EQUINE WELFARE: A STUDY OF DERMATOPHILOSIS AND THE MANAGEMENT OF DATA RELEVANT TO THE HEALTH AND WELLBEING OF HORSES

By

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LIST OF ACRONYMS

AA	Arachidonic acid
ALA	Alpha linolenic acid
CHD	Coronary heart disease
DGLA	Dihomogammalinolenic acid
EFAs	Essential fatty acids
EPA	Eicosapentanoic acid
EPO	Evening primrose oil
GLA	Gamma-linolenic acid
Hb	Haemoglobin
Ig	Immunoglobulin
LA	Linoleic acid
MCH	Mean cell haemoglobin
MCHC	Mean cell haemoglobin concentration
MCV	Mean cell volume
PCV	Packed cell volume
PG	Prostaglandin
Pl	Platelets
PUFAs	Polyunsaturated fatty acids
RCC	Red blood cell count
WCC	White blood cell count

SUMMARY

This thesis considers aspects of equine welfare which have received little attention in the U.K.

Skin disease, particularly bacterial skin disease, was highlighted as an area giving rise to concern with respect to equine welfare. Dermatophilosis was examined in detail as one of the commoner bacterial skin conditions responsible for animal suffering, and one for which management is often difficult.

Essential fatty acids (EFAs) were evaluated as a dietary supplement in an alternative approach to the management of equine dermatophilosis. The pharmacokinetics of EFAs in the horse were investigated, with EFAs supplemented as evening primrose oil (EPO), containing linoleic acid (LA) and gamma-linolenic acid (GLA). A very slow conversion of LA to its active metabolites was found in the horse compared to other species. A daily dose regime of 20g of 80% EPO and 20% fish oil and vitamin E was adopted for the consequent treatment and prophylactic studies.

In a placebo-controlled, double blind treatment study no significant effect was seen on severity or extent of distribution of lesions of dermatophilosis when horses received EFAs orally. When EFAs were supplemented over the traditional autumn high dermatophilosis risk period in a controlled prophylactic study, they did not prevent development of lesions or reduce incidence of infection. No significant improvement was afforded by EFAs on the condition of the coat, mane, tail or hooves, nor on general body condition. EFAs were not harmful and exerted no effect, adverse or beneficial, on haematological or biochemical parameters.

The characteristics of *D. congolensis* were examined in relation to the site and severity of lesions of dermatophilosis, but no correlation was found. All isolates were different when examined by differential bacteriological growth characteristics and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Proteolytic enzyme production by *D. congolensis* was investigated with regard to the virulence of the organism, and several isolates

demonstrated extracellular protease activity.

The clinical and haematological consequences of bleeding horses at regular intervals were monitored in a group of animals maintained for commercial blood production. No adverse effect was recorded on clinical, protein or haematological profiles when 8 litres of blood were removed every three weeks. Thoroughbred animals supported regular bleeding better than non-Thoroughbred animals.

A relational database system was created as a management tool for the manager of the horse herd. The information contained within the system, regarding horse details, bloodroom records and farm laboratory records, could be constantly updated. Rapid detection of poor performers or anaemic animals could permit prompt instigation of corrective action, avoiding undue animal distress.

It is hoped that some of the work within this thesis has made a worthwhile contribution to the extension of knowledge concerning the welfare of horses in the U.K.

INTRODUCTION

BACKGROUND

SKIN DISEASE IN THE HORSE

A PERSPECTIVE OF DERMATOPHILOSIS IN THE HORSE

ESSENTIAL FATTY ACIDS (EFAs): THE RATIONALE FOR POSSIBLE USE TO MANAGE EQUINE DERMATOPHILOSIS

BACKGROUND

This thesis considers aspects of welfare of horses which to date have been given little attention in the United Kingdom. Skin disease, and in particular dermatophilosis, is a common, debilitating problem in horses, and a new approach to management of the condition using essential fatty acids was investigated. In order to put the condition into perspective the problem of skin disease in the horse as a whole is reviewed prior to a more detailed consideration of dermatophilosis in subsequent chapters. In addition, the characteristics of *Dermatophilus congolensis* were examined in relation to the site and severity of lesions, leading to an investigation of an extracellular protease.

The horses under study were being kept for commercial blood production and as such were subjected to regular, repeated blood harvest, thus presenting an unique opportunity to assess the welfare consequences of such a procedure. To aid and improve the management of the large numbers of horses encountered in the work of this thesis, an equine management database was created.

SKIN DISEASE IN THE HORSE

The horse's skin is the largest organ in its body (Scott, 1988) and it is the point of contact between internal and external environments. The skin is a good indicator of general health and it performs the important functions of protection from trauma and prevention of invasion by micro-organisms or chemical compounds and it allows sensation of external stimuli such as temperature, touch, itch, pain and pressure. Skin pigmentation protects against solar radiation and vitamin synthesis occurs in the skin in response to solar radiation. Temperature regulation in the body is provided by evaporation of sweat from the skin.

Disease of the skin is readily noticed and is frustrating for the animal, the owner and the veterinary surgeon as it often causes discomfort, disfigurement and may preclude use for work, showing or riding (Fadok and Mullowney, 1983).

The horse's comfort is grossly affected for example by generalised pruritus and self inflicted trauma and excoriation resulting from allergic reactions to foods, drugs, insects, plants and numerous inhaled allergens. Localised pruritus can be inflicted by a range of ectoparasites, such as mane and tail infestation by lice or *Culicoides*, tail region affected by *Oxyuris equi*, extremities by harvest mites, and localised areas affected by ringworm. Discomfort to the extent of hyperaesthesia and pain, with secondary bacterial infection, can be a consequence of dermatophilosis, folliculitis and furunculosis, fly infestations, contact dermatitis, or ulceration of the surface of cutaneous neoplasms.

The appearance of the horse, and consequently its use as a show animal, can be drastically affected by self-inflicted trauma after pruritic episodes, by the alopecia and crusting after ectoparasite or ringworm infection, and permanent scarring may result from folliculitis, furunculosis or dermatophilosis. Cutaneous neoplasms: sarcoids, papillomas, melanomas and squamous cell carcinomas; nodular skin disease; environmental diseases such as

photosensitisation; seborrhoea; purpura haemorrhagica vasculitis and *Streptococcus equi* infection can all render an animal unsightly.

A horse's use as a riding or working animal is affected by conditions such as ringworm, dermatophilosis, contact dermatitis, nodular skin disease and sarcoids, as they can prohibit the use of tack, depending on site of lesions.

Skin conditions that can be transferred to other animals or to man prevent useful work, e.g., a horse suffering from zoonotic ringworm infection may be prohibited from racing and from shows (Thomsett, 1979). Dermatophilosis can be spread from horse to horse where there is close contact, and is also a zoonosis. *S. equi* infection, "strangles", is highly contagious to other horses and systemic involvement precludes useful work.

Skin conditions of very different aetiologies may have a similar clinical appearance (Evans and Stannard, 1986). Also, the skin has limited responses to external and internal challenge and the development of secondary lesions which often accompany chronic skin disease tend to complicate diagnosis (August, 1986). Early recognition and instigation of therapy in equine skin disease is thus highly important (Thomsett, 1979).

Specific equine dermatoses can be bacterial, parasitic, viral, fungal, neoplastic, allergic or immune-mediated, miscellaneous, environmental or congenital. The commoner conditions from each category are now reviewed.

Parasitic Skin Disease

Ectoparasitic infections of the skin are the most common skin diseases of large animals, including the horse (Scott, 1988). There is often a great degree of suffering by annoyance, irritation, itch, disfigurement, secondary infection and fly strike. Concentration loss owing to discomfort may preclude the horse's use for work and weight loss can result from lowered food

intake. Viruses, bacteria, fungi, helminths and protozoa may be transmitted by ectoparasites (Scott, 1988). As with endoparasite infection, ectoparasitic infection is a group problem, rather than one of individual animals. Healthy, properly fed, well-managed animals have increased resistance to the invasion and establishment of parasites. Improper feeding and grazing, overstocking, insanitary conditions and poor attention to illness in their early stages all favour ectoparasitic infection (Naviaux, 1985).

Some of the commoner parasitic skin conditions encountered in the U.K. include lice, *Culicoides spp.* hypersensitivity ("sweet itch"), fly infestations, harvest mites, and *Oxyuris equi* infection.

Biting (*Damalinia equi*) and sucking (*Haematopinus asini*) lice cause clinical signs which are more obvious during the winter when animals gather for warmth, when the haircoats are longer and when animals are stressed by the cold (Fadok and Mullowney, 1983; Fadok, 1987). In southern regions of the U.K. infestations can occur at any time of year. The presenting sign is pruritus seen as self-inflicted trauma, erythema and hair loss (Pascoe, 1973), and the coat is frequently dull with scales present. Affected animals are often restless and weight loss may result as appetite can be poor. *D. equi*, the biting louse, is small in size and pale brown in colour and prefers the dorsolateral trunk (Scott, 1988). Heavy infestations by the blue-grey coloured sucking louse, *H. asini*, may cause anaemia. Sucking lice tend to prefer the fetlocks and the mane and tail (Scott, 1988).

Nits, the eggs, are cemented to the host's hair by the adult females and hatch to nymphs. Lice spend their entire life on the host and are extremely host specific. Infection is by close contact with other infested animals or via infested boxes, tack, blankets and grooming kit, as the adults can live off the host for up to two weeks (Soulsby, 1982).

Culicoides hypersensitivity, or "sweet itch" is a common, well-recognised clinical condition which occurs all over the world

as well as in the U.K.. The incidence varies within countries and between geographical locations (Fadok, 1987; McCaig, 1973). It is a recurring seasonal dermatitis of individual horses and ponies during the warmer times of the year (Soulsby, 1982).

Affected animals develop allergic reactions to the bites of *Culicoides* species, biting midges (Baker and Quinn, 1978; Mellor, 1974; Pascoe, 1973). The individual species of *Culicoides* which feed on horses causing the pruritic condition have been identified and correlated with the site and seasonal occurrence of lesions, and geographical location. In the U.K. there are over 20 species of *Culicoides*, according to Baker & Quinn (1978), each with a characteristic habitat and preferred host for blood meals. Generally, *Culicoides* breed in still or slowly running water, typically lakes, marshy areas and small pools of water. The insects inhabit decaying plant material and manure (McMullan, 1982), particularly on sandy or alkaline soils (Fadok & Mullowney, 1983).

A British Equine Veterinary Association survey carried out between 1962 and 1963 placed the U.K. incidence at 2%, while McCaig (1973) found it to be 2.79% in ponies. McCaig (1973) reported regional variations in incidence with few cases in regions over 1000 feet.

Typically there is localised pruritus characterised by rubbing of the mane and tail. Excoriations, crusts, scaling, alopecia and erythema are seen on the forehead, withers, shoulders, rump and tailbase regions (Fadok & Mullowney, 1983). Mane and tail hairs are often broken and matted as a result of self-inflicted trauma. Severity of the disease varies between horses, and worsens with age. The condition has been seen in one and two year olds, but most ponies were noted by McCaig (1973) to show signs of the condition during their third or fourth summer.

A complex hypersensitivity to bites of dorsally feeding *Culicoides spp.* is the pathogenesis of the condition (Pascoe, 1973; Mellor, 1974; Baker and Quinn, 1978). There is an hereditary predisposition (Fadok and Mullowney, 1983). Initially the

condition coincides with the fly season, usually between April and November in the U.K., with peaks in May and November in Southern regions (McCaig, 1973).

Affected horses spend a large proportion of their time rubbing against stationary objects. Some horses show involvement of the entire dorsal midline, and cases involving pruritus of the ventral midline, pectoral and inguinal regions and lower limbs have been reported. When the condition becomes chronic, lesions involve larger body areas. Initially hyperaesthesia is marked, hair becomes tufted and papules are seen. Lesions heal and hair grows back the first winter, however the papules and pruritus return with warmer weather (McMullan, 1982). Characteristically, with recurrent attacks affected areas become alopecic with transverse ridging of the skin, hyperkeratinisation, marked acanthosis and diffuse scaliness. The proximal third of the tail and the mane are often denuded. According to Baker and Quinn (1978) the disease should be regarded as an unsoundness, as animals suffering from severe infections cannot be worked.

Many different flies attack horses, often leading to irritation, especially in young horses which may take fright and cause themselves injury (Naviaux, 1985; Pascoe, 1973). There is reduced concentration for work as a consequence of irritation, and bites, often painful, tend to induce allergic reactions (Fadok, 1987). When flies are present in large numbers they can cause significant blood loss (Naviaux, 1985).

Bite lesions are most often seen during the summer and are usually pruritic (Rollins, 1981) and result in self-inflicted trauma evidenced by alopecia, excoriation and lichenification. Bites from stable flies, *Stomoxys calcitrans*, are painful, reflecting the vicious mouthparts of the species (Pascoe, 1973); and robust horse flies, *Tabanids* and *Hybometra*, and deer flies, *Chrysops*, also inflict painful bites which develop into large wheals (Fadok, 1987). Horn flies, *Haematoba irritans*, cause the focal ventral midline dermatitis syndrome in the horse, characterised by alopecia, erythema, depigmentation, scaling, serous oozing crusting and excoriation which is localised to the umbilical

region (Rollins, 1981). *Simulium*, or black flies, attack other sparsely-haired ear, intermandibular, pectoral and inguinal regions; and *Habronema microstoma* larvae, which cause cutaneous habronemiasis or "summer sores", use stable flies as an intermediate host (Fadok, 1987).

Harvest mite infestations, or trombiculiasis, coincide with the late summer and autumn harvest season (Fadok and Mullowney, 1983; Soulsby, 1982) and are seen mainly in horses kept at pasture or exercised through infested fields and woods, particularly on chalky soils (Thomsett, 1979). Papular lesions become crusted and tend to occur on areas in contact with vegetation, typically on the extremities, face, neck and sometimes the thorax. Lesions may be very itchy. *Neotrombicula autumnalis* larvae parasitize small rodents while nymphs and adults are free-living (Soulsby, 1982). Larvae attach to the horse and salivary components hydrolyse the epidermis allowing tissue fluid extraction (Soulsby, 1982). Larvae hatch to adults off the host. According to Thomsett (1979), thin-skinned breeds and types are at highest risk of infection.

Oxyuriasis infection tends to be seen only amongst young horses on a poor anthelmintic programme (Foreman, 1987). Dermatological signs secondary to anal pruritus are caused by infection by the nematode parasite, *Oxyuris equi*, commonly known as the pinworm. Adult female worms reside in the caecum and colon and feed on intestinal contents, migrating out of the anus to deposit eggs in the perineal region. There is rapid development of the eggs, within three to five days, and pruritus is the result of skin irritation initiated by the gelatinous material which encases the eggs. Infection is by ingestion of infective eggs in contaminated feed or bedding (Soulsby, 1982).

Constant rubbing of the tail base lead to broken, matted tail hairs, erosions and ulcers, producing a "rat tail" appearance in severely affected animals (Fadok and Mullowney, 1983).

Bacterial Skin Disease

In the absence of agents or disease causing suppression of the immune system, and when no defect occurs in the protective barrier, the skin of healthy animals is not normally susceptible to infection by the extensive range of micro-organisms with which it contacts daily (Scott, 1988). Organisms may penetrate through damaged skin via hair follicles or sweat or sebaceous gland ducts, or they may reach the skin by haematogenous or lymphatic routes (Kral, 1962).

The main bacterial infections of skin in horses in the U.K. are dermatophilosis, folliculitis and furunculosis, and *S. equi* infection, "strangles" (Thomsett, 1979). While it is generally considered that these are less common than parasitic disease, they are an increasing welfare problem because they are seen more commonly under poorer management systems and can be difficult to treat and manage, in particular dermatophilosis. As dermatophilosis is a major component of this thesis it is dealt with only briefly as part of bacterial skin disease and is reviewed at length in a later section of this introduction.

Dermatophilosis is an infection of the epidermis which results in exudative epidermitis with scab formation (Van Saceghem, 1915). It is worldwide in distribution and clinical infection is most severe in tropical climates, but it is also a major problem in more temperate regions, particularly where rainfall is high (Stewart, 1972a).

Suppurative inflammation of the hair follicles is known as folliculitis and when the suppurative process extends into the surrounding dermis and subcutis the condition is referred to as furunculosis (Dietz and Wiesner, 1984). Bacterial folliculitis and furunculosis are often caused by bacteria, most commonly *D. congolensis* and *Staphylococcus aureus* (Dietz and Wiesner, 1984); but Pascoe (1984) reported involvement of *Corynebacterium pseudotuberculosis*, *Rodococcus equi* and *Bacillus spp.* and *S. hyicus* has also been isolated from such lesions (Devriese, Vlaminck, Nuytten and De Keersmaecker, 1983). Fungi and parasites

can also be causative agents. Mechanical trauma, resulting from biting insects and tack, warmth, moisture and unhygienic environmental conditions predispose animals to folliculitis and furunculosis.

Bacterial folliculitis and furunculosis is most frequently encountered in spring and summer, and is also referred to as acne, heat rash or summer rash (Mullowney and Fadok, 1984a). Infection is seen commonly in children's ponies which are not carefully tended, and where tack is badly maintained, and tends to be more severe and widespread in horses that have a long or poorly groomed haircoat (Pascoe, 1973). Once infection becomes established in a stable, well attended horses are also susceptible. Furunculoses can be very resistant to treatment, and healing may take weeks or months, and recurrences and reinfections are likely (Dietz and Wiesner, 1984).

Pascoe (1984) found single abscesses at the commissures of the lips and on the cheeks, but reported multiple lesions affecting the saddle, loin and chest areas as common. The area between the hindlegs may also be involved (Kral, 1962). Initial lesions are small, painful papules, enlarging over a couple of days to 5 or 10 mm diameter with serum exudation and scab formation. Although sometimes there is significant oedema which can lead to larger, painful swellings, little pus is reported (Pascoe, 1984). Owing to acute pain, the affected animal is often unfit for ridden work (Pascoe, 1973; Roberts, 1967a).

Streptococcus equi infection, or "strangles", although not a true skin infection, can cause submaxillary and pharyngeal lymph node abscesses. There may be thinning and loss of hair, focal necrosis, and subsequent rupture of lymph nodes to discharge thick, creamy pus through the skin (Thomsett, 1979).

Fungal Skin Disease

Infection of keratinised tissue, nail, hair or stratum corneum by a fungal species, such as *Microsporum* or *Trichophyton*, is known as

dermatophytosis (Kral, 1962). Dermatophytes are believed to be localised to keratinised tissues owing to lack of available iron elsewhere (Biberstein, 1990). Fortunately, of the vast numbers of fungi present in the horse's environment, few are pathogenic (Scott, 1988). Diagnosis of fungal skin infection is hampered by the huge variation in clinical manifestation.

Fungal infections can be transmitted between animals, from animals to humans, and from humans to animals. The progression of infection by zoophilic, or animal-related fungi, such as *Trichophyton equinum*, *T. mentagrophytes*, or *Microsporum equinum*, is often prevented by an acute inflammatory reaction in a human host. In the animal host, however, the fungus exists in harmony with its host and inflammation is rare (Scott, 1988).

Ringworm is one of the most common skin conditions (Saunders, 1981) and is one of the most important infectious diseases of the horse in the U.K. (Thomsett, 1979). It is also an important zoonosis. Infection often precludes useful work as the animal may be prohibited from racing, from shows and from sale rings (Thomsett, 1979). Outbreaks in riding and racing stables are common and most cases occur in autumn and winter, often where the environment is unhygienic, overcrowded, warm, damp, and where there is little sunshine (Pascoe, 1984). Once infection is established in a stable it can be very difficult to manage. Zoophilic species, such as *T. equinum* and *T. mentagrophytes*, are most frequently diagnosed, accounting for over half of equine ringworm cases, but *T. verrucosum*, *M. equinum* and *M.gypseum* may be involved (Johnston, 1986; Mullowney and Fadok, 1984b; Pascoe and Connole, 1974).

Fungal spores germinate near a follicular orifice, and hyphal strands grow into hair follicles along the outer root sheaths and invade growing hairs near the living root cells (Biberstein, 1990). Hyphae grow within the hair cortex. Colonisation may cause hypertrophy of the stratum corneum, resulting in accelerated keratinisation and exfoliation, producing a scurfy appearance and hair loss (Biberstein, 1990). It is usually by the second week that inflammation begins, at the margin of the parasitised area,

with manifestations ranging from erythema to vesiculopustular reactions and suppuration.

Abrasion by tack, especially by girths in the racing world, facilitate fungal invasion. The incubation period is normally between four and 30 days (Pascoe, 1979), and hair loss occurs after three to 10 days (Johnston, 1986).

Clinical appearance of the condition varies according to the response by the host as well as to the causative fungal species, as antibody- and cell-mediated hypersensitivities occur in the course of infection (Biberstein, 1990).

Transmission of *T. mentagrophytes* involves rodents (Kral 1962). Lesion distribution is mainly on the head, neck, tail base and extremities. Lesions are alopecic with heavy grey crusting (Kral, 1962). Hair regrowth begins in the lesion centre, and active papules, vesicles and pustules are seen peripherally. The degree of pruritus varies, but is less than that associated with *T. equinum* or "girth itch" lesions, which are intensely pruritic (Kral, 1962). *T. equinum* lesions range in diameter from 0.5 to 2 cm, and are alopecic and scaly. Vesicles are palpable at the edge of lesions.

T. verrucosum lesions are similar to those of *T. equinum* and are commonly seen in horses grazed with, or which have had access to buildings which housed, ringworm infected cattle (Johnston, 1986).

M. gypseum lesions are commonly found on the dorsal body areas, and tend to be associated with damp surroundings and biting insects (Pascoe and Connole, 1974). Inflammation around lesions is greater than for ringworm induced by other species, and crusting is heavy and grey. On removal of crust there is a moist, reddish ulcer (Johnston, 1986).

Lesions induced by *M. canis* often respond poorly to therapy and heal when the weather is warmer and sunnier (Kral, 1962). Areas under tack are those most commonly affected.

Ringworm infection may be directly or indirectly transmitted (Pascoe, 1973), as fungi may persist on tack, grooming kit, clippers and infected stalls and trailers or on human clothing (Kral, 1962). Lack of previous infection predisposes younger animals to infection, and age resistance develops so that although adults may suffer reinfection, infections are milder (Scott, 1983).

Neoplastic Skin Disease

Cutaneous tumours are the most common form of neoplasm in the horse, and there are only a limited number of important cutaneous neoplasms in the horse (Thomsett, 1979). In other species, skin tumours are associated with increasing age but this is not true for the horse (Scott, 1988). Sex and breed predilections exist, e.g., mastocytoma in male horses (Altera and Clark, 1970), and melanoma in Arabs and Percherons (Scott, 1988). Sarcoids, followed by papillomas, squamous cell carcinomas and then melanomas are the most frequently encountered cutaneous neoplasms in the horse (Pascoe, 1973; Pascoe and Summers, 1981).

The equine sarcoid is a locally aggressive fibroblastic tumour, and is the commonest tumour of the horse (Pascoe, 1973; Pascoe and Summers, 1981; Ragland, Keown and Spencer, 1968; Tarwid, Fretz and Clark, 1985), the donkey and the mule (Pascoe, 1984). Sundberg, Burnstein, Page, Kirkham and Robinson (1977) reported the tumour to account for over 43% of all equine neoplasms. In a 21-year survey of equine tumours (Pascoe and Summers, 1981), 142 out of 409 tumours were sarcoids. Sarcoids accounted for 55.6% of all neoplasms in horses and ponies in another survey over a 10-year period (Strafuss, Smith, Dennis and Anthony, 1973). No predilection exists for breed, sex, coat colour or season of the year, but there is a definite tendency for the tumour to be encountered in younger horses (six years old or less) compared to other malignancies that occur in horses (Tarwid, Fretz and Clark, 1985). The tumour has epithelial and fibroblastic components (Mullowney and Fadok, 1984a).

Aetiology is not certain but is considered to be viral (Tarwid, Fretz and Clark, 1985). Recent hybridization work confirms that the sarcoid contains papillomaviral DNA. Studies carried out on donkey derived sarcoid tumours have resulted in the identification of two types of viral DNA, one which is similar to bovine papillomavirus type 1 (BPV-1), and another similar to BPV-2 (Reid, unpublished data). The strongest evidence of papillomaviruses in the aetiology of the disease is the unique isolation of papillomaviral-like virions from an equine sarcoid. Although retroviral elements have been identified in a sarcoid derived cell line, the significance is unclear and the retrovirus is presumed to be endogenous (Reid, unpublished data). Familial tendencies have been observed (Strafuss, Smith, Dennis and Anthony, 1973) and equine leucocyte antigen (ELA) studies suggest connection of the predisposition to sarcoid with the major histocompatibility complex (Scott, 1988).

A horse can have a solitary sarcoid, but between one third and one half (Pascoe, 1984; Mullowney and Fadok, 1984a) up to over 80% (Tarwid, Fretz and Clark, 1985) of cases have multiple lesions. The most common locations are the extremities; the head, particularly periocularly, on the pinnae and on the commissures of the lips; and the ventral abdomen, on the prepuce and inner thighs (Strafuss, Smith, Dennis and Anthony, 1973). These areas are often subjected to trauma, and the tumour commonly occurs at scar sites (Mullowney and Fadok, 1984a; Tarwid, Fretz and Clark, 1985). Sarcoids do not metastasise but may spread by biting or rubbing of the lesion by the horse (Wyman, Rings, Tarr and Alden, 1977). Transmission is believed to be by contaminated tack, grooming equipment and surgical instruments, and epizootic forms have been seen within herds (Ragland, Keown and Spencer, 1968).

