinical internal parasite infection and elevated pepsinogen levels in deer. Live weight gain and egg- and larvae counts are considered the parameters for this subclinical parasite infection.

2. Materials and Methods

2.1 Data

The data of live weight gain, egg and larvae count over the period January 2007 to December 2007 were collected from a PhD study started by Jonna Swanson. The protocol is presented in Appendix I. Briefly the study includes two groups of weaner red deer grazed together on pasture. The suppressive treated group (n=20) contains deer that have been treated with a suppressive dose of Moxidectin at 0.5 mg/kg every 4 weeks. The trigger-treated monitor parasitized group (n=20) is a group of deer from within a trigger treated group (n=100) that were not drenched until an individual animal met trigger criteria. The trigger criteria were met on 26 March, 7 May and 2 July and all the animals were treated with albendazole at 20mg/kg. The deer are regularly weighed and sampled for blood and faeces. Blood samples were collected for a multitude of haematological and biochemical tests including serum stored frozen for pepsinogen.

2.3 Serum samples

The serum samples were selected based on the hypothesis that there should be a difference between serum pepsinogen before and after a treatment, if there is a relation between serum pepsinogen and parasitism. The serum samples of 26 March are considered pre-treatment and the samples of 21 May, 16 July post treatment. The sample of 23 April is the only sample between the treatment dates 26 March and 7 May. The first and last samples (January 29th and December 2nd) were added to monitor the serum pepsinogen level at the beginning and end of the trial and to possibly test for an age effect.

2.2 Pepsinogen

The method for measuring the pepsinogen concentration is based on the method of Paynter (1992). For every sample there are two eppendorf cups used, each containing 0.5 µl of plasma and 0.75µl of Glycine-buffered albumin. Both the tubes are incubated at 37°C, one for 30 minutes, the other for three and a half hour. After incubation the undigested substrate was precipitated by adding of 2,5µl 10 per cent perchloric acid and separated from the supernatant by centrifuging. The supernatant of each cup is then in quadruplicate pipetted on to a 96-well, flat-bottomed micro titre plate. Then a 190 µl solution of bicinochonic acid and copper sulphate was added and the plate was incubated for 30 minutes at 37°C. The plate was put in a micro plate reader and the optical density at 490 nm wavelength was measured and compared with known standards at 0, 1.8, 3.6, 5.4 mM tyrosine. This is as Scott (1995) described it, with minor modifications. Serum samples that gave negative pepsinogen values were tested a second time after all the other samples were tested to eliminate the effect of inexperience with the method. Hereafter, remaining negative values of serum pepsinogen were replaced by zero, assuming that negative values are a result of the error of the method. The standard operating procedure is listed in appendix II.

2.3 Statistical analysis

All data including zeros (undetectable) was included in all statistical analyses. To test if pepsinogen followed a normal distribution a Shapiro-Wilk Normality test was performed. To approach normality a transformation of the pepsinogen data to ln (pepsinogen+1) was done. A similar transformation was also performed on egg count and larvae count to make calculations of Pearson correlations coefficient possible. Pearson correlation coefficients were computed using Statistix 8 (Analytical Software, Tallahassee) for the following:

- Correlation between lnpepsinogen and ln FEC and Ln FLC within each treatment group at each sampling date
- Correlation between mean ln pepsinogen (each sampling date included) and live weight gain from January to December, for each treatment group
- Correlation between mean ln pepsinogen and sampling date for the suppressive treated control group

The difference between correlation coefficients for the relationship between pepsinogen and live weight gain was analysed using the Fisher test.

A logistic regression was performed to compare the proportion of animals positive for pepsinogen between the two treatment groups at each sampling date, and by sampling date for the suppressive treated group using SAS v9.1 (SAS Institute Inc., Cary NC). To make this possible dummy's where added to the dataset where there were missing data.

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Significant differences were declared at p <0.05. Appendix III contains the output of the statistical analysis.

3 Results

All the raw data are presented in appendix IV. At the start of the study 20 animals were selected for each trial group. Two animals were euthanized during the trial, one in the suppressive treated group, euthanized on 08/03 for septic arthritis in stifle joint, and one in the trigger treated group, euthanized on 09/03 because of an unstable fetlock fracture. The data of these animals was deleted and not used in the results.
The pepsinogen data are presented in Figure 1 and Table I.

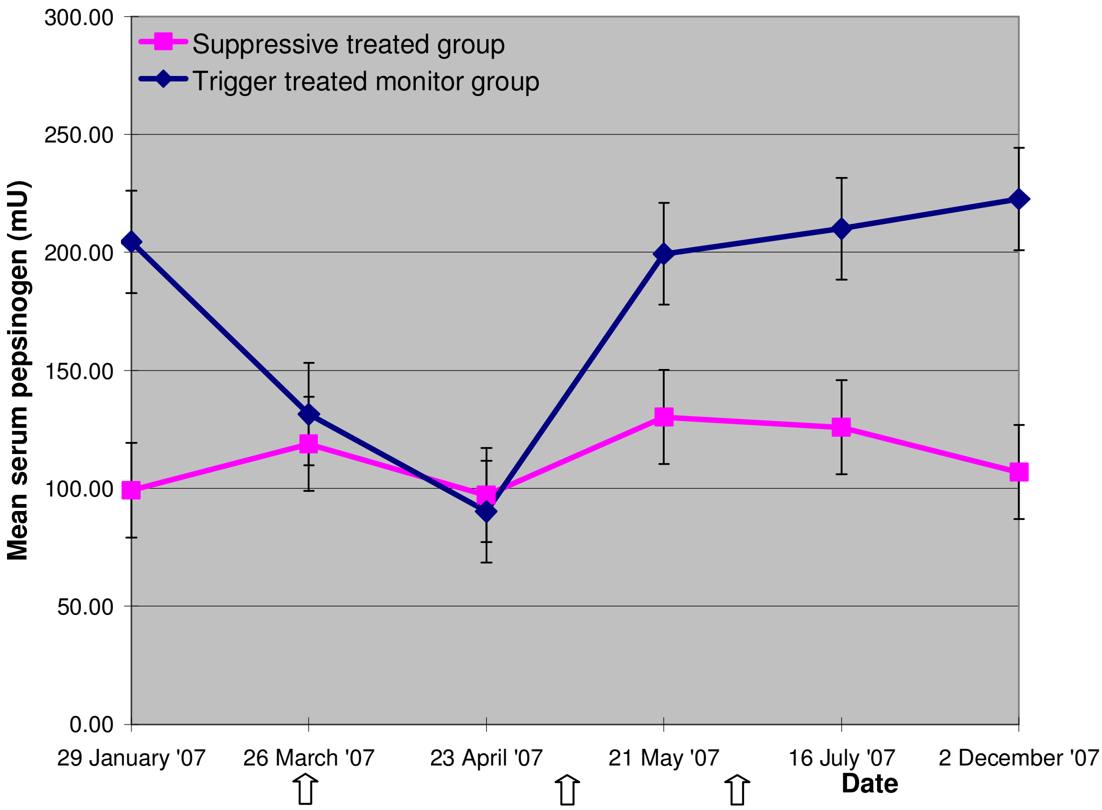

Figure 1. Mean (±SE) serum pepsinogen (mU) (including zeros) of both groups (n =19) on each sampling date. (Arrows indicate trigger treatment of the trigger treated group on 26 March, 7 May and 2 July)

Figure 1 shows that the mean serum pepsinogen of the suppressive treated group varied around 100 mU. The mean pepsinogen of the trigger treated group seemed to vary more than the suppressive treated group and looked higher on 29 January, Logistic regression showed no significant difference in the proportion positive for pepsinogen between groups at each date (Table II and Figure 2).

Logistic regression showed that in the suppressive treated group there was a significant difference ($p<0.005$) in the proportion positive for serum pepsinogen between on 29 January and 16 July (OR=1.81, C.I. 1.36-2.31) and January 29 and 2 December (OR=1.81, C.I.1.36-2.31) .

Table I. Geometrical mean, range and standard error (SEM) of serum pepsinogen (mU) of both groups (n=19) on each sampling date. (Group 1= Suppressive treated, Group 2 = Trigger treated)

Date	Group	Mean	Range	S.E.
29 January '07	1	104.4	0-473.1	34.0
	2	214.7	0-885.3	58.7
26 March '07	1	118.8	0-290.2	23.5
	2	131.4	0-762.9	43.3
23 April '07	1	97.2	0-377.5	24.5
	2	90.1	0-250.6	21.3
21 May '07	1	130.2	0-791.1	41.6
	2	199.4	0-510.7	34.3
16 July '07	1	125.8	10-330.0	19.4
	2	210.0	60-500.0	25.1
2 December ‘07	1	106.8	0-210.0	12.7
	2	222.6	20-570.0	41.5

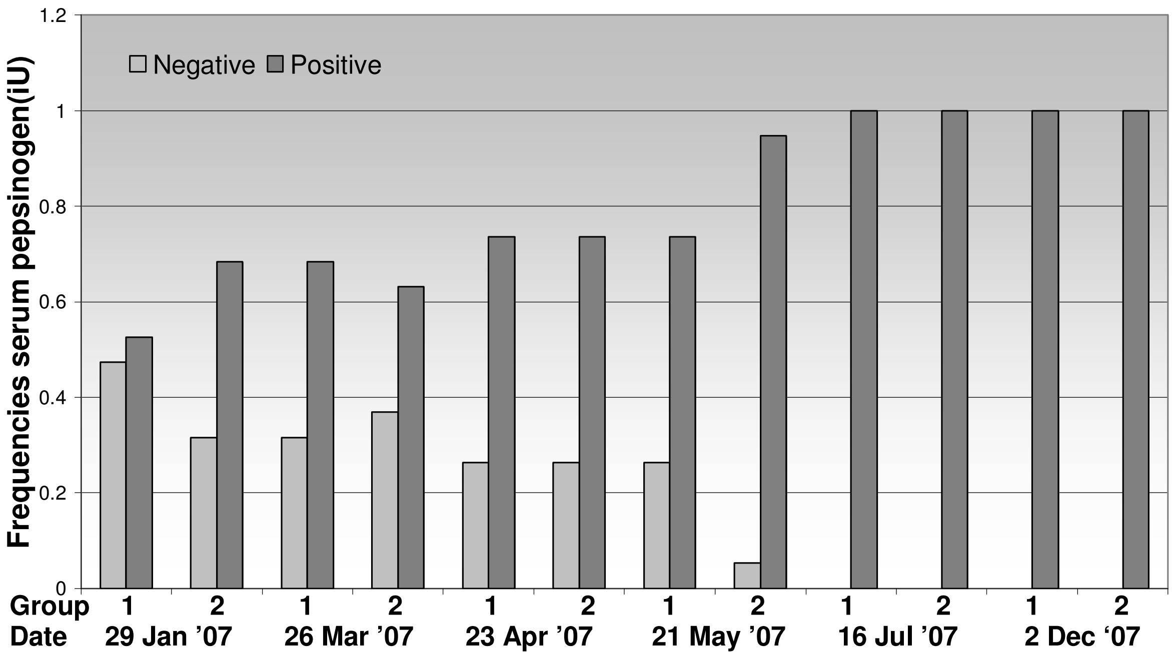

Figure 2. Frequencies of positive or negative (undetectable) serum pepsinogen of both groups on each sampling date. (Group 1= suppressive treated, group 2= trigger treated)

Table II Data for logistic regression analyses comparing proportion of animals with positive pepsinogen concentrations.

Date	Group	negative*	positive		OR1	C.I.1	OR2	C.I.2
16 July '07	1	0.05	0.95	b	1.81	1.36-2.31	1.50	1.17-1.59
16 July '07	2	0.05	0.95	b	1.81	1.36-2.31	1.50	1.17-1.59
2 December '07	1	0.05	0.95	b	1.81	1.36-2.31	1.50	1.17-1.59
2 December '07	2	0.05	0.95	b	1.81	1.36-2.31	1.50	1.17-1.59
21 May '07	2	0.0526	0.9474	b	1.80	1.36-2.27	1.50	1.17-1.56
23 April '07	1	0.2632	0.7368	ab				
23 April '07	2	0.2632	0.7368	ab				
21 May '07	1	0.2632	0.7368	ab				
29 January '07	2	0.3158	0.6842	ab				
26 March '07	1	0.3158	0.6842	ab				
26 March '07	2	0.3684	0.6316	a			1	
29 January '07	1	0.4737	0.5263	a	1			

(OD1= Odds ratio of 29 January '07, OD2= Odds ratio of 26 March '07, C.I. confidence interval of odds ratio's) * This table contains the dummies which were added to make the logistic regression possible for the groups on 16 July and 2 December.

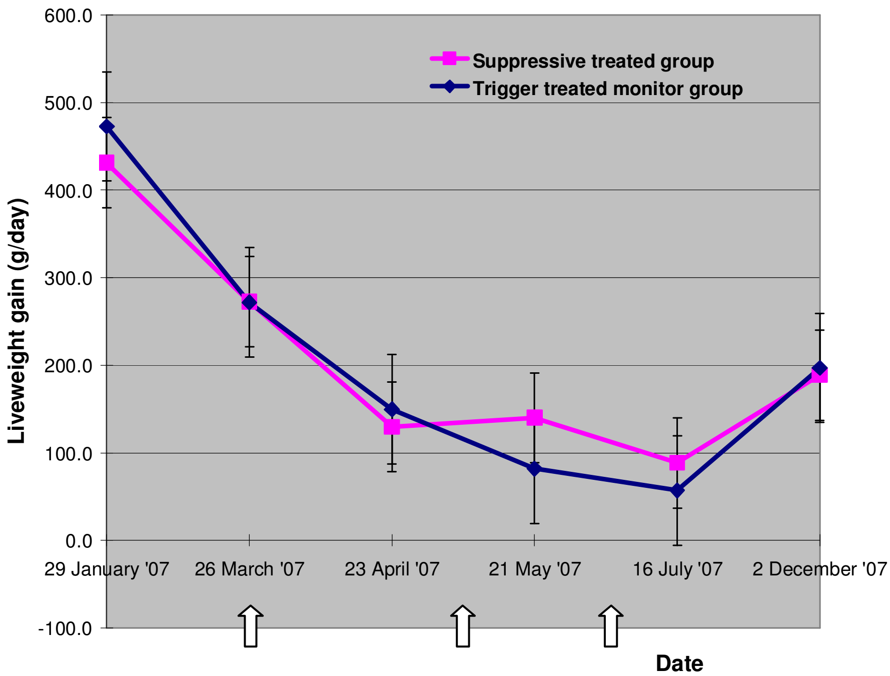

Figure 3. Mean liveweight gain (g/day) (± SEM) of both groups at sampling period (Arrows indicate treatment of the trigger treated group on 26 March, 7 May and 2 July)

Figure 3 shows the mean daily live weight gain (g/day) at each sampling period for both of the groups. Liveweight gain decreased until 16 July and increased after this date in the trigger treated group. The suppressive treated group followed a similar trend, except for the period of April till May which showed an increase instead of a decrease in liveweight gain.

