Role of Anorexia in Mediating Effects of Blowfly Strike on Wool

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ABSTRACT: Individually penned 18m.o. Merino wethers fed ad libitum on a maintenance diet were infected with 500 1st instar Lucilia cuprina larvae daily for 8 days (Fly Struck, FS, n=5), not infected (Control, C, n=6) or not infected, but pair-fed with fly struck sheep (Pair Fed Control, PFC, n=5). FS sheep developed moderate areas of cutaneous myiasis, high fever (40-41˚C) and exhibited depressed feed intake. While greasy fleece weight and yield were not affected by treatment, staple strength was significantly depressed in the FS, but not the PFC treatment. Similarly volumetric fibre growth determined by autoradiography was exhibited depressed feed intake. While greasy fleece weight and yield were not affected by treatment, staple strength was significantly depressed in the FS sheep relative to controls in the first 4 weeks after infection. PFC sheep exhibited intermediate values. Plasma concentrations of cortisol and IL-6, but not TNFα, IL-1β or IL-8 were significantly elevated in FS but not PFC sheep. Depression in feed intake accounted for 25.3% of the decline in staple strength and 55.0% of the decline in wool growth observed, indicating that other factors play a major role in mediating effects of blowfly strike on wool. Cortisol and/or IL-6 are likely candidates for this.

Key Words: blowfly, strike, wool quality, autoradiography, stress, anorexia, toxaemia, fever, cytokine

INTRODUCTION

Blowfly strike or cutaneous myiasis, is a serious disease problem with estimated annual costs to the Australian sheep industry of more than $160M. The proportion of these costs due to lost production has been estimated at 37% (Beck et al., 1985). Approximately 90% of fly strikes are initiated by the primary blowfly Lucilia cuprina.

Production losses occur through reductions in both wool quantity and quality. Fleece weights from flystruck sheep are 2-8% lighter than those from unaffected sheep (Raadsma and Baker, 1983; Broadmeadow et al., 1983a). However the most important aspect of production loss is a reduction in wool quality. Both fibre narrowing and complete breaks in individual wool fibres from struck sheep lead to a marked reduction in the tensile strength of the wool staple with adverse consequences for the processing attributes of the fibre. Up to 50% of affected sheep in field outbreaks of flystrike are likely to have frankly "tender" wool following infection.

Despite considerable research into the effects of blowfly strike on variables such as wool growth (Broadmeadow et al., 1983b), body temperature and feed intake (Broadmeadow et al., 1983b; 1986), cortisol concentrations (Shutt et al., 1988), and immune responses (Sandeman et al., 1985) the precise mechanisms by which the effects on wool are mediated remain unknown.

The main candidate mechanisms include reduced feed intake or anorexia, increased cortisol secretion and the actions of cytokines or other immune system mediators associated with generalised toxaemia and fever. In order to gain insight into the relative importance of these, an experiment was designed to test the following hypotheses:

1. The effects of flystrike on wool growth and quality are mediated primarily by the reduction in feed intake.

2. They are also associated with increases in plasma concentrations of cortisol and the cytokines IL-1β, IL-6, IL-8 and TNFα.

3. They vary inversely with distance from the site of the strike (ie. there are local as well as systemic effects).

MATERIALS AND METHODS

1. Experimental design

To test these hypotheses, 16 Merino wethers (18 months old) were individually housed in a fly-proof animal house and allocated to one of the following treatments after a 4-week adaptation period:

Flystruck (FS) – (n = 5): These animals were subjected to daily infections with 500 1st instar Lucilia cuprina larvae over an eight-day period. They were offered feed ad libitum throughout.

Pair-fed control (PFC) – (n = 5): Animals were matched on body weight with an animal in the FS group and offered the amount of feed consumed by their paired animal the previous day. These animals were not infected with L. cuprina.

Control (C) – (n = 6): These animals were not infected with L. cuprina larvae and were fed ad libitum for the duration of the experiment.

The experiment comprised four different stages:

Pre-experimental adaptation period (days -28 to day 0) prior to infection during which all animals were offered the experimental ration ad libitum.

Acute infection period (days 0-14). On days 0-7 of this period each sheep in the flystruck group was infected daily with 500 1st instar Lucilia cuprina larvae. Each sheep in the pair-fed group was fed only the amount eaten by its respective pair in the flystruck group on the previous day.