There are four gross types of equine sarcoid: the verrucous or warty type (type I); the fibroblastic or proud flesh-like type (type II); the mixed verrucous and fibroblastic (Type III), and the flat or occult type (type IV), (Pascoe and Summers, 1981; Tarwid, Fretz and Clark, 1985). Verrucous sarcoids are small, sessile or pedunculated, with a dry, horny cauliflower-like surface which frequently resembles a papilloma (Tarwid, Fretz and

Clark, 1985). The appearance of the fibroblastic sarcoid varies more, with some lesions circumscribed and nodular, occurring in the dermis and subcutis, while others may be over 25 cm in diameter with ulcerated, easily-traumatised surfaces. Occult sarcoids are found most often around the head, especially the ears and eyelids (Tarwid, Fretz and Clark, 1985). They may remain static for extended periods as circular, alopecic areas with scaling and crusting, then papules and nodules may develop within the hairless regions (Pascoe and Summers, 1981). Surgical intervention results in rapid transformation to the fibroblastic type of sarcoid (Tarwid, Fretz and Clark, 1985).

The equine sarcoid is notoriously difficult to treat (Murphy, Severin, Lavach, Hepler and Lueker, 1979): spontaneous regression is rare, and there is a very high recurrence rate following total surgical excision, radiation treatment and cryosurgery. Success of autogenous and heterologous vaccines has been of a varying degree (Wyman *et al.*, 1977). Reticuloendothelial stimulation using Bacille Calmette-Guerin (BCG), is a popular and effective treatment (Rebhun, 1987). When BCG is inoculated into the lesion the regression of tumour cells is dependent on the ability of the host to develop a delayed type hypersensitivity. This involves the stimulation of normal T and B lymphocytes and the generation of a population of activated macrophages with a potent cytolytic activity against sarcoid cells (Howarth, 1990). Unfortunately the treatment leads to intense inflammation, and it has caused anaphylactic deaths (Rebhun, 1987).

Papillomatosis, warts or infectious verrucae, caused by a host-specific DNA papovavirus (Scott, 1988), is primarily a condition of young horses (Duhaime, 1981), predominantly one and two year old animals (Fadok and Mullowney, 1984a; Pascoe and Summers, 1981), although older horses may be affected (Pascoe, 1984) and a congenital form has also been reported (Garma-Avina, Valli and Lumsden, 1981). A higher incidence of papillomatosis was reported when numbers of young stock were increased on brood mare farms during the breeding season and prior to sales (Pascoe, 1984).

Multiple lesions, often over 100 warts, are commonest, but single lesions may be seen. Transmission is via fomites and the virus, which is relatively resistant, uses skin abrasions and wounds as portals of entry (Fadok and Mullowney, 1984a). The primary site of lesions is the muzzle (Duhaime, 1981; Pascoe, 1984), followed by the legs, prepuce and ventral body wall. Involvement of the nose, eyelids, jowl and neck were reported by Pascoe (1984). Pascoe (1973) reported that warts usually appear suddenly, in small numbers which soon multiply rapidly to cover most of the nasal area. Warts that persist as multiple lesions on the inner surface of the ears and the legs and on the ventral body wall and prepuce may be a consequence of transfer of virus by grooming equipment or by rubbing of the face on the legs (Fadok and Mullowney, 1984a).

Thomsett (1979) described lesions beginning as raised, dome-like epidermal areas, single or in clusters, then proliferating to spherical masses up to 2 cm diameter. According to Pascoe (1984) they are grey with fibrous stalks. Surface of lesions may be rough, wrinkled or smooth, and grey red in colour (Fadok and Mullowney, 1984a). Papillomatous scratches may be a consequence of chronic inflammation initiated by mechanical or chemical irritants. Pascoe (1984) believed that maturation occurred over a three to four month period, with associated change in colour to pink, and that lesions regressed and sloughed three or four weeks later. Spontaneous regression of lesions tends to occur from the muzzle of young horses two to three months after appearance is first recorded (Duhaime, 1981; Pascoe and Summers, 1981).

A 60 to 90 day incubation period was reported during experimental infection by Fadok and Mullowney (1984a) with regression in most cases after 60 days. The solid immunity resulting after natural infection is not always induced by experimental infection.

Small raised areas of depigmented papillomatous skin are commonly seen in the ears of horses, of all breeds and of both sexes, causing no disturbance. They are seldom seen in horses under a year old (Stannard, 1972) and although aetiology is unconfirmed (Thomsett, 1979), papilloma virus has been isolated.

The greyish plaque-like lesions may coalesce from several mm diameter to cover a large proportion of the inner pinnal surface, and are easily dislodged. These plaques have never been proven to contribute to the head shaking syndrome, and although they tend to persist indefinitely, they are unimportant. Similar lesions have been found in the anal and vulval regions (Stannard, 1972).

Squamous cell carcinoma accounted for 20% of all equine neoplasms in one survey (Strafuss, 1976). Forty-three percent occurred on the head, eye and ocular adnexa, 45% were on the external male genitalia, and 12% were in the female perineal region. Pascoe and Summers (1981) found that squamous cell carcinoma accounted for 55 out of 409 equine tumours over a 21 year survey. Of these 55 lesions, 35 involved ocular tissues, and 16 were recorded on the external genitalia. It is the commonest penile tumour of the horse (Stafuss, 1976).

Predisposing factors are unpigmented skin (McMullan, 1982), and, when the tumour is located around the eye, chronic exudate from a weeping eye (Foreman, 1987). The cornea and sclera are less frequently affected than the eyelid, and there is often aggressive spread to the maxillary sinuses or lacrimal duct from the third eyelid. Development is usually at mucocutaneous junctions (McMullan, 1982) and areas such as the nasal cavity, tongue, stomach, maxillary sinus, tail, limbs, anal sphincter, hard palate, peritoneum, lips and nose have been invaded (Mullowney, 1985a). Carcinomas of the vulva and clitoris occur more frequently in older than in young mares (Pascoe and Summers, 1981).

Clinical appearance varies from papillomatous to ulcerated, cauliflower-like tumours, and growth rate is variable. Metastasis is rare but local invasion can be aggressive. The mean age affected is 12 years (Mullowney, 1985a; Strafuss, 1976; Sundberg *et al.*, 1977). Foreman (1987) reported a predisposition in Appaloosas, usually in horses over five years, and commonest in unpigmented areas.

Murray, Ladds and Campbell (1978) recorded a 6.7% incidence of squamous cell carcinoma, while Mullowney (1985a) found it to be

16.4% (329 of 2005 skin tumours) and Sundberg *et al.* (1977) described it as higher, at 24.6%.

Melanomas are common, easily-recognised neoplasms. Although encountered most frequently in greys, melanomas have been reported in horses of other colours, bays for example (Foreman, 1987; Pascoe and Summers, 1981). They are rare in animals under six years old, although congenital melanoma has been reported (Hamilton and Byerly, 1974). The high incidence of the tumour in Arabs and Percherons may be due to the high numbers of greys within these breeds. No sex predisposition occurs. Although melanomas are seen on the legs, neck, eyelids, ears and vulva, the primary sites are the parotid gland area and the perineum and underside of the tail (Dietz and Wiesner, 1984; Pascoe and Summers, 1981).

Melanomas may remain benign, growing very slowly, for as long as 20 years, but prognosis is hopeless once there is involvement of internal organs (McMullan 1982). Dystokia and problems in defaecation are likely consequences (Pascoe, 1984).

Sundberg *et al.* (1977) reported melanoma to account for 3.8% of all equine neoplasms diagnosed, with an age range of two to 20 years, and mean of just over eight years. Incidence of melanomas was 2% of all granulomatous and neoplastic lesions in equine skin in a survey by Murray, Ladds and Campbell (1978) and Mullowney (1985a) found an incidence of 5.3% (106 of 2005 skin tumours).

Allergic and Immune-Mediated Skin Disease

When an adaptive immune response occurs in an exaggerated or inappropriate form resulting in tissue damage, the term hypersensitivity is applied (Roitt, Brostoff and Male, 1989). Hypersensitivity is a characteristic of the individual and is expressed on second contact with the specific antigen inducing hypersensitivity. Allergic or hypersensitivity reactions have been classified into four types on an immunopathological basis by Gell and Coombs (1975): type I or immediate hypersensitivity, type

II or antibody-dependent hypersensitivity, type III or complex-mediated hypersensitivity, and type IV or delayed hypersensitivity. In practice these types do not always occur in isolation from each other (Roitt, Brostoff and Male, 1989). These reactions are simply manifestations of the beneficial immune responses causing inflammatory reactions and tissue damage expressions. Allergic or hypersensitivity reactions are often seen in the skin of the horse.

Allergic skin disease is a common, frustrating problem of individual horses, which can occur all year round, but during the summer months insect hypersensitivities are extremely prevalent.

Allergic contact dermatitis in the horse involves delayed, or type IV hypersensitivity (Tizard, 1987). The causative agents, which must contact the skin directly, range from drugs and chemicals, insecticide, heavy metal, soap or fly spray, for example; to blankets or wool bedding, and plants such as poison ivy (Tizard, 1987). These agents normally act as haptens which bind with skin protein to become complete antigens (Manning and Sweeney, 1986). The agent may have been present in the environment for years before allergic contact dermatitis develops (Stannard, 1972; Manning and Sweeney, 1986). After an incubation period of one to four weeks, hypersensitivity develops and persists indefinitely, with the result that inflammation will be seen within one to three days if the allergen is contacted (Mullowney, 1985b).

The lesions of allergic contact dermatitis vary greatly in severity, ranging from mild erythema to severe, erythematous vesiculation (Tizard, 1987). Erythematous, vesicular, plaque-like swellings are seen on the skin in early stages and if there is persistent allergen exposure alopecia, desquamation and lichenification result, usually confined to the epidermal and dermal areas (Manning and Sweeney, 1986). Because of the intense pruritus, self-trauma, excoriation, ulceration and secondary pyoderma often mask the true nature of the lesion (Tizard, 1987). Distribution of the dermatitis depends on the site of contact of allergens, but the head, extremities, ventral body surfaces and tail areas are frequently affected. Genetic predisposition, the

nature of the allergen, damage to the skin and mechanical factors, site on the body, sweat and sebaceous gland function, skin pH, and immunological competence are all believed by Mullowney (1985b) to be involved in the likelihood of a horse developing hypersensitivity reactions.

Acute but transient localised or generalised formation of wheals or plaques are characteristic of urticaria (Dietz and Wiesner, 1984). The nose, lips, neck and trunk are usually involved. Chemical mediators are produced as mast cells degranulate in response to this immediate or type I hypersensitivity reaction. Areas of dermal oedema are produced due to altered capillary permeability and pit on digital pressure.

Immunological, pharmacological, psychological and physical urticarias are recognised in the horse by Manning and Sweeney (1986). Immunological urticaria can then be subgrouped as reagin mediated (type I), cytotoxic antibody (type II) and immune complex (type III). The urticarial syndrome is a common problem but the responsible agent is often undetermined. Lesions are extremely irritating because of the histamine released and consequently the true nature of the lesion may be masked by scratching (Tizard, 1987). Urticaria is not always allergic, and when it is allergic it is not always IgE mediated (Manning and Sweeney, 1986).

Drugs such as glucocorticoids (Dietz and Wiesner, 1984), penicillin, and streptomycin (Kral, 1962), tetracycline, and pethidine (Pascoe, 1984); vaccines such as influenza and tetanus antitoxin (Pascoe, 1984); foods; additives; infections by bacteria (*S. equi*), fungi (*T. equinum*) and parasites (*Onchocerca cervicalis*) (Kral, 1962); bites; stings; inhaled allergens (Evans, 1987), and systemic disease such as purpura haemorrhagica (Manning and Sweeney, 1986), have all caused urticarial reactions in horses.

The most efficient reaginic or homocytotropic antibodies, responsible for type I hypersensitivity, belong to the IgE immunoglobulin class (Roitt, 1984). However recent studies in the horse demonstrate that certain IgG subclasses also fit this

description (Manning and Sweeney, 1986). IgE has been described specifically in the horse, but although experiments in cutaneous anaphylaxis have demonstrated the presence of reaginic antibody in horses, the immunoglobulin class has not been characterised (Manning and Sweeney, 1986).

Small, pruritic lumps predominantly affecting the thorax, but sometimes extending to the head and neck are often attributed to food allergy, and these signs may be accompanied by severe tail rubbing (Pascoe, 1973). Self inflicted trauma and secondary bacterial infection are possible consequences. Ingestion of excessively high protein diet (Pascoe, 1973), or of potatoes, distillery wastes, malt, beet pulp, clover, St. John's wort, glucose, wheat, oats, barley, bran and chicory (Mullowney, 1985b) often lead to urticarial reaction .

The mucous membranes of the nose, mouth, anus and vulva may become involved in urticarial lesions, and infrequently the pharynx and larynx are affected, with impaired swallowing and breathing (Thomsett, 1979).

Vasculitis may occur at any location in the body and is commonly a secondary sign of a disorder elsewhere (Morris, 1987). The most commonly diagnosed cutaneous vasculitic syndrome in the horse is purpura haemorrhagica (Morris, 1987). It is an uncommon sequel to *S. equi* or *S. zooepidemicus* infection (Manning and Sweeney, 1986), characterised by extensive oedematous and haemorrhagic swellings in subcutaneous tissues, accompanied by haemorrhages in the mucosae and viscera (Blood and Radostits, 1989a). It has classically been described as a nonthrombocytopaenic purpura, as platelet counts are normal in affected horses (Reef, 1987). It typically occurs between two and four weeks after respiratory infection of streptococcal origin, but may be associated with previous and/or concurrent beta-haemolytic streptococcal infection, influenza, or to drug-induced allergies (Reef, 1987). The condition is considered to be a significant problem although incidence is low (<10% of streptococcal respiratory infections and <1% of influenza cases)(Manning and Sweeney, 1986). Incidence is reported to be highest in large groups of horses used for military

purposes, or during and after shipment (Blood and Radostits, 1989a).

Clearly circumscribed oedematous areas of the dermis and subcutis are seen about the face and muzzle but are often present on other parts of the body and are not necessarily symmetrical in distribution (Blood and Radostits, 1989a). Cutaneous infarction, necrosis and serum exudation are likely consequences. At this point, the condition may increase in severity, or may resolve in a week. Great variation thus exists in the severity of purpura haemorrhagica (Manning and Sweeney, 1986). One or more legs may be affected, and the prepuce and ventral abdomen are other common sites. Progression of lesions and mortality rate are variable, with mild lesions healing in seven to 10 days, but recovery may extend to a week in severe cases, and to two or three months where skin necrosis occurs. Blood and Radostits (1989a) reported that most severely affected animals die of the disease.

Morris (1987) studied 19 horses showing cutaneous vasculitic syndrome and noted that almost three-quarters of cases were between three and 10 years old, and Reef (1987) also reported the condition as primarily one of young adults. Three-quarters of the cases studied by Morris (1987) were female. Just over one third of the cases had a history of *S. equi* infection, but for another third there was no history involving any of the generally accepted predisposing causes.

Although allergic reaction to antigens of streptococcal or viral origin is believed to cause purpura haemorrhagica, this has not been proven. Antigen-antibody complexes containing *S. equi* M protein and IgA have been identified in affected animals (Blood and Radostits, 1989a; Tizard, 1987). Some authors suspect involvement of a type II or cytotoxic hypersensitivity in addition to immediate, or type I, and immune complex, or type III, hypersensitivities (Manning and Sweeney, 1986).

Miscellaneous Skin Disease

Many skin conditions exist for which the cause and pathogenesis are complex or poorly defined. The most important of these is equine nodular skin disease, nodules being the most frequently encountered skin lesion of the species (Scott, 1987). Skin biopsy is the only definitive diagnostic method, and treatment of the condition may be problematic. Similarly, seborrhoea is another condition of uncertain and multifactorial aetiology where management can prove extremely difficult (Dietz and Wiesner, 1984).

Nodular collagenous granuloma or nodular necrobiosis, axillary nodular necrosis, unilateral papular dermatosis, sterile nodular panniculitis, mastocytoma and cutaneous amyloidosis are collectively classified as "nodular skin disease" (Scott, 1987).

Nodules are clearly demarcated, firm, normally spherical structures and are larger than a centimetre in diameter; that may appear raised or may be contained in the dermis or the subcutis. They may be a result of hyperplastic reaction by the dermis or epidermis, or they may be composed of inflammatory or neoplastic infiltrates or lipid (Scott, 1987). Although hypersensitivity to biting insects or migrating nematode larvae, and mechanical trauma by tack have been incriminated, aetiology is uncertain (McMullan, 1982).

Nodular necrobiosis or collagenolytic or eosinophilic granuloma tends to be seen most often in riding horses and ponies (Mullowney, 1985a), usually developing first in warmer weather (McMullan, 1982). Nodules begin as oedematous areas of the dermis in the upper thoracic and saddle regions, with possible extension to the neck, shoulder, flank, proximal hindlimbs and croup areas. The skin overlying the painless, freely-moveable nodules, often several cm in diameter, is normal and there is no itch (Scott, 1987).

Axillary nodular necrosis is a rare condition of unknown cause and pathogenesis (Scott, 1987), which comprises one or two clearly

demarcated, firm, painless nodules that are situated in the girth and axillary areas.

Unilateral papular dermatosis is another uncommon nodular condition which develops in warmer months, featuring multiple, 30 to 300, papules and nodules which affect only one side of the trunk (McMullan, 1982)

An uncommon, complex inflammatory condition of subcutaneous fat is known as sterile nodular panniculitis (Scott, 1987). Injury to the sensitive lipocytes induces release of lipid which is hydrolysed to fatty acids, which are powerful inflammatory agents. The nodules are deep-seated and they vary in appearance and consistency. They occur either singly or in groups in the region of the chest, the axillae and the shoulders. Systemic involvement, such as inappetence, dullness, lethargy and pyrexia, has been reported (Scott, 1987).

Mastocytoma is a misnomer because although the cause of the condition is unknown, a reactive, hyperplastic rather than a neoplastic pathogenesis is suspected (Altera and Clark, 1970). Transmission between horses has been unsuccessful (Scott, 1987).

Mastocytoma is nearly five times as likely to occur in male animals than in females (Altera and Clark, 1970), but no age or breed predilection is found (Scott, 1987).

Most cases exhibit single lesions, two to 20 cm in diameter, developing on the limbs and on the head (Altera and Clark, 1970). Head lesions tend to be clearly demarcated and soft, in contrast to the firm, poorly-defined forms seen on the limbs. Hair loss may occur over the nodules, which may be painful and pruritic. Nodules are self-limiting and are reported not to metastasise (McMullan, 1982).

The cause of equine mastocytoma is unknown, but has been suggested to be chronic inflammation, parasitic infestation or local antigen-antibody reaction (Nyrop, Coffman and Johnston, 1986).

Wheals which show temporary response to therapy then develop into nodules are typical of cutaneous amyloidosis (Mullowney, 1985a). The aetiology of the nodules, which contain granulomatous material interspersed with the fibrillar protein, amyloid, is not well understood (Scott, 1988).

Although it may occur as a primary atypical form, the condition is more commonly a secondary consequence of chronic purulent inflammation such as tuberculosis, "strangles", chronic gastroenteritis, or it may follow vaccination (Mullowney, 1985a).

The condition is slowly progressive, with the appearance of firm, painless nodules varying from 2 to over 10 cm diameter in the skin over the pectoral areas and the head and neck. Internal organs are not involved in the primary form of the condition (Scott, 1988).

Seborrhoea is hypersecretion of sebaceous secretion or sebum. Sebum is necessary to prevent cutaneous dehydration and to maintain skin softness (Mullowney, 1985a). It permeates over hair shafts from the sebaceous glands of the hair follicles and gives the coat its shine. Ectoparasites, systemic disorders, environmental, climatic (Mullowney, 1985a) or nutritional (Carpenter, 1981) factors may lead to a higher than normal level of sebum production, which may be localised or generalised, and which may be manifest as crusting or as oily exudate. The aetiology of seborrhoea is unknown, but genetic predisposition may be involved (Dietz and Wiesner, 1984).

Seborrhoea is not uncommon, and is an important condition in the horse (Carpenter, 1981). Most cases of equine seborrhoea are generalised (Kral, 1962), with a crusted rather than an oily appearance to the coat (Stannard, 1972). Foul-smelling, powdery greasy bran-like flakes are seen in the coat and the skin below is reddened, sometimes lichenified, and folded. Secondary bacterial infection may be caused by the build-up of sweat and keratinous material. In rare cases, seborrhoeic skin lesions are confined to the mane and tail (Kral, 1962).

The prognosis for the condition is guarded as the disease is

chronic in character and usually persists for life (Dietz and Wiesner, 1984). The horse gradually loses condition (Dietz and Wiesner, 1984; Kral, 1962) and is less capable of work. Response to treatment is generally poor and euthanasia often results (Dietz and Wiesner, 1984).

Environmental Skin Disease

Environmental skin conditions are often encountered among large animals including the horse (Scott, 1988). The clinical manifestations produced in cases of chemical and hepatic plant toxicities are often spectacular.

Photosensitisation is an aberrant reaction within the skin in response to ultraviolet (UV) light (Stannard, 1972). The pathogenesis of the syndrome is variable, but all types of photosensitisation have common features: the presence of a photodynamic agent in the skin concomitant with exposure to UV light of the A range (320 to 400 nm), the absorption of which is facilitated by deficiency of melanin pigment in the skin and haircoat (White, 1987).

Photodynamic agents (PDAs) are activated by a specific wavelength of light and transmit radiation to adjacent cells (Kral, 1962), causing inflammation and eruption of unpigmented skin, accompanied by oedema, congestion, necrosis, cracking and sloughing (Pascoe, 1973). There may be superimposed secondary bacterial infection in severe cases (Stannard, 1972).

PDAs can occur outwith or within the animals's body (Kral, 1962). Ingested feeds, components within plants such as St. John's wort, and drugs such as phenothiazines, tetracyclines and sulphonamides (McMullan, 1982) are the commonest exogenous agents; while phylloerythrin is the predominant endogenous PDA (Kral, 1962; White, 1987). In secondary or hepatogenous photosensitisation, the PDA phylloerythrin is formed when chlorophyll is degraded by intestinal bacteria. Excretion of phylloerythrin is greatly reduced where there is hepatic compromise, and it accumulates in

tissues and may reach levels in the skin which cause the skin to become light sensitive (Dietz and Wiesner, 1984). Approximately a quarter of horses suffering from liver disease have excessively high levels of phlloerythrin circulating to the cutaneous level. Ingestion of plants, such as ragwort, groundsel, tar- or rattle-weeds, which contain pyrrolizidine alkaloids, is one of the commonest causes of hepatic dysfunction with resultant accumulation of phylloerythrin, but hepatotoxic drugs and obstructive biliary diseases are other possibilities (White, 1987).

Rape, alfalfa and leguminous plants, particularly lucerne and aslike clover, have been connected with photosensitivity, but the PDA responsible and the pathogenesis are unclear (Dietz and Wiesner, 1984).

When skin is exposed to sunlight of the UV B range, 290 to 320 nm, excessively, the normal response is sunburn. Sunburn is normally seen only in white or lightly-pigmented horses, and depends on the length of time spent in the sun and on the level of melanin in the skin and haircoat (White, 1987).

The irritant form of contact dermatitis is seen more often than the allergic form of the condition (Stannard, 1972; Mullowney, 1985a). Despite similarity in clinical appearance, pathogenesis of the two forms of contact dermatitis is very different. There is no involvement of the immune mechanism in irritant contact dermatitis (Scott, 1988), rather the direct action of the irritant. The irritant may be a body secretion, a wound excretion, a strong acid or alkali, a plant such as the nettle, a topical drug such as mercury compounds used in blisters, or it may be crude oil, diesel, turpentine, or leather preservative (Scott, 1988). The head, extremities, ventral body surfaces and areas under tack are those most commonly affected (Mullowney, 1985a).

The skin becomes reddened and pruritic, with oozing or vesiculation, and crusting. Hair may be lost due to self mutilation or damage to hair follicles, and depigmentation of hair and skin may persist indefinitely (Mullowney, 1985a).

When the epidermal barrier is compromised by moisture there is greater contact between the irritant and the skin, so where horses experience muddy conditions underfoot there is increased risk of dermatitis of the feet. Previous skin damage, length of time of contact and the concentration of the irritant dose all influence the development of contact dermatitis (Scott, 1988).

Skin related toxicities have been reported after ingestion of excessive amounts of compounds such as selenium, mercury and pentachlorophenol (Mullowney, 1985a; Pascoe, 1973; Scott, 1988). Lesions of the skin and the hair are often the presenting signs, in the form of extensive hair loss from the mane and the tail, and in some cases, progressive generalised alopecia (Scott, 1988). Signs of systemic and gastrointestinal involvement are usually also seen, and in chronic selenium toxicity or alkali disease there is lameness; in severe cases the hooves may slough (Blood and Radostits, 1989b; Pascoe, 1973). Acute selenium toxicity may result in death (Mullowney, 1985a).

Ingestion of cereals grown on seleniferous soils, or of selenium-accumulating plants such as *Morinda, Astralgus* or *Xylorrhiza spp* are causes of selenium toxicity (Blood and Radostits, 1989b). Mercurial poisoning is usually a consequence of accidental feeding of cereal treated with antifungal organic mercurials (Pascoe, 1973), although overdosing of mercury-containing medicaments and percutaneous absorption from skin dressings and blisters have been known (Blood and Radostits, 1989b; Pascoe, 1973; Scott, 1988). Pentachlorophenols are used as fungicides, molluscicides, wood preservatives, and are components of waste motor oil. Signs of toxicity followed the use of waste oil to settle dust in a riding arena (Mullowney, 1985a). The resulting dermatitis was similar to contact dermatitis and involved the mucous membranes of the lips, mouth and nasal passages, resembling a chronic respiratory infection.