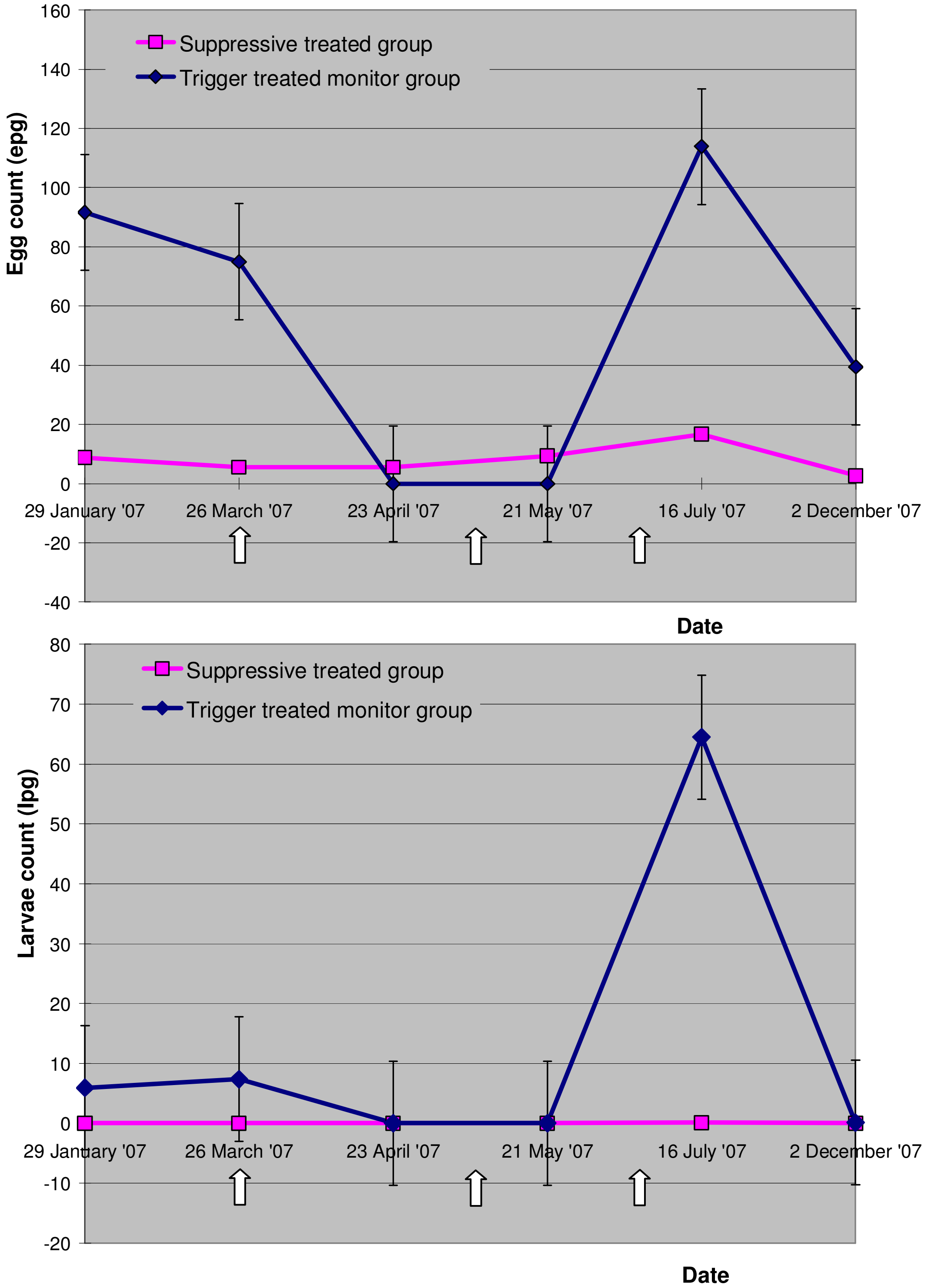

Figure 4. Mean egg count (epg) (top) and larvae count (lpg) (±SEM) (bottom) for each group on each sampling date. (Arrows indicate trigger treatment of the trigger treated group on 26 March, 7 May and 2 July)

There was a significant inverse relationship between the mean log pepsinogen within each animal and liveweight gain over the trial period from January until December ($r = -0.5177$, $p<0.001$) for the trigger treated group. There was a significant positive relationship between the mean log pepsinogen within each animal and liveweight gain over the trial period from January until December ($r = 0.2192$, $p=0.019$) for the suppressive treated group. The correlation coefficients were significantly different between groups ($p<0.001$).

The correlation between mean serum pepsinogen and sampling date (age) in the suppressive treated group was not significant ($r =0.028$, $p=0.767$).

Figure 4 shows the data of mean egg- and larvae counts. The animals in the suppressive treated group showed egg and larvae counts, despite that they were treated with a suppressive dose of anthelmintic regime every four weeks. The mean egg and larvae count of the trigger treated group showed a decrease after the trigger treatment of 26 March. This treatment was justified by the significant difference in liveweight gain between the both groups, which was one of the criteria. The same trigger criterion justified the treatment on 7 May. After 21st of May the mean egg and larvae count in the trigger treated group rose and resulted in another trigger treatment on the 2nd of July, when some egg counts reached > 500 eggs per gram (epg) together with a reduction in liveweight gain. After 16 July both the egg count and the larvae count decreased.

The correlations between serum pepsinogen, larvae count and egg count are presented in table III. There were no significant correlations between serum pepsinogen and egg or larval count in either group.

Table III. Correlations between log serum pepsinogen and log egg- or log larvae counts (Group 1 = suppressive treated group, group 2 = trigger treated group)

Date	Group	Egg count	Larvae count
29 January 2007	1	r = -0.043 (*p*=0.870)	-
	2	r = -0.095 (*p*=0.708)	r = -0.319 (*p*=0.197)
26 March 2007	1	r = 0.241 (*p*=0.335)	-
	2	r = 0.277 (*p*=0.266)	r = 0.026 (*p*=0.917)
23 April 2007	1	r = -0.127 (*p*=0.616)	-
	2	-	r = 0.101 (*p*=0.680)
21 May 2007	1	r = 0.166 (*p*=0.539)	-
	2	-	-
16 July 2007	1	r = -0.115 (*p*=0.650)	r = -0.298 (*p*=0.245)
	2	r = 0.352 (*p*=0.152)	r = 0.156 (*p*=0.538)
2 December 2007	1	r = 0.190 (*p*=0.435)	r = 0.190 (*p*=0.435)
	2	r = -0.216 (*p*=0.374)	r = -0.179 (*p*=0.463)

4. Discussion

In this study the relationship between pepsinogen values and parasitism was evaluated to investigate if serum pepsinogen level can be of interest as a diagnostic parameter. Parasitism was measured by the parameters egg count, larvae count and liveweight gain.
Several researchers state that pepsinogen levels increase when sheep get infected with GI nematodes, especially Ostertagia. (Lawton 1996; Stear 1999) In other ruminants such as cattle there is also an increase in pepsinogen levels seen after an infection with GI parasites. (Berghen 1993; Eysker 2000) Eysker and Ploeger (2000) state that in first year grazing cattle high pepsinogen values correlate with the occurrence of parasitic gastroenteritis. In calves a correlation between mean pepsinogen values and liveweight gain is described by Ploeger et al (1994). In first-grazing season calves a relation exists between adult *Ostertagia* worm burdens and serum pepsinogen levels.

Thus in sheep and cattle pepsinogen levels are used as an indicator of infections with GI parasites. Kloosterman and Falkena(1988) concluded that lungworm infections in calves also result in elevated pepsinogen levels, but this is only a small elevation in comparison to the elevation of serum pepsinogen levels after a *Ostertagia spp*. infection.

In deer pepsinogen levels also rise if there is an infection with GI nematodes. Johnston et al (1984) shows that *Heamonchus* significantly elevated pepsinogen levels in weaner stags. When weaners have been given different doses of lungworm and GI nematodes varying from low to high, an increase in pepsinogen levels has been observed.(Hoskin 2000). In research of Audigé et al. (1998) pepsinogen was correlated with summer growth rates suggesting that abomasal parasitism might influence growth in weaner deer. These data suggest that there might be an important role for pepsinogen as diagnostic tool for infections with GI nematodes in deer, prompting the present research to look in more detail at the relationships between worm burden, pepsinogen concentration and production parameters.

Paynters (1992) method of determination of serum pepsinogen was chosen because it shows the least day to day variation and it is quick simple and needs small volumes of serum and reagents (Scott 1995). Paynter makes the note that the detection limit of the assay is approximately 0.2U/L. Audigé et al (1998) and Hoskin et al (2000) use the technique described by Pomroy and Charleston (1989). This method is an modification of the method of

Uete, Wasa and Shimogami (1969). Johnston (1984) used in his estimation of the pepsinogen values in weaner stags a method described by Mylrea and Hotson (1969).

The mean serum pepsinogen levels in this study vary between 99 and 130 mU in suppressive treated deer and between 90 and 223 mU in trigger treated monitor deer. This seems quite low, compared to pepsinogen levels found in previous trials. Audigé (1998) found pepsinogen concentrations in naturally infected weaner deer varying from 154 mU in March to 531 mU in November, while the range can also vary from 80 to 150 mU in control animals, with peaks of 350 mU after infection with *Haemonchus* (Johnston 1984).

As mentioned before on the previous page Paynter (1992) himself placed a note to his method for the interpretation of values below 200 mU. Therefore the values of pepsinogen in this report should be read with caution and the few differences determined might be because of low pepsinogen levels per se.

The logistic regression on the positive and negative frequencies of serum pepsinogen shows there is no significant difference between the suppressive treated group and the trigger treated group on the same date (Table III). This differs from earlier data describing significant difference in mean serum pepsinogen between an uninfected control group and infected groups in research where animals were infected with different dose rates of lungworm and gastrointestinal nematode larvae (Hoskin 2000). Those animals were not treated during the 12/13 weeks the experiment was carried out.

It has to be noted that the animals in the suppressive treated group, which should not have egg- and larvae counts at all, did have low egg and larvae counts. This could explain the absence of a significant difference between groups. This raises the question why the animals that got a suppressive dose of anthelmintics show egg- and larvae counts.

The increase within the suppressive treated group might suggest an increase in serum pepsinogen level with increasing age.(Audigé 1998). When the Pearson correlation coefficient is computed, no significant correlation between mean serum pepsinogen and age was found. The logistic regression did show a significant difference between the positive proportion in the first sample of 29 January and the samples of 16 July and 2 Decemberin the suppressive treated group This might indicate a seasonal or age effect. This would support the previous finding of Audigé (1998) that mean pepsinogen concentrations tend to be higher later in the year.

Brunsdon (1972) gives several reasons for an elevated pepsinogen level in calves; an increase in level with age, the long period pepsinogen levels take to return to normal following abomasal damage and the influence of age, size and conditions of the host. In calves that get exposed to infection over a longer period, the abomasal mucosa may take more time to recover and therefore pepsinogen levels might stay elevated over a longer period.
In cows, elevated serum pepsinogen levels can also be found in clinically healthy animals probably as a result of hypersensitivity because of a previously experienced infection (McKellar 1984-1985).

It is previously reported that deer of all ages shed a higher proportion of lungworm larvae then GI parasite eggs (Audigé 1998). Wilson (1981) mentions the low correlation between worm burdens and faecal egg count and caution should be taken to use egg count as a diagnostic tool.
Hoskin et al. (1997) found that egg- and larvae counts vary in individual deer in groups with different doses of lungworm- and GI nematode larvae and that there is no significant difference in faecal egg or larvae counts between the different groups. There was a difference of mean liveweight gain between the control group and infected animals increasing with time. Audigé et al. (1998) found a negative relationship between weaner faecal larval index and farm mean weaning weight, suggesting that the presence of internal parasites may be a contributory factor in the lower weaning weights. Also deer with high mean lungworm and GI worm numbers showed a reduction in voluntary food intake and liveweight gain (Hoskin 1997). Both Audigé et al (1998) and Hoskin et al (2000) suggest that a reduction in liveweight gain is associated with sub-clinical GI parasitism in deer.

Statistical analysis shows there is a significant inverse relationship between mean serum pepsinogen and liveweight gain in the trigger treated group. This would support previous observations (Audigé 1998; Hoskin 2000) .

This study does not show significant correlations between serum pepsinogen and egg counts or larvae counts. In the animals of the trigger treated group the serum pepsinogen level tends to rise, but no correlation was found with egg- and larvae counts. This suggests that the infection level is very low or that the present infection results in undetectable tissue damage. On 26 March and 7 May the animals were drenched based on the a significant difference in liveweight gain between the suppressive treated and trigger treated groups and both Audigé et al (1998) and Hoskin et al (2000) suggest that reduction in weight gain is associated with subclinical infection.

It might even be that the counts are unreliable as an indicator of infection as Brundson (1972) states. In his article he says that potentially, the most important role of pepsinogen determinations lies in the indication of the level of abomasal infection in calves at times when faecal egg counts are expected to be unreliable. This has been the case in his trial were anthelmintic untreated control calves had considerable abomasal worm burdens determined at slaughter date, which were not suggested by mean faecal egg counts. In other research it was said that for the diagnosis of ostertagiosis in cattle the pepsinogen values always have to be in conjunction with clinical and parasitological data (Eysker 2000).

Further the biological relevance should be considered. The statistical power for the serum pepsinogen was calculated per date and varies because of the lack of a significant difference between the groups per date. The power of the total trial was then calculated and this gives a value of 42%, which means that the number of animals in the trial is to low or that the differences in serum pepsinogen are too small to be biologically significant.

Before the standard pepsinogen assay can be used as a tool to diagnose subclinical gastrointestinal parasite infection, further research is necessary. In this research no significant differences in proportion positive between groups at any sampling date were seen. There was a relation between liveweight gain and serum pepsinogen, but no significant relation between serum pepsinogen and the measured egg or larvae counts. Serum pepsinogen can serve as an additional diagnostic tool in subclinical parasite infections, but until more is known about the dynamics of pepsinogen in deer, it is not suitable on its own. Considering the results of this research and the literature, pepsinogen seems to be a better indicator for parasite infections when the infection levels are higher (Audigé 1998; Hoskin 2000)

Liveweight gain was one of the chosen parameters for subclinical parasite infection. It appeared that even parasitism associated with low egg and larvae counts result in a reduction of liveweight gain. So in clinical situations it appears that liveweight gain is a more reliable indicator for subclinical parasite infection when counts are low than serum pepsinogen levels.

5. Acknowledgments

First I want to thank Jorien Druijf for al the energy she put in this research. Not only did she help me performing the assays but also her ability to give feedback and motivation helped me to complete this internship. (Dank je voor de geweldige tijd die we hier samen hebben gehad!)

If it was not for Peter Wilson I would not even have been in New Zealand for my research internship. I want to thank him for giving me the opportunity to work with deer, which was an unknown species to me, and for giving me the possibility to also do research in other areas. (Appendix V)

With the help of Nicholas Lopez-Villalobos and his never ending enthusiasm (even when I asked his help for the 5th time that day) I managed to complete the statistical part of this research. I can now say that I know a lot more about it, then when I finished my statistical course.

For all those moments I just needed to get away from the computer and out of the office to clear my head, I want to thank Geoff Purchas for informing me about the practical deer work that needed to be done at the Massey Deer Unit.

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Appendix I Research Proposal

Research Proposal:

Epidemiology and Evaluation of Diagnostic Parameters of Parasitism in Young Farmed Deer in New Zealand

A Collaborative Effort between AgResearch Invermay and Massey University

Participating Researchers:

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Massey University, Palmerston North

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Prof. Peter Wilson BVSc, PhD, MACVSc
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Institute of Veterinary, Animal and Biomedical Sciences
Massey University, Palmerston North

Dr. Marion Johnson BAgrSc, MSc, PhD
AgResearch Invermay

Dr. Colin Mackintosh BVSc, PhD
AgResearch Invermay

A study designed to:

- Investigate the effect of parasitism on the production of young growing deer
    - Determine the effect of parasitism, throughout the production cycle, on young deer using live-weight gain as an indicator of productivity

- Evaluate a number of diagnostic parameters of sub-clinical and clinical parasitism for their potential use in routine herd-health monitoring systems for future on- farm parasite control programs and research
    - These diagnostic tools include serum pepsinogen levels, serum albumin and total protein levels, eosinophil counts, faecal egg counts, faecal lungworm larvae counts, faecal larval cultures, acute phase proteins (haptoglobin and fibrinogen)

- Analyze the relationship between worm burdens, diagnostic markers of parasitism, and production effects

- Investigate the dynamics of parasite infections in young growing deer throughout a production cycle
    - Determine periods of seasonal change in faecal egg and larvae shedding
    - Evaluate changes in genera composition throughout the production cycle
    - Investigate the pathogenic nematode species responsible for sub-clinical effects leading to a loss of production

- Justification
    - In order to achieve rational and sustainable control of nematode parasites in the deer industry, comprehensive knowledge of the epidemiology of internal parasites and their interactions with the host is a prerequisite
    - Diagnostic and monitoring tools used to detect losses due to parasitism need to be evaluated before proper on-farm management decisions can be implemented
    - Evaluation of the nature of the pathogenic worm burdens, responsible for production losses, allows for improved control strategies aimed specifically at the parasites responsible for disease

Research Design:

- Longitudinal study
    - From January of 2007 to January of 2008
- Concentrating on young-stock population

Trial Design Idea:

- Research and commercial farms evaluated
    - Massey Deer Research Farm
    - Hindon farm located on South Island, ½ hour west of Invermay

On each farm:

- 2 treatment groups of weaner deer – grazed together on pasture
- All hinds will remain untreated with anthelmintic until weaning
    - Suppressively treated group (n=20)
        - This group of weaners will provide "worm free" comparison animals for evaluation of diagnostic parameters
        - 20 animals will be assigned to this group based on sex, liveweight gain and genotype on day 1 of the trial and will be representative of the entire group of weaners
        - Will be treated with a suppressive dose of anthelmintic; Moxidectin at 0.5 mg/kg every 4 weeks
            - These animals will be isolated for approximately 2 hours after administration of this pour-on anthelmintic to prevent spread to non-treated animals

    - Trigger-treated group – the rest of weaner herd will not be drenched until specific trigger criteria are met by **an individual animal** in the herd. Once an individual animal in the herd meets the trigger criteria, the entire mob will be treated with albendazole at 20 mg/kg
        - We will need approximately 80 animals to make up this trigger-treated herd. Consequently, a total of 100 weaners will be required for the trial (80 receiving trigger treatment and 20 receiving suppressive treatment).
        - A group of 20 animals from within the trigger treated group will be randomly selected based on live-weight, sex, genotype, initial faecal egg and initial faecal larvae counts at the beginning of the trial to be monitored as representative animals for comparison to the suppressive group.
        - These animals will be called the trigger-treated monitor group (n=20)

        - Trigger treatment:

            - Albendazole at 20 mg/kg will be administered to all animals in the trigger-treated group when:
                - (a). One animal from this group exhibits clinical signs of parasitism (coughing, scouring, respiratory distress) and/or a reduction of live-weight is observed (no net

gain or loss of bodyweight observed over a 2 week period) together with either i). lungworm larvae / g faeces exceeding 500 and/or ii). strongyle eggs / g faeces exceeding 500.