Recovery period (days 14-42) of four weeks after the infection period, during which all sheep were maintained in individual pens and pair-fed feeding was continued.

Pasture period (days 42-126) of twelve weeks duration, where the sheep were returned to pasture.
2. Location, animals and diet

The experiment took place at the University of New England (UNE), Armidale (30.52°S, 151.67°E). Day 0 was 3 Feb. 1998. The wethers used were fine wool Merinos aged 16 months, with 6 months of wool and weighing (+SEM) 31±1.1 kg at day -28. The sole diet used comprised wheaten chaff with 2% urea and 0.2% mineral mix (Lutavit® ruminant premix, BASF).

3. Infection with blowfly larvae

Blowfly larvae were produced in Lucilia cuprina colonies established at UNE. In the afternoon of day 0 all sheep in the experiment had a 2x4cm midside patch clipped and the site punched 10 times with a Mantoux test punch. Sheep in the FS treatment then had approximately 500 freshly hatched larvae (~35mg) in 1ml of water applied to a moistened site before covering with a moist swab held in place by a rubber band in the surrounding wool. This procedure was repeated for 8 days (days 0-7) with the swab no longer required after 2 days. Animal well-being and strike development were monitored closely with flystrike lesions scored for larval numbers, size and activity, lesion size and the severity of skin damage within the struck area. On day 10 infection was terminated by the administration of an insecticide (diazinon) to the fleece of all sheep in the experiment.

4. Measurements

Between days -14 and 42 liveweight was measured weekly. Feed intake was recorded daily and the animals fed ad libitum were offered 10-20% more feed than that consumed the previous day (20% during periods of variable intake). Pair-fed animals received the same amount of feed as their pair consumed the previous day, between days 1 and 42.

Wool growth was determined at 3 sites; 10cm posterior to the right midside infection site (site 1), 20 cm posterior to the infection site (site 2) and at the mirror of site 1 on the opposite side of the animal (site 3). Staple strength was determined on samples clipped from sites 1 and 3 on day 164, using 10 staples per site and an Agritest Staple Breaker Model II. The animals were shorn on day 170 and greasy fleece weights recorded. On days -14, 0, 14, 28 and 42, 0.01 μCi of 35-S-cysteine in 0.2 ml saline was injected intradermally at sites 1,2 and 3 of all sheep. Wool from these sites was harvested on day 56 and prepared for autoradiography using a modification of the method described by Downes et al., (1967). Using Kodak 1 scientific imaging film (BIOMAX MR) an exposure time of 3 weeks proved optimal. Fibre length and diameter (in 4 places) between each labelled site were determined using a Leica Quantimet 500MC image analysis system. Fifty fibres per site for each sheep were mounted.

During the acute infection period body temperature was monitored twice daily (~9am and ~4pm) using a rectal digital thermometer probe. Blood samples (5ml) were collected regularly during the adaptation, acute infection and recovery periods to assay for plasma cortisol (RIA kit, Orion Diagnostica) and selected cytokines (indirect ELISA). The assay limits of sensitivity were 0.7ng/ml for cortisol, 0.13ng/ml for IL-1β, 0.03ng/ml for IL-6, 0.05ng/ml for IL-8 and 0.1ng/ml for TNFα.

5. Derived variables and statistical analysis

Voluntary feed intake was expressed on an "as fed" basis (of a diet of 87.5%DM) and in terms of metabolic weight (LW0.75). Volumetric fibre growth for each period was calculated from autoradiographic data (Length x π x diameter²/4). Data were investigated using analysis of variance with treatment and site as fixed effects (SuperANOVA, Abacus Concepts, Inc. Berkeley, Ca.). Repeated measures analysis was used where appropriate. Significant interactions with time were examined using one-way analysis of variance within time periods and mean separation with Duncan’s New Multiple Range test. Results are expressed as mean ± SEM.

RESULTS

1. Flystrike induction and body temperature

Flystrike was successfully initiated in all 5 FS sheep resulting in moderate areas of myiasis (452±102 cm²) at the end of the infection period. Animals in the flystruck treatment exhibited a significant increase in rectal temperature on day 1 of the experiment (P<0.001). Temperatures remained significantly elevated above those from the control and pair-fed treatment groups until day 11 when they began to drop to around the basal level (Fig. 1).