Congenital Skin Disease

Although congenital abnormalities involving the skin of the horse

are rare, they are striking and are often disfiguring, precluding use as show animals.

Albinism refers to a congenital absence of melanin that also affects the hooves and the eyes. The occurrence of true equine albinism is disputed (Dietz and Wiesner, 1984), and partial albinism only is seen, as most horses have pigmented irides, although the coat is white. The condition is believed to be related to an autosomal dominant gene (Scott, 1988).

Angiomatosis or verrucous haemangioma, which is similar to the "strawberry birthmark" of humans, is an easily traumatised lesion which bleeds profusely if damaged. It is an alopecic, usually solitary lesion, tending to occur on an extremity. In contrast to the human lesion, regression is unlikely (Mullowney, 1985a).

Firm, clearly-demarcated painless nodular lesions up to 12 cm in diameter are occasionally found in the subcutis adjacent to joints or tendon sheaths, commonly lateral to the stifle joint (McMullan, 1982; Scott, 1988). These are known as calcinosis circumscripta or tumoural calcinosis. They are calcified deposits which induce a fibrous granuloma and are normally seen in animals younger than four years old. Aetiology is suggested to be repeated trauma (McMullan, 1982), or an autosomal recessively transmitted defect of phosphorous metabolism (Mullowney, 1985a).

Congenital and hereditary cysts of the skin have been reported in the horse (Scott, 1988). These include epidermoid or epithelial cysts, and dermoid cysts, which are clinically similar. Both are characterised by an epithelial wall and keratinous contents. Neither type of cyst is thought to be neoplastic or preneoplastic (Mullowney, 1985a). In one survey (Pascoe and Summers, 1981), all cases were Thoroughbreds, but there is no age predilection (Scott, 1988).

Epidermoid or epithelial cysts result from first branchial cleft abnormality (Mullowney, 1985a), in the form of unilateral pouch-like outgrowths which contain embryonic teeth, hair, keratin and hair and sebaceous and sweat gland secretions. Epithelium

becomes aberrantly situated, either by congenital ectopic development or by traumatic implantation or hair follicle occlusion (Pascoe, 1973). The size of the cyst increases as keratinous material builds up inside it. They are situated at the ear base, firmly adherent to underlying bone or cartilage, and tend to become fistulous (Mullowney, 1985a).

Dermoid cysts occur along the dorsal midline between the withers and the rump (Pascoe and Summers, 1981). The cysts are characterised histologically by an epidermal lining that contains adnexa (Scott, 1988).

Conclusion

Even today skin disease in the horse is poorly diagnosed and frequently mistreated, resulting in undue suffering. As a consequence skin disease must be considered a major welfare problem in the horse.

Skin disease in the horse was reviewed in this chapter. It was seen as a significant, common complaint which often precludes use of the animal for the purpose for which it is kept. Various causes of skin disorders have been considered, and attempt was made to highlight the most important conditions, although information regarding the relative incidences of the equine skin disorders is not readily available. Bacterial skin disease was shown to be common in the horse and dermatophilosis has been put into perspective as one of the most frequently encountered equine bacterial skin infections.

A PERSPECTIVE OF DERMATOPHILOSIS IN THE HORSE

Skin disease, and in particular bacterial skin disease, was shown in the preceding chapter to be an increasing equine welfare problem. Many cases result from poor management, unhygienic living conditions, dirty tack and lack of close attention, particularly if animals are housed outside for most of the year.

Dermatophilosis is one of the commonest bacterial skin conditions of the horse, and is encountered most often in horses and ponies, of all types and of all breeds, which are outwintered. These animals are exposed to prolonged, excessive wetting, particularly in the West of Scotland, conditions which are known to predispose to dermatophilosis infection. Under these circumstances of outwintering close inspection may be infrequent, and early cases of dermatophilosis, where the coat simply appears soaked, are often missed or overlooked. Infection is likely to be advanced before it gives cause for concern.

By the time the severity of the condition is appreciated lesions may be extremely painful, and there may be complicating secondary bacterial infection, particularly where lesions of the lower limbs are involved and where the horses are constantly standing in damp, muddy conditions underfoot. This obviously leads to concern for the welfare of such animals. Housing, which, as described in the following chapter, often leads to spontaneous healing of the lesions,is not always possible. The site of the lesions, with the typical dorsal distribution where the coat is wettest, and their painful nature, often prohibits the use of a saddle and so the horse can not be used for ridden work.

This chapter describes equine dermatophilosis in more detail and the features of the condition in the horse are compared to those found in other species. Consideration is then given to the nature and the efficacy of the regimes which are currently practised in treatment, control and prophylaxis of the disease.

The clinical manifestations of *Dermatophilosis congolensis* infection vary greatly in the wide range of species which the organism infects (Stewart, 1972a). There are few veterinary pathogens which are encountered quite as universally or where infection advances as imperceptibly as *D. congolensis*. Dermatophilosis was thought for decades to be a condition of horses, cattle, sheep and goats, found only in the tropics. Reports frequently originate from the African continent, but the disease is much more common than originally thought in more temperate regions such as the U.K., Europe, Eire, Australia, New Zealand and North and South America. The disease is worldwide in distribution, although the intensity of clinical infection tends to be most severe in countries with a less temperate climate (Stewart, 1972a).

Dermatophilosis is a major, debilitating problem that is difficult to manage in the horse. Although the mortality is low, morbidity may be as high as 80%, particularly under crowded stable conditions (Ford, Cairns and Short, 1974). Considerable weight losses have been recorded, and even death has been reported which was attributed to widespread alopecia and epithelial destruction subsequent to the infection (Ford, Cairns and Short, 1974). The disease is also of enormous economic importance in countries where raising livestock is a primary industry, e.g. ovine infection in countries such as Australia. Downgrading of fleeces, particularly in fine-wooled breeds is a major loss of revenue (Austwick and Davies, 1958). Bovine dermatophilosis, although worldwide in distribution, is of greatest importance economically in tropical regions, especially Madagascar and Central and Western Africa, where huge deficits are incurred each year owing to damaged hides, chronic illthrift, reduced production, increased culling and mortality (Lloyd, 1976; Bida, unpublished data). In Nigeria, it was estimated that, annually, around 11 per cent of a total cattle population of 11 million (Food and Agriculture Organisation, 1973) were clinically affected during the rainy season and approximately five per cent during the dry season (Oduye, 1975; Lloyd, 1976). The economic losses from spoilage of leather from cattle, sheep, swine and goats is in the region of $ 37.4 million per annum in Nigeria (Arowolo, Amakiri and Nwufoh, 1987). According to Macadam

(1964b), during the rains morbidity reached 50 per cent and the mortality five per cent. Bovine dermatophilosis has also become more important in recent years due to its disastrous effect on imported, high producing breeds. It drastically hinders selective crossbreeding and improvement of indigenous cattle under intensive systems of management (Ilemobade, Gyang, Bida & Addo, 1979).

The spectacular lesions of bovine dermatophilosis first drew the attention of clinicians (Stewart, 1972a). *D. congolensis* infection was initially described in cattle in the then Belgian Congo, now Zaire, by Van Saceghem (1915). He reported an exudative dermatitis with small raised scabs containing embedded hair, epidermal cells, leucocytes and coagulated serum. Removal of these crusts revealed a concave pus-filled area beneath the scab. These crusts and the pus harboured the organisms which appeared as branching mycelial elements. Hyphae divided in a transverse fashion without separating, to form coccoid elements. As the filament widened, longitudinal and transverse divisions were seen, often up to eight cocci across. Thompson (1954), cited by Stewart (1972b), reported formation of motile spores from these cocci. Broth culture growths showed initial hyphal formation, with later growth predominantly coccoid in form. *D. congolensis* was established as aerobic, gram-positive, and non-acid fast, and was found to possess limited capacity to ferment sugars (Thompson, 1954).

The Organism

Classification of the organism is: Class: *Schizomycetes* (Cruickshank, 1955); Order: *Actinomycetales* (Austwick, 1958, cited by Stewart, 1972b); Family: *Dermatophilaceae* (Austwick, 1958, cited by Stewart, 1972b); Genus: *Dermatophilus* (Van Saceghem, 1915); Species: *Congolensis* (Gordon, 1964; Roberts, 1965). Edwards and Gordon (1962), carried out electron microscopical studies which showed that the *Actinomycetales* have no structural relationship with the *Eumycetes*, or true fungi, and that they show similarities to the *Eubacteriales*.

Scanning electron microscopy (SEM) studies by Abu-Samra and Walton (1977) indicated that germinating zoospores developed within certain hyphae and were subsequently released, whilst the coccoid forms were produced by budding from a different type of hyphae. Colonies were found to have both vegetative and aerial hyphae. The various patterns of mycelial activity and structure that were seen raised serious doubts about the earlier ideas on the method of growth and therefore the classification of *Dermatophilus* within the *Actinomycetales* (Abu-Samra and Walton, 1977).

The Disease

Although much of the literature records cases in cattle and sheep, the infection has been described in the horse from the earliest of reports. The disease was first reported in horses and cattle by Van Saceghem (1915); then in sheep in South Africa (Bekker, 1928) and in Australia (Bull, 1929; Seddon, 1929, cited by Austwick and Davies, 1958). In the U.K. the first report in sheep was from Scotland by Harriss (1948). Beaton (1928), cited by Stewart, 1972a, described the condition in goats. Cases are recorded in a wide variety of domestic animals and wildlife species. Dogs (Chastian, Carithers, Hogle, Abou-Gabal, Graham and Branstetter, 1976) and a cat (Carakostas, Miller and Woodward, 1984) have been infected, and Lomax and Cole (1983) reported the condition in pigs. Wildlife species infected include, deer (Salkin, Gordon and Stone, 1975); owl monkeys (Fox, Campbell, Reed, Snyder and Soave, 1973); titi monkeys (Migaki & Seibold, 1976, Washington); rabbits (Shotts and Kistner, 1970); hares, hedgehogs and gerbils (Kusel'tan, 1967); chamois (Nicolet, Klinger and Fey, 1967, Switzerland); Australian bearded lizards (Montali, Smith, Davenport and Bush, 1975, Washington); polar bears (Newman, Cook, Appelhof and Kitchen, 1975, Detroit); wild raccoons (Salkin, Gordon and Stone, 1976, New York), and camels have been experimentally infected (Abu-Samra, Imbabi and Mahgoub, 1976). The domestic fowl, on the other hand, appears to be completely resistant to dermatophilosis infection (Abu-Samra, Imbabi and Mahgoub, 1976).

Man has also been infected, from infected deer (Dean, Gordon, Severinghaus, Kroll and Reilly, 1961, cited by Stewart, 1972a); experimentally, from cultures (Memery and Thiery, 1960, cited by Stewart, 1972a) and from experimental exposure to infected horses (Ford, Cairns and Short, 1974).

Van Saceghem (1915) first identified the disease in horses in Africa. Hudson (1937) observed lesions in the horse similar to those in cattle, again in Africa, and the disease was reported in the U.K. in the same year by Stableforth (1937). Since then, there have been many reports of the disease affecting equines. Scarnell (1961) again drew attention to the condition in British horses and transmitted the condition to other horses. Dermatophilosis was identified among mares and foals in Australia by Edgar and Keast (1940) while on the North American continent Bentinck-Smith, Fox and Baker (1961) and Searcy and Hulland (1968) diagnosed the disease in horses in America and Canada, respectively. Green (1960) identified lesions seen on donkey and zebra skins in Kenya, and Lloyd (1971) made clinical observations on streptothricosis in the domestic donkey in Nigeria. The disease was reported for the first time in East or South East Asia in horses in Hong Kong by Munro (1977). Bussieras, Chermette and Marchand recorded the disease in horses in France in 1978.

Stableforth (1937) described lesions which developed over the rump and hindquarters as "paintbrush" formations of matted hairs with scab formation and large cornified areas. In a review of 10 natural and 48 experimental cases, Scarnell (1961) noted acute and chronic forms of the disease. In the acute form lesions often went unnoticed for long periods, particularly in pastured horses. Small lesions developed and there was insidious spread over the body until the coat looked soaked. Crusty lesions could be palpated below the haircoat. Scabs thicker than 0.5 cm and over 2.5 cm in diameter were noted. These were frequently grey, sometimes confluent, with tufts of protruding hair. The underside of the scab was typically concave with protruberant hairs and a club-shaped hair root. Bilaterally symmetrical lesions were seen most often on the dorsal surfaces of the body, from the poll to the tail root. The limbs, particularly the distal areas, were also

affected. The muzzle and face were affected by the non-pruritic lesions (Edgar and Keast, 1940; Macadam, 1964c), and the underside of the abdomen was involved in some cases (Macadam, 1964c). Bentinck-Smith, Fox and Baker (1961) described lesions as an exudative dermatitis with hyperaesthesia, affecting the back and croup, and also noted the absence of pruritus.

The chronic form of the condition consisted mainly of plaque-like hard lesions of raised hair and flaking skin. Scarnell (1961) suggested that these reduced skin elasticity and contributed to "cracked heels". Chronic lesions were common over the saddle area and on the pasterns. He believed there was connection with "mud fever". McCaig (1967) also described the localised form of the disease in horses. Lesions affected the lower limbs up to the region of the fetlock, in particular the front legs in hunters. The lesions were more noticeable where there was white hair. In severe cases the area under the body, where mud splashed, was also affected. Lameness may be the presenting sign in "mud rash", owing to deep fissures and heavy scabbing of skin around the coronet, heels and pastern, as a consequence of the inflammation. Oedema, which was very slow to resolve, was reported by Bentinck-Smith, Fox and Baker (1961).

The lesions described by Scarnell (1961) began to develop after persistent wet weather and the acute form resembled "rain scald". Pascoe (1971) also noted the major contributing cause to the severity of dermatophilosis in horses to be excessive wet, cloudy weather (Pascoe, 1971) and the condition occurs most commonly in horses and ponies kept outdoors. In the U.K. the largest number of cases are seen in autumn and winter (Mullowney and Fadok, 1984a).

Untreated horses have become anorexic and debilitated, and have lost as much as 130 kg in weight (Ford, Cairns and Short, 1974). Approximately 60 per cent of the horses studied by the same authors developed dermatophilosis during outbreaks in two successive years. The latter authors state that there is potential for annual outbreaks within large horse groups which are stabled.

Lesions in untreated horses can persist for up to six months or

longer, depending on the severity (Ford, Cairns and Short, 1974). According to Lloyd (1971), lesions in the donkey are similar, involving very thick scabs that are firmly adherent to the mane and tail. Pinnal margins and the muzzle are often affected.

D. congolensis infection in cattle is seen mainly on the African continent where it is of major importance (Stewart, 1972a). The West African N'dama and Muturu are believed to be highly resistant to infection (Coleman, 1967; Macadam, 1970; 1976) whereas European breeds are particularly susceptible (Lloyd, 1976). Jersey cattle imported to Africa are very susceptible and, if tick infested, readily die (Stewart, 1972a). Up to 70 per cent of a herd can be affected (Stewart, 1972a). The resistance of Brahman cattle was compared with that of African Zebus and their crosses, and it was shown that Brahmans were highly susceptible, and that Brahman bulls transferred varying susceptibility to their progeny (Dumas, Lhoste, Chabeuf and Blancou, 1971). An hereditary predisposition has been suggested to exist among Shorthorns (Stewart, 1972a).

Cattle of all ages are affected, but great variability exists within infected herds (Stewart, 1972a). The characteristic lesion is an exudative epidermitis which causes crust formation. Lesions become alopecic and are often so thick that they resemble horn. This typical scab formation was described by Zlotnik (1955) as "crocodile skin". Lesions are papular and pustular initially, and later coalesce to form extensive scabs. Four forms of bovine lesion were described by Mornet and Thiery (1955). The ichthyotic form is the commonly seen scab-like lesion; while the nodular form is invariably associated with concurrent *Demodex bovis* infestation, and nodules may be as large as 12 cm and 2 cm thick. A tumourous form, originating from histioma development around a hair follicle bulb is described, and a leproid form exists where dermal infiltration by inflammatory cells, with folliculitis, is seen. Tongue lesions, hyperplastic lymph nodes, toxic hepatitis and nephritis have been recorded in cattle by Mornet and Thiery (1955). Considerable loss of condition is often reported as a consequence of reduced mobility in severe cases, and of difficulty in prehension of food owing to lesions involving the lips and muzzle (Plowright, 1956). Weight gains in recovered animals are

often very poor (Macadam, 1970).

Lesions of dermatophilosis typically affect the dorsal surfaces: along the neck, the back, and the hindquarters, and there is often chest and flank involvement. This distribution reflects the range of environmental skin insults which facilitate permeability by the organism. Lesions coincide with tick predilection sites, sites damaged by vegetation, maceration, and biting flies (Scott, 1988). Transmission of infection has occurred by biting and non-biting flies (Richard and Pier, 1966) and has been associated with ticks (Macadam, 1962; Plowright, 1956).

In Africa, bovine lesions may persist for many months and may carry over to the next rainy season (Macadam, 1964a). In the U.K. lesions in cattle lasted eight months (Roberts and Vallely, 1962).

In sheep, although most breeds seem to be susceptible, fine wooled breeds are more severely affected (Smith and Austwick, 1975; Hart, 1976). In Australia, Merino-cross lambs suffer severely and there may be high mortality (Stewart, 1972a). Dermatophilosis is not always readily detected: in one report in the U.K. (Austwick and Davies, 1958), nearly 80 per cent of one flock of ewes was affected, and the owners of 30 affected flocks were unaware of the existence of the disease.

Lesions in sheep are variable, and depend largely on the geographical location, climatic factors, pathogenicity of the strain of the organism and the exact part of the animal affected. Three forms of the condition are seen: lesions on the wool-covered parts, mainly the back, body sides and neck regions are known as "mycotic dermatitis" (Seddon, 1929, cited by Austwick and Davies, 1958), synonymous with "lumpy wool" (Bekker, 1928). Lesions may also occur on the hair-covered areas of the head, especially the ears, commissures of the lips and the hair over the nasal bones, and in rams there may be scrotal involvement. Alternatively, if lesions are associated with the hair of the legs from the coronet or hock joints the condition is known as "strawberry foot rot" (SFR), first described by Harriss (1948).

The lesions of these forms are basically the same, but the appearance is altered by the degree of covering of hair or wool (Stewart, 1972a). Lesions begin as small, hyperaemic areas of up to two weeks duration (Seddon, 1929, cited by Austwick and Davies, 1958), which progress to result in exudation and encrustation. Exudation is continued by lateral extension of the lesion, cornification takes place and scab material separates from the underlying epidermis. Re-infection occurs in the newly-formed epidermal layer and the process is perpetuated. In chronic cases the crust detaches from the skin and is held in the adjacent fleece as a conical mass. Sometimes wool fibres will break off revealing alopecic areas with black pigmentation of the skin. In affected animals there is frequently a dry, white appearance to the fleece, and disordered wool fibres are seen around lesions (Austwick and Davies, 1958).

Lesions of the haired areas are flatter than fleece lesions and develop from amber-coloured scabs. They are thickened, alopecic regions of skin, with greasy appearance to the surface. These lesions are often seen in lambs of a few days old (Hudson 1937), where secondary bacterial invasion may follow and death may result.

In "strawberry foot rot" (SFR), described by Harriss (1948), all four legs may be affected simultaneously. The typical small, thick scab develops and extends to form a rough, verrucous growth, which eventually extends over the entire leg. Further exudation forms a firm casing which can lead to swelling and inflammation of the coronet. If scabs are removed shallow, haemorrhagic ulcers which resemble strawberries are seen. Pruritus and lameness are present only if secondary bacterial infection develops. In the absence of secondary infection there is no pruritus and no lameness. Sheep of all ages were affected (Harriss, 1948). In pasture where there were no abrasive whins, Harriss (1948) found SFR to be nonexistent.

SFR lesions are clinically similar to the lesions of contagious pustular dermatitis (CPD) or orf (Harriss, 1948). Severe lesions similar to field SFR infections were produced when lambs were

experimentally infected simultaneously with orf virus and *D. congolensis*. These lesions were not seen, however, when only one organism was inoculated, suggesting that in field circumstances, *D. congolensis* acts synergistically with other organisms to produce severe generalised lesions (Abu-Samra and Walton, 1981).

In natural dermatophilosis cases in sheep, duration of lesions has varied from one week to over two months before the "lumpy wool" scabs have lifted off (Austwick and Davies, 1958). Harriss (1948) stated that the duration of SFR cases was up to 25 weeks, with most lesions healing in five to six weeks.

In caprine infections, lesions in kids may be seen as early as four to five days after birth, as scabs which are detected on the inner surface of the pinna, or as crusty lesions on the smooth skin on the ventral aspect of the tail (Munro, 1978). Lesions enlarge to two or three centimetres in diameter and reach approximately one millimetre thick. Scabs, which are readily removed to reveal smooth light coloured areas, are dark in colour and may resemble warts (Munro, 1978).

Adult goats may have lesions on their ears, similar to those seen in kids, and adherent scabs are found on the nose. Removal of scabs causes discomfort and leaves a raw, bleeding surface (Munro, 1978).

Two three week old piglets presented with multiple to confluent yellow crusts and scabs on the ears, face and over the dorsum (Lomax and Cole, 1983). *S. hyicus* and *D. congolensis* organisms were identified from the lesions. The piglets were housed with their dams in farrowing crates in a concrete-floored farrowing house, and the moist and disrupted epidermal surface was thought to have provided a sufficiently favourable environment for secondary infection with *D. congolensis* (Lomax and Cole, 1983).

Natural infection (Chastain, Carithers, Hogle, Abou-Gabal, Graham and Branstetter, 1976) and experimental infection (Richard, Pier and Cysewski, 1973) in the dog have resulted in similar lesions: erythematous, suppurative ulceration, which was encrusted. Of the

natural infections, one dog demonstrated posterior dorsal midline lesions which had been present for two months (Chastain *et al.*, 1976). The dog was anorexic, depressed and had become progressively emaciated over a six month period. Another dog suffering from natural infection presented with a history of patches of alopecia and skin encrustation (Chastain *et al.*, 1976). The lateral femoral and dorsal scapular regions were involved and lesions appeared as thick, hard, encrusted lesions up to four centimetres in diameter. No sign of systemic illness was evident. In neither case was the source of infection traced.

Mild focal pruritus was reported in the experimental dermatophilosis infections (Richard *et al.*, 1973), and although no systemic signs of infection were seen, there were deaths following progressive emaciation.

Contamination of injuries or inoculation by an infected claw was assumed to be the origin of infection in a case of subcutaneous dermatophilosis in a cat (Carakostas, Miller and Woodward, 1984). The cat presented with two draining, firm, subcutaneous masses, in the phalanx and popliteal lymph node regions, respectively. Thick, grey, purulent material drained through tracts which extended to the skin surface. The surrounding areas were hairless.

Raised, golden-brown cutaneous nodules were described on the heads, bodies and extremities of three Australian bearded lizards on arrival at a Washington zoo after a lengthy journey (Montali, Smith, Davenport and Bush, 1975). Lesions were up to five millimetres in diameter, although several were larger and confluent, particularly at pressure points on the limbs. *D. congolensis* organisms were isolated. The generalised nature of the infection was believed to be associated with stress prior to arrival at the zoo (Montali *et al.*, 1975).

In a study by Ford, Cairns and Short (1974), four people were experimentally exposed to active lesions of infected horses, while four control persons were exposed to skin and hair of non-infected horses. Within 36 to 48 hours after exposure, the four people exposed to active lesions developed discrete, erythematous,

pruritic pustules, from which *D. congolensis* was isolated. Lesions persisted from two weeks to two months. One case of natural transmission occurred from horse to man over the two-year study: numerous discrete pustules developed over the handler's forearm 48 hours after handling a severely infected horse.

Ford *et al.* (1974) obtained biopsies from human and equine infections. Lesions in both hosts were consistent with a mixed nonspecific epidermitis; the dermis was only superficially involved.

After scarification of the skin, *D. congolensis* culture was applied to the skin of the forearm by Memery and Thiery (1960), cited by Stewart (1972a), and pustules appeared in two days. Scab formation was then seen, and after eight days antibiotics were administered.

Two men who had dressed an infected deer carcase, and two others who had handled it, developed furunculosis of the dorsal surfaces of the hands (Dean *et al.*, 1961, cited by Stewart, 1972a). Incubation ranged from two to seven days, and lesions began as multiple, non-painful white pimples or pustules up to 0.5 cm diameter with a serous, yellowish exudate. Pustules were surrounded by a hyperaemic zone and later ruptured leaving a red crater-like cavity. There was no spread or coalescence, as seen in animals. After three to 14 days lesions healed spontaneously. No systemic illness was recorded, nor was the condition contagious to human in-contacts.

Pathology and Pathogenesis

According to Lloyd (1984), natural immunity to dermatophilosis exists in varying degrees in domestic animals, and is a heritable characteristic. Variability in resistance has been recorded within a species, between breeds. N'dama and Muturu cattle, for example, are believed to be highly resistant to infection (Coleman, 1967), whereas Jersey cattle (Stewart, 1972a) and Brahman cattle were found to be highly susceptible, and susceptibility was transferred

from Brahman bulls to their progeny (Dumas, Lhoste, Chabeuf and Blancou, 1971). Species resistance is believed to exist: the domestic fowl is believed to be completely resistant to dermatophilosis (Abu-Samra, Imbabi and Mahgoub, 1976).