-OR-

  - (b). The trigger-treatment will apply when a statistically significant difference ($P<0.05$) in liveweight gain or liveweight is detected between the suppressively treated and the trigger-treated groups

- Sentinel animals:
  - Six sentinel animals will be sacrificed from the trigger-treated group once the trigger-treatment criteria have been met.
  - The six sentinel animals will be randomly chosen from the trigger-treatment group based on live-weight gain, sex, genotype, faecal egg counts, and faecal larvae counts. These sentinel animals will NOT be selected from the trigger treatment monitor group, rather they will be chosen from the rest of the trigger treatment herd and will be representative of the 20 animals in the monitor group.

NOTE: The research plan has budgeted for 4 kills a year. If the trigger-treatment criteria have not been met by the following time periods, 6 sentinel animals will be sacrificed from the trigger-treatment group at these times:

- Early April
- July
- Late September
- Early December

If the deer are sent to slaughter before these planned kill dates, every effort will be made to obtain samples from the slaughter facilities.

Research Methods:

- **Sentinel worm counts:**
  - 6 weaners from the trigger treatment herd will be sacrificed once the effects of parasitism are observed. These sentinel animals will provide us with information on actual worm burdens, which can be compared to production data along with data obtained to investigate markers of sub-clinical parasitism.
- **Faecal egg counts:**
  - Faecal egg counts will initially be performed every 2 weeks on both the suppressively treated group (n=20) and the trigger-treated monitor group(n=20). Once increased signs of parasitism are observed in the herd, faecal samples will be obtained and analyzed weekly. In addition, faecal egg counts will be performed on any sentinel deer prior to slaughter. Evaluation will be made according to a modified McMaster technique where a count of 1 egg is equivalent to 50 epg. Further evaluation may be performed using the FECPAK where a count of 1 egg is equal to 10 epg.
- **Faecal larvae counts:**
  - Faecal larvae counts will be performed every 2 weeks on both the suppressive treatment group (n=20) and the trigger-treated monitor group (n=20). Once increased signs of parasitism are observed in the herd, faecal samples will be obtained and analyzed weekly. In addition, faecal larvae counts will be obtained from any sentinel deer prior to slaughter. Evaluation will be made using a modified Baermann technique.
- **Faecal larval cultures:**
  - Pooled larval cultures from the trigger treated group will be performed every 2 weeks and larvae will be identified to the genus level.
- **Live-weight gain:**
  - All of the weaner deer in the herd will be weighed every 2 weeks. Once increased signs of parasitism are observed in the herd, all animals will be weighed weekly. Live-weight gain in terms of grams / day will be evaluated.
- **Blood haematology**:
  - Enough blood to fill two 10 ml purple-topped EDTA tube will be obtained from all deer in both the suppressive (n=20) and the trigger treated monitor group (n=20). In addition, blood haematology will be performed on any sentinel deer prior to slaughter. Blood samples will be obtained using jugular venipuncture every 4 weeks.
- **Serum Chemistry**
  - Enough blood to fill two 10 ml red-topped serum tube will be obtained from all deer in both the suppressive and the trigger treated monitor group. In addition, serum chemistry will be performed on any sentinel deer prior to slaughter. Blood samples will be obtained using jugular venipuncture every 4 weeks.
- **Molecular analysis:**
  - A database of material will be collected for future molecular analysis. Eggs, larvae, and adult worms will be placed in isopropyl alcohol solution for storage.
- **Histology:**
  - Small sections of the gastrointestinal tract will be collected and preserved in formulin for future histological evaluation.

- **Climate data:**
  - Weather conditions will be obtained for each monitor farm region. Weekly average high temperatures, low temperatures, rainfall, and humidity will be recorded throughout the trial period.

TIME TABLE:

- **Suppressive Group:**
  - Treat once every 4 weeks with Moxidectin at 0.5 mg/kg
  - Weigh every 2 weeks*
  - Faecal sample every 2 weeks*
  - Blood sample every 4 weeks

* Animals may need to be weighed and faecal samples may need to be obtained at weekly rather than bi-weekly intervals during periods of peak parasite burden

Date	**15/01/07**	**29/01/07**	**12/02/07**	**26/02/07**	**12/03/07**	**26/03/07**	**09/04/07**	**23/04/07**	**07/05/07**	**21/05/07**	**04/06/07**	**18/06/07**	**02/07/07**	**16/07/07**	**30/07/07**
No. in Suppressive Group	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Treat all in Group	X		X		X		X		X		X		X		X
Weigh	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Faecal sample	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood Sample		X		X		X		X		X		X		X	

Date	**13/08/07**	**27/08/07**	**10/09/07**	**24/09/07**	**08/10/07**	**22/10/07**	**05/11/07**	**19/11/07**	**03/12/07**	**17/12/07**
No. in Suppressive Group	20	20	20	20	20	20	20	20	20	20
Treat all in Group		X		X		X		X		X
Weigh	X	X	X	X	X	X	X	X	X	X
Faecal sample	X	X	X	X	X	X	X	X	X	X
Blood Sample	X		X		X		X		X	

Trigger treated monitor group:

- All animals in the herd will be drenched once an individual animal exhibits the trigger-treatment criteria
- 20 animals from the trigger-treated herd will be randomly chosen to be part of a trigger-treated monitor group. These animals will be weighed, faecal sampled, and blood sampled for comparison to the suppressively treated group
- In addition, all sentinel animals will be faecal sampled and blood sampled prior to slaughter
- Weigh every 2 weeks*
- Faecal sample every 2 weeks*
- Blood sample every 4 weeks

*Animals may need to be weighed and faecal samples may need to be obtained at weekly rather than bi-weekly intervals during periods of peak parasite burden

Date	**15/01/07**	**29/01/07**	**12/02/07**	**26/02/07**	**12/03/07**	**26/03/07**	**09/04/07**	**23/04/07**	**07/05/07**	**21/05/07**	**04/06/07**	**18/06/07**	**02/07/07**	**16/07/07**	**30/07/07**
No. in Trigger-Treatment Monitor Group	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Weigh	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Faecal sample	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood Sample		X		X		X		X		X		X		X	

Date	**13/08/07**	**27/08/07**	**10/09/07**	**24/09/07**	**08/10/07**	**22/10/07**	**05/11/07**	**19/11/07**	**03/12/07**	**17/12/07**
No. in Trigger Treatment Monitor Group	20	20	20	20	20	20	20	20	20	20
Weigh	X	X	X	X	X	X	X	X	X	X
Faecal sample	X	X	X	X	X	X	X	X	X	X
Blood Sample	X		X		X		X		X	

GENERAL BUDGET FOR SOUTH ISLAND HINDON FARM:

Epidemiology and Evaluation of Diagnostic Parameters of Parasitism in Young Farmed Deer in New Zealand

Item	**Cost per Item**	**Quantity Needed per Farm**	**Total Cost per Item**
DEER			
*Weaner Deer	$300 / deer	24 deer	$7200
LAB TESTS			
Serum total protein / albumin	$10 / sample	504 samples	$5040
Automated WBC differential – for eosinophil count	$15 / sample	504 samples	$7560
Haptoglobin, fibrinogen, plasma viscosity	$20 / sample	504 samples	$10,080
TRAVEL			
Travel to and from farm	$100 / visit	25 visits	$2500
COURIER COSTS			
Faeces – Inv to Mas Plasma – Inv to Mas	$38.50 / trip	25 trips	$962.50
Guts and lungs – Inv to Mas	$462 / trip	4 trips	$1848
CONSUMABLES			
Moxidectin Pour-On	$110 / L	2 liter	$220
Albendazole	$200 / L	4 liter	$800
Serum collection tubes	$24 / box of 100	1200 tubes	$288
EDTA collection tubes	$30 / box of 100	1200 tubes	$360
Vacutainer needles	$23 / box of 100	1200 needles	$276
Specimen containers for faecal samples and for starage of material for histopathology and molecular database	$98 / bag of 500	1500 containers	$294
Latex gloves for faecal collection	$6 / box of 100	500 gloves	$30
Ependorf tubes for storing serum samples	$33 / bag of 1000	1000 tubes	$33
TOTAL COST FOR SOUTH ISLAND FARM			$37,491.50 ($30,291.50 if weaner deer donated)

*Budgeting for 4 slaughter periods, 6 sentinel animals slaughtered at each period. These deer may be donated by Hindon Farm on South Island.

GENERAL BUDGET FOR NORTH ISLAND MASSEY UNIVERSITY FARM: Epidemiology and Evaluation of Diagnostic Parameters of Parasitism in Young Farmed Deer in New Zealand

Item	Cost per Item	Quantity Needed per Farm	Total Cost per Item
DEER			
*Weaner Deer	$300 / deer	24 deer	$7200
LAB TESTS			
Serum total protein / albumin	$10 / sample	504 samples	$5040
Automated WBC differential – for eosinophil count	$15 / sample	504 samples	$7560
Haptoglobin, fibrinogen, plasma viscosity	$20 / sample	504 samples	$10,080
COURIER COSTS			
Plasma and Serum – Mas to Inv	$38.50 / trip	12 trips	$462
CONSUMABLES			
Moxidectin Pour-On	$110 / L	2 liter	$220
Albendazole	$200 / L	4 liter	$800
Serum collection tubes	$24 / box of 100	1200 tubes	$288
EDTA collection tubes	$30 / box of 100	1200 tubes	$360
Vacutainer needles	$23 / box of 100	1200 needles	$276
Specimen containers for faecal samples and for storage of histopathology and molecular samples for database	$98 / bag of 500	1500 containers	$294
Latex gloves for faecal collection	$6 / box of 100	500 gloves	$30
Ependorf tubes for storing serum samples	$33 / bag of 1000	1000 tubes	$33
Screw topped tubes to send plasma and serum for viscosity / fibrinogen / and haptoglobin analysis		504 tubes to hold approximately 1 ml serum 504 tubes to hold 5 to 6 mls plasma	
TOTAL COST FOR NOUTH ISLAND FARM			$32,643

*Budgeting for 4 slaughter periods, 6 sentinel animals slaughtered at each period

Other expenses not included:

- Slaughter costs – will most likely equal gain from carcasses
- Overhead costs – estimated $6000 to $8000 per trial
- Purchase of reagents required to run pepsinogen assay
- Ear tags – to label suppressive treatment groups
- Freezer – for storing organs, serum, etc. until processing - $500.00

Notes:

Order screw-topped tubes for plasma samples to be sent for fibrinogen and viscosity
Liquid EDTA tubes preferable

<u>Things to discuss / add:</u>

- BUDGET (new for s. vs n. island) also marion's budget comments
- Option; not performing all blood tests once a month to save on costs (maybe some performed once q. 2 months)
- Ideal treatment for weaners and hinds on Hindon Farm
- Perform drench efficiency study at some point before the trial (talk to Simone about best time to evaluate)
- Talk to FECPAK / Ancare about donating drenches
- Need to discuss arrangements w. Martin Regarding buying in new animals, cost of farm, etc.

Appendix II Data

1=female, 2 = male, 1 = suppressive group, 2 = monitor trigger treatment group

			Pepsinogen (iU)					
deer ID	Sex	Group	29/01/07	26/03/07	23/04/07	21/05/07	16/07/07	02/12/07
611	1	1	-0.11	0.10	0.11	-0.01	0.13	0.10
615	1	1	0.08	0.10	0.12	-0.27	0.19	0.15
616	2	1	-0.23	0.26	0.06	0.19	0.01	0.10
617	2	1	0.04	0.13	0.09	0.18	0.19	0.16
626	1	1	-0.08	0.16	0.00	0.18	0.20	0.09
628	2	1	0.09	0.10	0.19	-0.44	0.17	0.16
633	1	1	0.39	-0.40	0.19	0.14	0.01	0.21
636	1	1	-0.27					
638	1	1	0.10	0.22	0.04	-0.03	0.33	0.19
640	2	1	-0.36	-0.27	0.38	0.13	0.08	0.02
644	2	1	0.47	0.20	0.16	0.02	0.10	0.05
649	2	1	0.04	0.29	0.15	0.79	0.14	0.06
655	1	1	-0.32	0.05	0.00	0.05	0.10	0.00
657	2	1	-0.02	-0.26	-0.36	0.07	0.12	0.08
659	1	1	0.29	0.17	0.27	0.05	0.25	0.11
662	1	1	0.21	0.24	-0.03	0.31	0.03	0.11
669	2	1	-0.18	-0.28	-0.08	0.12	0.11	0.13
674	1	1	-0.43	-0.54	-0.09	-0.15	0.08	0.06
676	2	1	-0.15	-0.08	-0.06	0.15	0.01	0.10
677	2	1	0.27	0.24	0.09	0.11	0.14	0.15
		Mean	**-0.01**	**0.02**	**0.06**	**0.08**	**0.13**	**0.11**
			-8.14	22.45	64.54	82.81	125.79	106.84
605	2	2	0.49	0.12	0.04	0.07	0.17	0.14
610	2	2	0.17	0.05	-0.46	0.02	0.06	0.07
613	1	2	0.14	0.23	0.23	0.20	0.50	0.19
614	2	2	0.67	0.07	0.01	0.20	0.36	0.11
619	2	2	0.28	-0.03	0.05	0.36	0.22	0.09
620	1	2	0.50	0.25	0.13	0.23	0.30	0.57
621	1	2	0.30	-0.13	-0.14	0.03	0.19	0.55
623	2	2	-0.07	-0.49	-0.10	0.41	0.11	0.11
627	1	2	0.89	0.18	0.25	0.17	0.20	0.44
632	1	2	0.01					
634	1	2	-0.11	0.13	0.09	0.36	0.16	0.15
641	2	2	-0.16	0.08	0.11	0.12	0.28	0.36
642	1	2	0.13	0.42	0.23	0.00	0.09	0.08
652	1	2	0.04	0.76	-0.28	0.15	0.20	0.14
656	2	2	-0.19	-0.79	-0.04	0.34	0.21	0.02
661	2	2	-0.18	0.10	0.00	0.31	0.13	0.03
664	1	2	0.15	-0.50	0.08	0.11	0.06	0.35
668	2	2	0.07	0.12	0.24	0.51	0.27	0.08
672	1	2	-0.13	-0.24	0.17	0.19	0.31	0.49
683	2	2	0.24	-0.22	0.09	-0.25	0.17	0.26
		Mean	**0.16**	**0.01**	**0.04**	**0.19**	**0.21**	**0.22**
			162.48	5.09	36.44	186.23	210.00	222.63