Figure 1. Rectal temperature (mean±SEM) before and during the acute infection period (* P<0.05).

2. Voluntary feed intake, liveweight and growth.

Feed intake during weeks 1 and 2 (acute infection period) was significantly depressed in the FS and PFC relative to the C group (P<0.05, Figure 2).
There were no significant treatment effects on liveweight at any stage. Over the period 0-42 days animals lost weight slightly (-11.8 g/day).

There was a significant effect of treatment on growth during the first week of infection with greater weight loss in the FS (-534±120 g/d) than C (-160±30 g/d) group (P<0.05). The PFC group showing an intermediate level of weight loss (-329±122 g/d).

Figure 2. Voluntary feed intake (mean±SEM) during the experiment. (* P<0.05).

3. Wool variables

Total greasy fleece weight (3.17±0.07 kg), yield (70±0.9%) and staple length (86±1.7 mm) were not affected by the treatments applied. Staple strength did not differ between the two sites measured in any treatment (overall means for sites 1 and 3, 22.4±2.5 and 21.6±2.4 N/ktx respectively; P= 0.80), but was profoundly influenced by treatment (P=0.002). Only 4 of the 5 FS sheep had measurable staple strength (mean 12.8±2.6 N/ktx), significantly lower than for PFS or Control sheep (23.3±1.7 and 27.0±2.5 N/ktx respectively). These data show reductions in staple strength of 13.4% and 52.7% for PFC and FS treatment respectively with decline in feed intake accounting for 25.3% of the reduction seen in FS sheep.

Figure 3. Volumetric fibre growth (mean±SEM) relative to pre-treatment values (%).

There was no effect of site on wool growth variables so data for all sites were pooled. Only sheep with complete data on more than 15 complete fibres were included (4 sheep/treatment used with a mean of 36±4 fibres/sheep). Treatment had no significant effect on mean fibre diameter (15.3±0.29 μm) or longitudinal fibre growth (279±8 μm/day) but for each variable there was a decreasing trend over the first 4 weeks after infection in FS and PFC sheep. When the two were combined to produce volumetric fibre growth, FS sheep had significantly lower wool growth relative to pre-treatment growth than C sheep over days 1-14 (75±4.6 v 95±2.9%) and 15-28 (68±9.5 v 93±2.1%) post infection (P<0.05, Figure 3).

The PFC group showed nonsignificant declines over the same period. By days 28-42 wool growth had returned to normal in all treatments. Over the 4-week period following infection, volumetric fibre growth in the C, PFC and FS groups was reduced by 6.1, 18.3 and 28.4% respectively. Removing the non-specific reduction seen in the C group leaves a specific reduction of 12.3% and 22.3% for PFC and FS respectively. This suggests that 55% of the reduction was due to reduced feed intake and 45.5% is due to other causes.

4. Plasma cortisol and cytokine concentrations

Flystruck sheep exhibited significant elevations in cortisol concentrations on days 2, 3, 4 and 6 post infection (P<0.05) before declining towards basal levels by day 8 (Fig. 4).

No treatment effects were detected for TNFα, IL-1β or IL-8. TNFα concentrations were low and around the limit of assay sensitivity with only 35% of samples reaching this level (overall mean 0.11±0.06 ng/ml). This was also true for IL-1β (21% positive samples and mean of 0.41±0.16 ng/ml).

IL-8 concentrations were well above the limit of assay sensitivity but there were no treatment effects (overall mean 10.0±2.8 ng/ml respectively). IL-6 concentrations in control and PFC sheep were around or below the limit of assay sensitivity, in the FS group they were significantly elevated on days 2, 3, 4 and 6 after infection (P<0.05, Fig. 5).

Figure 4. Plasma cortisol (mean±SEM) during the acute infection period. (* P<0.05).
Growth in mice have also been reported (Turksen 1996). Specific inhibitory effects of IL-6 on hair differences in autoradiography variables between sites to site of infection) and 3 (midside on opposite side) or 1, 2 and 3 do not support our third hypothesis and demonstrate that the major effects of blowfly strike on wool growth are systemic rather than local.

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