The nature of the haircoat, the structure of the skin, and the inherent ability of the host animal to mount a rapid immune response following infection, are factors determining natural immunity to dermatophilosis (Lloyd, 1984). When *D. congolensis* is inoculated into the epidermis the main barrier to infection is the outer stratum corneum and the sebaceous lipid which permeates it (Roberts, 1967a; Lloyd and Jenkinson, 1980). *Dermatophilus* is believed not to be a commensal bacterium (Macadam, 1970) and it cannot penetrate intact corneocytes and films of sebum (Roberts, 1967a). Although sebaceous lipid from sheep is toxic to *D. congolensis in vitro*, the protective effect of the lipid was shown to be physical (Roberts, 1967a). Recent work in cattle demonstrated that this protection was very superficial and possibly associated with changes in the nature of the lipid when it reached the skin surface (Lloyd and Jenkinson, 1980). Barrier function may be compromised by trauma, ectoparasites, prolonged wetting and heavy rain or sprays (Macadam, 1970). Alterations in atmospheric temperature and humidity do not directly influence skin resistance (Lloyd and Jenkinson, 1980).

"Mycotic dermatitis" and "cutaneous streptothricosis" are misnomers as the causal agent is bacterial, not fungal, in origin (Roberts, 1967a). *D. congolensis* penetrates the stratum corneum and invades living epidermis causing an acute inflammatory reaction with neutrophil accumulation, accelerated keratinisation and epidermal proliferation immediately below the infected area (Roberts, 1965). The penetration of epidermal cells was believed by Roberts (1967a) to be due to mechanical force, and to be independent of known haemolysin, protease and phospholipase; he believed that no toxin was produced by the organism. The accumulated neutrophils act to prevent invasion by *Dermatophilus* but in the initial infection stages the organism colonises the newly-formed epidermis by lateral extension from infected sites. The organism multiplies in and destroys the hair follicle sheaths

(Abu-Samra, Imbabi and Mahgoub, 1976) resulting in infiltration of the dermis by lymphocytes, macrophages, proliferating fibroblasts and connective tissue formation. Delayed hypersensitivity reactions to experimental inoculation of *D. congolensis* have been recorded in domestic animals and in the rabbit (Roberts, 1966; Makinde and Wilkie, 1979; Abu-Samra, Imbabi and Mahgoud, 1976). However, skin lesions are concluded to be the consequence of nonspecific inflammation, resulting from the products of cellular damage diffusing from the infected epidermis (Roberts, 1967a).

Flagellar and somatic agglutinins are produced in response to *Dermatophilus*; precipitins are only present after repeated or prolonged infection or vaccination (Roberts, 1967b). Antibody has been identified on the skin surface in cattle after intradermal vaccination (Lloyd and Jenkinson, 1981) and has also been demonstrated in the milk of infected cows (Makinde, 1981). Repeated infection or vaccination stimulates an anamnestic response (Makinde and Ezeh, 1981).

High serum antibody titre does not confer immunity to natural dermatophilosis (Perreau and Chambron, 1966) and although zoospores are agglutinated and immobilised *in vitro* by such antibody, it does not kill them (Roberts, 1964). Zoospore destruction following neutrophil phagocytosis is enhanced by the presence of high titre specific antibody in sheep and guinea pigs (Roberts, 1966) and the specific antibody greatly increases resistance to experimental infection of scarified skin where there is contact between inoculum and invading leucocytes.

Recent studies following the dose-response of rabbits to *D. congolensis* infection (How and Lloyd, 1990) indicate a degree of strain-specificity in immunity. Further work is needed examine the antigenic variability of isolates and to determine strain-specific antigens. To date (1990) there is no recognised method for serological or biochemical classification of *D. congolensis* isolates. Cross-reacting antibody between two strains following vaccination was demonstrated by enzyme-linked immunosorbent assay (ELISA) of serum (How and Lloyd, 1990) but no direct evidence exists that antibody is protective.

Diagnosis

Clinical lesions are quite distinctive in all species. In the horse no other organism induces the classic "paintbrush lesions". The distribution of lesions, coinciding with the areas most in contact with rain, is equally distinctive.

Diagnosis is usually based upon the identification of characteristic morphological elements in Giemsa stained smears from crusts and scabs, and growth of bacteria with the cultural features of *D. congolensis*. Direct smear is made from pus on the underside of scabs, or from serous exudate after scab removal, or from dried crusts emulsified in physiological saline or distilled water. Gram-positive hyphal elements, which branch transversely and longitudinally, are seen, and consist of up to eight rows of cocci (Roberts, 1961). Organisms are often described as "railroad tracks" on smear.

If the organism is demonstrated in smears, it can usually be cultured. Grinding and powdering of scab material prior to culture is reported to improve isolation of *D. congolensis*, by allowing the release of the organism from between the layers of hard scab before inoculation on to culture medium (Abu-Samra and Walton, 1977). Selective medium containing polymixin B sulphate reduces contamination from field samples but does not completely eliminate it (Abu-Samra and Walton, 1977). Pre-incubation with CO_2 was not found to be essential for growth. The optimal temperature for growth was 37°C (Edgar and Keast, 1940).

The use of fluorescent antibody techniques has greatly facilitated the detection of *D. congolensis* in suspensions of exudate (Pier, Richard and Farrell, 1964).

Serum antibody titres to *D. congolensis* were demonstrated by ELISA in cattle in the west of Scotland (Lloyd, 1981) allowing screening of sera for exposure to infection which may have otherwise gone unnoticed.

When counterimmunoelectrophoresis, passive haemagglutination, and

agar gel diffusion were compared, counterimmunoelectrophoresis, which is specific and sensitive, was found to be the most satisfactory method of screening large numbers of serum samples, with an antibody detection rate of over 80 per cent (Makinde and Majiyagbe, 1982). Cellular antigens, particularly cell wall extract, were more successful than extracellular antigen in antibody detection in sera.

A monoclonal antibody, produced from murine hybridoma cultures, was used to demonstrate *D. congolensis* in clinical material from confirmed bovine and ovine cases of dermatophilosis by indirect immunofluorescent staining (How, Lloyd and Lida, 1988).

Management

Two points cited by Stewart (1972b), put treatment of dermatophilosis into perspective. In severe cases the thickness of the crusts is too great, and active hyphae are embedded too deep in the follicle sheaths, for topical treatment to be either practical or effective (Roberts, 1967b); and to allow topical treatment to penetrate even to the superficial areas of the lesion, scabs should be removed, and wholesale removal is too harsh, very painful and inhumane (Coleman, 1967).

Roberts (1967b) stated that rational treatment should be large doses of antibiotic to which the organism is sensitive, remembering that many cases heal spontaneously. Dermatophilosis is reported, however, by some authors (Mullowney and Fadok, 1984a) to be highly sensitive to most antibacterial agents, and in mild cases to require only topical antiseptic therapy.

In the horse, Searcy and Hulland (1968) used topical application of iodine solution in addition to parenteral penicillin-streptomycin combination treatment, and recovery was recorded within three weeks. Animals recovered but comparison to untreated cases made assessment of the efficacy of treatments difficult. Horses were treated topically with 0.1% solution of chlorhexidine digluconate with good results (Dykstra and Osinga,

1969). Only partial response was recorded, however, to spraying of lesions with magnesium fluosilicate in 70 severely affected equine cases (Pascoe, 1971). Parenteral penicillin-streptomycin gave best results as judged by hair regrowth and loss of irritation in affected areas. In a further outbreak involving 278 horses, Pascoe (1972) reported that recovery of most cases occurred within seven days, whether treated with systemic or locally applied antibiotics, particularly 0.5 % chloramphenicol used topically. Resistant cases were described as showing a better response to an oily preparation of chloramphenicol, and parenteral antibiotics (Pascoe, 1972).

Ford, Cairns and Short (1974) studied a herd of over a hundred pleasure horses over a two year period, and recorded regression of active lesions within four days of first treatment after cleansing of infected skin using povidone-iodine solution or commercial shampoo. Copper sulphate was an effective topical (Watson and Walton, 1973), when used after daily currying and washing with water. Horses were treated once weekly for two weeks. Iodophors, 0.5% zinc sulphate, 0.2% copper sulphate and 1% potassium aluminium sulphate (alum) are commonly used topical solutions (Scott, 1988). Solutions are applied as total body washes, sprays or dips for three to five days and are then continued weekly until healing occurs.

Infections of unpigmented areas such as the muzzle or lower extremities commonly crack or fissure, resulting in cellulitis causing severe pain or lameness in the horse. These respond best to combined antibiotic-steroid ointments (Scheidt and Lloyd, 1987), but astringents such as white lotion (20 g zinc sulphate and 30 g lead acetate in 50 ml water) applied daily for five days reduce secondary swelling and inflammation of the lower extremities (McCaig, 1967). McCaig (1967) also recommended triamcinolone acetonide and halquinol in a cream base in treatment, and the application of emolient cream or Vaseline to the legs prior to riding. He recorded efficacy of antihistamines in cases where there was severe inflammation of the lower limbs.

For severe generalised infections, parenteral antibiotics are

required, such as procaine penicillin G (22,000 IU/kg) and streptomycin (11 mg/kg) intramuscularly twice daily for seven days (Scott, 1988), although some authors find a three to five day course to be adequate (Scheidt and Lloyd, 1987).

Successful therapy in horses depends also on removal predisposing factors (Scheidt and Lloyd, 1987): affected animals should be confined to dry places, kept stabled, out of rain and wet pastures, or at least rugged. If the rainy season is not prolonged then the disease is self limiting. Chronically affected animals are the primary source of infection (Roberts, 1967a; Mullowney and Fadok, 1984a), and mechanical transmission of the disease occurs by both biting and non-biting flies (Macadam, 1964a, 1964b; Richard and Pier, 1966), ticks (Macadam, 1961), and fomites such as grooming equipment and electric clippers. Softening with povidone-iodine soaks or chlorhexidine solution facilitates removal of the painful scabs which, along with crust and hair, should be eliminated from disinfected premises to reduce environmental contamination and therefore reduce the source of re-infection (Scheidt and Lloyd, 1987). Other recommendations for control of dermatophilosis include improved hygiene, nutritional and management practices; insect and arthropod control; isolation or culling of infected animals, and evasion of mechanical cutaneous trauma (Scott, 1988).

The earliest recorded topical treatments for dermatophilosis in cattle include 3% sodium arsenite solution (Van Saceghem, 1915), and that prescribed by Armfield (1918), who claimed "moderate success for the following treatment in some cases: moisten crusts with soft soap and warm water. Then mix the following: quick lime, 1 lb; sulphur 2 lb; and water, 2 gallons. Simmer over a slow fire for three hours and apply to the skin while warm". This treatment was to no avail in advanced cases. The use of dressings was decried, as they spread the disease from one part of the skin to another, and rather removing scabs and painting of the underlying areas with one per cent picric acid was recommended (Armfield, 1918).

More recently, a single dose of antibiotics has proved effective

in treatment of the bovine condition, 75,000 IU/kg penicillin and 75 mg/kg streptomycin (Blancou, 1969). Recoveries of over 80 per cent with treatment, but only 14 per cent without, have been recorded. In Africa a single intramuscular injection of long-acting oxytetracycline (20 mg/kg) was reported to be curative in over 90 per cent of animals treated (Ilemobade, Gyang, Bida and Addo, 1979; Gyang, Ilemobade and Shannon, 1980). Control measures, such as six week quarantine of new animals; isolation of affected animals and slaughter if there is no response after the animal has been housed for six weeks, as the animal is likely to be a permanent reservoir of infection; dehorning; avoidance of skin branding; and segregation from sheep and horse; and ectoparasite control, were recommended in tropical countries by Macadam (1970). However, the traditional migration patterns and nomadic management of cattle in countries like Nigeria render measures such as isolation and ectoparasite control virtually impossible (Macadam, 1970). The discordant results obtained for single and combination systemic treatments lead to a situation which calls for a scientific search for better drugs (Arowolo, Amakiri and Nwufoh, 1987). Other methods of control or prevention are obviously desperately needed.

In problem flocks of sheep where elimination of dermatophilosis is impractical, routine summer and autumn protection dips with 0.5% zinc sulphate or 1% potassium aluminium sulphate are reported to be effective (Lofstedt, 1983; Martin, 1983). Resistant cases were treated with either a single parenteral dose of 70,000 IU procaine penicillin G/kg and 70 mg/kg streptomycin, or five days treatment at 5,000 IU procaine penicillin G and 5mg/kg streptomycin daily (Lofstedt, 1983).

Dermatophilosis is a disease which can be controlled by the consistent use of effective husbandry practices. However, where improvement of management is impractical or impossible, a situation which may be relatively common in poorly managed horses which are permanently at pasture, prophylaxis in the form of vaccination would be a very desirable method of control.

Also, for domestic animals kept in large numbers such as large

cattle herds in the tropics and large flocks of sheep in countries such as Australia and New Zealand, and where losses of productivity is of major economic importance, and where control of transmission factors is impossible, vaccination is the only realistic hope of disease control (Lloyd, 1984).

Blancou (1976), cited by Lloyd (1984), evaluated the use of live intradermal vaccines in a herd of Brahman and Brahman-cross cattle, comparing matched experimental and control groups, and concluded that whilst this approach reduced the disease severity, it had minimal effect on the incidence of infection. There have been no reports indicating the successful use vaccine of this type since then.

It is uncertain whether superficial epidermal defences or deeper secondary immune responses are more influential in the prevention of clinical dermatophilosis. Following intradermal vaccination specific antibodies to *D. congolensis* are secreted into the stratum corneum and protection may be mediated partly by the immobilisation of infective zoospores by flagellar agglutinin (Lloyd and Jenkinson, 1981). Local cutaneous humoral immune mechanisms may therefore be important and might explain the superior results obtained after intradermal vaccination. Flagellar antigen variability between strains of *D. congolensis* may therefore be significant (Roberts, 1964). Live intradermal vaccination stimulates considerable flagellar agglutinin production (Lloyd, Jenkinson, Nimmo and Mackie, unpublished observations). Therefore the antigenic composition of vaccinal strains may require to be tailored to predominant strains in a specific area. If it is crucial that superficial defences should be avoided by vaccination through the stratum corneum, then other antigens may be more important in the stimulation of an appropriate immune response (Lloyd, 1984).

The promise of live intradermal vaccination as an effective prophylactic have not then been fulfilled (Arowolo, Amakiri, and Nwufoh, 1987; Lloyd, 1984), and the need for a new approach to management of dermatophilosis is greater than ever.

Conclusion

Dermatophilosis is now a significant welfare problem in the horse in the U.K. It is seen most commonly where horses are exposed to the elements, without provision for shelter. It tends therefore to be a disease of the poorly managed horse. Debility and considerable weight loss often accompany generalised cases of dermatophilosis.

Owing to the insidious onset of infection in horses kept at pasture, cases are often advanced before detection, and are particularly refractive to treatment. Spontaneous recovery can occur in horses on housing, but this is often impossible. Although numerous therapies exist, response varies, particularly to topical treatment. Application of such treatments, especially washes, can be difficult and time consuming and, where the weather is inclement or when large numbers of animals are involved in an outbreak, it can be impractical. Treatments are often required for prolonged periods during which zoonotic infection may occur, and the recovery period for dermatophilosis in horses is often protracted.

The fact that live intradermal vaccination has not proved to be an effective means of prophylaxis, the potential for annual outbreaks within a stable, and the possibility of transmission of infection to humans and to domestic pets further strengthen the need for investigation of alternative methods of management of dermatophilosis in horses, a need which was addressed in this thesis.

ESSENTIAL FATTY ACIDS (EFAs): THE RATIONALE FOR POSSIBLE USE TO MANAGE EQUINE DERMATOPHILOSIS

Background

Essential fatty acids (EFAs), in particular gamma linolenic acid (GLA), are becoming increasingly widely used in clinical medicine, including treatment and management of skin disorders in man and in domestic animals. The metabolism and functions of EFAs, and their current relevance in medicine, with are reviewed in this chapter. Emphasis is placed on their role in the skin and in dermatological disorders, as an introduction to the justification for use as an alternative approach to management of dermatophilosis in horses.

EFAs are dietary factors which like vitamins or essential amino acids cannot be manufactured by the mammalian host but must be ingested with food (Horrobin, 1990a and 1990b). They are polyunsaturated fats, which means that they contain at least two double bonds. All EFAs are polyunsaturated fatty acids (PUFAs) but many PUFAs are not EFAs, and those which are not may have adverse effects (Horrobin, 1990b). There are two series of EFAs, the n-6 series derived from linoleic acid (LA), and the n-3 series derived from alpha-linoleic acid (ALA). LA, 18:2n-6, is present in most seed oils, sunflower oil for example contains over 50% of fatty acids as LA; and ALA, 18:3n-3, is the main fatty acid in leaves, grass contains 64% of fatty acids in this form. The nomenclature of EFAs is given in shorthand, e.g. LA is 18:2n-6. The first number, 18, is the number of carbon atoms in the molecule, and the number after the colon is the number of double bonds in the molecule. The last n-3 or n-6 defines whether the fatty acid is from the n-3 or n-6 series, indicating the position of the first double bond from the methyl end of the molecule: this is between the 3rd and 4th carbon atoms in the n-3 series and between the 6th and 7th carbon atoms in the n-6 series (Horrobin, 1990b).

The n-6 series seems to be considerably more important, as when n-6 and n-3 EFAs are deficient, abnormalities are quickly

corrected by n-6 EFAs alone, however the supplementation of n-3 EFAs correct none of the abnormalities, and make several worse (Ziboh and Chapkin, 1987). In the zebra, whose EFA intake is almost all n-3 ALA from grass, the ratio of n-6 to n-3 EFAs in tissues is between 3 and 9:1. Although the metabolic enzymes appear to be very similar, the n-3 and the n-6 series EFAs are not interchangeable.

LA and ALA have no biological activity beyond oxidation to provide energy, and most EFA function requires their metabolism by alternating slow desaturation and rapid elongation steps, shown in Figure 1, resulting in the formation of active metabolites. The limited effects of LA and ALA are shown by the stunted growth of animals fed large amounts of LA and ALA. If the rate-limiting step of 6-desaturation is bypassed by dietary supplementation with n-6 gamma-linolenic acid (GLA) and n-3 eicosapentanoic acid (EPA) then normal growth is again observed. The principal functions of LA itself are maintenance of the integrity of the impermeable barrier formed by the epidermis to water, as well as an anti-thrombotic effect of endothelium (Horrobin, 1990b).

Considerable species variation exists in EFA metabolism. In particular, the regulation of the first, rate limiting desaturation step by delta-6-desaturase enzyme activity varies in rate from one species to another, as indicated by the ratio of LA to its metabolites in body tissues (Horrobin and Manku, 1990).

The 6-desaturation reaction is most rapid in the rat, and slowest in the rabbit and in the guinea-pig (Horrobin and Manku, 1990). In cats, the 6-desaturation is either absent or exceedingly slow (Frankel & Rivers, 1978). In humans the rate of LA conversion is closest to rabbits and guinea-pigs (Horrobin, Huang, Cunnane and Manku, 1984; Sinclair, 1985). As far as can be determined, no information is available on EFA metabolism in the horse.

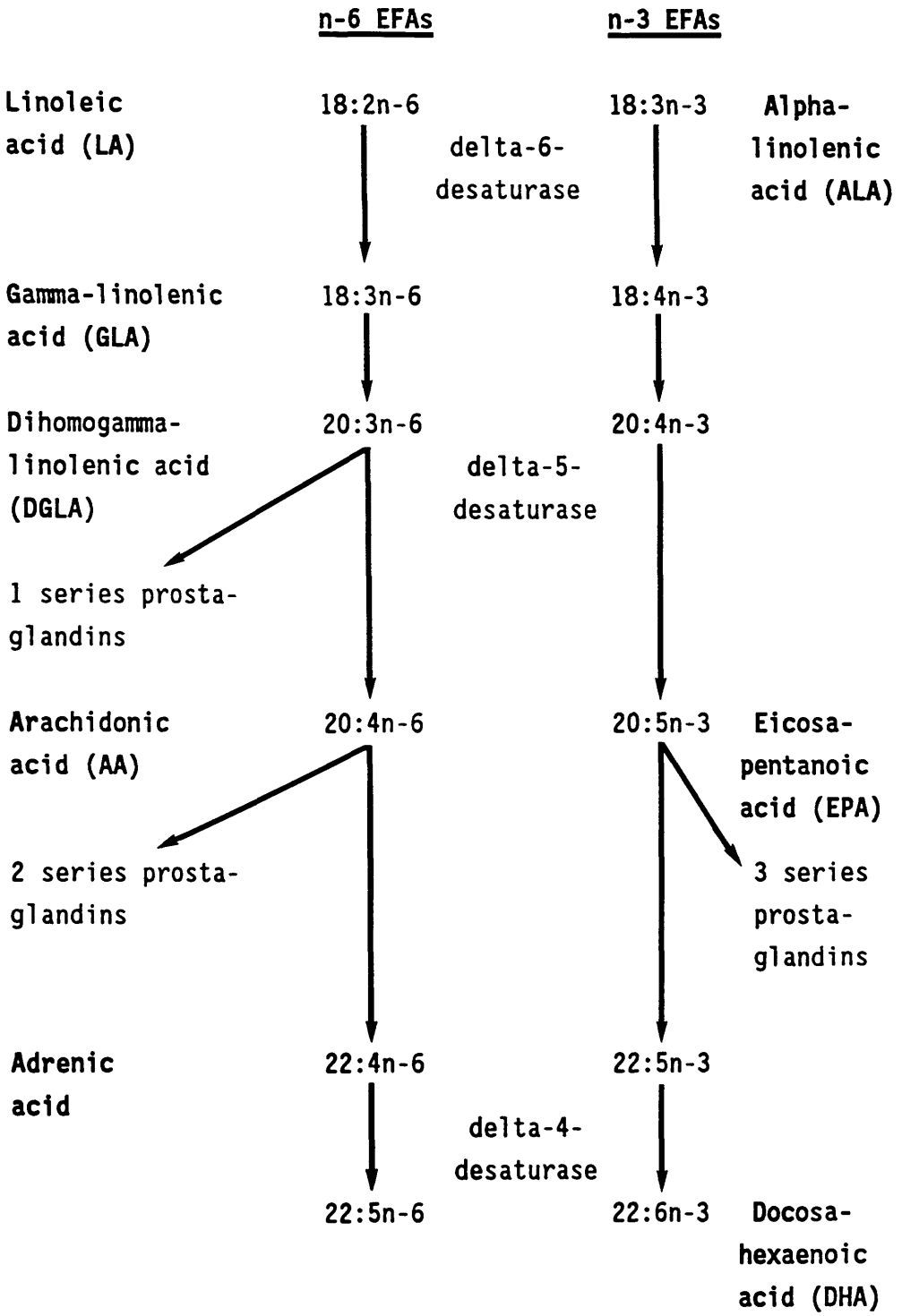

Figure 1. Pathways of metabolism of the n-6 and n-3 essential fatty acids (EFAs)

Functions of EFAs

Primary EFA functions relate to their roles in membrane structure and in biosynthesis of short lived derivatives which regulate cellular activity (Sinclair, 1990).

Owing to their unsaturation EFAs confer fluidity, flexibility, and permeability on all membranes in the body. EFA deficiency has, for example, led to reduced oxygenation of tissues due to failure of inflexible red blood cells to pass through capillaries (Simpson, 1988). EFAs modulate the function of membrane-bound proteins such as receptors, ATPases and ion channels and in EFA deficiency exaggerated effects are seen when normal concentrations of ligands such as oestrogens and progestins, angiotensin or opioids bind to receptor sites in membranes (Horrobin, 1990b).

The EFAs are the precursors for short lived regulatory molecules, known as the eicosanoids, which are derived from 20 carbon EFAs, namely arachidonic acid (AA) and DGLA of the n-6 series, and eicosapentanoic acid (EPA) of the n-3 series (Sinclair, 1990). These eicosanoids include prostaglandins (PGs), leukotrienes (LTs), and hydroxy-acids, which are all produced and destroyed locally, according to tissue requirements, and are involved in regulation of calcium contractions within cells, in smooth muscle contraction and relaxation, in control of chemotaxis and in modulation of cytokine production. The pathway for AA metabolism is shown in Figure 2.

The main enzymes involved in the metabolism of the EFAs to eicosanoids are the cyclo-oxygenase and related systems, which give rise to prostaglandins and thromboxanes; and the 5-, 12- and 15-lipoxygenases, which give rise to a variety of oxygenated metabolites including the leukotrienes (Horrobin and Manku, 1990). AA derivatives formed by cyclo-oxygenase and by 5- and 12-lipoxygenases are believed to be of particular importance in inflammation.