1=female, 2 = male, 1 = suppressive group, 2 = monitor trigger treatment group

deer ID	Sex	Group	Pepsinogen (iU) negatives replaced by zero 29/01/07	26/03/07	23/04/07	21/05/07	16/07/07	2/12/07
611	1	1	0.00	0.10	0.11	0.00	0.13	0.10
615	1	1	0.08	0.10	0.12	0.00	0.19	0.15
616	2	1	0.00	0.26	0.06	0.19	0.01	0.10
617	2	1	0.04	0.13	0.09	0.18	0.19	0.16
626	1	1	0.00	0.16	0.00	0.18	0.20	0.09
628	2	1	0.09	0.10	0.19	0.00	0.17	0.16
633	1	1	0.39	0.00	0.19	0.14	0.01	0.21
636	1	1	0.00					
638	1	1	0.10	0.22	0.04	0.00	0.33	0.19
640	2	1	0.00	0.00	0.38	0.13	0.08	0.02
644	2	1	0.47	0.20	0.16	0.02	0.10	0.05
649	2	1	0.04	0.29	0.15	0.79	0.14	0.06
655	1	1	0.00	0.05	0.00	0.05	0.10	0.00
657	2	1	0.00	0.00	0.00	0.07	0.12	0.08
659	1	1	0.29	0.17	0.27	0.05	0.25	0.11
662	1	1	0.21	0.24	0.00	0.31	0.03	0.11
669	2	1	0.00	0.00	0.00	0.12	0.11	0.13
674	1	1	0.00	0.00	0.00	0.00	0.08	0.06
676	2	1	0.00	0.00	0.00	0.15	0.01	0.10
677	2	1	0.27	0.24	0.09	0.11	0.14	0.15
		Mean	**0.10**	**0.12**	**0.10**	**0.13**	**0.13**	**0.11**
			99.21	118.76	97.17	130.18	125.79	106.84
605	2	2	0.49	0.12	0.04	0.07	0.17	0.14
610	2	2	0.17	0.05	0.00	0.02	0.06	0.07
613	1	2	0.14	0.23	0.23	0.20	0.50	0.19
614	2	2	0.67	0.07	0.01	0.20	0.36	0.11
619	2	2	0.28	0.00	0.05	0.36	0.22	0.09
620	1	2	0.50	0.25	0.13	0.23	0.30	0.57
621	1	2	0.30	0.00	0.00	0.03	0.19	0.55
623	2	2	0.00	0.00	0.00	0.41	0.11	0.11
627	1	2	0.89	0.18	0.25	0.17	0.20	0.44
632	1	2	0.01					
634	1	2	0.00	0.13	0.09	0.36	0.16	0.15
641	2	2	0.00	0.08	0.11	0.12	0.28	0.36
642	1	2	0.13	0.42	0.23	0.00	0.09	0.08
652	1	2	0.04	0.76	0.00	0.15	0.20	0.14
656	2	2	0.00	0.00	0.00	0.34	0.21	0.02
661	2	2	0.00	0.10	0.00	0.31	0.13	0.03
664	1	2	0.15	0.00	0.08	0.11	0.06	0.35
668	2	2	0.07	0.12	0.24	0.51	0.27	0.08
672	1	2	0.00	0.00	0.17	0.19	0.31	0.49
683	2	2	0.24	0.00	0.09	0.00	0.17	0.26
		Mean	**0.20**	**0.13**	**0.09**	**0.20**	**0.21**	**0.22**
			204.48	131.41	90.12	199.39	210.00	222.63

Weight
1=female, 2 = male, 1 = suppressive group, 2 = monitor trigger treatment group

			Weight (kg)							
deer ID	Sex	Group	11-Jan	15/01	29-Jan	12-Feb	19-Feb	26-Feb	12-Mar	26-Mar
611	1	1	20.5	23.0	29.0	34.5	36.5	39.0	41.5	42
615	1	1	28.0	29.5	34.5	39.5	38.5	44.0	47	47
616	2	1	30.5	32.0	37.5	43.5	46.5	47.5	50.5	51
617	2	1	22.5	25.0	28.0	37.5	40.5	43.5	45.5	48
626	1	1	29.0	31.0	36.0	42.0	44.0	46.0	49.5	51.5
628	2	1	29.5	31.5	36.5	43.0	45.0	47.0	50.5	50.5
633	1	1	24.5	26.0	30.0	36.5	38.5	40.5	44	45
636	1	1	25.5	26.5	29.0	31.5	32.0	32.5	.	.
638	1	1	26.0	26.0	32.0	38.0	40.0	41.5	45	45
640	2	1	35.5	38.0	43.5	50.5	54.5	56.0	60	60.5
644	2	1	23.0	25.0	29.5	34.5	36.0	39.0	42	43.5
649	2	1	36.5	38.5	44.5	51.5	54.0	56.0	57.5	60.5
655	1	1	28.5	31.0	36.0	42.0	43.5	47.5	50.5	51
657	2	1	26.5	29.0	35.0	42.0	44.5	47.5	48.5	49
659	1	1	26.0	28.0	32.5	39.5	42.0	42.5	45	47.5
662	1	1	32.5	35.0	40.0	46.0	49.0	50.5	52.5	55.5
669	2	1	23.5	26.0	31.5	37.5	41.0	44.0	46	47.5
674	1	1	35.0	37.4	43.0	50.0	53.0	53.5	58.5	57
676	2	1	31.5	34.0	39.5	45.5	48.0	50.5	54.5	53.5
677	2	1	33.0	35.5	40.5	48.0	51.5	54.5	56.5	57.5
605	2	2	24.5	27.0	32.5	39.5	42.0	45.0	48.5	47
610	2	2	35.0	37.5	43.0	51.0	53.0	56.0	58	59
613	1	2	29.0	30.5	34.5	39.5	42.0	44.5	46	45.5
614	2	2	33.5	36.0	41.0	47.5	50.5	53.0	55.5	56.5
619	2	2	28.5	28.5	36.5	43.0	44.5	49.5	50.5	50
620	1	2	31.0	33.5	38.5	45.0	47.5	49.5	54	54
621	1	2	31.0	33.5	37.5	43.5	46.0	47.5	50.5	51
623	2	2	29.5	32.0	37.5	44.5	47.0	50.5	53	54
627	1	2	26.0	28.5	33.0	39.0	41.0	43.0	45	45.5
632	1	2	25.5	28.5	31.5	35.0	38.0	39.0	.	.
634	1	2	30.0	32.0	36.5	42.5	44.5	47.0	49.5	50
641	2	2	30.5	32.5	37.0	43.0	44.0	47.0	50	49
642	1	2	21.5	24.0	30.0	36.0	39.0	41.5	43.5	44
652	1	2	34.5	37.0	41.5	47.5	50.0	51.0	53.5	53.5
656	2	2	37.0	40.0	46.0	53.5	57.0	60.5	63.5	64
661	2	2	23.0	25.5	31.0	38.0	40.5	43.5	46.5	47
664	1	2	29.5	32.5	36.0	42.5	44.5	46.5	50	49
668	2	2	32.5	35.5	41.0	47.5	50.5	54.0	57	57
672	1	2	24.5	27.0	32.0	38.0	40.0	42.5	44.5	44.5
683	2	2	28.0	30.5	34.0	40.0	42.0	45.0	46.5	47.5

deer ID	weight (kg) 02/04	9-Apr	23-Apr	07/05	21-May	4-Jun	18-Jun	2-Jul	16-Jul	30-Jul	13-Aug
611	43.5	43.5	48	50.5	51.5	56.5	55.5	54.5	53	55.5	56
615	46.5	48	50.5	53	53.5	55.5	55	56.5	56	57	57.5
616	51.5	53.5	55	58	59.5	63	62.5	65	64	67	69
617	48.5	50.5	54	56.5	55.5	62	63	63	64	65.5	67.5
626	52	53.5	55.5	59	61	63.5	62	64	62.5	64.5	65
628	50.5	53.5	56	59.5	61	64	65	66	65	67.5	69
633	44.5	48	48.5	51	53	56	54	56.5	54.5	56.5	56
636	.	.	.	.	.	.	.	.	.	.	.
638	45.5	47.5	50	52.5	53.5	55.5	55.5	57	56.5	57	57.5
640	59	60	65	66	66	72	71	74.5	75	76	77.5
644	43.5	44.5	48.5	51.5	52.5	56.5	57.5	59	60	62.5	64
649	62	64.5	68.5	71	74	78	77.5	79.5	81	84	86.5
655	53.5	54	57	60.5	64	67	66	71	69	71	73
657	49	50.5	52.5	55.5	54	57.5	56	58	59.5	60.5	63.5
659	47.5	49.5	53	54.5	55	59.5	58.5	61	61.5	62.5	63.5
662	54.5	58	60.5	64	66	70	64	70.5	69.5	72	74
669	47	49	52	55	57.5	60	60.5	64.5	65.5	69	70.5
674	57	59.5	62	66	65.5	71.5	70	70.5	70	78	72.5
676	54	55.5	58.5	61	62	63.5	64.5	66.5	65	68	69.5
677	57.5	60.5	63.5	64	68	74	73	74.5	75.5	76.5	78.5

605	47.5	48.5	51	50.5	52.5	57.5	58	55.5	56.5	59	61
610	59	61	65.5	66	68	70.5	70	72.5	73.5	75	76.5
613	46	49	53	54	53.5	56	57	59.5	57	60	61.5
614	58	60	63	66	68	70.5	68.5	71	71	73	73.5
619	49.5	51.5	54.5	56.5	59.5	61.5	62.5	62.5	64.5	66.5	68.5
620	54.5	57	59	60	63	65.5	63.5	66.5	63	67.5	67
621	51	52	55	58.5	59.5	62.5	61	63	61.5	62.5	63.5
623	52	56	58.5	57	58	63	63	63	62	65	66.5
627	46.5	48	50.5	53.5	54.5	57.5	55.5	57	56.5	58.5	60
632	.	.	.	.	.	.	.	.	.	.	.
634	51.5	53.5	56.5	58	60	62.5	62	62	60.5	62	62
641	49.5	50	52	52	55	56.5	55	60.5	60	61	62.5
642	44.5	46.5	49.5	51	51	55.5	55	56	55.5	58.5	59.5
652	54.5	57.5	60.5	61	62	63.5	62.5	63	60.5	62	63.5
656	64	69	73	74.5	71.5	78	76.5	79	78	82.5	82.5
661	47.5	50	53.5	55.5	56	60	61.5	60.5	61	63	65
664	50	52.5	54.5	57	58.5	61.5	61.5	62	62	64	66
668	57.5	59.5	64	65.5	61.5	62.5	62	64	64	69.5	73
672	44.5	46	48	50	51.5	54.5	53.5	53.5	51.5	54.5	55.5
683	47	49.5	53	55	54.5	59	61	62.5	60	62.5	66.5

deer ID	Weight (kg) 27/08	10-Sep	24-Sep	8-Oct	23-Oct	5-Nov	19-Nov	2-Dec
611	58	60.5	61.5	64	68	70	73.5	76
615	60	62	63.5	65	69	71	72.5	75.5
616	73.5	76	76	74.5	79	82.5	88	89
617	72	75	77.5	83.5	88.5	92	93.5	95.5
626	68	68	72	72	76.5	78	81	82
628	71	74.5	80	81.5	86.5	89	90.5	94.5
633	58	61.5	62	63	68	68.5	71.5	73
636	.	.	.	.	.	.	.	.
638	60.5	62.5	64	64	68	71.5	72	73.5
640	82.5	87	90.5	92.5	99	101.5	104	106.5
644	68.5	71	75	79	86.5	87	93.5	98
649	92	94	98	100.5	108.5	110	112.5	118.5
655	74	77	75	78.5	84.5	86.5	89	88.5
657	68.5	70.5	71.5	76	82.5	87	89	91.5
659	65	68	68	70.5	75.5	75.5	80.5	81.5
662	78	79	80	83	90.5	91	94	95.5
669	74	77	80.5	83	90	94	95	97
674	73.5	76.5	78.5	80	84	88.5	89.5	91.5
676	72	76.5	78.5	79.5	86	90	91.5	95
677	81.5	83.5	88	89	95.5	101	103	106.5
605	61	67	68.5	74.5	79	82.5	88.5	92
610	79.5	85	85	90	97.5	102	103.5	111.5
613	62.5	63.5	66	68	72.5	74.5	76.5	78.5
614	75	79	83.5	85.5	94	95	93	97
619	70	78.5	81	80	85.5	87	91.5	93.5
620	69.5	71.5	74	76.5	81	83	86.5	87.5
621	66	67	69.5	71.5	77	79.5	81	83.5
623	68.5	76.5	81	80.5	86	87	92	95.5
627	61.5	64	64	64.5	70	71.5	75	77.5
632	.	.	.	.	.	.	.	.
634	64	66.5	68.5	70	75	79.5	84.5	83.5
641	65.5	68.5	73	75.5	82	85	88.5	88.5
642	61	63	64	68	71	75	78	78
652	64.5	65	68	68	71.5	73.5	75	77.5
656	84.5	92	97	97.5	105.5	109	111.5	114
661	66.5	68	71	74.5	80	85	88.5	91.5
664	67	70	72.5	74	77	79	82	84.5
668	77.5	81.5	84.5	88.5	95.5	97.5	102	106.5
672	56.5	58.5	61	62.5	67.5	67.5	69.5	71.5
683	69	72	74.5	78.5	82.5	85.5	89	90

deer ID	Live weight gain (g/day) 11/01 to 15	15/01 to 29	11/01 to 29/01	29/01 to 12/02	12/02 to 19/02	12/02 to 26/02
611	625.0	428.6	526.8	392.9	285.7	321.4
615	375.0	357.1	366.1	357.1	-142.9	321.4
616	375.0	392.9	383.9	428.6	428.6	285.7
617	625.0	214.3	419.6	678.6	428.6	428.6
626	500.0	357.1	428.6	428.6	285.7	285.7
628	500.0	357.1	428.6	464.3	285.7	285.7
633	375.0	285.7	330.4	464.3	285.7	285.7
636	250.0	178.6	214.3	178.6	71.4	71.4
638	0.0	428.6	214.3	428.6	285.7	250.0
640	625.0	392.9	508.9	500.0	571.4	392.9
644	500.0	321.4	410.7	357.1	214.3	321.4
649	500.0	428.6	464.3	500.0	357.1	321.4
655	625.0	357.1	491.1	428.6	214.3	392.9
657	625.0	428.6	526.8	500.0	357.1	392.9
659	500.0	321.4	410.7	500.0	357.1	214.3
662	625.0	357.1	491.1	428.6	428.6	321.4
669	625.0	392.9	508.9	428.6	500.0	464.3
674	600.0	400.0	500.0	500.0	428.6	250.0
676	625.0	392.9	508.9	428.6	357.1	357.1
677	625.0	357.1	491.1	535.7	500.0	464.3
	505.0	357.5	431.3	446.4	325.0	321.4
605	625.0	392.9	508.9	500.0	357.1	392.9
610	625.0	392.9	508.9	571.4	285.7	357.1
613	375.0	285.7	330.4	357.1	357.1	357.1
614	625.0	357.1	491.1	464.3	428.6	392.9
619	0.0	571.4	285.7	464.3	214.3	464.3
620	625.0	357.1	491.1	464.3	357.1	321.4
621	625.0	285.7	455.4	428.6	357.1	285.7
623	625.0	392.9	508.9	500.0	357.1	428.6
627	625.0	321.4	473.2	428.6	285.7	285.7
632	750.0	214.3	482.1	250.0	428.6	285.7
634	500.0	321.4	410.7	428.6	285.7	321.4
641	500.0	321.4	410.7	428.6	142.9	285.7
642	625.0	428.6	526.8	428.6	428.6	392.9
652	625.0	321.4	473.2	428.6	357.1	250.0
656	750.0	428.6	589.3	535.7	500.0	500.0
661	625.0	392.9	508.9	500.0	357.1	392.9
664	750.0	250.0	500.0	464.3	285.7	285.7
668	750.0	392.9	571.4	464.3	428.6	464.3
672	625.0	357.1	491.1	428.6	285.7	321.4
683	625.0	250.0	437.5	428.6	285.7	357.1
	593.8	351.8	472.8	448.2	339.3	357.1

deer ID	lwg (g/day) 26/02 to 12/0	lwg (g/day) 12/03 to 26/0	lwg (g/day 29/01 to 26/0	lwg (g/day) 26/03 to 02/04	lwg (g/day) 26/03 to 09/04	lwg (g/day) 09/04 to23/04
611	178.6	35.7	304.2	214.3	107.1	321.4
615	214.3	0.0	227.5	-71.4	71.4	178.6
616	214.3	35.7	334.8	71.4	178.6	107.1
617	142.9	178.6	412.4	71.4	178.6	250.0
626	250.0	142.9	336.5	71.4	142.9	142.9
628	250.0	0.0	319.0	0.0	214.3	178.6
633	250.0	71.4	331.7	-71.4	214.3	35.7
636	.	.	239.4	.	.	.
638	250.0	0.0	308.7	71.4	178.6	178.6
640	285.7	35.7	404.3	-214.3	-35.7	357.1
644	214.3	107.1	309.7	0.0	71.4	285.7
649	107.1	214.3	358.2	214.3	285.7	285.7
655	214.3	35.7	323.5	357.1	214.3	214.3
657	71.4	35.7	335.7	0.0	107.1	142.9
659	178.6	178.6	347.9	0.0	142.9	250.0
662	142.9	214.3	366.3	-142.9	178.6	178.6
669	142.9	107.1	385.3	-71.4	107.1	214.3
674	357.1	-107.1	350.4	0.0	178.6	178.6
676	285.7	-71.4	338.9	71.4	142.9	214.3
677	142.9	71.4	398.5	0.0	214.3	214.3
	204.9	67.7	336.6	30.1	152.3	206.8
605	250.0	-107.1	333.0	71.4	107.1	178.6
610	142.9	71.4	339.8	0.0	142.9	321.4
613	107.1	-35.7	292.6	71.4	250.0	285.7
614	178.6	71.4	358.3	214.3	250.0	214.3
619	71.4	-35.7	299.6	-71.4	107.1	214.3
620	321.4	0.0	347.4	71.4	214.3	142.9
621	214.3	35.7	323.7	0.0	71.4	214.3
623	178.6	71.4	359.8	-285.7	142.9	178.6
627	142.9	35.7	300.9	142.9	178.6	178.6
632	.	.	399.1	.	.	.
634	178.6	35.7	314.0	214.3	250.0	214.3
641	214.3	-71.4	273.5	71.4	71.4	142.9
642	142.9	35.7	345.1	71.4	178.6	214.3
652	178.6	0.0	311.0	142.9	285.7	214.3
656	214.3	35.7	407.0	0.0	357.1	285.7
661	214.3	35.7	360.2	71.4	214.3	250.0
664	250.0	-71.4	313.0	142.9	250.0	142.9
668	214.3	0.0	373.2	71.4	178.6	321.4
672	142.9	0.0	308.4	0.0	107.1	142.9
683	107.1	71.4	322.2	-71.4	142.9	250.0
	182.3	9.4	334.1	48.9	184.2	216.2

deer ID	lwg (g/day) 23/04 to 21/05	lwg (g/day) 21/05 to 04/06	lwg (g/day) 04/06 to 18/06	lwg (g/day) 18/06 to 02/07	lwg (g/day) 02/07 to 16/07	lwg (g/day) 21/05 to 16/07
611	125.0	357.1	-71.4	-71.4	-107.1	26.8
615	107.1	142.9	-35.7	107.1	-35.7	44.6
616	160.7	250.0	-35.7	178.6	-71.4	80.4
617	53.6	464.3	71.4	0.0	71.4	151.8
626	196.4	178.6	-107.1	142.9	-107.1	26.8
628	178.6	214.3	71.4	71.4	-71.4	71.4
633	160.7	214.3	-142.9	178.6	-142.9	26.8
636		.	.	.	.	
638	125.0	142.9	0.0	107.1	-35.7	53.6
640	35.7	428.6	-71.4	250.0	35.7	160.7
644	142.9	285.7	71.4	107.1	71.4	133.9
649	196.4	285.7	-35.7	142.9	107.1	125.0
655	250.0	214.3	-71.4	357.1	-142.9	89.3
657	53.6	250.0	-107.1	142.9	107.1	98.2
659	71.4	321.4	-71.4	178.6	35.7	116.1
662	196.4	285.7	-428.6	464.3	-71.4	62.5
669	196.4	178.6	35.7	285.7	71.4	142.9
674	125.0	428.6	-107.1	35.7	-35.7	80.4
676	125.0	107.1	71.4	142.9	-107.1	53.6
677	160.7	428.6	-71.4	107.1	71.4	133.9
	140.0	272.6	-54.5	154.1	-18.8	88.3
605	53.6	357.1	35.7	-178.6	71.4	71.4
610	89.3	178.6	-35.7	178.6	71.4	98.2
613	17.9	178.6	71.4	178.6	-178.6	62.5
614	178.6	178.6	-142.9	178.6	0.0	53.6
619	178.6	142.9	71.4	0.0	142.9	89.3
620	142.9	178.6	-142.9	214.3	-250.0	0.0
621	160.7	214.3	-107.1	142.9	-107.1	35.7
623	-17.9	357.1	0.0	0.0	-71.4	71.4
627	142.9	214.3	-142.9	-35.7	107.1	35.7
632		.	.	.	.	
634	125.0	178.6	-35.7	0.0	-107.1	8.9
641	107.1	107.1	-107.1	392.9	-35.7	89.3
642	53.6	321.4	-35.7	71.4	-35.7	80.4
652	53.6	107.1	-71.4	35.7	-178.6	-26.8
656	-53.6	464.3	-107.1	178.6	-71.4	116.1
661	89.3	285.7	107.1	-71.4	35.7	89.3
664	142.9	214.3	0.0	35.7	0.0	62.5
668	-89.3	71.4	-35.7	142.9	0.0	44.6
672	125.0	214.3	-71.4	0.0	-142.9	0.0
683	53.6	321.4	142.9	107.1	-178.6	98.2
	81.8	225.6	-32.0	82.7	-48.9	56.9

deer ID	lwg (g/day) 21/05 to 16/0	lwg (g/day) 16/07 to 30/0	lwg (g/day) 30/07 to 13/0	lwg (g/day) 13/08 to 27/08	lwg (g/day) 27/08 to 10/09	lwg (g/day) 10/09 to 24/09
611	26.8	178.6	35.7	142.9	178.6	71.4
615	44.6	71.4	35.7	178.6	142.9	107.1
616	80.4	214.3	142.9	321.4	178.6	0.0
617	151.8	107.1	142.9	321.4	214.3	178.6
626	26.8	142.9	35.7	214.3	0.0	285.7
628	71.4	178.6	107.1	142.9	250.0	392.9
633	26.8	142.9	-35.7	142.9	250.0	35.7
636		.	.	.	.	.
638	53.6	35.7	35.7	214.3	142.9	107.1
640	160.7	71.4	107.1	357.1	321.4	250.0
644	133.9	178.6	107.1	321.4	178.6	285.7
649	125.0	214.3	178.6	392.9	142.9	285.7
655	89.3	142.9	142.9	71.4	214.3	-142.9
657	98.2	71.4	214.3	357.1	142.9	71.4
659	116.1	71.4	71.4	107.1	214.3	0.0
662	62.5	178.6	142.9	285.7	71.4	71.4
669	142.9	250.0	107.1	250.0	214.3	250.0
674	80.4	571.4	-392.9	71.4	214.3	142.9
676	53.6	214.3	107.1	178.6	321.4	142.9
677	133.9	71.4	142.9	214.3	142.9	321.4
	88.3	163.5	75.2	225.6	186.1	150.4
605	71.4	178.6	142.9	0.0	428.6	107.1
610	98.2	107.1	107.1	214.3	392.9	0.0
613	62.5	214.3	107.1	71.4	71.4	178.6
614	53.6	142.9	35.7	107.1	285.7	321.4
619	89.3	142.9	142.9	107.1	607.1	178.6
620	0.0	321.4	-35.7	178.6	142.9	178.6
621	35.7	71.4	71.4	178.6	71.4	178.6
623	71.4	214.3	107.1	142.9	571.4	321.4
627	35.7	142.9	107.1	107.1	178.6	0.0
632		.	.	.	.	.
634	8.9	107.1	0.0	142.9	178.6	142.9
641	89.3	71.4	107.1	214.3	214.3	321.4
642	80.4	214.3	71.4	107.1	142.9	71.4
652	-26.8	107.1	107.1	71.4	35.7	214.3
656	116.1	321.4	0.0	142.9	535.7	357.1
661	89.3	142.9	142.9	107.1	107.1	214.3
664	62.5	142.9	142.9	71.4	214.3	178.6
668	44.6	392.9	250.0	321.4	285.7	214.3
672	0.0	214.3	71.4	71.4	142.9	178.6
683	98.2	178.6	285.7	178.6	214.3	178.6
	56.9	180.5	103.4	133.5	253.8	186.1

deer ID	lwg (g/day) 24/09 to 08/10	lwg (g/day) 08/10 to 23/1	lwg (g/day) 23/10 to 05/1	lwg (g/day) 5/11to 19/11	lwg (g/day) 19/11 to 02/1	lwg (g/day) 16/07 to 2/	Total lwg (g/day) 11-01to02-
611	178.6	285.7	142.9	250.0	178.6	204.9	170.25
615	107.1	285.7	142.9	107.1	214.3	182.5	145.71
616	-107.1	321.4	250.0	392.9	71.4	218.3	179.45
617	428.6	357.1	250.0	107.1	142.9	260.6	223.93
626	0.0	321.4	107.1	214.3	71.4	183.5	162.58
628	107.1	357.1	178.6	107.1	285.7	248.6	199.39
633	71.4	357.1	35.7	214.3	107.1	177.7	148.77
636	.	.	.	.	.		.
638	0.0	285.7	250.0	35.7	107.1	168.4	145.71
640	142.9	464.3	178.6	178.6	178.6	262.7	217.79
644	285.7	535.7	35.7	464.3	321.4	305.3	230.06
649	178.6	571.4	107.1	178.6	428.6	302.5	251.53
655	250.0	428.6	142.9	178.6	-35.7	186.2	184.05
657	321.4	464.3	321.4	142.9	178.6	267.5	199.39
659	178.6	357.1	0.0	357.1	71.4	189.8	170.25
662	214.3	535.7	35.7	214.3	107.1	229.0	193.25
669	178.6	500.0	285.7	71.4	142.9	265.4	225.46
674	107.1	285.7	321.4	71.4	142.9	200.9	173.31
676	71.4	464.3	285.7	107.1	250.0	256.3	194.79
677	71.4	464.3	392.9	142.9	250.0	262.8	225.46
	146.6	402.3	182.3	186.1	169.2	230.2	0.00
							0.00
							0.00
							0.00
							0.00
605	428.6	321.4	250.0	428.6	250.0	285.5	207.06
610	357.1	535.7	321.4	107.1	571.4	302.2	234.66
613	142.9	321.4	142.9	142.9	142.9	195.3	151.84
614	142.9	607.1	71.4	-142.9	285.7	224.6	194.79
619	-71.4	392.9	107.1	321.4	142.9	244.6	199.39
620	178.6	321.4	142.9	250.0	71.4	215.5	173.31
621	142.9	392.9	178.6	107.1	178.6	199.3	161.04
623	-35.7	392.9	71.4	357.1	250.0	274.2	202.45
627	35.7	392.9	107.1	250.0	178.6	193.4	157.98
632	.	.	.	.	.		.
634	107.1	357.1	321.4	357.1	-71.4	207.0	164.11
641	178.6	464.3	214.3	250.0	0.0	243.3	177.91
642	285.7	214.3	285.7	214.3	0.0	204.5	173.31
652	0.0	250.0	142.9	107.1	178.6	169.7	131.90
656	35.7	571.4	250.0	178.6	178.6	293.4	236.20
661	250.0	392.9	357.1	250.0	214.3	258.1	210.12
664	107.1	214.3	142.9	214.3	178.6	206.5	168.71
668	285.7	500.0	142.9	321.4	321.4	336.7	226.99
672	107.1	357.1	0.0	142.9	142.9	191.0	144.17
683	285.7	285.7	214.3	250.0	71.4	256.9	190.18

1=female, 2 = male, 1 = suppressive group, 2 = monitor trigger treatment group

			Egg counts epg						
Deer ID	Sex	Group	11/01/07	15/01/07	29/01/07	12.02.07	26.02.07	12.03.07	26.03.07
611	1	1	0	0	0	0	0	0	0
615	1	1	0	50	0	0	0	0	0
616	2	1	0	0	0	0	0	0	0
617	2	1	.	0	0	0	0	0	50
626	1	1	.	.	0	0	50	0	0
628	2	1	0	0	0	0	0	0	0
633	1	1	0	0	0	0	0	0	0
638	1	1	0	0	0	0	0	0	50
640	2	1	0	0	0	50	50	0	0
644	2	1	0	0	0	0	0	0	0
649	2	1	0	0	0	0	0	0	0
655	1	1	0	0	0	0	0	0	0
657	2	1	0	0	.	.	0	0	0
659	1	1	0	50	0	0	0	0	0
662	1	1	0	0	50	0	0	0	.
669	2	1	0	0	0	0	0	0	0
674	1	1	0	0	100	0	0	0	0
676	2	1	0	0	.	0	0	0	0
677	2	1	0	0	0	0	0	0	0
			0	5.555556	8.823529	2.7778	5.2632	0	5.55556
605	2	2	.	.	0	0	50	100	0
610	2	2	0	100	450	100	0	0	100
613	1	2	0	0	150	.	0	200	50
614	2	2	0	0	50	200	200	100	0
619	2	2	0	0	0	0	50	0	50
620	1	2	0	0	50	400	0	100	.
621	1	2	0	0	400	350	0	300	50
623	2	2	0	0	50	100	150	0	0
627	1	2	0	.	0	100	350	0	100
634	1	2	0	0	50	0	0	0	250
641	2	2	0	0	100	50	0	200	100
642	1	2	0	0	0	0	150	250	50
652	1	2	0	0	150	200	250	0	250
656	2	2	0	0	0	50	50	350	0
661	2	2	0	0	0	100	0	400	50
664	1	2	.	0	.	300	200	100	50
668	2	2	0	50	50	200	100	0	0
672	1	2	0	0	0	150	0	50	150
683	2	2	0	0	150	250	250	100	100
			0.00	8.82	91.67	141.67	94.74	118.42	75.00

	Egg counts epg								
Deer ID	09.04.07	23.04.07	07.05.07	21.05.07	04.06.07	18.06.07	02.07.07	16.07.07	30.07.07
611	0	50	0	0	0	0	200	.	50
615	0	.	0	.	0	50	100	0	0
616	0	0	0	.	0	50	300	0	50
617	0	0	50	0	0	150	350	100	0
626	0	0	0	0	0	50	250	0	100
628	0	0	50	0	0	0	50	0	0
633	0	0	0	0	.	0	150	0	0
638	.	0	0	0	0	50	487	0	100
640	0	0	100	0	0	0	200	50	0
644	0	0	0	0	0	0	200	0	0
649	0	0	0	0	0	0	259	50	0
655	50	0	250	0	0	50	0	0	0
657	0	50	0	0	.	.	350	0	0
659	0	0	0	0	0	0	450	0	.
662	50	0	0	50	0	0	0	0	50
669	100	0	150	0	0	0	100	50	50
674	.	0	0	.	0	50	.	0	0
676	.	0	0	50	0	0	250	50	0
677	50	0	0	50	0	50	150	0	0
	15.625	5.5556	31.579	9.375	0	27.778	213.667	16.6667	22.2222
605	50	0	50	0	0	50	250	50	50
610	0	0	150	0	0	.	100	150	0
613	0	0	50	0	.	0	100	100	50
614	0	0	.	0	50	50	210	150	0
619	0	0	50	0	0	50	50	150	0
620	0	0	0	0	0	0	306	150	0
621	0	0	100	0	0	50	500	350	150
623	0	0	.	0	0	.	400	0	0
627	0	0	50	0	0	50	535	.	0
634	0	0	0	0	.	.	.	150	0
641	0	0	150	0	0	0	400	50	0
642	0	0	150	0	0	.	0	150	0
652	0	0	50	0	.	0	200	150	0
656	0	0	0	.	0	0	550	100	0
661	0	0	50	0	50	50	150	150	.
664	0	0	0	0	0	.	.	0	0
668	0	0	0	.	0	100	400	50	.
672	0	0	50	0	0	0	50	100	0
683	.	0	50	.	0	50	109	50	0
	2.78	0.00	55.88	0.00	6.25	32.14	253.53	113.89	14.71