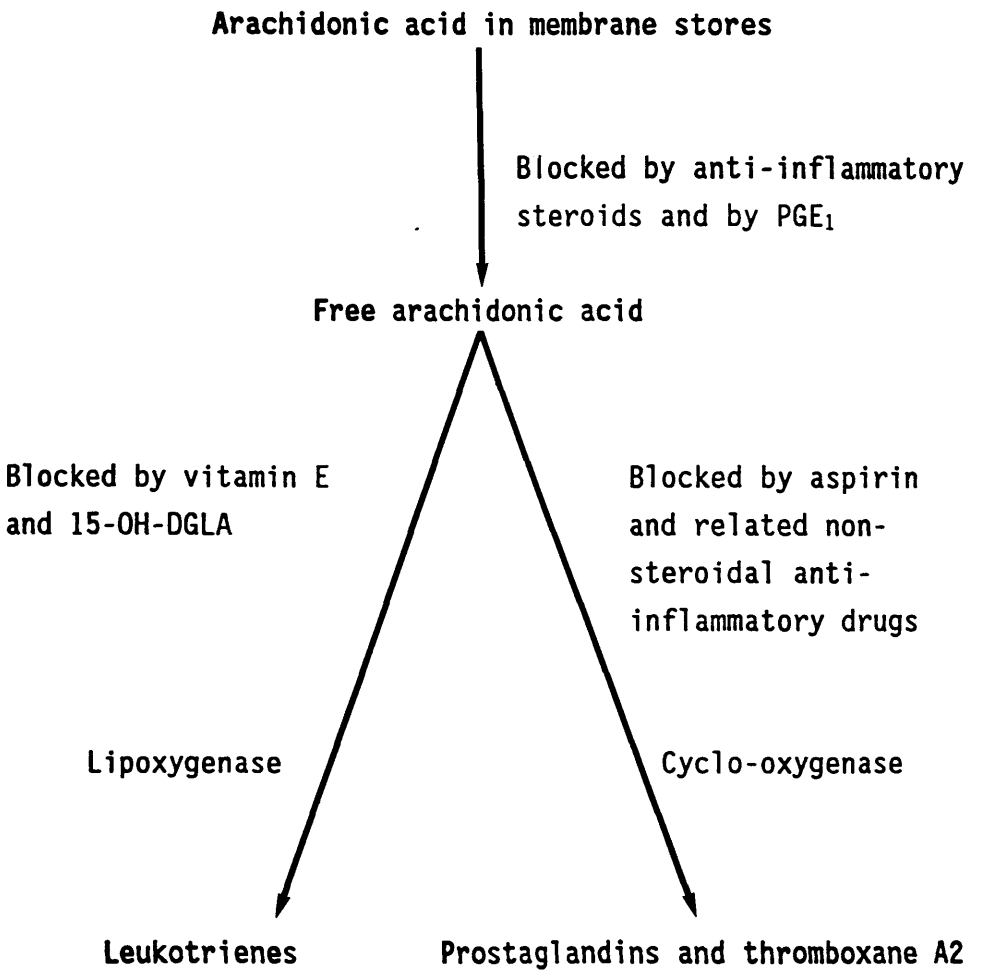

Figure 2. Pathway showing the formation of prostaglandins and leukotrienes from arachidonic acid

PGE_1, which is derived from DGLA, has a wide range of desirable effects including inhibition of platelet aggregation and inflammation, vasodilatation, reduction of blood pressure, elevation of cyclic AMP levels, and inhibition of phospholipases. 15-OH-DGLA, also derived from DGLA, inhibits 5- and 12-lipoxygenases and therefore the formation of proinflammatory metabolites from AA (Horrobin and Manku, 1990).

AA derivative PGI_2 (prostacyclin) and EPA derivative PGI_3 both have effects similar to those of PGE_1. AA derivative thromboxane A_2, a potent proaggregatory and vasoconstrictive agent is rapidly converted to a relatively inert metabolite, thromboxane $(Tx)B_2$ (Horrobin and Manku, 1990). Leukotrienes from AA, which contract smooth muscle are strongly proinflammatory.

The n-6 EFAs are required for maintenance of the integrity of the impermeable barrier to water which the skin provides. LA and GLA are the only EFAs capable of this, probably by metabolism to 13-hydroxy derivatives, which may also regulate permeability of other barriers throughout the body (Horrobin, 1990b). The skin is permeable to the passage of water and the transport of many other substances, and when there are insufficient EFAs present water quickly leaks through the skin, and the skin becomes waterlogged by osmosis when surrounded by water (Horrobin and Manku, 1990).

EFAs are involved in the transport and metabolism of cholesterol, which is moved around the body predominantly as fatty acid esters (Horrobin, 1990b). EFA esters are more soluble and more easily dispersed than unsaturated fatty acid. EFAs of the n-6 series consistently lower plasma cholesterol, probably with effect mediated by LA metabolites (Horrobin, 1990b).

It is evident, thus, that the detrimental effects of EFA deficiency are likely to manifest in all body tissues (Horrobin, 1990b).

EFA Deficiency in Animals

Poor growth, unlike that seen in other deficiencies, was observed in the rat as one of the initial manifestations of EFA deficiency

(Sinclair, 1952). When pair-fed rats were studied after weaning onto a fat-free diet, male rats grew virtually normally for the first five weeks post weaning, and females for the first 10 weeks. After a plateau at 13 weeks, the stunted rats survived for long periods but showed increased susceptibility to infections. Hyperkeratosis and acanthosis of the epidermis were seen, with hypertrophy and atrophy of the sebaceous glands. The lesions in transitional epidermal layers led to increased water permeability, with transepidermal water loss and scaliness shown to result from the same lesion (Sinclair, 1990). The signs were reversed by topical application of AA or LA.

Renal dysfunction has been observed in EFA deficiency in animals, with haematuria and renal tubule calcification, induced by prostanoid deficiency (Sinclair, 1990). Connective tissue involvement has been noted, cartilage was greatly altered and osteoporosis was seen, and erythrocyte fragility was increased, along with capillary permeability. Oxidative and phosphorylative reactions were uncoupled. Recent work has demonstrated a definite requirement of animals and humans for the n-3 ALA series EFAs: abnormalities have been demonstrated in retinal and brain function and in growth arising from n-3 deficiency only (Neuringer, Anderson and Connor, 1988).

EFA Deficiency in Man

It was first proven that EFAs were required by humans when oils were used in the treatment of infantile eczema (Hansen, Adam & Wiese, 1958). The rarity of EFA deficiency in humans has frequently been confirmed. Relative EFA deficiency, a low ratio of EFAs to antagonistic long-chain saturated fatty acids and trans isomers, has been suggested by Sinclair (1990) to account for Western diseases such as coronary heart disease and atherosclerosis, producing a different syndrome than absolute deficiency: for example, elevated plasma cholesterol levels are recorded in relative, but not absolute deficiency.

Rationale for Administration of GLA

Three main reasons exist for GLA supplementation (Horrobin, 1990b); in summary, if there is inadequate LA intake, or

inadequate rate of GLA formation, rate of GLA formation from LA may be insufficient to supply enough GLA and further metabolites. Excessive consumption of LA metabolites may occur, for example adequate oxygen and inadequate anti-oxidants result in EFA consumption and conversion to lipid peroxides; and where there is excessive inflammation AA is converted to PGs and LTs at an abnormally high rate. If cell division is rapid EFA provision may be inadequate to match the requirements of dividing cells. The rate of loss may be greater than the rate of LA metabolism (Horrobin, 1990b).

In addition, at least two GLA metabolites have desirable actions. These are prostaglandin E_1 (PGE_1), formed by cyclo-oxygenase from DGLA, and 15-OH-DGLA, formed by 15-hydroxylation of DGLA. PGE_1 inhibits inflammation, regulates the immune system, causes vasodilatation and lowers blood pressure, fluidises red cell membranes, causes induction of insulin receptors and inhibits platelet aggregation and thrombosis. 15-OH-DGLA inhibits 5- and 12- lipoxygenases, which lead to pro-inflammatory metabolites from AA (Horrobin, 1990b).

Forms of GLA Administration

GLA can be administered as triglycerides either derived from natural oils or artificially manufactured as free fatty acids, ethyl or methyl esters or as phospholipids (Horrobin, 1990b). The nature of other fatty acids present, and the exact nature of the molecule into which the fatty acids are incorporated can both have important effects on biological activity (Lawson and Hughes, 1988). Triglyceride structure is important in determining biological activity.

Evening primrose oil is a particularly desirable source of GLA: 7 to 10% of its fatty acids consist of GLA, and is more effective than other oils at increasing production of desirable PGE_1 and prostacyclin. EPO is unique in containing no n-3 fatty acids and almost no saturated fatty acids, both of which interfere with n-6 EFA metabolism and biological activity.

Methyl and ethyl esters are thought to be less effectively

absorbed than triglycerides or free fatty acids (Horrobin, 1990b).

The Use of EFAs in Treatment of Skin Conditions in Man and in Companion Animals

The use of EFAs in the management of skin conditions is better documented for humans than for our animals. The dermatological changes observed in atopic eczema bear a superficial resemblance to n-6 EFA deficiency (Wright, 1990); there is inherited slow 6-desaturation rate, with low levels of LA and ALA metabolites in umbilical cord blood, red cells and plasma, milk, and adipose tissue (Horrobin, 1990b). There is no evidence of reduced intake, absorption, or plasma levels of LA in atopic eczema, rather LA levels are often elevated (Horrobin, 1990b). In cord blood, the severity of the LA abnormality is positively correlated with the degree of elevation of IgE, which indicates risk of development of atopy (Horrobin, 1990b). The fundamental aetiology of atopy may be defective EFA metabolism, exerting different influences on the development of the disorder.

Prophylactic measures, by ensuring adequate intake of pre 6-desaturated EFAs by infants at risk from atopy, by adding GLA to artificial infant milks, already practised in Japan, or by increasing consumption of GLA as EPO by the breast-feeding mother, are proposed (Horrobin, 1990b).

IgE production is controlled by T-lymphocytes (Wright, 1990). Deficiency of 6-desaturated EFAs, as seen in atopy, may lead to an upset in the T-helper to T-suppressor cell ratio, because this balance is normally regulated by these EFAs. The T-suppressor to T-helper ratio is increased in atopic patients, usually due to low total T-lymphocyte numbers (Wright, 1990). In combination with abnormal permeability of the skin and the gut, T-cell imbalance will result in the adverse response by atopic patients to antigens which cause no response in normal individuals (Wright, 1990).

Atopic manifestations may therefore be associated directly with 6-desaturated EFA deficiency on skin, membranes and smooth muscle,

and indirectly with the induction of abnormal immune response consequent of EFA deficiency.

In treatment of atopic eczema using EPO, the mode of action is uncertain (Burton, 1990), but improvement is thought to be due partly to lowered production of the pro-inflammatory 2 series PGs, and also lineolate forms an integral part of the ceramide molecule, essential for the epidermal permeability barrier which is defective in atopic eczema (Burton, 1990). The increased transepidermal water loss in atopic eczema, and consequent drying of the skin, may be due to defective epidermal ceramides. EFAs might also modulate inflammation by an effect on the cell-signalling system (Burton, 1990).

Evening primrose oil (Epogam or Efamol, Scotia Pharmaceuticals) has recently received a product licence in the U.K. for the treatment of atopic eczema. In randomised double-blind placebo controlled trials to ameliorate all features of the disease, especially itch, and to reduce skin roughness, and to allow substantial reduction in topical steroids, and oral steroids, antihistamines and antibiotics in some patients (Schalin-Karrila, Mattila, Jansen and Uotila, 1987). The treatment is very safe compared to conventional parenteral treatments such as prednisolone or azathioprine (Burton, 1990). Preliminary investigations using Efamol Marine, a combination of n-6 and n-3 EFAs, 80% EPO and 20% fish oil, have shown better results than n-6 EPO alone (Horrobin, 1990b).

Abnormal keratinisation, classical of EFA deficiency, along with inflammation are seen in acne and psoriasis, two very common, but clinically diverse skin conditions (Allen, 1990).

Acne vulgaris, inflammation of the cutaneous pilosebaceous unit, has a complex aetiology, with active sebum production and abnormal duct keratinisation as prerequisites (Allen, 1990). There may be absolute, relative, or local EFA deficiency, and as precursors of the proinflammatory leukotrienes and prostaglandins, specific manipulation of EFAs may modify inflammation (Allen, 1990).

Aetiology of psoriasis is uncertain, but evidence exists that AA metabolism is upset, with elevated free AA and LT levels (Allen, 1990). GLA, supplemented in the diet or applied topically, might lead to amelioration by cyclo-oxygenase activity inhibition, or PGE1 or 15-OH-DGLA mediated specific anti-inflammatory effect (Allen, 1990).

EFAs can improve skin texture (Marshall and Evans, 1990). Thirty seven patients with atopic eczema were recruited into a randomized, double-blind, parallel trial to investigate the improvement of skin roughness, using Efamol EPO compared to a placebo. Patients received EPO daily for three months and were assessed monthly. Thirty one patients completed the trial. Objective measurements to assess skin smoothness showed marked, consistent improvement, within one month and thereafter maintained throughout the trial, in patients receiving Efamol treatment (Marshall & Evans, 1990).

EFAs have been found to be helpful in the management of anhidrosis in horses. Anhidrosis is related to the inability to sweat in response to an adequate stimulus (Correa and Calderin, 1966). It is encountered most frequently in hot, humid tropical climates such as Puerto Rico, Australia and New Zealand, Panama, South America and Hong Kong (Correa and Calderin, 1966).

Sweating is the major method of thermoregulation in the horse, and 75 per cent of body heat is lost in this way. In anhidrotic horses the body temperature may rise to 107 or 108°F (42°C), and animals may collapse following exercise. Prevalances of up to 20 per cent in Thoroughbred horses in some areas render the condition economically significant (Mullowney, 1985b). Although several theories have been postulated regarding aetiology, this is uncertain and treatment gives variable results. Treatments for the condition include sodium or potassium iodides, iodinated casein or thyroid hormone, oral vitamin E, minimising concentrates in the diet, and adrenocorticotrophic hormone (ACTH) injections. Provision of a cooler environment with reduced humidity eases the distress, and soaking the horse to allow cooling by evaporation, and restriction of exercise to the cooler times of the day are all

beneficial (Mullowney, 1985b).

However, in Florida three chronically anhidrotic horses were supplemented with EPO and safflower oil, at daily doses of 8g and 2 oz, respectively. All three horses had raised temperatures at rest. The owners of the horses reported greatly improved sweating ability, and also noted improved condition of hair and skin throughout the late summer, autumn and winter (Mayhew, 1985, personal communication).

Scurviness of the skin, a dry, dull coat, and loss of hair are seen when dogs are deprived of fats. Skin then becomes itchy, oily and susceptible to infection. The epidermis peels, there is otitis externa, exudation between the digits, and skin becomes thickened (Hansen and Wiese, 1951). Cats show similar signs (Frankel and Rivers, 1978). The signs resemble skin disorders in the dog and the cat, but EFA deficiency is uncommon in these species. If there is inadequate antioxidant in dried food, or high fatty acid oxidation after prolonged storage or accidental exposure to high temperatures, the EFA supply in commercial pet foods which is usually ample may be depleted (Lloyd, 1990). Intestinal malabsorption and hepatic disorders may also lead to EFA deficiency. Low levels of cofactors in EFA metabolism, vitamin E or zinc, can induce clinical signs similar to experimentally-induced EFA deficiency (Scott and Sheffy, 1987).

Cats lack 6-desaturase enzyme, and are therefore more dependent than the dog on GLA and its metabolites. Contrary to expectation, EFA deficiency is extremely uncommon in the domestic cat, and skin disease in the domestic cat is rarer than in the dog (Muller, Kirk and Scott, 1983).

Results of EFA supplementation in human atopic eczema have stimulated studies into the use of EFAs in treatment of skin disorders in companion animals. Allergic skin disease and seborrhoea in dogs and cats have been treated by EFA supplementation, and EPO, cold water marine fish oil and EPO/fish oil combinations have improved allergic skin disease in open studies in dogs (Scott & Buerger, 1988). EPO was shown to be

effective against atopy in dogs in a double-blind, placebo-controlled, crossover study (Scarff & Lloyd, 1989, in preparation).

Results of a study to investigate the efficacy of EPO supplementation in a multi-centre placebo controlled study in general practice (Scarff, Harvey and McEwan, in preparation) mirrored extensive human studies in that initial improvements in canine atopic dermatitis, for example improvements in scaling, pruritus, erythema, oedema, coat and overall condition, on high dose EPO therapy were substantial but tend to plateau by 12 weeks. It was thought likely that maintenance of the plateau might be achieved by lower dose long-term therapy.

EFA supplements containing mineral and vitamin cofactors such as zinc, magnesium, niacin, and vitamins A and C, have also proved beneficial in the treatment of pruritic and seborrhoeic conditions in clinical practice (Lloyd, 1990). Although anecdotal reports indicate efficacy of EFAs in seborrhoea and miliary eczema, studies in cats have been limited (Lloyd, 1990).

Other Clinical Uses of EFAs

Cardiovascular Disease

Elevated cholesterol levels, elevated triglyceride levels, hypertension and platelet aggregation are associated with increased risk of coronary heart disease (CHD) and peripheral vascular disease, and diabetes enhances risks at lower levels of these factors (Rosgengren, Welin, Tsipogianni and Wilhelmsen, 1989).

Abnormal EFA intake and metabolism may be equally or more important than these risk factors, because low intake and low plasma and adipose tissue concentrations of LA have been associated with high CHD risk (Oliver, Riemersma, Thomson, Fulton, Abraham and Wood, 1990). There may be more direct involvement of LA metabolites than of LA itself, as low plasma and adipose DGLA and AA levels are strong markers of CHD. EFA levels were lower in

patients who developed CHD, although no differences were seen in cholesterol, triglyceride or blood pressure (Wood, Butler, Riemersma *et al.* 1984).

The current EFA hypothesis of CHD is that the primary disorder is an inadequate, imbalanced n-6 EFA source (Horrobin, 1990b). Consequently there is increased cholesterol and triglyceride biosynthesis, increased platelet aggregation, raised blood pressure and reduced coronary stability with a higher risk of arrhythmia, with possible spasm of the coronary vessels. These factors are consequent of abnormal EFA metabolism and result in a higher risk of death from coronary failure. Supplementation of 6-desaturated n-6 and n-3 EFAs might correct the primary problem, whereas single factor therapies will be only moderately effective (Horrobin, 1990b).

Rheumatoid Arthritis

Elevated PG and LT levels found in cells and inflammatory exudates in rheumatological disorders suggests that inhibition of their synthesis might ameliorate the signs and symptoms of these disorders (Belch, 1990). PGs and LTs can be inhibited at numerous stages of AA metabolism. A diet rich in AA leads to the formation of the 2 series PGs and 4 series LTs with proinflammatory effects, and EFA manipulation in the diet can modify some of these effects (Belch, 1990). Evening primrose oil (EPO), a source of GLA, will elevate DGLA levels, resulting in increased production of the 1 series PGs, including PGE_1, which has been shown to inhibit AA mobilisation, to inhibit chemotaxis, to enhance T lymphocyte function, and consequently to suppress inflammation in a variety of animal models (Horrobin, 1990b).

In a prolonged EPO study in humans (Belch, Ansell, Madhock, O'Dowd & Sturrock, 1988) a proportion of patients were able to reduce or stop adjunctive non-steroidal anti-inflammatory (NSAI) therapy after three months, and after a double blind phase for 12 months there was significant difference between placebo and EPO or EPO and fish oil treated groups: in active treatment groups 90 per cent of patients reported improvement compared to only 30 per cent in the placebo group.

The use of GLA and EPA to inhibit AA metabolites thus enabled many patients to appreciate subjective improvement while simultaneously reducing NSAID dose, particularly important where there is renal compromise or gastric irritation.

Psychiatric Disorders and Neuropathies

The EFAs comprise approximately one fifth of the dry weight of the brain and peripheral nerves, and in contrast to other tissues, almost all of the EFAs present in nerve tissue are 6-desaturated (Horrobin, 1990a). The EFAs and their eicosanoid derivatives modulate nerve conduction, transmitter release, transmitter reuptake, and post synaptic transmitter effects, and varying abnormalities of EFA metabolism in blood and other tissues have been recorded in alcoholism, schizophrenia, depression and hyperactivity (Horrobin, 1990a). Particularly in alcoholism, schizophrenia, and childhood depression and hyperactivity, attempts to modulate EFA levels have produced very mixed results. A preparation containing zinc, pyridoxine, niacin and vitamin C, co-factors known to be important in EFA metabolism, in supplement to EFAs, produced marked increase in n-6 and n-3 incorporation into red cell membranes, producing significant clinical improvements in memory and schizophrenic symptoms (Vaddadi, Gilleard, Courtney and Horrobin, 1990).

In diabetes mellitus, hepatic cirrhosis, and alcoholism, where there is abnormal EFA metabolism peripheral neuropathies are common, and some neurological damage may be a consequence of abnormal EFA biochemistry. Strong evidence exists, for example, of acquired reduction in EFA 6- and 5- desaturation in diabetes, and reduced 6-desaturated EFA flow has been proposed to account for many long term adverse diabetic consequences (Horrobin, 1990b).

Cancer

Marked consistency now exists in work on the effects of 6-desaturated EFAs on cancer (Horrobin, 1990a). They have been demonstrated to kill human and animal cancer cells *in vitro* at concentrations safe to normal cells, and can control the growth of animal cancers (Horrobin, 1990a). Growth of human cancers implanted into nude mice can be regulated by EFAs which may kill

primary and metastatic cancer cells, or may inhibit the development of metastases, or enhance the efficacy of radiation and drugs (Pritchard and Mansel, 1990).

In order to monitor the effects of dietary lipids on growth of human tumour xenografts athymic mice were studied (Pritchard and Mansel, 1990). A diet high in fat (20% corn oil) enhanced growth of breast tumours compared with control diets of 5% and 10% corn oil. Both n-6 and n-3 series EFAs were supplemented as EPO and fish oil in the diet and tumour growth was significantly inhibited. The growth of human melanoma in mice was prevented by EPO and 5% fish oil 5% dietary supplement. Human tumours are thus reported to be sensitive to lipids and nutritional manipulation may influence the management of some human malignancies (Pritchard & Mansel, 1990).

Renal Disease

The renal medulla is especially rich in the E series PG precursor, DGLA (Nissen and Bojesen, 1969), which is thought to play an important role in the maintenance of high blood flow in the kidney. Failure of this control may result from PG inhibition by long term administration of analgesics (Horrobin, 1990b). Toxic, cyclosporin-associated renal damage appears to be related to excess thromboxane A_2 production without E series PG balance (Elzinga, Kelley, Houghton and Bennet, 1987).

EFA deficiency consistently leads to urinary tract tumour development (Monis and Eynard, 1980). GLA, administered to enhance PGE_1 synthesis has prevented or attenuated renal damage in animal models, and EPA in fish oil has reduced cyclosporin induced renal damage (Elzinga, Kelley, Houghton and Bennet, 1987).

Hepatic Disease

In patients suffering from hepatic disease it is not surprising that LA concentration is significantly elevated whilst LA metabolite levels are reduced, because the largest amount of 6-desaturase in the body is found in the liver (Horrobin, 1990b). Hepatic compromise would be expected to lower total body 6-desaturase activity (Biagi, Hrelia, Stafanini, Zurarelli and

Bordoni, 1990).

GLA metabolite PGE_1 is being used increasingly in treatment of liver transplant patients and to promote the ability of the liver to eliminate viral infections (Horrobin, 1990b). Additional GLA would lengthen the duration of these beneficial effects.

EPO (Efamol) inhibited hepatic fatty change following carbon tetrachloride administration (Cunnane and Horrobin, 1983), and plasma liver enzyme levels were normalised more rapidly in alcoholics following Efamol administration compared to controls. Pruritus was significantly reduced in primary biliary cirrhosis, and reduced n-6 EFA concentrations seen in plasma phospholipids and triglycerides in these patients were restored to normal by administration of Efamol (Triger, 1990).

Premenstrual Syndrome, Mastalgia (Breast Pain) and Prostatic Hypertrophy

Although the premenstrual syndrome (PMS), mastalgia and prostatic hypertrophy are only observed in the presence of gonadal hormones, abnormalities have not been recorded, and there is thought to be excessive end organ sensitivity to normal hormone levels (Horrobin, 1990b). High absolute or high saturated fat intake relative to EFAs may be relevant in pathogenesis (Horrobin, 1990b). Esterification of steroid hormones to fatty acids occurs in target tissues, and saturated fatty esters are more potent oestrogens than EFA esters, so if lipids in target tissue have high saturated fat to EFA ratio, normal circulating oestrogen levels will have exaggerated actions (Larner, Eisenfeld and Hochberg, 1985). Saturated fats have higher receptor affinity than unsaturated fats, so in membranes where steroid receptors are EFA deficient, receptors bind steroid more avidly, again with exaggerated effects despite levels being normal. In DGLA or PGE_1 deficiency, actions of prolactin, trophic for the breast and the prostate, are not modulated and normal levels have excessive effects (Horrobin, 1990b).

The pattern seen for these conditions is similar to that of atopic eczema, and epidemiological association exists between atopic

disorders and atopic eczema. In placebo controlled studies physical and psychological aspects of PMS improved for patients receiving GLA (Puolakka, Makarainen, Viinikka and Ylikorkala, 1985).

Viral Infections and Post-Viral Fatigue Syndrome

Viral infections are associated with low levels of LA and impaired desaturation (Horrobin, 1990b). Reduced DGLA and AA formation result in reduced ability of interferon to exert anti-viral effects. EFAs also have direct virucidal actions, particularly against lipid envelopes. Supplementation of 6-desaturated EFAs may therefore assist response to viral infections (Horrobin, 1990b). Red cell membrane levels of n-3 and n-6 EFAs were found to be lowered, whereas elevated levels of saturated and monounsaturated fats were recorded in post-viral fatigue syndrome (PVFS) patients. Symptoms of muscle weakness, aches, pains, loss of memory and concentration, exhaustion, depression, dizziness and vertigo were reported to improve when patients received 80% EPO and 20% fish oil (Efamol Marine), compared to placebo (Behan and Behan, 1990). Patients showed normalisation of saturated and monounsaturated fats and n-6 EFAs in red cell membranes, and elevation of n-3 EFAs to levels above normal. GLA and EPA may thus be valuable adjuncts to anti-viral therapy (Horrobin, 1990b).