	Egg count epg								
Deer ID	13.08.0	27.08.07	10.09.07	24.09.07	08.10.07	23.10.07	05.11.07	19.11.0	02.12.07
611	0	0	0	0	0	0	0	0	0
615	0	0	0	0	57.803	0	50	0	0
616	0	.	0	0	.	0	0	0	0
617	0	50	0	0	0	0	0	0	0
626	0	0	0	0	0	0	0	0	0
628	0	0	0	0	100	0	0	0	0
633	0	0	0	0	0	0	0	0	0
638	0	0	0	0	0	0	0	0	0
640	0	0	0	0	0	0	0	0	0
644	0	50	0	0	0	0	0	0	0
649	0	0	0	0	0	0	.	.	0
655	0	50	0	0	0	50	0	0	0
657	0	0	0	0	0	0	0	0	0
659	0	0	0	0	0	0	0	50	0
662	0	100	0	0	150	0	50	0	0
669	0	0	0	0	0	0	0	.	0
674	0	50	0	0	0	0	0	0	0
676	0	50	0	0	0	0	0	0	0
677	0	50	.	0	0	0	0	0	50
	0	22.2222	0	0	17.1	2.63158	5.5556	2.941	2.63158
605	150	50	0	0	0	0	0	50	0
610	50	50	0	0	50	0	50	0	150
613	0	0	0	0	0	0	0	0	0
614	0	0	0	0	0	0	.	50	50
619	0	100	0	0	0	100	0	0	100
620	117.65	0	0	0	0	0	0	0	0
621	0	50	0	0	50	0	0	100	0
623	0	50	0	0	0	0	0	0	200
627	0	0	0	0	50	0	0	0	50
634	0	50	0	0	0	0	0	150	0
641	0	50	0	50	50	0	50	0	0
642	0	0	0	0	0	50	0	0	0
652	0	0	0	0	0	50	0	0	0
656	50	0	0	0	0	0	0	0	0
661	0	50	0	0	50	0	0	50	50
664	0	150	0	0	0	0	0	100	50
668	0	0	0	0	0	50	0	50	0
672	0	0	0	0	0	50	0	0	0
683	0	.	.	0	71.429	50	0	0	100
	19.35	33.33	0.00	2.63	16.92	18.42	5.56	28.95	39.47

1=female, 2 = male, 1 = suppressive group, 2 = monitor trigger treatment group,

Larvae counts (lpg)

Deer ID	Sex	Group	11/01/07	15/01/07	29/01/07	12.02.07	26.02.07	12.03.07	26.03.07	09.04.07
611	1	1	0	0	0	0	0	0	0	0
615	1	1	0	3.25	0	0	0	0	0	0
616	2	1	0	0	0	0	0	0	0	0
617	2	1	.	0	0	0	0	0	0	0
626	1	1	.	.	0	0	0	0	0	0
628	2	1	0	0	0	0	0	0	0	0
633	1	1	0	0	0	0	0	0	0	0
636	1	1	0	0	0	0	.	.	.	.
638	1	1	0	0	0	0	0	0	0	.
640	2	1	0	0	0	0	0	0	0	0
644	2	1	0	0	0	0	0	0	0	0
649	2	1	0	0	0	0	0	0	0	0
655	1	1	0	0	0	0	0.25	0	0	0
657	2	1	0	0	.	.	0	0	0	0
659	1	1	0	0	0	0	0	0	0	0
662	1	1	0	0	0	0	0	0	.	0
669	2	1	0	0	0	0	0	0	0	0
674	1	1	0	7.75	0	0	0	0	0	.
676	2	1	0	0	.	0	0	0	0	.
677	2	1	0	0.25	0	0	0	0	0	0
					0	0	0.01316	0	0	0

605	2	2	.	.	0	0	0	0.5	5.5	0
610	2	2	0	0	0	5.25	0	0	1	0
613	1	2	.	0	5	.	0.25	2	0	0
614	2	2	0	0	0	6.75	0	0.75	6.25	0
619	2	2	0	0	0	0	0	2	6.75	0
620	1	2	0.75	7.25	0	26.25	0.66667	2.75	.	0
621	1	2	0	8.75	2	6.75	2.25	8.25	25.5	0
623	2	2	0	0	0	0	1.75	12.75	29.25	0
627	1	2	0	.	0	8	6.5	0	0.75	0
632	1	2	0	0	22.22	23.75	27	.	.	.
634	1	2	0	0	9.25	6	0	4	0.25	0
641	2	2	0	0.5	0.25	0	0	0	0	0
642	1	2	0	0	0	0	27.5	10.75	5.75	0
652	1	2	0	14.3	34.5	40	14.5	0	17.25	0
656	2	2	0	0	22.5	11.25	1	2	2.25	0
661	2	2	0	0	0	0	0	0	5.25	0
664	1	2	.	0	.	20	0	3.75	7.5	0
668	2	2	0	0	0	0	2.5	0	4.75	0
672	1	2	0	0	0	0	0	5.25	1.25	0
683	2	2	0	0	17.25	13.75	4.75	23.25	13.5	.
					5.95	8.83	4.43	4.11	7.38	0.00

1=female, 2 = male, 1 = suppressive group, 2 = monitor trigger treatment group,

	Larvae count (lpg)								
Deer ID	23.04.07	07.05.07	21.05.07	04.06.07	18.06.07	02.07.07	16.07.07	30.07.07	13.08.07
611	0	0	0	.	0	0	.	0	0
615	.	0	.	0	0	0	0	0	0
616	0	0	.	0	0	0	0	0	0
617	0	0	0	0	0	0	0	0	0
626	0	0	0	0	0	0	0	0	0
628	0	0	0	0	0	0	0	0	0
633	0	0	0	.	0	0	0	0	0
636	.	.	.	.	.	.	.	.	.
638	0	0	0	0	0	0	0	0	0
640	0	0	0	0	0	0	0	0	0
644	0	0	0	0	0	0	0	0	0
649	0	0	0	0	0	0	0	0	0
655	0	0	0	0	0	0	0	0	0
657	0	0	0	.	.	0	0	0.33333	0
659	0	0	0	0	0	0	0	.	0
662	0	0	0	0	0	0	0	0	0
669	0	0	0	0	0	0	0.75	0	0
674	0	0	.	0	0	.	0	0	0
676	0	1	0	0	0	0	1	0	0
677	0	0	0	0	0	0	.	0	0
	0	0.05263	0	0	0	0	0.10294	0.01852	0

605	0	41	0	1.75	10	23.75	54.25	0	0
610	0	21	0	0	.	5.75	21	0	0
613	0	93	0	.	128.5	43.75	102.75	0	0.5
614	0	.	0	0	5	5.25	18	.	1.5
619	0	177	0	0	101	126.75	240	0	0
620	0.25	0	0	0	56	27	41.25	0	0
621	0	81	0	0	14	20	14.25	0	0
623	0	.	0	0	.	91.25	201.25	0	0
627	0	41	0	0	5	43	.	0	0
632	.	.	.	.	.	.	.	.	.
634	0	16	0	.	.	.	62	0	9
641	0	129	0	0	0	18.75	68	0	0
642	0	137	.	0	.	1	29.75	0	0
652	0	40	0	.	102.25	6.25	197.25	0	0.5
656	0	53	.	0.25	7.5	10.25	30	0	2
661	0	31	0	4.25	1.5	15.25	1	.	0
664	0	50	0	0	.	.	10	0	5
668	0	0	.	0	29	136.5	8.25	.	0
672	0	2	0	0	0	10.25	10.5	0	0
683	0	10	.	0	5.75	2	51.25	0	0
	0.01	54.24	0.00	0.39	33.25	34.51	64.49	0.00	0.97

1=female, 2 = male, 1 = suppressive group, 2 = monitor trigger treatment group,

Larvae count (lpg)

Deer ID	27.08.07	10.09.07	24.09.07	08.10.07	23.10.07	05.11.07	19.11.07	02.12.07
611	0	0	0	0	0	0	0	0
615	0	0	0	0	0	0	0	0
616	.	0	0	.	0	0	0	0
617	0	0	0	0	0	0	0	0
626	0	0	0	0	0	0	0	0
628	0	0	0	0	0	0	0	0
633	0	0	0	0	0	0	0	0
636	.	.	.	.	.	.	.	.
638	0	0	0	0	0	0	0	0
640	0	0	0	0	0	0	0	0
644	0	0	0	0	0	0	0	0
649	0	0	0	0	0	.	.	0
655	0	0	0	0.25	0	0	0	0
657	0	0	0	0	0	0	0	0
659	0	0	0	0	0	0	0	0
662	0	0	0.25	0	0	0	0	0
669	0	0	0	0	0	0	.	0
674	0	0	0	0	0	0	0	0
676	0	0	0	0	0	0	0	0
677	0	.	0	0	0	0	0	0.25
	0	0	0.01316	0.0139	0	0	0	0.01316

605	41.75	0	0	0	4	3.75	0	2
610	3.75	0	6.25	2	1.75	0.25	0	0
613	28	0	1.25	8.5	9.75	1.5	0	0
614	17	0	0	0	0	.	0	0
619	8.75	0	1.75	0	0	0	0	0
620	5	0	0	0	0.25	0	0	0
621	9.25	0	0	0	0	0	0	0
623	8.5	0	9.67742	4	3.25	0	0	0
627	5.75	0	0.5	3	0.25	0	0	0
632	.	.	.	.	.	.	.	.
634	0	0	3.125	0	0	0	0	0
641	21.25	0	0	1.75	3.75	0	0	0
642	31	0	0	0.75	0.75	3.25	0	0.25
652	35.2	0	0.75	0.5	2	0	0	0
656	3.25	0	1.75	0.25	0	0.25	0	0
661	129	1	17	7	38	0.25	0	0
664	9	0	0.25	1	3.5	0	0	0
668	10	0	0	4.25	0	0	1.5	0.25
672	2	0	0	0	0	0	0	0
683	.	.	0	0	1	0	0	0
	20.47	0.06	2.23	1.74	3.59	0.51	0.08	0.13

Appendix III Standard operating procedure Serum Pepsinogen Assey

Matherial & Methods:
Substrate
3.2% Bovine Serum Albumin (BSA)
1% Glycine
pH 1.6

Weigh out 3.2 g BSA and 1g glycine into a 100ml beaker. Drop in a magnetic flea and add approximately 70ml of deionised water. Place on a magnetic stirrer and leave until totally dissolved.

Adjust pH to 1.6 using 1M hydrochloric acid at the same time as more deionised water is added until the volume is 100ml.

Precipitating solution
Dilute 70% perchloric acid solution- 1 part to 9 parts deionised water.

Tyrosine standards
10 mM L-Tyrosine stock solution

Dissolve 0.1812g L-Tyrosine in a further diluted solution of perchloric acid (1 Part precipitating solution to 4 parts deionised water) – final volume 100ml

0, 10, 20 and 30iU standards are then prepared as follows

	Vol perchloric acid	vol 10mM Tyrosine
0 iU	1000µl	0
10 iU	820	180
20 iU	640	360
30 iU	460	` 540

Colour reagent
Bicinchoninic acid (BCA)
4% Copper(II) Sulphate solution

Immediately prior to use, add 1 part Copper sulphate solution to 49 parts BCA.

Equipment:
1.5 ml conical microcentrifuge tubes (Eppendorfs)
96-well, flat-bottemed microtitre plates
Waterbath incubator (grant Y28 Tank and VFP temperature control unit, Grant Instruments Ltd, Cambridge, England)
Eppendorf Centrifuge (Eppendorf centrifuge 5415, Eppendorf geratebau, Germany).
Microplate Reader (V max, Kinetic microplate reader, Molecular devices, USA)

Procedure

1. Label 2 eppendorf tubes for each serum sample – one labelled N, the other I, plus the sample number*. In addition, label 4 eppendorf tubes as the tyrosine standards (e.g. 0T, 10T, 20T and 30T)
2. Into each eppendorf add 75µl of the substrate solution.
3. Add 50 µl of each tyrosine standard into the standard eppendorfs.
4. Add 50 µl of each serum sample into the two labelled eppendorfs.
5. Shake all tubes on a vortex mixer.
6. Incubate all tubes in the waterbath at 37°C.
7. After 30 minutes remove the tyrosine standard eppendorfs and all the N-labelled eppendorfs from the bath and immediately add 250 µl of the precipitating solution. Shake and leave to stand for 10 mins.
8. Centrifuge the precipitated eppendorfs for 10 min at 14,000 rpm.
9. After a further 3 hours of incubation (=total incubation of 3 hours 30 mins), remove all I tubes an precipitate as in 7. Centrifuge these as in 8.
10. Prepare the BCA/copper sulphate solution.
11. The microtitre plate consists of wells in 8 rows (A-H) by 12 columns (1-12). Pipette 10 µl of fluid from the 0iU tyrosine standard eppendorf into wells A1, B1, C1 and D1. Similarly, pipette 10µl of the 10, 20 and 30iU standards into A2-D2, A3-D3 and A4-D4.
12. Pipette 10 µl of fluid from the N-eppendorf of the first serum sample into A5-D5.
13. Pipette 10 µl of fluid from the I-eppendorf of the first serum sample into A6-D6.
14. repeat until the first plate is finisched. Each plate has enough wells for the 4 standards and the duplicate tubes of 10 serum samples.
15. Set up the second plate as before with the tyrosine standards in A1-D1 through to A4-D4 and the next 10 serum samples.
16. Into each well of each plate add 190 µl of the BCA/copper sulphate mixture.
17. Incubate each plate at 37°C for 30 minutes then place in the microplate reader and read Optical densities (OD) at 490nm wavelength. Engage the autoshaker to shake the plate before the readings are made
18. With the printout of the results scan for any wells with abnormally high readings. These likely resulted from contamination (e.g. dust). Ignore these
19. Average the 4 OD values for each standard and N and I tube for each sample. Can be easily done in Microsoft Excell.
20. Calculate a standard curve from the 4 standards. This gives you an equation in de form of y=mx+c and a R2 value. The R2 value should be >0.98 and as close to 1.0 as possible. This can be done using the regression function in Excell or in any statistical software (e.g. Prism)
21. Using the equation, the average OD values for each N and I sample can be converted to iU by the following formula:

$$iU = \frac{(\text{average OD} - c)}{m}$$

22. Subtract the value for the N eppendorf from that for the I eppendorf to get the pepsinogen activity for each sample.

*If you have 50 serum samples it is generally easier to label the eppendorfs 1 to 50. Record the actual sample id (animal number and sample date) in a separate list.

Appendix IV Statistics

```
Statistix 8.0                  Statistixwithoutcros..., 2/9/2009,
11:53:15 AM
```

Shapiro-Wilk Normality Test

Variable	N	W	P
PEPSINOGE	228	0.8294	0.0000

The W statistics should approach 1 for normally distributed data. The null hypothesis that the data is normally distributed is rejected because the p-value is small (e.g. smaller then 0.05)

```
Statistix 8.0                  Statistixwithoutcros..., 2/24/2009,
9:03:05 AM
```

Descriptive Statistics

DATEGROUP = 1

	PEPSINOGE
N	19
Lo 95% CI	0.0330
Mean	0.1044
Up 95% CI	0.1759
SD	0.1482
Variance	0.0220
SE Mean	0.0340
C.V.	141.93
Minimum	0.0000
Median	0.0400
Maximum	0.4731

DATEGROUP = 2

	PEPSINOGE
N	19
Lo 95% CI	0.0914
Mean	0.2147
Up 95% CI	0.3381
SD	0.2559
Variance	0.0655
SE Mean	0.0587
C.V.	119.19
Minimum	0.0000
Median	0.1417
Maximum	0.8853

DATEGROUP = 3

	PEPSINOGE
N	19
Lo 95% CI	0.0694
Mean	0.1188
Up 95% CI	0.1682
SD	0.1025
Variance	0.0105
SE Mean	0.0235
C.V.	86.318
Minimum	0.0000
Median	0.1014
Maximum	0.2902

DATEGROUP = 4

	PEPSINOGE
N	19
Lo 95% CI	0.0403
Mean	0.1314
Up 95% CI	0.2225
SD	0.1889
Variance	0.0357
SE Mean	0.0433
C.V.	143.77
Minimum	0.0000
Median	0.0780
Maximum	0.7629

DATEGROUP = 5

	PEPSINOGE
N	19
Lo 95% CI	0.0457
Mean	0.0972
Up 95% CI	0.1486
SD	0.1067
Variance	0.0114
SE Mean	0.0245
C.V.	109.83
Minimum	0.0000
Median	0.0900
Maximum	0.3775

DATEGROUP = 6

	PEPSINOGE
N	19
Lo 95% CI	0.0454
Mean	0.0901
Up 95% CI	0.1348
SD	0.0928
Variance	8.610E-03

SE Mean	0.0213
C.V.	102.96
Minimum	0.0000
Median	0.0800
Maximum	0.2506

DATEGROUP = 7

	PEPSINOGE
N	19
Lo 95% CI	0.0427
Mean	0.1302
Up 95% CI	0.2176
SD	0.1814
Variance	0.0329
SE Mean	0.0416
C.V.	139.35
Minimum	0.0000
Median	0.1087
Maximum	0.7911