Adverse Events and Toxicology

Animal toxicity studies on Efamol EPO at doses of up to 10 ml/kg/day have covered reproductive performance, teratogenicity, carcinogenicity and long term toxicology in dogs, rats, mice and rabbits (Horrobin, 1990b). No toxic effects attributable to EPO were found, and in particular there was no evidence of carcinogenicity (Everett, Perry and Bayliss, 1988). In placebo-controlled studies involving over 3,000 patients receiving Efamol for three months or more, no specific adverse event can be attributed with any confidence to Efamol (Scotia Pharmaceuticals, 1990, data on file).

Conclusion

This review details the rationale for the use of EFAs in the management of skin disorders such as atopic eczema, which probably involves a defect common to that which occurs in equine dermatophilosis, namely, impaired epidermal barrier function. LA and GLA are required for the maintenance of the integrity of the impermeable barrier formed by the skin to water; and GLA metabolites inhibit inflammation, regulate the immune system, and inhibit the formation of pro-inflammatory metabolites from AA. Favourable results have been obtained using oral supplementation of n-3 and n-6 EFAs in the treatment of the condition in man and in the dog. At the same time, improvement occurred in condition of the coat of horses which received EPO (containing LA and GLA) for the management of anhidrosis. EFAs have been shown to be non-toxic and non-carcinogenic, and free from adverse events. On the basis of the foregoing evidence an investigation was carried out on the effects of the use of EFAs, as a supplement to normal dietary intake, on the management of dermatophilosis in horses.

MATERIALS AND METHODS

Horses and Site

The horses used in this thesis were part of a herd of around 200 mature horses kept at two farms situated in the West of Scotland. Horses have been managed by the owner at Gartmorn Hill Farm, which spans 195 acres close to Dollar, since 1978, and at Burnhouse Farm which covers 125 acres near Bonnybridge, since 1981. Figure 3 shows Burnhouse Farm. Both farms are within an hour's drive of the University of Glasgow Veterinary School.

The horses are in good health, but are unsound or retired. Most of the horses belong to the owner of the farms, but some are loaned for temporary periods from other horse owners around the country. The owner of the farm uses several of the mares in the herd as brood mares. Most animals are Thoroughbred or Thoroughbred crosses, but there are also cobs and heavier types of animal. The horses are mature and are over 14.2 hands in height. The herd comprises approximately equal numbers of mares and geldings. The age ranges from four to over 20 years.

Blood Harvesting Procedures

The horses are kept for commercial blood production. Most of the blood is dispensed in the form of defibrinated whole blood, for incorporation into microbiological culture media. Serum is also used for the production of media. The customers for blood products are hospital laboratories and public health and private clinical laboratories also have a requirement for blood for diagnostic purposes.

Blood is harvested every three weeks. Individuals donate approximately 8 litres of blood at each harvest. This is around 20% of blood volume for a 500kg horse (Schalm, 1986). Blood is collected via the jugular vein in the mid third region of the neck. Alternate jugulars are used at each harvest. Prior to harvest local anaesthetic is infiltrated around the point of insertion of the harvest needle. The harvest volume is drawn into a sterile collection vessel and defibrination is performed.

Figure 3. Burnhouse Farm, by Bonnybridge, the home of part of the horse herd

The horses are tethered quietly and allowed to eat concentrates in a stall in the blood collection room throughout the harvest. Immediately after blood collection a loose cotton wool dressing or antiseptic spray is applied over the jugular area and the animals are tethered in an adjacent collection paddock to recover before returning to the covered yard or to pasture.

In 1987 both farms were licensed by the Home Office, permitting blood harvest by the owner of the herd, and by several of the grooms. Each time blood is collected, the procedure is overseen by the local veterinary surgeon. All premises are regularly examined by a Home Office Veterinary Inspector.

Management of Horses

The horses are managed together in groups of 30 to 40 animals, housed in large, covered sheds at Burnhouse Farm as routine during the winter, usually between September or October and March or April, depending on the weather. There are several loose boxes for new horses and for animals requiring special attention. While inside, animals are bedded on deep litter straw, and were fed top quality round baled silage daily on an *ad lib.* basis, and some hay and quality feeding straw. Figures 4, 5, 6 and 7 illustrate this group management system under conditions of housing.

During the summer months the horses are grazed together in groups of 20 to 30 on a pasture rotation system over the combined 320 acres of the farms. Figure 8 shows part of the Burnhouse herd at grass, and one of the brood mares is photographed with her foal in Figure 9.

Veterinary Attention

Veterinary attention for the treatment of disease and injury, and vaccination of the herd against tetanus, is provided by the local veterinary surgeons, who visit the farm several times each week. Individual health record cards are kept for each horse.

Figures 4 and 5 show the group management system of the horse herd during housing at Burnhouse Farm

Figures 6 and 7 show the group management system of the horse herd at housing, with the deep litter bedding and silage and hay feeding

Figure 8. Part of the Burnhouse horse herd at grazing during the summer

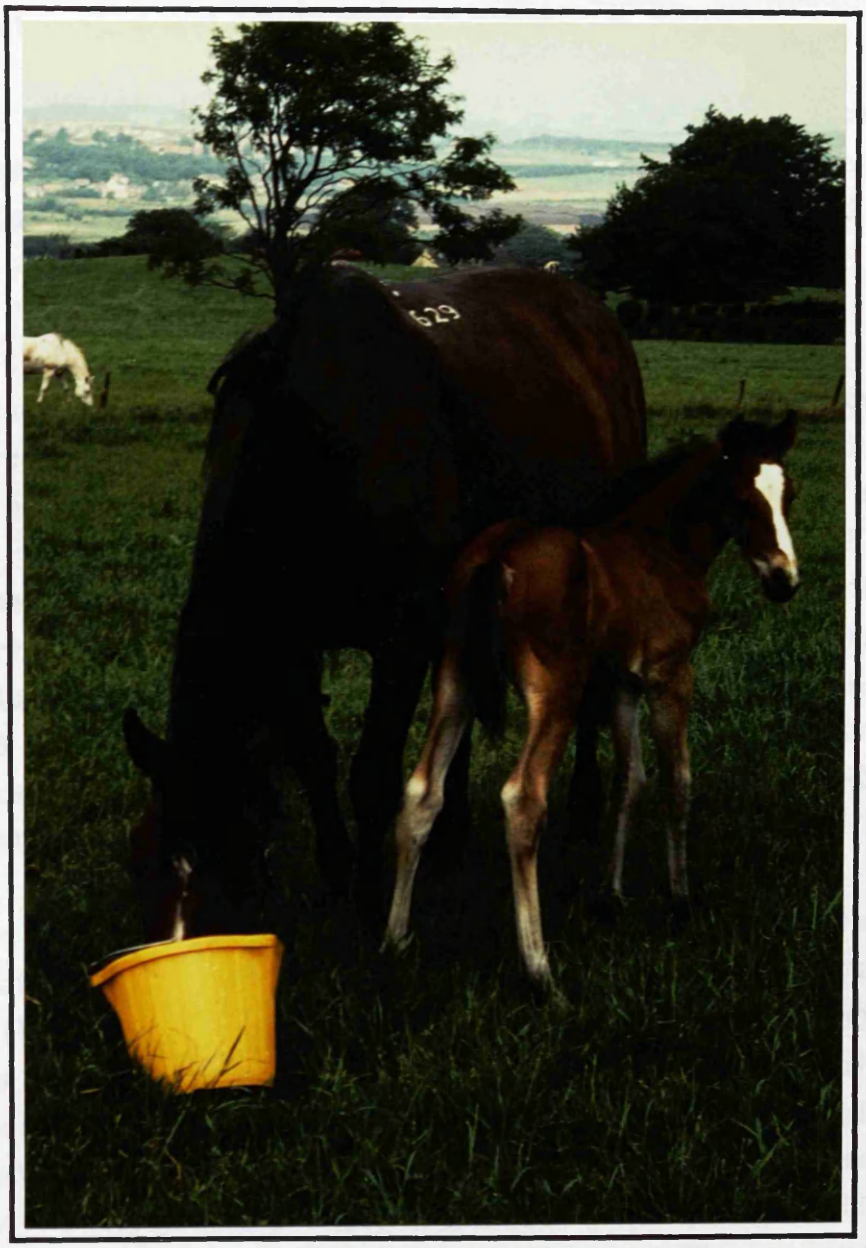

Figure 9. One of the Burnhouse Farm brood mares at grass with her foal

An anthelmintic programme has been designed, and is monitored, by the Department of Parasitology in the University of Glasgow Veterinary School.

Parameters Assessed in the Current Study

Values for all clinical, haematological and biochemical parameters, and for all EFA estimations are presented with standard deviations and means for each time point, and group means, in the appendices described in the text.

Clinical Monitoring

The condition of the coat, the mane, the tail and the hooves was recorded using subjective 1 to 10 numerical scales, with 1 being the poorest condition, and 10 being optimal condition. General body condition score was also monitored, on a 1 to 5 scale (Henneke, 1985). Assessment was based on palpation of the fat covering of the lower neck, thoracic and tailhead regions, similar to condition scoring in cattle. Optimal condition score was 3 to 3.5, with 1 being an emaciated animal, 2 a thin animal, 3 indicating moderate condition, 4 a fleshy animal, and 5 indicating obesity.

Where appropriate, the severity and distribution of dermatophilosis lesions were regularly recorded by means of a profile mapping system, as shown in Figure 10. A dermatophilosis index was designated, on a 0 to 10 numerical scale, calculated by recording the presence in each case of features typical of dermatophilosis: "paintbrush" lesions, scabs, hyperaesthesia, pain, secondary bacterial infection, oedema and cellulitis. Severity of lesions then was graded from 0 to 10, a score of 0 indicated absence of infection, and a score of 10 indicated the most severe infection. The distribution of lesions was recorded on the profile, using a grid to give crude percentage of body surface area affected, from 0 to 100 per cent. The dorsum: withers, saddle area and the rump, and the dorsal surfaces of the hindlimb pasterns were the areas most likely to be invaded by *D. congolensis*. The presence of any incidental skin lesions was

Figure 10. The horse profile mapping system used in clinical assessment

noted, such as those caused by trauma, external parasites, fungi, viruses or other bacteria.

All the clinical measurements were made by the same clinical assessor, to minimise variation in results.

Haematology and Clinical Biochemistry

Peripheral blood samples were routinely collected by the farm owner from all horses on the farms for monitoring of values for packed cell volume. In the present studies these blood samples were examined by additional haematological test and biochemically, and were used for EFA analyses.

Peripheral blood samples, 5 ml volume, were collected from the jugular vein, without the use of a tourniquet, via 20 g needles into EDTA Vacutainer tubes. Routine haematological examination was performed on the blood in the University of Glasgow Veterinary School Haematology Laboratory. Analyses performed were total red cell count (RCC), haemoglobin (Hb), packed cell volume (PCV), mean cell volume (MCV), mean cell haemoglobin concentration (MCHC), platelet count (Pl) and total white cell count (WCC).

Peripheral venous samples were collected for clinical biochemistry as for haematology, into 10 ml volume Lithium heparinised Vacutainer tubes. Routine assays, performed in the University of Glasgow Veterinary School Department of Biochemistry, were urea, sodium, potassium, calcium, chloride, magnesium, inorganic phosphate (i.phosphate), alkaline phosphatase (AP), aspartate aminotransaminase (AST), bilirubin, total plasma proteins (TPP), albumin and globulins. Gamma glutamyl transpeptidase (GGT), triglycerides (TG) and cholesterol were also assayed.

EFA Estimations

On return to the Veterinary School blood samples were centrifuged at 2500 rpm for 20 min at 4°C, and plasma removed. Red cells were then washed in equal volume 0.9% saline and centrifuged at 2500 rpm at 4°C, and saline wash and buffy coat discarded. Plasma and red cells were stored separately at -20°C and sent as batches at the conclusion of the studies to the Efamol Research Institute in

Nova Scotia, Canada, for measurement of fatty acids in plasma phospholipids and in red cell membrane phospholipids. The fatty acid composition of plasma triglycerides and cholesterol esters was also determined. Samples were sent for analysis uncoded with regard to controls and to baseline samples.

Lipids were extracted from plasma and red cells, and phospholipid and other lipid fractions were separated by thin layer chromatography, then fractions methylated and prepared by gas chromatography. Sampling was fully automated. The amount of each fatty acid present was calculated and printed out by computer, and transcribed on to standard data forms and returned to the clinical investigator.

Data Collected by the Farm Owner

Each horse is identified to the farm owner by name, and by number: most horses are freeze-branded. Details of individual horses are recorded, including the sex, age, breed or type, height, date and source of acquisition, and any relevant information on health or temperament.

At each blood harvest, PCV and the volume of blood collected are recorded, and any relevant comments, e.g., the demeanour of the animal during the harvest, and the appearance of the blood collected, are noted. The volume of blood dispensed from the farm laboratory after each harvest, and the percentage of blood lost during clotting, the waste, are recorded for each individual. The average weekly PCV is calculated for the herd as a whole, to allow weekly and monthly comparisons to be drawn for herd production from year to year.

Statistical Analyses

A repeated measures analysis of variance design was undertaken on the clinical indices, on haematological and biochemical parameters, and on results of EFA estimations. The design provided

tests for differences between treatment groups, differences between times and interaction between treatment and time (Ott, 1988). Significance levels of 5% were used unless otherwise indicated.

RESULTS

SECTION 1. THE USE OF ESSENTIAL FATTY ACIDS (EFAs) IN THE MANAGEMENT OF DERMATOPHILOSIS IN HORSES

SECTION 2. CHARACTERISTICS OF *DERMATOPHILOSIS CONGOLENSIS* IN RELATION TO SITE AND SEVERITY OF LESIONS, LEADING TO INVESTIGATION OF AN EXTRACELLULAR PROTEASE

SECTION 3. THE CLINICAL AND HAEMATOLOGICAL CONSEQUENCES OF BLEEDING HORSES AT REGULAR INTERVALS

SECTION 4. THE CREATION OF A MANAGEMENT EQUINE DATABASE

SECTION 1. THE USE OF ESSENTIAL FATTY ACIDS (EFAs) IN THE MANAGEMENT OF DERMATOPHILOSIS IN HORSES

Background

As reviewed in the introduction, essential fatty acids (EFAs) are dietary factors which like vitamins and essential amino acids cannot be made by the mammalian host but must be ingested with food. As essential constituents of all membranes in all body tissues, including the skin, they are vital in the determination of biological properties of these membranes. EFA deficiency leads to profound disturbances in all tissues, and it is known that EFAs are important in maintaining healthy skin and coat in several mammalian species including the horse, the dog and the cat, laboratory animals and man, as detailed in the introduction.

Lipids and EFAs play an essential role in the maintenance of the epidermal barrier (Elias, 1981). Lamellar bodies are ovoid organelles, synthesised in the stratum spinosum and stratum granulosum, which contain a variety of materials including lipids. One of their most important functions appears to be the deposition of lipid-rich intercellular materials, such as ceramide, necessary for maintenance of the epidermal permeability barrier (Burton, 1990). By providing the lipids that impede the outward movement of water and water soluble components (Elias, 1981), lamellar bodies therefore contribute greatly to the regulation of skin permeability. In EFA deficiency, detailed in the introduction, lamellar bodies are secreted in normal quantities but are largely devoid of lipid (Elias and Brown, 1978), and consequently the intercellular spaces of the stratum granulosum are porous and allow the passage of water soluble material.

In many dermatoses, such as atopic eczema, the epidermal permeability barrier function has been shown to be defective (Burton, 1990), and the integrity of this barrier has been shown to be dependent upon the availability of EFAs, particularly linoleic and gamma-linolenic acids (Burton, 1990; Elias, 1981; Elias and Brown, 1978; Wright, 1990). Fatty acids also play an important role in host defence mechanisms in that they are known to be toxic to many pathogenic microorganisms that come in contact with the skin (Biberstein, 1990).

It is possible that the excessive wetting and humidity predisposing to dermatophilosis in horses could lead to a deficiency of skin lipids and resultant defective barrier function. By correction of this deficiency it might be possible to restore skin integrity, normalise barrier function and prevent establishment of *D. congolensis* infection.

PGE_1 derived from DGLA inhibits inflammation, and PGI_2, derived from AA, and EPA derivative PGI_3, have similar effects to PGE_1. 15-OH-DGLA, formed by 15-hydroxlation of DGLA, inhibits 5- and 12-lipoxygenases, which lead to pro-inflammatory metabolites from AA. These mechanisms are shown in Figures 1 and 2 of the introductory section on EFAs. Thus, supplementation of GLA in the form of EPO, with fish oil and vitamin E (Efamol Marine, Scotia Pharmaceuticals), may cause inhibition of the local inflammation involved in dermatophilosis lesions by supplementing levels of DGLA and by preventing the formation of proinflammatory mediators from AA. As the lesions of dermatophilosis are believed to be the result of a nonspecific inflammatory response to *D. congolensis* organisms, once the epidermis is traumatised, the anti-inflammatory effects of EFAs may be particularly relevant in treatment of the condition.

EFA supplementation reduced pruritus, scaling, erythema, oedema and improved coat condition when used to treat canine atopic dermatitis (Scarff, Harvey and McEwan, unpublished data). When EFAs were used to treat anhidrotic horses in California, improvement was also recorded in the condition of skin and of the haircoat (Mayhew, 1985, personal communication). In humans and in animals, better results have been obtained when EFAs of the n-3 and n-6 series have been used in combination, as Efamol Marine, 80% EPO and 20% fish oil and vitamin E, than when EPO is used alone (Horrobin, 1990b).

The foregoing evidence would appear to justify an investigation on the effects of the use of EFAs, as a supplement to normal dietary intake, on the management of dermatophilosis in horses. Also, as discussed in the introductory section, EFAs have been shown to be non-toxic and non-carcinogenic, and free from adverse events

(Scotia Pharmaceuticals, data on file).

On two farms located approximately 30 miles northeast of the University of Glasgow Veterinary School there are around 200 horses, of all types and ages. Regular examination of these horses identified the prevalence and relative importance of naturally-occurring skin disorders. During the autumn and winter of 1986 the prevalence of *Dermatophilus congolensis* infection was approximately 60 per cent on these two farms. In around one third of these cases the infection was severe, affecting the dorsum and the lower limbs in particular.

The high prevalence of *D. congolensis* infection occurred even though the rainfall during the summer and autumn period of 1986 was not above average for the West of Scotland. The summer and autumn of 1985, however, was one of the wettest in the recorded history of Scotland and the owner of the two farms stated that the prevalence and severity of dermatophilosis in the horses was even more severe than in 1986.

During the survey animals of all ages were affected with dermatophilosis, and the condition was seen to recur, with individual horses appearing to be particularly susceptible. Moreover, although the infection was most severe during the autumn and winter periods, lesions due to *D. congolensis* were recognised throughout the year. The horses on both farms are at pasture during the summer and early autumn, but are housed in groups of 30 to 40 in large covered yards during late autumn and the winter months.

Around 200 outwintered ponies in the same region were studied during the same period and were found to have dermatophilosis during the winter of 1986. Many horses and most ponies in Scotland are kept unstabled during the winter.

It was thought that if the use of EFAs could prevent the establishment or even reduce the severity of infection, the welfare of the horse population in the West of Scotland and subsequently throughout Britain would be greatly improved.

Several studies were designed, therefore, with the initial aim of establishing baseline information concerning EFA metabolism in the horse, and comparing it to that in other species. The effects of EFAs, in the form of Efamol Marine (80% EPO and 20% fish oil and vitamin E), were studied first as a treatment and then as a prophylactic approach to the management of dermatophilosis in horses.

The size of the horse herd, at around 200, and the system of group management of the horses, along with the history of a recurrently high prevalence of dermatophilosis, were thought to provide a unique opportunity for a series of studies of this type.

The Pharmacokinetics of Essential Fatty Acids (EFAs) in Horses

Introduction

In the literature which was reviewed in the introductory section of this thesis it was revealed that linoleic acid (LA) must be converted to its metabolites to exert the full range of biological actions of essential fatty acids (EFAs) (Horrobin, 1990b). The first step in EFA metabolism is the 6-desaturation of LA, which is rate limiting, and is controlled by the 6-desaturase enzyme. The rate of this reaction is known to vary between species as indicated by the ratio of LA to its metabolites. Of the species which have been studied, the reaction rate is known to be fastest in the rat, and slowest in the rabbit and the guinea pig (Horrobin, 1990b). As far as can be determined, there is no information available regarding EFA metabolism in the horse. The reaction rate of 6-desaturation, for example, is unknown. This study was designed to ascertain the basal levels of EFAs in the horse, to investigate the ratio of LA to its active metabolites, dihomogammalinolenic (DGLA) and arachidonic acids (AA), and thus to estimate the 6-desaturase conversion rate in the horse. The objective of the study was to determine a suitable dose rate for subsequent investigation into the use of EFAs in the management of dermatophilosis in horses.

Materials and Methods

Method of Administration of EFAs

EFAs and placebo treatments were formulated as gelatin capsules, of 5 g weight. As the capsules were not readily tolerated by the horses without food, they were cut open and the contents disguised in a handful of coarse mix. This proved an acceptable method of supplementation to the animals, and each individual was fed the treatment and coarse mix from a hand-held scoop to ensure that the full EFA or placebo dose was received.

EFA treatment capsules contained 80% evening primrose oil (EPO) and 20% fish oil and vitamin E (Efamol Marine, Scotia Pharmaceuticals). Efamol Marine contains EFAs of both series: n-6 linoleic acid (LA) and gamma-linolenic acid (GLA) in the EPO, and n-3 eicosapentanoic acid (EPA) in the fish oil.

Experimental Design

Forty-eight horses were randomly assigned to four groups of 12 animals, each receiving a different dose or dose regimen of EFAs. One group received 5g EPO and 15g olive oil daily (olive oil was used to conserve equal dose volumes between groups); a second group received 10g EPO and 10g olive oil every other day; a third group received 20g EPO daily and a fourth group received 40g EPO every other day. The supplementation period spanned six weeks, from June through to July, while the horses were at grass. Horses did not receive any form of topical or systemic medication throughout the study.

Parameters Monitored

Clinical indices including the condition of the coat, mane, tail and hooves, haematological and biochemical changes, and EFA estimations, were recorded at weeks 0, 3 and 6. Details are contained in the general Materials and Methods section. Details of statistical analyses performed on the parameters are given in the general Materials and Methods section.

Results

Clinical Indices

Improvement of condition of the coat, mane, tail and hooves, was seen over the period of study, as indices increased. Means for the indices assigned at weeks 0, 3 and 6 are shown in Table 1. It is evident that most values for each of the indices fell between 5 and 6 on the 1 to 10 numerical scale, indicating average or above average condition, and that only very small variations were seen between groups.

Table 1. Mean values for clinical indices of condition of coat, mane, tail and hooves at weeks 0, 3 and 6 of four groups of 12 horses receiving different oral doses of n-3 and n-6 EFAs

Clinical index	coat condition			mane condition		
Week	0	3	6	0	3	6
Horse group						
5g daily	5.0	5.8	7.0	4.7	5.9	6.0
10g EOD	5.3	5.5	5.5	5.2	6.1	6.4
20g daily	4.9	5.8	6.6	4.8	5.9	6.3
40g EOD	4.4	6.4	6.9	4.4	5.7	6.4

Clinical index	tail condition			hoof condition		
Week	0	3	6	0	3	6
Horse group						
5g daily	4.8	5.8	6.7	4.6	5.4	6.7
10g EOD	5.2	6.3	6.8	4.8	6.3	7.1
20g daily	4.8	5.9	6.8	4.4	5.4	6.6
40g EOD	4.5	6.2	6.9	4.7	5.8	6.7

A full set of clinical results, with standard deviations indicated, is found in Tables I to IV in Appendix I.

The improvements recorded over time for condition of the coat, mane, tail and hooves were statistically significant.

The improvement of the coat condition was significantly less, however, for the 10g EPO alternate day group than for the other treatment groups. Analysis of variance (ANOVA) tables used in statistical analyses of the clinical parameters are given in Appendix V.

Haematology

Although differences were found for over time, and between the different EFA dose rate groups over time, for some haematological parameters, these changes were very small. Mean values for haematological parameters for the four treatment groups at weeks 0, 3 and 6 are given in Table 2. Values were within normal ranges with the exception of several elevated white cell counts, recorded for one horse in the 5g EPO daily group at week 3, three horses in the 10g EPO alternate day therapy group at week 0, and one horse in the 40g EPO alternate day group at week 0.

The values for red cell counts, haemoglobin and packed cell volumes for the horses receiving 40g EPO increased between week 0 and week 6, whereas the values for other groups dipped at week 3. This difference for time patterns between groups was statistically significant.

Values for mean cell volume and mean cell haemoglobin for all groups fell over time, and these time differences were statistically significant. Mean cell haemoglobin concentration values increased slightly at week 3, then dropped by week 6. Statistical analysis showed values for white cell counts to be significantly higher for the group of animals receiving 10g EPO than for the other groups. Platelet values fell over time for animals receiving 10g and 20g doses of EPO; and values for the 5g group dipped at week 3 then rose towards week 6, while those for the 40g group peaked at week 3 then fell towards week 6.

ANOVA tables used in statistical analyses of haematological values are shown in Appendix V.