DATEGROUP = 8

	PEPSINOGE
N	19
Lo 95% CI	0.1273
Mean	0.1994
Up 95% CI	0.2715
SD	0.1495
Variance	0.0224
SE Mean	0.0343
C.V.	74.995
Minimum	0.0000
Median	0.1883
Maximum	0.5107

DATEGROUP = 9

	PEPSINOGE
N	19
Lo 95% CI	0.0851
Mean	0.1258
Up 95% CI	0.1665
SD	0.0845
Variance	7.137E-03
SE Mean	0.0194
C.V.	67.160
Minimum	0.0100
Median	0.1200
Maximum	0.3300

DATEGROUP = 10

	PEPSINOGE
N	19
Lo 95% CI	0.1573
Mean	0.2100
Up 95% CI	0.2627
SD	0.1094
Variance	0.0120
SE Mean	0.0251
C.V.	52.092
Minimum	0.0600
Median	0.2000
Maximum	0.5000

DATEGROUP = 11

	PEPSINOGE
N	19
Lo 95% CI	0.0801
Mean	0.1068
Up 95% CI	0.1335
SD	0.0554
Variance	3.067E-03
SE Mean	0.0127
C.V.	51.836
Minimum	0.0000
Median	0.1000
Maximum	0.2100

DATEGROUP = 12

	PEPSINOGE
N	19
Lo 95% CI	0.1355
Mean	0.2226
Up 95% CI	0.3098
SD	0.1808
Variance	0.0327
SE Mean	0.0415
C.V.	81.195
Minimum	0.0200
Median	0.1400
Maximum	0.5700

Statistix 8.0 Statistixwithoutcros..., 2/9/2009,
10:57:38 AM

Descriptive Statistics

DATEGROUP = 1

	LNPEPS
N	19
Lo 95% CI	0.0312
Mean	0.0916
Up 95% CI	0.1519
SD	0.1251
Variance	0.0157
SE Mean	0.0287
C.V.	136.68
Minimum	0.0000

Median	0.0392
Maximum	0.3874

DATEGROUP = 2

	LNPEPS
N	19
Lo 95% CI	0.0833
Mean	0.1759
Up 95% CI	0.2686
SD	0.1922
Variance	0.0369
SE Mean	0.0441
C.V.	109.22
Minimum	0.0000
Median	0.1325
Maximum	0.6341

Power both groups January =59%

DATEGROUP = 3

	LNPEPS
N	19
Lo 95% CI	0.0641
Mean	0.1083
Up 95% CI	0.1524
SD	0.0915
Variance	8.378E-03
SE Mean	0.0210
C.V.	84.551
Minimum	0.0000
Median	0.0965
Maximum	0.2548

DATEGROUP = 4

	LNPEPS
N	19
Lo 95% CI	0.0422
Mean	0.1125
Up 95% CI	0.1828
SD	0.1459
Variance	0.0213
SE Mean	0.0335
C.V.	129.71
Minimum	0.0000
Median	0.0751
Maximum	0.5669

Power both groups March= 4%

DATEGROUP = 5

	LNPEPS
N	19
Lo 95% CI	0.0435
Mean	0.0885
Up 95% CI	0.1335
SD	0.0934
Variance	8.730E-03
SE Mean	0.0214
C.V.	105.58
Minimum	0.0000
Median	0.0862
Maximum	0.3203

DATEGROUP = 6

	LNPEPS
N	19
Lo 95% CI	0.0427
Mean	0.0830
Up 95% CI	0.1232
SD	0.0834
Variance	6.957E-03
SE Mean	0.0191
C.V.	100.55
Minimum	0.0000
Median	0.0770
Maximum	0.2236

Power both groups April=100%

DATEGROUP = 7

	LNPEPS
N	19
Lo 95% CI	0.0464
Mean	0.1125
Up 95% CI	0.1786
SD	0.1372
Variance	0.0188
SE Mean	0.0315
C.V.	121.97
Minimum	0.0000
Median	0.1032
Maximum	0.5829

DATEGROUP = 8

	LNPEPS
N	19
Lo 95% CI	0.1151
Mean	0.1746
Up 95% CI	0.2340
SD	0.1233
Variance	0.0152
SE Mean	0.0283
C.V.	70.649
Minimum	0.0000
Median	0.1726
Maximum	0.4126

Power both groups May= 49%

DATEGROUP = 9

LNPEPS

N	19
Lo 95% CI	0.0802
Mean	0.1159
Up 95% CI	0.1515
SD	0.0740
Variance	5.480E-03
SE Mean	0.0170
C.V.	63.892
Minimum	9.950E-03
Median	0.1133
Maximum	0.2852

DATEGROUP = 10

	LNPEPS
N	19
Lo 95% CI	0.1445
Mean	0.1869
Up 95% CI	0.2293
SD	0.0879
Variance	7.728E-03
SE Mean	0.0202
C.V.	47.035
Minimum	0.0583
Median	0.1823
Maximum	0.4055

Power both groups July= 96%

DATEGROUP = 11

	LNPEPS
N	19
Lo 95% CI	0.0761
Mean	0.1003
Up 95% CI	0.1245
SD	0.0502
Variance	2.521E-03
SE Mean	0.0115
C.V.	50.054
Minimum	0.0000
Median	0.0953
Maximum	0.1906

DATEGROUP = 12

	LNPEPS
N	19
Lo 95% CI	0.1229
Mean	0.1912
Up 95% CI	0.2596
SD	0.1418
Variance	0.0201
SE Mean	0.0325
C.V.	74.153
Minimum	0.0198
Median	0.1310
Maximum	0.4511

Power both groups december = 98%

```
Statistix 8.0                    Statistixwithoutcros..., 2/9/2009,
11:02:54 AM
```

Correlations (Pearson) All animals Total liveweight gain and average lnpeps

```
             MEAN
TOTAL      -0.2127
  P-VALUE   0.0012

Cases Included 228    Missing Cases 0

Statistix 8.0                    Statistixwithoutcros..., 2/9/2009,
11:12:17 AM
```

Correlations (Pearson)group=1 total liveweight gain and average lnpeps

```
             MEAN
TOTAL       0.2192            P-VALUE   0.0191

Cases Included 114    Missing Cases 0

Statistix 8.0                    Statistixwithoutcros..., 2/9/2009,
11:13:56 AM
```

Correlations (Pearson)Group=2 total liveweight gain and average lnpeps

```
             MEAN
TOTAL      -0.5177          P-VALUE   0.0000
Cases Included 114    Missing Cases 0
```

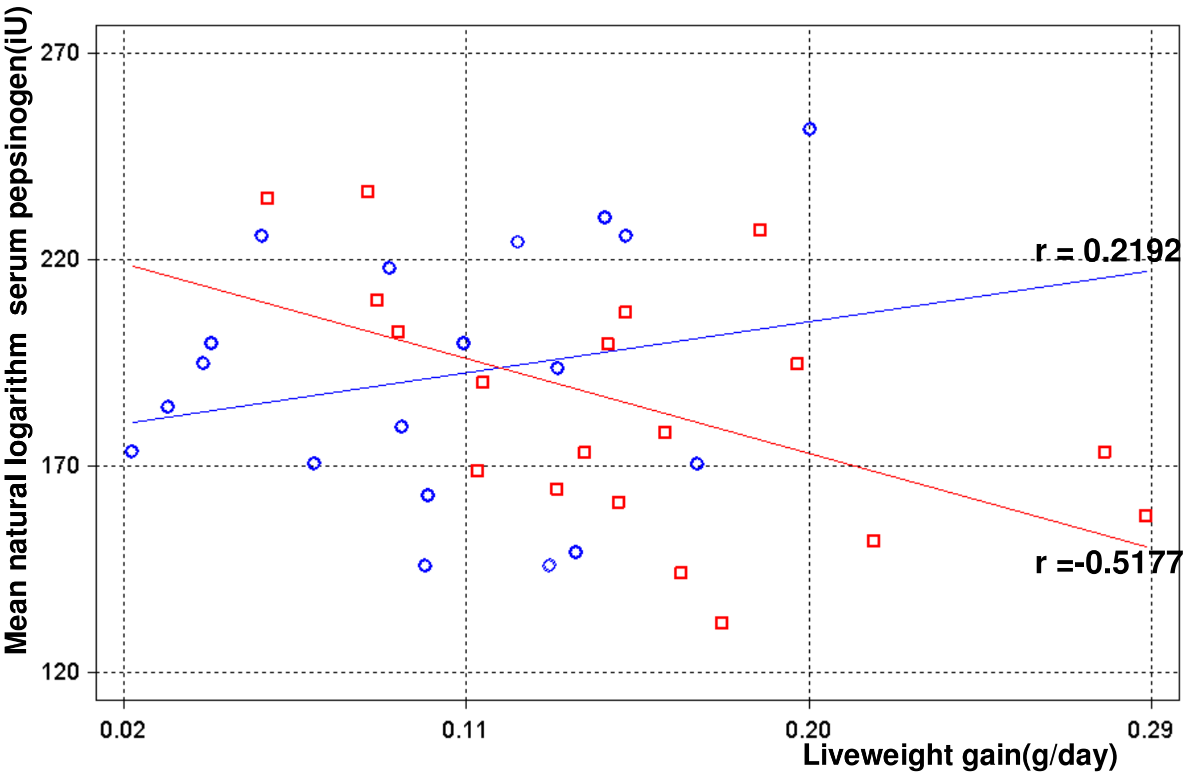

Figure 2. Correlation between mean natural logarithm serum pepsinogen (iU) and liveweight gain (g/day)
○ group 1, suppressive treated group, □ group 2, trigger treated monitor group

Correlations between ln pepsinogen and ln FEC or ln FLC per date per group (19 animals)

```
Statistix 8.0                   Statistixwithoutcros..., 2/10/2009,
10:03:09 AM
```

Correlations (Pearson)date1 group1

```
           LNPEPS
LNFEC     -0.0428
  P-VALUE  0.8704

Cases Included 17    Missing Cases 2

Statistix 8.0                   Statistixwithoutcros..., 2/10/2009,
10:03:33 AM
```

Correlations (Pearson)date1 group1

```
           LNPEPS
LNFLC           M
  P-VALUE       M

Cases Included 17    Missing Cases 2

Statistix 8.0                   Statistixwithoutcros..., 2/10/2009,
10:04:47 AM
```

Correlations (Pearson)date1 group2

```
           LNPEPS
LNFEC     -0.0948
  P-VALUE  0.7081

Cases Included 18    Missing Cases 1

Statistix 8.0                   Statistixwithoutcros..., 2/10/2009,
10:04:24 AM
```

Correlations (Pearson)date1 group2

```
           LNPEPS
LNFLC     -0.3192
  P-VALUE  0.1966

Cases Included 18    Missing Cases 1

Statistix 8.0                   Statistixwithoutcros..., 2/10/2009,
10:07:39 AM
```

Correlations (Pearson)group1 date2

```
           LNPEPS
LNFEC      0.2410
  P-VALUE  0.3354

Cases Included 18    Missing Cases 1

Statistix 8.0                   Statistixwithoutcros..., 2/10/2009,
10:08:08 AM
```

Correlations (Pearson)group1 date2

	LNPEPS
LNFLC	M
P-VALUE	M

Cases Included 18 Missing Cases 1

Statistix 8.0 Statistixwithoutcros..., 2/10/2009, 10:09:11 AM

Correlations (Pearson) group2 date2

	LNPEPS
LNFEC	0.2769
P-VALUE	0.2660

Cases Included 18 Missing Cases 1
Statistix 8.0 Statistixwithoutcros..., 2/10/2009, 10:08:53 AM

Correlations (Pearson) group2 date2

	LNPEPS
LNFLC	0.0263
P-VALUE	0.9174

Cases Included 18 Missing Cases 1

Statistix 8.0 Statistixwithoutcros..., 2/10/2009, 10:12:40 AM

Correlations (Pearson) group1 date3

	LNPEPS
LNFEC	-0.1267
P-VALUE	0.6164

Cases Included 18 Missing Cases 1

Statistix 8.0 Statistixwithoutcros..., 2/10/2009, 10:13:03 AM

Correlations (Pearson) group1 date3

	LNPEPS
LNFLC	M
P-VALUE	M

Cases Included 18 Missing Cases 1
Statistix 8.0 Statistixwithoutcros..., 2/10/2009, 10:14:03 AM

Correlations (Pearson)group2 date3

	LNPEPS
LNFEC	M
P-VALUE	M

Cases Included 19 Missing Cases 0

Statistix 8.0 Statistixwithoutcros..., 2/10/2009, 10:13:45 AM

Correlations (Pearson) group2 date3

	LNPEPS
LNFLC	0.1011
P-VALUE	0.6804

Cases Included 19 Missing Cases 0

Statistix 8.0 Statistixwithoutcros..., 2/10/2009, 10:16:42 AM

Correlations (Pearson) group1 date4

	LNPEPS
LNFEC	0.1658
P-VALUE	0.5394

Cases Included 16 Missing Cases 3

Statistix 8.0 Statistixwithoutcros..., 2/10/2009, 10:17:07 AM

Correlations (Pearson) group1 date4

	LNPEPS
LNFLC	M
P-VALUE	M

Cases Included 16 Missing Cases 3
Statistix 8.0 Statistixwithoutcros..., 2/10/2009, 10:18:22 AM

Correlations (Pearson) group 2 date4

	LNPEPS
LNFEC	M
P-VALUE	M

Cases Included 16 Missing Cases 3

Statistix 8.0 Statistixwithoutcros..., 2/10/2009, 10:18:00 AM

Correlations (Pearson) group 2 date4

	LNPEPS
LNFLC	M
P-VALUE	M

Cases Included 15 Missing Cases 4

Statistix 8.0 Statistixwithoutcros..., 2/10/2009, 10:19:14 AM

Correlations (Pearson) group1 date5

LNPEPS

```
LNFEC     -0.1149
  P-VALUE  0.6499

Cases Included 18    Missing Cases 1

Statistix 8.0                   Statistixwithoutcros..., 2/10/2009,
10:19:45 AM
```

Correlations (Pearson) group1 date5

```
           LNPEPS
LNFLC     -0.2980
  P-VALUE  0.2453
Cases Included 17    Missing Cases 2
Statistix 8.0                   Statistixwithoutcros..., 2/10/2009,
10:21:16 AM
```

Correlations (Pearson) group2 date5

```
           LNPEPS
LNFEC      0.3520
  P-VALUE  0.1519

Cases Included 18    Missing Cases 1

Statistix 8.0                   Statistixwithoutcros..., 2/10/2009,
10:20:55 AM
```

Correlations (Pearson) group2 date5

```
           LNPEPS
LNFLC      0.1555
  P-VALUE  0.5378

Cases Included 18    Missing Cases 1

Statistix 8.0                   Statistixwithoutcros..., 2/10/2009,
10:23:08 AM
```

Correlations (Pearson) group 1 date6

```
           LNPEPS
LNFEC      0.1902
  P-VALUE  0.4354

Cases Included 19    Missing Cases 0

Statistix 8.0                   Statistixwithoutcros..., 2/10/2009,
10:23:39 AM
```

Correlations (Pearson) group 1 date6

```
           LNPEPS
LNFLC      0.1902
  P-VALUE  0.4354

Cases Included 19    Missing Cases 0

Statistix 8.0                   Statistixwithoutcros..., 2/10/2009,
10:24:39 AM
```

Correlations (Pearson)group2 date6

```
            LNPEPS
LNFEC      -0.2161
  P-VALUE   0.3743

Cases Included 19    Missing Cases 0

Statistix 8.0                    Statistixwithoutcros..., 2/10/2009,
10:24:18 AM
```

Correlations (Pearson) group2 date6

```
            LNPEPS
LNFLC      -0.1791
  P-VALUE   0.4631

Cases Included 19    Missing Cases 0
```

Correlation between ln pepsinogen and date/age in animals group 1

```
Statistix 8.0                      Statistixwithoutcros..., 2/9/2009,
4:44:31 PM
```

Correlations (Pearson)group1

```
            LNPEPS
DATUM       0.0280
  P-VALUE   0.7672

Cases Included 114    Missing Cases 0
```

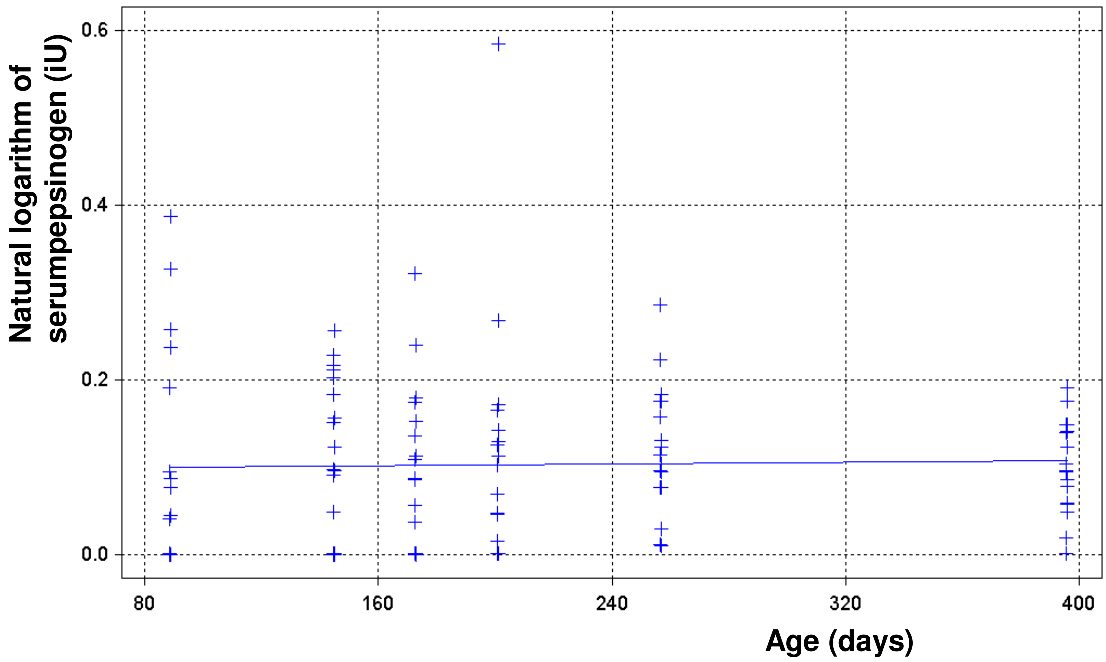

Figure 4. Correlation between natural logarithm of serum pepsinogen and age in the suppressive treated group

For the power calculation, the powercalculator on the massey site was used.
http://research.massey.ac.nz/massey/fms/Animal%20Ethics/Documents/Power%20calculator.xls

Power Calculator	Source:	Snedecor&Cochran, p.69
(entry fields are blue)	**Mean**	**Proportion**
ENTER GROUP 1:		
The mean of group 1 is:	0.1028	0.12
The common stddev of both groups is:	0.11775	
The sample size is:	43.25370532	150
The acceptable alpha error group 1 is:	0.05	0.05
ENTER GROUP 2:		
The mean of group 2 is:	0.154	0.06
One (1) or two (2) tailed test?	2	1
Power	**80%**	**73%**

The stdev of group 1 proportion is:		0.32496154
The stdev of group 2 proportion is:		0.24
Tails multiplier	1	2
alpha	0.05	0.1
OUTPUT GROUP 1:		
t (df) of group 1:	2.02	1.66
Z right	-0.842	3.916
Z left	-4.878	0.606
P(Z) right	0.800	0.000
P(Z) left	0.000	0.728

Power calculation of the trial for pepsinogen

```
The SAS System                                                     09:39 Monday,
February 9, 2009   8

The FREQ Procedure

Table of Dategroup by Pepsposneg

Dategroup(Dategroup)
          Pepsposneg(Pepsposneg)

Frequency,
Percent  ,
Row Pct  ,
Col Pct  ,       0,       1,  Total
ƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒˆ
       1 ,      9 ,     10 ,     19
         ,   3.88 ,   4.31 ,   8.19
         ,  47.37 ,  52.63 ,
         ,  18.75 ,   5.43 ,
```

```
ƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒˆ
       2 ,      6 ,     13 ,     19
         ,   2.59 ,   5.60 ,   8.19
         ,  31.58 ,  68.42 ,
         ,  12.50 ,   7.07 ,
ƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒˆ
       3 ,      6 ,     13 ,     19
         ,   2.59 ,   5.60 ,   8.19
         ,  31.58 ,  68.42 ,
         ,  12.50 ,   7.07 ,
ƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒˆ
       4 ,      7 ,     12 ,     19
         ,   3.02 ,   5.17 ,   8.19
         ,  36.84 ,  63.16 ,
         ,  14.58 ,   6.52 ,
ƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒˆ
       5 ,      5 ,     14 ,     19
         ,   2.16 ,   6.03 ,   8.19
         ,  26.32 ,  73.68 ,
         ,  10.42 ,   7.61 ,
ƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒˆ
       6 ,      5 ,     14 ,     19
         ,   2.16 ,   6.03 ,   8.19
         ,  26.32 ,  73.68 ,
         ,  10.42 ,   7.61 ,
ƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒˆ
       7 ,      5 ,     14 ,     19
         ,   2.16 ,   6.03 ,   8.19
         ,  26.32 ,  73.68 ,
         ,  10.42 ,   7.61 ,
ƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒˆ
       8 ,      1 ,     18 ,     19
         ,   0.43 ,   7.76 ,   8.19
         ,   5.26 ,  94.74 ,
         ,   2.08 ,   9.78 ,
ƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒˆ
       9 ,      1 ,     19 ,     20
         ,   0.43 ,   8.19 ,   8.62
         ,   5.00 ,  95.00 ,
         ,   2.08 ,  10.33 ,
ƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒˆ
      10 ,      1 ,     19 ,     20
         ,   0.43 ,   8.19 ,   8.62
         ,   5.00 ,  95.00 ,
         ,   2.08 ,  10.33 ,
ƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒˆ
      11 ,      1 ,     19 ,     20
         ,   0.43 ,   8.19 ,   8.62
         ,   5.00 ,  95.00 ,
         ,   2.08 ,  10.33 ,
ƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒˆ
      12 ,      1 ,     19 ,     20
         ,   0.43 ,   8.19 ,   8.62
         ,   5.00 ,  95.00 ,
         ,   2.08 ,  10.33 ,
ƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒˆ
Total          48      184      232
            20.69    79.31   100.00
```

Statistics for Table of Dategroup by Pepsposneg

Statistic	DF	Value	Prob
Chi-Square	11	29.8649	0.0017
Likelihood Ratio Chi-Square	11	32.5618	0.0006
Mantel-Haenszel Chi-Square	1	25.5606	<.0001
Phi Coefficient		0.3588	
Contingency Coefficient		0.3377	
Cramer's V		0.3588	

WARNING: 50% of the cells have expected counts less than 5. Chi-Square may not be a valid test.

Sample Size = 232

The GENMOD Procedure

Model Information

Data Set	WORK.HELENA	
Distribution	Binomial	
Link Function	Logit	
Dependent Variable	Pepsposneg	Pepsposneg

Number of Observations Read	232
Number of Observations Used	232
Number of Events	48
Number of Trials	232

Class Level Information

Class	Levels	Values
Dategroup	12	1 2 3 4 5 6 7 8 9 10 11 12

Response Profile

Ordered Value	Pepsposneg	Total Frequency
1	0	48
2	1	184

PROC GENMOD is modeling the probability that Pepsposneg='0'. One way to change this to model the probability that
Pepsposneg='1' is to specify the DESCENDING option in the PROC statement.

Parameter Information

Parameter	Effect	Dategroup
Prm1	Intercept	
Prm2	Dategroup	1
Prm3	Dategroup	2
Prm4	Dategroup	3
Prm5	Dategroup	4
Prm6	Dategroup	5
Prm7	Dategroup	6
Prm8	Dategroup	7
Prm9	Dategroup	8
Prm10	Dategroup	9
Prm11	Dategroup	10
Prm12	Dategroup	11
Prm13	Dategroup	12

Criteria For Assessing Goodness Of Fit

Criterion	DF	Value	Value/DF
Deviance	220	203.9927	0.9272
Scaled Deviance	220	203.9927	0.9272
Pearson Chi-Square	220	232.0000	1.0545
Scaled Pearson X2	220	232.0000	1.0545
Log Likelihood		-101.9964	

Algorithm converged.

Analysis Of Parameter Estimates

Parameter		DF	Estimate	Standard Error	Wald 95% Confidence Limits		Chi-Square	Pr > ChiSq
Intercept		1	-2.9444	1.0260	-4.9553	-0.9336	8.24	0.0041
Dategroup	1	1	2.8391	1.1242	0.6358	5.0424	6.38	0.0116
Dategroup	2	1	2.1712	1.1385	-0.0602	4.4027	3.64	0.0565
Dategroup	3	1	2.1712	1.1385	-0.0602	4.4027	3.64	0.0565
Dategroup	4	1	2.4054	1.1309	0.1890	4.6219	4.52	0.0334
Dategroup	5	1	1.9148	1.1507	-0.3405	4.1701	2.77	0.0961
Dategroup	6	1	1.9148	1.1507	-0.3405	4.1701	2.77	0.0961
Dategroup	7	1	1.9148	1.1507	-0.3405	4.1701	2.77	0.0961
Dategroup	8	1	0.0541	1.4520	-2.7917	2.8999	0.00	0.9703
Dategroup	9	1	-0.0000	1.4510	-2.8438	2.8438	0.00	1.0000
Dategroup	10	1	-0.0000	1.4510	-2.8438	2.8438	0.00	1.0000
Dategroup	11	1	-0.0000	1.4510	-2.8438	2.8438	0.00	1.0000
Dategroup	12	0	0.0000	0.0000	0.0000	0.0000	.	.
Scale		0	1.0000	0.0000	1.0000	1.0000		

NOTE: The scale parameter was held fixed.

LR Statistics For Type 3 Analysis

Source	DF	Chi-Square	Pr > ChiSq
Dategroup	11	32.56	0.0006

Least Squares Means

Effect	Dategroup	Estimate	Standard Error	DF	Chi-Square	Pr > ChiSq
Dategroup	1	-0.1054	0.4595	1	0.05	0.8186
Dategroup	2	-0.7732	0.4935	1	2.45	0.1172
Dategroup	3	-0.7732	0.4935	1	2.45	0.1172
Dategroup	4	-0.5390	0.4756	1	1.28	0.2571
Dategroup	5	-1.0296	0.5210	1	3.91	0.0481
Dategroup	6	-1.0296	0.5210	1	3.91	0.0481
Dategroup	7	-1.0296	0.5210	1	3.91	0.0481
Dategroup	8	-2.8904	1.0274	1	7.91	0.0049
Dategroup	9	-2.9444	1.0260	1	8.24	0.0041
Dategroup	10	-2.9444	1.0260	1	8.24	0.0041
Dategroup	11	-2.9444	1.0260	1	8.24	0.0041
Dategroup	12	-2.9444	1.0260	1	8.24	0.0041

Differences of Least Squares Means

Effect	Dategroup	_Dategroup	Estimate	Standard Error	DF	Chi-Square	Pr > ChiSq
Dategroup	1	2	0.6678	0.6743	1	0.98	0.3220
Dategroup	1	3	0.6678	0.6743	1	0.98	0.3220
Dategroup	1	4	0.4336	0.6613	1	0.43	0.5120
Dategroup	1	5	0.9243	0.6947	1	1.77	0.1833
Dategroup	1	6	0.9243	0.6947	1	1.77	0.1833
Dategroup	1	7	0.9243	0.6947	1	1.77	0.1833
Dategroup	1	8	2.7850	1.1255	1	6.12	0.0133
Dategroup	1	9	2.8391	1.1242	1	6.38	0.0116
Dategroup	1	10	2.8391	1.1242	1	6.38	0.0116
Dategroup	1	11	2.8391	1.1242	1	6.38	0.0116
Dategroup	1	12	2.8391	1.1242	1	6.38	0.0116
Dategroup	2	3	0.0000	0.6980	1	0.00	1.0000
Dategroup	2	4	-0.2342	0.6854	1	0.12	0.7326
Dategroup	2	5	0.2564	0.7176	1	0.13	0.7209
Dategroup	2	6	0.2564	0.7176	1	0.13	0.7209
Dategroup	2	7	0.2564	0.7176	1	0.13	0.7209
Dategroup	2	8	2.1172	1.1398	1	3.45	0.0632
Dategroup	2	9	2.1712	1.1385	1	3.64	0.0565
Dategroup	2	10	2.1712	1.1385	1	3.64	0.0565
Dategroup	2	11	2.1712	1.1385	1	3.64	0.0565
Dategroup	2	12	2.1712	1.1385	1	3.64	0.0565

Dategroup	3	4	-0.2342	0.6854	1	0.12	0.7326
Dategroup	3	5	0.2564	0.7176	1	0.13	0.7209
Dategroup	3	6	0.2564	0.7176	1	0.13	0.7209
Dategroup	3	7	0.2564	0.7176	1	0.13	0.7209
Dategroup	3	8	2.1172	1.1398	1	3.45	0.0632
Dategroup	3	9	2.1712	1.1385	1	3.64	0.0565
Dategroup	3	10	2.1712	1.1385	1	3.64	0.0565
Dategroup	3	11	2.1712	1.1385	1	3.64	0.0565
Dategroup	3	12	2.1712	1.1385	1	3.64	0.0565
Dategroup	4	5	0.4906	0.7054	1	0.48	0.4867
Dategroup	4	6	0.4906	0.7054	1	0.48	0.4867
Dategroup	4	7	0.4906	0.7054	1	0.48	0.4867
Dategroup	4	8	2.3514	1.1321	1	4.31	0.0378
Dategroup	4	9	2.4054	1.1309	1	4.52	0.0334
Dategroup	4	10	2.4054	1.1309	1	4.52	0.0334
Dategroup	4	11	2.4054	1.1309	1	4.52	0.0334
Dategroup	4	12	2.4054	1.1309	1	4.52	0.0334
Dategroup	5	6	0.0000	0.7368	1	0.00	1.0000
Dategroup	5	7	0.0000	0.7368	1	0.00	1.0000
Dategroup	5	8	1.8608	1.1519	1	2.61	0.1062
Dategroup	5	9	1.9148	1.1507	1	2.77	0.0961
Dategroup	5	10	1.9148	1.1507	1	2.77	0.0961
Dategroup	5	11	1.9148	1.1507	1	2.77	0.0961
Dategroup	5	12	1.9148	1.1507	1	2.77	0.0961
Dategroup	6	7	0.0000	0.7368	1	0.00	1.0000
Dategroup	6	8	1.8608	1.1519	1	2.61	0.1062
Dategroup	6	9	1.9148	1.1507	1	2.77	0.0961
Dategroup	6	10	1.9148	1.1507	1	2.77	0.0961
Dategroup	6	11	1.9148	1.1507	1	2.77	0.0961
Dategroup	6	12	1.9148	1.1507	1	2.77	0.0961
Dategroup	7	8	1.8608	1.1519	1	2.61	0.1062
Dategroup	7	9	1.9148	1.1507	1	2.77	0.0961
Dategroup	7	10	1.9148	1.1507	1	2.77	0.0961
Dategroup	7	11	1.9148	1.1507	1	2.77	0.0961
Dategroup	7	12	1.9148	1.1507	1	2.77	0.0961
Dategroup	8	9	0.0541	1.4520	1	0.00	0.9703
Dategroup	8	10	0.0541	1.4520	1	0.00	0.9703
Dategroup	8	11	0.0541	1.4520	1	0.00	0.9703
Dategroup	8	12	0.0541	1.4520	1	0.00	0.9703
Dategroup	9	10	-0.0000	1.4510	1	0.00	1.0000
Dategroup	9	11	0.0000	1.4510	1	0.00	1.0000
Dategroup	9	12	-0.0000	1.4510	1	0.00	1.0000
Dategroup	10	11	0.0000	1.4510	1	0.00	1.0000
Dategroup	10	12	-0.0000	1.4510	1	0.00	1.0000
Dategroup	11	12	-0.0000	1.4510	1	0.00	1.0000

Appendix V Additional work

During my stay at Massey University I was not only occupied with my own research, but I also participated in other researches.
I listed my occupations sorted by descending amount of time I spend doing it.

Jorien Druijfs research about postparturiant rise of parasites in red deer hinds -18 days
Sampling of deer (rectal or by collecting samples from the paddock), counting eggs and larvae by flotac method and counting larvae in a Bearmann.

Lesley Stringers research about Johne's disease in deer
Weighing and keeping record of deer -3 days
Selecting, measuring and sampling of mesenterial lymph nodes in deer plants - 13 days

Ongoing research at the deer unit of Massey University
Weighing deer/fawns - 1 day
DNA sampling of deer - 1 day
Faecal sampling of deer - 1 day
Drenching fawns – ½ day