Table 2. Mean values of haematological parameters of red cell count (RCC), haemoglobin (Hb), packed cell volume (PCV), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), platelets and white cell count (WCC), at weeks 0, 3 and 6, of four groups of 12 horses receiving different oral doses of n-3 and n-6 EFAs

Parameter	RCC			Hb		
Week	0	3	6	0	3	6
5g daily	6.82	6.43	7.46	12.87	12.16	12.68
10g EOD	7.28	6.47	8.07	13.31	11.76	13.12
20g daily	6.95	6.40	7.58	12.75	11.72	12.48
40g EOD	6.11	6.77	7.77	11.45	12.52	13.00

Parameter	PCV			MCV		
Week	0	3	6	0	3	6
5g daily	0.377	0.354	0.381	53.34	55.13	51.17
10g EOD	0.392	0.342	0.401	53.94	53.00	49.80
20g daily	0.375	0.343	0.379	54.03	53.76	50.16
40g EOD	0.336	0.365	0.394	55.17	54.17	50.52

Parameter	MCH			MCHC		
Week	0	3	6	0	3	6
5g daily	18.92	18.93	17.04	34.15	34.33	33.32
10g EOD	18.31	18.22	16.33	33.98	34.38	32.81
20g daily	18.49	18.38	16.68	34.03	34.14	33.01
40g EOD	18.78	18.58	16.70	34.05	34.29	33.08

Parameter	Platelets			WCC		
Week	0	3	6	0	3	6
5g daily	162.3	157.7	165.3	8.43	9.65	8.45
10g EOD	176.5	175.8	158.8	10.65	12.69	9.86
20g daily	172.8	160.8	138.1	9.40	9.15	9.83
40g EOD	166.0	172.5	149.3	9.53	10.28	8.40

A full set of haematological results is found in Tables V to XII in Appendix I. Normal ranges and units of measurement for haematological parameters are given in Appendix IV.

Biochemistry

As for the haematological parameters, small changes were recorded over time and between groups over time, for some biochemical parameters. Minor deviations from normal ranges were recorded for several biochemical parameters. Mean values at weeks 0, 3 and 6 for biochemical parameters are shown in Table 3.

Several sodium values were marginally low: at week 0, two horses in the 40g group (127 and 129 mmol/l); at week 3 two horses in each in the 5g and 10g groups (both 128 mmol/l); and at week 6 one horse from the 10g (131 mmol/l), and two horses from the 20g groups (131 and 129 mmol/l) and one from the 40g group (128 mmol/l). Magnesium values were low for several animals: at week 3, the value for one horse in the 5g group, and one horse in the 10g group at 0.49 mmol/l were marginally below the lower normal limit. Group means for triglyceride values were all elevated above the normal range of between 0.12 and 0.35 mmol/l. High alkaline phosphatase values were recorded for several horses in each of the groups at each time point, but group means were within normal ranges. Total plasma proteins were elevated for three animals in the 40g group at week 0 (103, 100 and 87 g/l); for four animals in the 5g group (102, 91, 85 and 90 g/l), five animals in the 10g group (85, 89, 93, 96 and 90 g/l), one in the 20g group (91g/l) and two in the 40g group at week 3 (90 and 117 g/l); and for two horses in the 5g group (98 and 102 g/l) and three in the 10g group (87, 95 and 89 g/l) at week 6. These results reflected increased globulin values.

Differences were found in the pattern over time between groups for values for urea, magnesium, inorganic phosphate, AST, globulins and cholesterol. These changes were statistically significant. The 40g dose of EFAs on alternate days led to increase in urea, magnesium, inorganic phosphate and cholesterol values over time. The urea values for each group dropped between week 0 and week 3, and increased again towards week 6, and the 40 g group showed a sharper incline to week 6 than the other groups. A similar trend was seen for magnesium values, with decreases for the 5g, 10g, and 20g groups, whereas the 40g group showed a pattern of increase between week 0 and week 6. Values for the 40g group for inorganic

Table 3. Means values of biochemical parameters of urea, sodium, potassium, calcium, chloride, magnesium, inorganic phosphate, alkaline phosphatase, aspartate aminotransaminase, bilirubin, total plasma proteins, albumin, globulins, triglycerides and cholesterol, at weeks 0, 3 and 6, of four group of 12 horses receiving different oral doses of n-3 and n-6 EFAs

Parameter	urea			sodium		
Week	0	3	6	0	3	6
Horse group						
5g daily	6.36	4.68	5.84	136.8	135.3	135.9
10g EOD	5.89	4.03	5.84	135.8	134.3	133.8
20g daily	6.57	4.69	6.37	135.6	135.4	133.6
40g EOD	6.17	5.91	7.10	134.8	137.2	133.5

Parameter	potassium			calcium		
Week	0	3	6	0	3	6
Horse group						
5g daily	4.72	3.93	3.68	3.00	2.87	2.94
10g EOD	4.48	3.99	3.59	2.95	2.88	2.99
20g daily	4.71	4.23	3.72	2.95	2.90	2.94
40g EOD	4.44	4.14	3.81	2.93	2.89	3.05

Parameter	chloride			magnesium		
Week	0	3	6	0	3	6
Horse group						
5g daily	97.3	95.7	96.3	0.63	0.60	0.67
10g EOD	96.1	93.7	94.6	0.62	0.58	0.70
20g daily	96.8	95.2	94.4	0.62	0.62	0.68
40g EOD	94.7	96.5	95.1	0.61	0.67	0.71

Parameter	i.phosphate			AP		
Week	0	3	6	0	3	6
Horse group						
5g daily	1.12	1.11	0.97	383.3	327.8	329.4
10g EOD	1.15	1.18	0.95	343.3	329.8	355.3
20g daily	1.19	1.16	0.88	370.1	349.6	336.5
40g EOD	1.11	0.93	1.04	344.3	354.7	323.2

Table 3 (continued)

Parameter	AST			bilirubin		
Week	0	3	6	0	3	6
Horse group						
5g daily	296.4	299.3	270.0	8.9	14.0	9.7
10g EOD	337.1	246.2	251.9	13.3	14.8	10.9
20g daily	300.3	320.3	282.3	8.6	12.1	10.3
40g EOD	265.0	328.4	300.9	9.2	9.6	11.9

Parameter	TPP			albumin		
Week	0	3	6	0	3	6
Horse group						
5g daily	70.2	78.0	74.4	31.6	31.9	29.8
10g EOD	71.3	83.3	79.1	31.1	30.8	28.8
20g daily	69.6	76.3	69.8	31.1	30.0	29.8
40g EOD	77.8	78.3	72.1	29.8	31.6	33.3

Parameter	globulins			TG		
Week	0	3	6	0	3	6
Horse group						
5g daily	38.6	44.5	44.6	0.46	0.31	0.39
10g EOD	40.3	52.5	50.3	0.41	0.32	0.42
20g daily	38.5	45.4	39.3	0.43	0.47	0.36
40g EOD	48.0	46.7	38.8	0.37	0.34	0.33

Parameter	cholesterol		
Week	0	3	6
Horse group			
5g daily	2.41	2.50	2.33
10g EOD	2.11	1.97	1.86
20g daily	2.33	2.40	2.22
40g EOD	1.97	2.14	2.40

A full set of biochemical results is found in Tables XIII to XXVII in Appendix I. Normal ranges and units of measurement for biochemical parameters are given in Appendix IV.

phosphate showed a different pattern to other groups once more: values rose, while they fell for other groups. Aspartate aminotransaminase values increased between week 0 and week 3, then fell between weeks 3 and 6 for the 5g, 20g, and 40g dose groups; in contrast, the 10g group values fell between week 0 and week 3, then rose between week 3 and week 6. Globulin values for the 5g group remained static between week 3 and week 6, whereas values fell between these time points for the other groups. Cholesterol values fell over time for the 10g group, but increased over time for the 40g group.

Differences were found between EFA treatment groups for values of urea and cholesterol. Values for the group receiving 10g were lower than for other groups for both parameters, as seen by the group means: for 10g group the cholesterol value in mmol/l was 1.979, compared to 2.415, 2.315 and 2.171 of the 5g, 20g and 40g treatment groups, respectively. Similarly, urea values were lower for this group, with a group mean of 5.25 mmol/l as compared to 5.63 for the 5g group, 5.88 for the 20g group, and 6.39 for the 40g group. These differences proved to be statistically significant.

No significant differences were seen for chloride, bilirubin, albumin, or triglyceride levels. Significant differences over time were seen for all groups for the other parameters: sodium, potassium, calcium, alkaline phosphatase, inorganic phosphate, total plasma proteins, globulins, urea and magnesium. For all groups total protein values rose at week 3, then fell back at week 6. Inorganic phosphate values for the 40g group dipped at week 3, and increased at week 6, whereas values for other groups fell over time. Although changes over time for sodium, globulins and alkaline phosphatase values were statistically significant, no specific trends were seen. The drop which was recorded in potassium values for all groups over time was statistically significant.

Results of statistical analyses of biochemical parameters are recorded in ANOVA tables in Appendix V.

EFA Estimations

The different dose rates of EFAs were not influential on LA, AA or EPA values in circulatory fractions. DGLA values, however, were significantly lower when horses received 10g EFAs on alternate days than when the dose was 5g or 20g daily, or 40g on alternate days.

The mean values for LA, DGLA, AA and EPA at weeks 0, 3 and 6, in red blood cell phospholipids, plasma phospholipids, and plasma cholesteryl esters are given for the different dose rate groups in Tables 4, 5 and 6, respectively.

From the results of EFA estimations of red blood cell phospholipids, presented in Table 4, increases in values between week 0 and week 3, then again between week 3 and week 6, were found for all groups for LA, DGLA, AA and EPA.

Assays of plasma phospholipids in Table 5 reveal that there were increases for LA between week 0 and week 6 for all groups. DGLA values increased over this time span for the 5g, 20g and 40g groups. Horses in the 5g, 10g and 20g groups showed increases in AA values between week 0 and week 6, but values for the 40g group dropped over the six weeks. A pattern of decrease over time was found for EPA values for horses receiving 5g and 10g EPO, but there was no definite trend for the 20g and 40g groups.

In plasma cholesteryl esters increases in LA values between were recorded for all the groups between week 0 and week 6, shown in Table 6. The groups of horses which received 10g, 20g and 40g of EFAs showed increases in DGLA levels between week 0 and week 6. Values for AA for the same groups also rose over this period. Elevation of EPA values for the 5g, 10g and 40g groups were recorded between weeks 0 and 6.

Statistical analyses of these results revealed, however, that none of the differences which occurred in LA, AA or EPA values either between groups between week 0 and week 3, or between week 0 and week 6, were significant. Similarly, no significant differences were found between groups for the values for DGLA

Table 4. Mean values for linoleic acid (LA), dihomogammalinolenic acid (DGLA), arachidonic acid (AA) and eicosapentanoic acid (EPA) in red blood cell phospholipids at weeks 0, 3 and 6, of four group of 12 horses receiving different oral doses of n-3 and n-6 EFAs

Parameter	LA			DGLA		
Week	0	3	6	0	3	6
Horse group						
5g daily	13.33	26.38	34.48	0.00	0.08	0.12
	(10.80)	(7.86)	(3.86)	(0.00)	(0.08)	(0.11)
10g EOD	12.48	18.27	33.85	0.00	0.00	0.09
	(8.58)	(7.33)	(5.57)	(0.00)	(0.00)	(0.09)
20g daily	15.03	24.92	34.48	0.01	0.08	0.17
	(11.41)	(11.49)	(4.65)	(0.03)	(0.07)	(0.06)
40g EOD	19.29	25.88	35.93	0.00	0.05	0.10
	(15.09)	(11.30)	(4.59)	(0.00)	(0.09)	(0.13)

Parameter	AA			EPA		
Week	0	3	6	0	3	6
Horse group						
5g daily	0.73	0.94	1.30	0.36	0.39	0.97
	(0.44)	(0.25)	(0.36)	(0.43)	(0.22)	(1.27)
10g EOD	0.73	0.79	1.33	0.35	0.30	0.43
	(0.34)	(0.31)	(0.59)	(0.25)	(0.17)	(0.34)
20g daily	0.81	0.99	1.45	0.43	0.37	0.64
	(0.44)	(0.30)	(0.23)	(0.27)	(0.22)	(0.14)
40g EOD	0.49	1.16	0.98	0.16	0.23	0.35
	(0.58)	(0.62)	(0.74)	(0.24)	(0.29)	(0.33)

Values are given as % of total EFAs
Values in brackets are standard deviations

Table 5. Mean values for linoleic acid (LA), dihomogammalinolenic acid (DGLA), arachidonic acid (AA) and eicosapentanoic acid (EPA) in plasma phospholipids at weeks 0, 3 and 6, of four group of 12 horses receiving different oral doses of n-3 and n-6 EFAs

Parameter	LA			DGLA		
Week	0	3	6	0	3	6
Horse group						
5g daily	37.03	42.37	42.13	0.33	0.41	0.41
	(2.72)	(5.83)	(6.23)	(0.05)	(0.06)	(0.08)
10g EOD	40.54	46.75	44.70	0.35	0.42	0.29
	(4.92)	(4.90)	(7.35)	(0.13)	(0.06)	(0.14)
20g EOD	46.24	53.75	52.47	0.35	0.45	0.42
	(4.19)	(3.92)	(3.40)	(0.07)	(0.09)	(0.14)
40g daily	41.17	45.64	45.16	0.32	0.46	0.47
	(6.76)	(5.88)	(6.93)	(0.12)	(0.07)	(0.07)

Parameter	AA			EPA		
Week	0	3	6	0	3	6
Horse group						
5g daily	1.43	1.57	1.57	0.54	0.51	0.44
	(0.51)	(0.71)	(0.61)	(0.09)	(0.08)	(0.11)
10g EOD	1.86	1.86	1.91	0.64	0.60	0.58
	(0.75)	(0.52)	(0.67)	(0.16)	(0.16)	(0.10)
20g daily	1.56	1.67	1.69	0.75	0.76	0.75
	(0.33)	(0.47)	(0.26)	(0.13)	(0.21)	(0.15)
40g EOD	1.98	1.87	1.85	0.69	0.74	0.62
	(0.60)	(0.44)	(0.42)	(0.34)	(0.14)	(0.24)

Values are given as % of total EFAs
Values in brackets are standard deviations

Table 6. Mean values for linoleic acid (LA), dihomogammalinolenic acid (DGLA), arachidonic acid (AA) and eicosapentanoic acid (EPA) in plasma cholesteryl esters at weeks 0, 3 and 6, of four group of 12 horses receiving different oral doses of n-3 and n-6 EFAs

Parameter	LA			DGLA		
Week	0	3	6	0	3	6
Horse group						
5g daily	43.69	50.51	52.45	0.14	0.03	0.04
	(9.05)	(10.95)	(13.01)	(0.33)	(0.07)	(0.15)
10g EOD	41.36	48.83	45.95	0.10	0.11	0.14
	(10.00)	(11.44)	(10.55)	(0.27)	(0.27)	(0.23)
20g daily	45.93	44.51	46.96	0.12	0.16	0.18
	(9.72)	(16.24)	(8.06)	(0.13)	(0.21)	(0.21)
40g EOD	44.64	52.30	53.95	0.05	0.14	0.20
	(13.63)	(10.18)	(14.76)	(0.12)	(0.27)	(0.33)

Parameter	AA			EPA		
Week	0	3	6	0	3	6
Horse group						
5g daily	6.62	6.42	6.55	0.18	0.10	0.25
	(2.32)	(3.65)	(2.85)	(0.17)	(0.10)	(0.55)
10g EOD	7.48	8.29	7.77	0.00	0.00	0.03
	(2.41)	(2.61)	(2.96)	(0.00)	(0.00)	(0.10)
20g daily	4.73	3.96	5.60	0.08	0.04	0.02
	(1.65)	(2.05)	(2.26)	(0.08)	(0.07)	(0.06)
40g EOD	3.78	3.53	4.49	0.13	0.21	0.19
	(3.60)	(2.70)	(7.13)	(0.14)	(0.10)	(0.16)

Values are given as % of total EFAs
Values in brackets are standard deviations

between weeks 0 and 3. Multiple range analysis revealed that between weeks 0 and 6, the DGLA values were significantly lower for the 10g group than for the other groups.

Results of statistical analyses of EFA values are presented in Appendix V.

The basal levels of LA, and its metabolites DGLA and AA in plasma phospholipids in the horse are illustrated in Figure 11. Comparative levels in the dog, the cat and in man are shown.

Discussion

Basal levels of EFAs were established for the horse, and the metabolism of EFAs was found to be different in the horse to that in other species. The percentage of total EFAs comprised by LA in the horse is more than twice the percentage in the dog, the cat and in man. In contrast, the percentages of total EFAs of LA metabolites, DGLA and AA, in the horse are very much lower than in other species. The percentage of total EFAs formed by DGLA is approximately one fifteenth of that in man; and the percentage of total EFAs comprised by AA is less than one tenth of the percentage found in the dog.

The ratio of LA to its metabolites was examined for the horse, and was found to be very much lower than the ratio found in the dog, the cat, or in man. It was considered likely that the low ratio in this species was due to slow 6-desaturation of LA by the 6-desaturase enzyme. The rate of the 6-desaturation reaction was slower, for example, in the horse than in man; and the ratio in man is closer to that of herbivores such as the rabbit and guinea pig, than it is to that of the rat. The conversion rate of LA to its biologically active metabolites for the horse was therefore found to be similar to the conversion rate for other herbivores.

Although the improvements recorded in the condition of the coat, mane, tail or hooves of the horses were statistically significant with respect to time, no significant difference was found for time

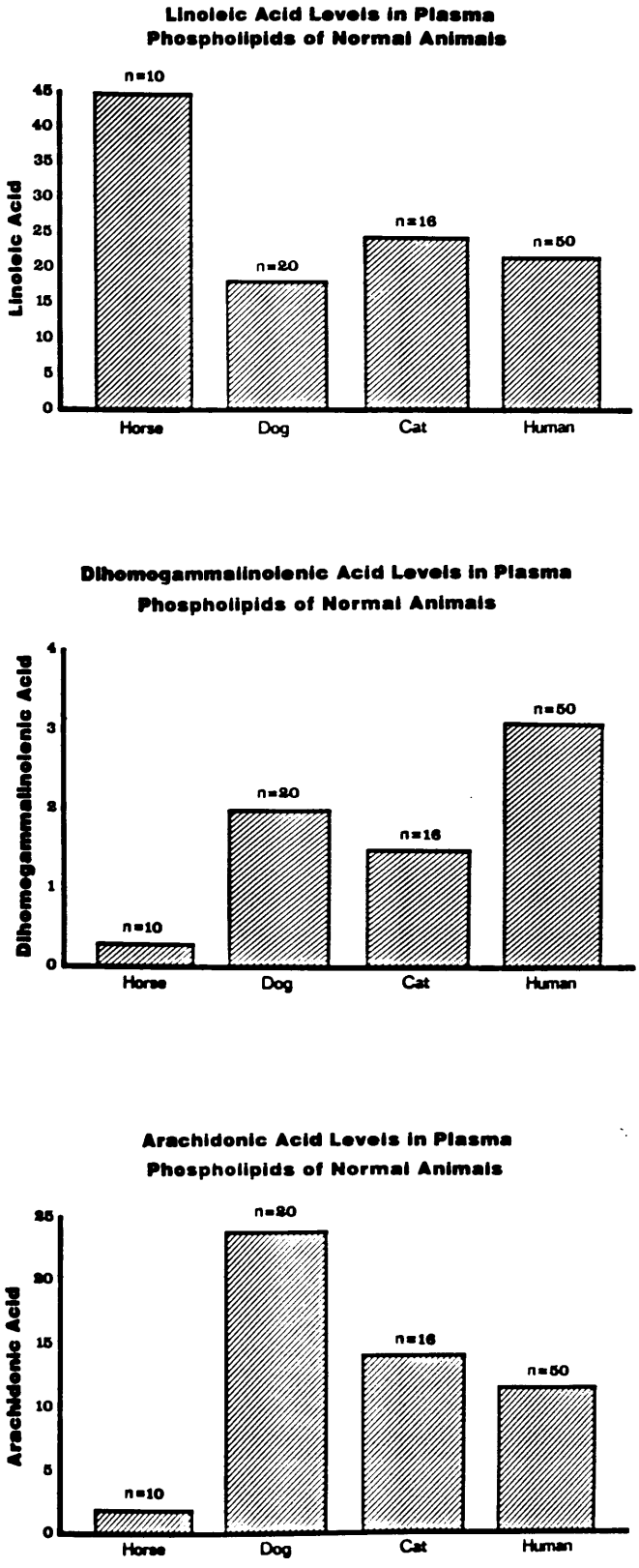

Figure 11. Basal levels of linoleic acid (LA), and its metabolites dihomogammalinolenic acid (DGLA) and arachidonic acid (AA) in the horse, with comparison to levels in other species. All values are given as % of total EFAs.

pattern between groups. As this was a titration study, a control group was not used, and it could not therefore be assumed that these changes over time for clinical parameters were attributable to the EFA treatments. Moreover, it is likely that the improvement in these clinical parameters was attributed to the improving plane of nutrition of the grazing between June and July when the study was performed, rather than to the EFA treatments.

Fluctuations over time were seen for haematological and biochemical parameters within groups and between groups during the study. Despite the fact that statistical analyses revealed several of these changes to be significant, no distinct patterns were found which were considered relevant to, or attributable to, the EFA treatments. Fluctuations in these parameters were thought to reflect nutritional factors. Thus, no harmful effect was evident on either haematological or biochemical parameters at any of the EFA dose rates studied.

Statistical analyses of results of EFA estimations indicated that no particular dose regimen was significantly more efficacious than another. In order to ensure maximum opportunity for uptake, it was decided that the dose level of 20 g of Efamol Marine daily should be administered.

The Use of Essential Fatty Acids (EFAs) in the Treatment of Dermatophilosis in Horses

Introduction

The need for an alternative approach to the treatment of dermatophilosis in the horse was emphasised in the introductory chapters. Evening primrose oil (EPO) with added fish oil and vitamin E, formulated as Efamol Marine (Scotia Pharmaceuticals), was used to try to treat dermatophilosis in horses. EPO is a rich source of linoleic acid (LA) and gamma-linolenic acid (GLA), the EFAs which are vital to the maintenance of the epidermal barrier to water. Fish oil contains n-3 eicosapentanoic acid (EPA), derivatives of which inhibit inflammation; and vitamin E blocks conversion of arachidonic acid (AA) to the pro-inflammatory leukotrienes.

Active EFA treatment was to be compared to placebo, which was hydrogenated coconut oil with added vitamin E. EFA and placebo treatments were to be administered at the period when prevalence of dermatophilosis infection was found to be highest, in late autumn and early winter. This study followed the autumn period when the rainfall recorded in the West of Scotland is traditionally highest. The dose rate for EFA supplementation to ensure maximum opportunity for uptake was determined in a pharmacokinetic study, detailed in this section. Improvement in the severity of lesions of dermatophilosis was sought, and lesion material was to examined microscopically to determine the activity of such lesions. The condition of the haircoat, the mane, tail and hooves and general body condition were to be monitored. There were reports of improvement of these parameters when EFAs were used to treat anhidrotic horses, described in the EFA review. It was hoped that these findings could be substantiated by the use of EFAs in this study. Haematological and biochemical parameters were also to be monitored for any systemic effect or adverse event.

Materials and Methods

Method of Administration of EFAs

The method of administration of treatments, and details of the EFA treatment capsules, Efamol Marine, are described in the Materials and Methods for the pharmacokinetic study in this section of the results. Placebo capsules contained hydrogenated coconut oil and vitamin E.

Experimental Design

A group of randomly selected horses received active, EFA supplementation, and were compared to placebo and control groups. Three groups of 12 animals were randomly identified. One group received 20 g of a combination of n-3 and n-6 EFAs (Efamol Marine) daily, the dose rate which was found in the pharmacokinetic study to allow maximum opportunity for uptake; and a second group received 20g placebo daily. The third group, the control animals, received no treatment.

The supplementation period spanned 16 weeks, from early November to late February, during which the horses were housed, but were most likely to be infected by *D. congolensis*, based on the experience of previous years on the two farms, and on the typical disease pattern in the West of Scotland. The trial was run double-blind, the investigator was unaware of which horses received which, if any, treatment.

Parameters Monitored

Dermatophilosis lesions

The severity and the extent of distribution of the lesions of dermatophilosis were recorded at weeks 0, 2, 4, 6, 8, 10, 12, 14, and 16 during the study. Details are provided in the general Materials and Methods section.

Bacteriology

Where present, paintbrush lesions and scab material from *D. congolensis* infected horses were subjected to bacteriological examination at regular intervals during the study. Material was collected into sterile bijoux, and where secondary bacterial

infection was evident, lesions were swabbed and material transferred in Ames transport medium (Transwab, The Medical Wire and Equipment Company) to the laboratory. Paintbrush lesions and scabs were soaked overnight in sterile physiological saline to soften them and to allow release of zoospores, and stored at 4°C to reduce the risk of contamination. Thick impression smears were made from soaked scabs, then methanol fixed and stained by Giemsa. Material were cultured on sheep blood agar and MacConkey's agar, and on mannitol salt agar to screen for *Staphylococci*. Culture was also made on medium selective for *D. congolensis*, containing polymixin B sulphate at a concentration of 1000 IU/ml (160 mg/ml) in blood agar base No.2 (Oxoid), supplemented with 5% sheep blood, according to the method of Abu-Samra (1977). Cultures were incubated at 37°C for 48 h.

D. congolensis and any other significant bacteria were sought. Giemsa stained smears were examined under oil immersion at x100 magnification for the presence of Gram-positive cocci and filamentous hyphal growths showing the transverse and longitudinal divisions, the "railroad tracks" considered characteristic of *D. congolensis*. Smears were graded in severity: + was mild infection, ++ was moderate, and +++ was severe infection, as judged by the density of organisms seen. Cultures considered positive for *D. congolensis* were dry, beta-haemolytic, grey-yellow coloured, pinprick sized colonies which were embedded in the agar. Bacteriological examination was performed at week 0, before EFA supplementation was begun; at week 10, during supplementation, and at week 16, when supplementation was stopped.

Clinical monitoring system

Clinical indices of condition of the coat, mane, tail and hooves, and general body condition were recorded at weeks 0, 2, 4, 6, 8, 10, 12, 14 and 16 during the study. Details of the measurement of these parameters are provided in the general Materials and Methods section.

Haematological and biochemical parameters were examined, and EFA estimations were performed, at weeks 0, 4, 8, 12 and 16. Details of these measurements are provided in the general Materials and

Methods section.

Statistical analyses were undertaken on the clinical indices, on haematological and biochemical parameters, and on EFA estimations using a repeated measures design, which is described in the general Materials and Methods section.

Results

The Effect of EFAs on the Lesions of Dermatophilosis

Dermatophilosis lesions became less severe for all the groups, indicated by the drop in the dermatophilosis index in most cases; and the distribution of dorsum and hindlimb lesions became less widespread with time. Lesions were more severe and more widespread on horses in the placebo and EFA groups than for the controls. Most of the horses showed mild infections, with low dermatophilosis indices, group means of less then 3, but each group did contain severely affected animals. In the control group one horse scored 8, and another 7 for severity of lesions; in the placebo group one scored 8 and another 7; and in the EFA group one horse scored 9, the most severe infection in the study. The most widespread distribution occurred for the horse which had scored 9 on the dermatophilosis index, Henry, in the EFA group: at week 0 lesions affected 60% of his body, and 60% of his hindlimbs. It is seen from the values presented in Table 7 that lesions affected a far lesser area of the body and hindlimbs than this in most horses.

An example of the typical "paintbrush" dermatophilosis lesions, of matted exudate in the haircoat, is shown in Figure 12. These lesions were situated on the rump region of this particular animal. Figure 13 illustrates the painful scab lesions of dermatophilosis on the dorsum of one of the study horses. Grey-green pus is seen on the undersides of the uplifted scabs, and there are ulcer-like raw areas below the scabs. The scab lesions found on the lower hindlimbs of infected horses are seen in Figure 14. These "mud fever" lesions were identified on the dorsal pasterns, and around the fetlock and coronary band regions.

Table 7. Mean indices at weeks 0, 8 and 16 for the severity and the extent of distribution of dermatophilosis lesions on the dorsum and on the hindlimbs of three groups of horses in a treatment study. Eleven horses received no treatment, 12 received 20g daily placebo of hydrogenated coconut oil and vitamin E, and 12 animals received 20g of n-3 and n-6 EFAs daily.

Clinical index	dermatophilosis index		
Week	0	8	16
Horse group			
Controls	3.2	1.3	1.1
Placebo	4.2	2.1	1.7
EFAs	4.3	2.9	1.5

Clinical index	dorsum distribution			hindlimb distribution		
Week	0	8	16	0	8	16
Horse group						
Controls	18.1	7.1	2.5	17.5	2.1	2.5
Placebo	19.8	9.4	5.5	25.0	4.2	8.5
EFAs	20.5	8.6	6.8	19.0	5.3	2.8

A full set of results of severity and distribution of lesions of dermatophilosis is found in Tables I to III in Appendix II.

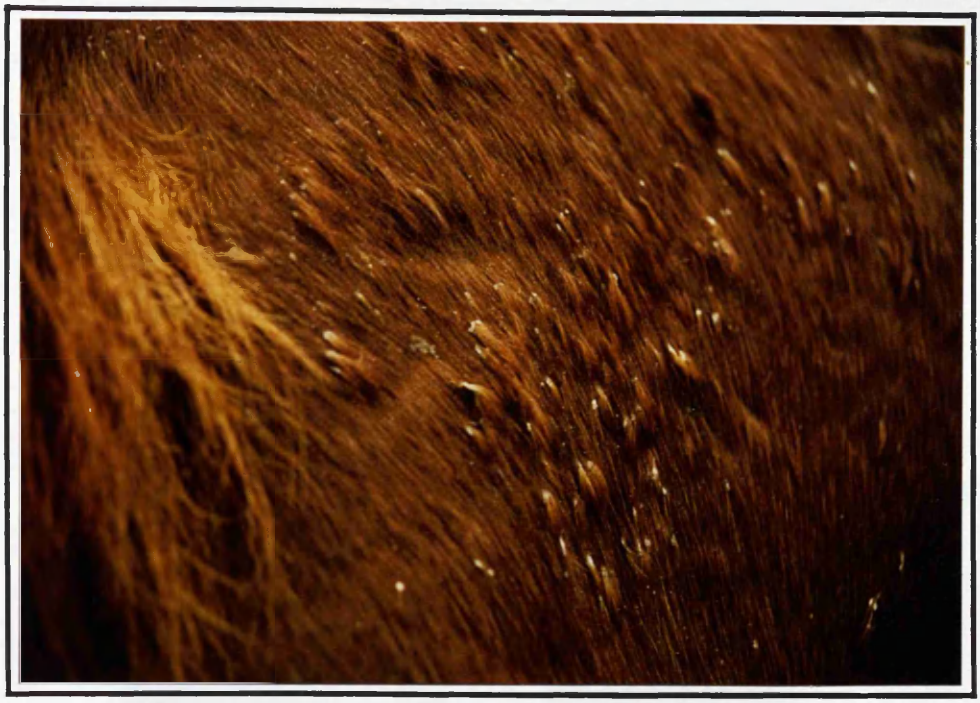

Figure 12. The typical "paintbrush" lesions of dermatophilosis seen on the dorsum of a horse during the EFA treatment study

Figure 13. Painful scab lesions of dermatophilosis found on the dorsum of a horse during the EFA treatment study

Figure 14. Dermatophilosis lesions involving the lower hindlimbs of a horse during the EFA treatment study

The reduction in dermatophilosis lesion severity and in the distribution of dorsum and hindlimb lesions were statistically significant. These improvements occurred, however, for all groups: improvement was not statistically greater for the treatment than for placebo or for untreated groups.

Bacteriology

Although *D. congolensis* organisms were isolated from over 60% of lesions examined, they were more difficult to culture. Throughout the study the prevalence of dermatophilosis infection, according to clinical observation, Giemsa smears, and cultures, was not significantly lower for the animals which received EFA than for those which received placebo, or were untreated.

Of the 65 clinical dorsum lesions sampled over the duration of the study, 48 (73.8%) proved positive on Giemsa smear, and 27 (41.5%) were confirmed positive on culture. Of 56 clinical lesions of the lower hindlimbs which were sampled, 36 (64.3%) were positive on Giemsa smear and 7 (12.5%) were positive on culture. Where secondary infection and pus were present on dorsum lesions, swabs were taken. Seven of the 10 (70%) of the lesions swabbed proved positive on culture for *D. congolensis*. The results of bacteriological findings are summarised in Table 8. Each entry in the table for each horse shows results of detection of clinical lesions, and of detection of *D. congolensis* on smear and on culture.

One horse from the control group and one from the EFA group, both of which were negative for clinical lesions at the study onset, developed dorsum lesions which were confirmed positive on culture during the trial. Three horses, one each from control, placebo and EFA groups, and having no lesions at the study onset, developed lower hindlimb lesions. Two of these were positive for *D. congolensis* organisms by Giemsa smear, although none could be cultured.

Pathogenic *Staphylococci* were isolated from one of the EFA group horses at the onset of the study, and from two animals in the placebo group at the end of the study. The organism was recovered

from lesions involving the lower hindlimbs in each case.

These organisms were confirmed to be pathogenic by subculture from mannitol salt agar to DNase medium. Other incidental findings were that two animals had lice infestations at the conclusion of the trial, one control and one EFA group animal. One of the placebo group horses showed evidence of a mite infection of the fetlocks during the trial, but a species could not be identified. Owing to the fact that the horses were run together in large groups, most animals showed minor skin trauma at some point during the trial due to kicking and biting.

Chi-square analyses were undertaken on the prevalence of dermatophilosis in different treatment groups as determined according to observation on each of clinical lesions, Giemsa smears and cultures. Results are presented in Table 8. In each case analysis was undertaken on pre-treatment (Week 0), mid-treatment (Week 10) and post-treatment (Week 16) points in time. No significant differences between groups were detected, with the exception of clinical lower hindlimb lesions post-treatment. Only two out of 10 horses in the control group had lesions, whereas six out of eight and six out of 10 in the placebo and EFA groups, respectively, had lesions.

Clinical Indices

For most animals in all the groups the indices for coat, tail and hooves increased over time, i.e. the condition improved. Although body condition scores fluctuated, no definite upward or downward trends were identified. Mean values, at weeks 0, 8, and 16, for the clinical indices of coat condition, mane condition, tail condition, hoof condition, and body condition are contained in Table 9.

From the results, condition indices for coat, mane, tail and hooves around the middle of the scale, 5 or 6, average condition, were found at the study onset, whilst by the end of the study values were moving toward the upper end of the scale, at 7 or 8. Values showed particularly marked improvement for all groups for coat and tail condition. Very small differences between group

Table 8. Bacteriological results, comparing results of clinical examination, Giemsa smears and cultures for detection of *Dermatophilus congolensis* organisms.

Clinical dorsum lesions

	Before treatment		During treatment		After treatment	
	present	absent	present	absent	present	absent
Group						
Controls	7	4	4	7	6	4
Placebo	7	4	6	6	4	4
EFAs	7	4	7	4	7	3
Total Chisquares:	0.00		1.64		0.75	
Results	NS	NS	NS	NS	NS	NS

Clinical lower hindlimb lesions

	Before treatment		During treatment		After treatment	
	present	absent	present	absent	present	absent
Group						
Controls	9	2	3	8	2	8
Placebo	9	2	5	7	6	2
EFAs	8	3	8	3	6	4
Total Chisquares:	0.36		4.78		6.00	
Results	NS	NS	NS	NS	NS	Sig

Giemsa Smear of Infective Material

Dorsum lesions

	Before		During		After	
	+	-	+	-	+	-
Group						
Controls	5	6	3	9	4	6
Placebo	6	5	6	6	4	4
EFAs	6	5	6	5	6	4
Total Chi-squares:	0.24		2.43		NTP	
Results	NS		NS			

Lower hindlimb lesions

	Before		During		After	
	+	-	+	-	+	-
Group						
Controls	5	6	1	10	2	8
Placebo	6	5	4	8	3	5
EFAs	7	4	5	6	3	7
	0.73		3.64		0.68	
	NS		NS		NS	

Table 8 (continued)

Culture of Infective Material

Dorsum lesions

	Before		During		After	
	+	-	+	-	+	-
Group						
Controls	0	11	2	9	4	6
Placebo	1	10	3	9	2	6
EFAs	2	9	3	8	3	7
Total Chi-squares:	2.20		0.27		0.49	
Results	NS		NS		NS	

Lower hindlimb lesions

	Before		During		After	
	+	-	+	-	+	-
Group						
Controls	1	10	0	11	1	9
Placebo	0	11	1	11	1	7
EFAs	0	11	2	9	1	9
	NTP		NTP		NTP	

Key:

Before, during and after indicate before, during and after supplementation with active treatments.

+ indicates the presence of *D. congolensis* organisms

- indicates the absence of *D. congolensis* organisms

NTP indicates no test possible, as numbers were too small

NS indicates result was not significant

Table 9. Mean values at weeks 0, 8 and 16 for clinical indices of condition of coat, mane, tail and hooves, and of general body condition of three groups of horses during a treatment study. Eleven horses received no treatment, 12 received 20g daily placebo of hydrogenated coconut oil and vitamin E, and 12 animals received 20g of n-3 and n-6 EFAs daily

Clinical index	coat condition			mane condition		
Week	0	8	16	0	8	16
Horse group						
Controls	5.3	6.9	7.3	5.6	6.9	7.7
Placebo	5.4	7.3	6.9	5.1	7.3	7.9
EFAs	5.5	7.0	7.2	5.5	6.8	7.7

Clinical index	tail condition			hoof condition		
Week	0	8	16	0	8	16
Horse group						
Controls	5.4	7.1	6.8	6.7	7.3	7.1
Placebo	5.3	7.3	7.9	6.4	7.3	7.1
EFAs	5.6	7.0	7.5	6.5	6.9	6.9

Clinical index	body condition		
Week	0	8	16
Horse group			
Controls	3.1	3.2	3.0
Placebo	3.0	3.3	3.1
EFAs	3.1	3.3	3.1

A full set of clinical results is found in Tables IV to VIII in Appendix II.

means were seen for coat, mane, tail and hoof condition.

The improvements recorded for coat, tail and hoof condition were all statistically significant. These improvements occurred, however, for all groups: improvement was not statistically greater for EFA treatment than for placebo or for untreated groups.

Haematology

The pattern for animal groups for haematological parameters in this treatment study resembled that of the groups in the pharmacokinetic study, in that values fluctuated over time. The values for all haematological parameters dropped for the three groups over time, and the reductions were statistically significant.

The administration of EFA and placebo treatments was not influential on the haematological parameters, and no adverse effect was found. There were no statistically significant differences for time patterns for the parameters between EFA, placebo and control groups.

The values for haematological parameters for each horse are given in Tables IX to XVI of Appendix II. Values were within normal ranges for haematological parameters with the exception of several low platelet counts, for three horses in the placebo group and for two in the EFA group. Normal ranges for hot and cold blooded animals, Thoroughbreds and ponies, and the units used in measurement of each parameter are indicated in Appendix IV.

Biochemistry

As for haematological parameters, differences in time patterns were recorded for biochemical parameters which were found to be statistically significant. Examination of these differences failed to reveal specific trends for parameters over time. Values were within normal ranges for biochemical parameters. Although a statistically significant difference between groups was recorded for the time pattern for magnesium values, no definite upward or downward trends were found for the values. For all other biochemical parameters, there were no significant differences for

time patterns between groups.

EFA and placebo treatments caused no harmful effect on biochemical parameters. Tables XVII to XXXII of Appendix II show values for biochemical parameters. Normal ranges and the units used in measurement of each parameter are indicated in Appendix IV.

EFA Estimations

No pattern of increase over time for any EFA measured in circulatory fractions was found for the group of horses which received oral n-3 and n-6 EFAs compared to placebo or control groups.

Group means at weeks 0, 8 and 16 for n-6 series EFA, LA, and its metabolites DGLA and AA; and n-3 series EPA in plasma phospholipids, plasma cholesteryl esters and red cell phospholipids are contained in Tables 10, 11 and 12, respectively.

Results of statistical analyses of EFAs indicated significant differences over time for LA, AA and EPA values in red cell phospholipids. For all groups LA values dropped markedly between weeks 0 and 8, and were at the lower value at week 16. AA and EPA values increased over time for all groups.

In plasma phospholipids and in plasma cholesteryl esters there were statistically significant differences over time for LA, DGLA and AA values. No definite trends could be detected, however, for changes in EFAs in plasma phospholipids over time. In plasma cholesteryl esters no trend was detected for LA values over time, but AA values increased over time for all groups. No significant difference was recorded for EPA values in plasma cholesteryl esters. Statistical analyses revealed that mean EPA values in plasma phospholipids for the EFA group were significantly higher, with group mean of 0.721, than for the control or placebo groups, of group means 0.610 and 0.573,

Table 10. Mean values at weeks 0, 8 and 16 for linoleic acid (LA), dihomogammalinolenic acid (DGLA), arachidonic acid (AA) and eicosapentanoic acid (EPA) in red cell phospholipids of three groups of horses during a treatment study. Twelve animals received no treatment, 12 received 20g coconut oil and vitamin E placebo daily, and 11 received 20g n-3 and n-6 EFAs daily.

	LA			DGLA		
Week	0	8	16	0	8	16
Horse group						
Control	14.98	4.85	5.02	0	0.01	0
	(7.03)	(1.09)	(1.34)	(0)	(0.02)	0
Placebo	12.83	5.12	5.26	0	0.03	0
	(4.84)	(2.07)	(1.35)	(0)	(0.05)	(0)
EFAs	12.65	5.11	5.13	0.43	0.05	0
	(8.40)	(1.14)	(0.68)	(0.96)	(0.09)	(0)
	AA			EPA		
Week	0	8	16	0	8	16
Horse group						
Control	0.40	1.09	1.33	0.16	0.68	0.73
	(0.31)	(0.45)	(0.27)	(0.33)	(0.51)	(0.32)
Placebo	0.52	1.11	1.41	0.25	0.54	1.20
	(0.34)	(0.53)	(0.18)	(0.29)	(0.31)	(0.94)
EFAs	0.65	0.97	1.27	0.26	0.65	0.86
	(0.49)	(0.49)	(0.55)	(0.24)	(0.26)	(0.55)

Values are given as % of total EFAs
Values in brackets are standard deviations

Table 11. Mean values at weeks 0, 8 and 16 for linoleic acid (LA), dihomogammalinolenic acid (DGLA), arachidonic acid (AA) and eicosapentanoic acid (EPA) in plasma phospholipids of three groups of horses during a treatment study. Twelve animals received no treatment, 12 received 20g coconut oil and vitamin E placebo daily, and 11 received 20g n-3 and n-6 EFAs daily.

	LA			DGLA		
Week	0	8	16	0	8	16
Horse group						
Control	48.74	46.79	48.08	0.55	0.54	0.39
	(13.90)	(3.29)	(5.43)	(0.35)	(0.13)	(0.14)
Placebo	51.74	48.46	51.15	0.38	0.60	0.44
	(4.67)	(3.03)	(2.43)	(0.25)	(0.17)	(0.09)
EFAs	54.05	48.45	50.94	0.31	0.70	0.59
	(3.98)	(2.40)	(3.37)	(0.30)	(0.15)	(0.12)

	AA			EPA		
Week	0	8	16	0	8	16
Horse group						
Control	2.25	2.08	1.94	0.83	0.54	0.57
	(0.38)	(0.35)	(0.36)	(0.32)	(0.14)	(0.21)
Placebo	2.24	2.02	1.80	0.86	0.53	0.54
	(0.37)	(0.40)	(0.32)	(0.33)	(0.13)	(0.12)
EFAs	1.94	2.08	1.97	0.78	0.71	0.69
	(0.66)	(0.24)	(0.29)	(0.40)	(0.14)	(0.18)

Values are given as % of total EFAs
Values in brackets are standard deviations

Table 12. Mean values at week 0, 8 and 16 for linoleic acid (LA), dihomogammalinolenic acid (DGLA), arachidonic acid (AA) and eicosapentanoic acid (EPA) in plasma cholesteryl esters of three groups of horses during a treatment study. Twelve animals received no treatment, 12 received 20g coconut oil and vitamin E placebo daily, and 11 received 20g n-3 and n-6 EFAs daily.

	LA			DGLA		
Week	0	8	16	0	8	16
Horse group						
Control	68.28	68.14	59.39	0	0.01	0
	(5.67)	(5.37)	(9.86)	(0)	(0.02)	(0)
Placebo	67.72	68.15	66.06	0	0.03	0
	(4.10)	(3.47)	(4.29)	(0)	(0.05)	(0)
EFAs	69.65	70.46	63.06	0.43	0.05	0
	(5.33)	(3.50)	(5.76)	(0.96)	(0.09)	(0)

	AA			EPA		
Week	0	8	16	0	8	16
Horse group						
Control	0.40	1.09	1.33	0.16	0.68	0.73
	(0.31)	(0.45)	(0.27)	(0.33)	(0.51)	(0.32)
Placebo	0.52	1.11	1.41	0.25	0.54	1.20
	(0.34)	(0.53)	(0.18)	(0.29)	(0.31)	(0.94)
EFAs	0.65	0.97	1.27	0.26	0.65	0.86
	(0.49)	(0.49)	(0.55)	(0.24)	(0.26)	(0.55)

Values are given as % of total EFAs
Values in brackets are standard deviations

respectively. No difference found between groups over time was statistically significant for values for any EFA in any blood fraction.

Statistical Analyses

The tables detailing statistical analyses by analysis of variance (ANOVA) for dermatophilosis indices, clinical condition indices, haematological and biochemical parameters, and individual EFAs in the different circulatory fractions are given in Appendix VI.

Exclusions

The horse named Sandy Lad, (EFA treatment group) was removed from the trial before the midway point, owing to generalised debility. Data were not included in statistical analysis. Natasha, (placebo group) left the farm at week 12. Horses named Temple, (control); Jack, (placebo); Big Ben, (placebo); Rainbeam, (placebo); Officer, (placebo) and Heidi, (EFA) were removed from the trial at week 14, for commercial reasons. Data from these animals were included in statistical analyses.

Discussion

In this controlled study the oral administration of 20g daily of n-3 and n-6 series EFAs had no clinical or bacteriological effect on the severity or on the distribution of lesions of dermatophilosis. The progression of infection was not influenced by 80% EPO and 20% fish oil and vitamin E (Efamol Marine) or by hydrogenated coconut oil and vitamin E placebo, when comparison was made to untreated controls. At the same time, EFAs did not cause significant improvement in the condition of the haircoat, mane, tail or hooves of the general body condition. Although values for haematological and biochemical parameters fluctuated, these changes were attributed to other factors, such as changing nutrition, and not to EFAs. No adverse effect was recorded during the study, and EFAs were not therefore found to be harmful. The fall over time for haematological parameters over this period, just before winter housing of the horses, may reflect the fall in the nutritional value of grazing at this time. The PCV values are

observed by the farm owner to drop every year at this time.

Oral supplementation of n-3 or n-6 EFAs did not lead to significant increases in levels of these EFAs in circulatory fractions when treated animals were compared to placebo and control groups.

The reason for the failure of EFAs to produce significant clinical effects, either on the severity and distribution of the lesions of dermatophilosis or on the condition of the haircoat, mane, tail or hooves was unclear. The EFA dose rate may have been incorrect, but the adopted 20g daily dose was selected on the results of the pharmacokinetic study to allow maximum opportunity for EFA uptake. Any higher a dose would not have been practical from the point of view of administration or of cost.

It was considered that the failure of EFAs in respect of treatment of dermatophilosis was possibly due in part to the fact that dermatophilosis lesions were already established prior to the initiation of EFA supplementation. The epidermis would already have been disrupted, facilitating invasion by *D. congolensis* and the subsequent development of lesions. The start of treatment may thus have been too late.

The Use of Essential Fatty Acids (EFAs) in the Prophylaxis of Dermatophilosis in Horses

Introduction

In the previous study, oral supplementation of EFAs at the 20g daily dose rate determined in the pharmacokinetic study was not effective in the treatment of dermatophilosis in horses. No reduction in the severity nor in the extent of distribution of lesions of dermatophilosis was afforded by the treatment regime used. The failure of significant effect may have been due to the fact that lesions were already established prior to initiation of EFA supplementation, and so the epidermis was already traumatised allowing invasion of *D. congolensis* organisms and development of lesions.

The aim of this study was to supplement EFAs in a prophylactic approach to management of dermatophilosis in the horse. EFAs were administered in the same form, at the same 20g daily dose rate, and supplementation was started before the period of highest infection risk. In this way, it was hoped to combat infection before any lesions were established, when the epidermis was still intact.

In reviewing accepted treatments and control measures in dermatophilosis in horses in the introductory section it was evident that no effective prophylactic exists at present, as vaccination is not commercially available. The use of EFAs was thought to provide a possible new prophylactic for the management of equine dermatophilosis.

Materials and Methods

Method and Administration of EFAs

The method of administration and the details of the EFA supplementation are provided in the Materials and Methods of the pharmacokinetic study in this section.

Experimental Design

Two groups of 12 animals were randomly selected. One group received 20 g of a combination of n-3 and n-6 EFAs (Efamol Marine) daily, and the control group received no treatment. Supplementation spanned 16 weeks when the horses were believed to be at highest risk of dermatophilosis, beginning in September when the horses were still at pasture, and continuing through to January, by which time they had been housed for several months. The animals were monitored clinically, haematologically and biochemically for 8 weeks following supplementation, and EFA estimations were made 8 weeks after the end of supplementation, in an attempt to determine the persistence, if any, of the effects of EFAs in the horse beyond the end of supplementation.

Parameters Monitored

The severity and distribution of dermatophilosis lesions, were recorded repeatedly, at weeks 0, 3, 6, 9, 12, 15, 20 and 24 of the study. The indices of the condition of the coat, the mane, the tail, the hooves, and general body condition were also monitored at these times. Haematological and biochemical parameters were recorded at weeks 0, 8 and 16 of the study. EFA estimations were made at weeks 0, 8, 16 and 24. Details of measurement of parameters are detailed in the general Materials and Methods section. Values for all clinical, haematological and biochemical parameters, and EFAs, were statistically analysed using a repeated measures analysis of variance design, described in the general Materials and Methods section.

Results

Clinical Indices

The mean values for dermatophilosis index, and the dorsal and hindlimb distribution indices for lesions are very low, indicating mild, localised infections. Very few horses in either treatment or control groups were infected. The mean values for indices denoting dermatophilosis lesion severity, and dorsal and hindlimb lesion distributions at weeks 0, 9, 15 and 24 are presented in Table 13. One horse in each group developed dorsal lesions during the study;

Table 13. Mean values at weeks 0, 9, 15 and 24 weeks for the severity and extent of distribution of dermatophilosis lesions on the dorsum and on the hindlimbs of two groups of horses in a prophylactic study; 12 animals received no treatment, and 11 animals received 20g of n-3 and n-6 EFAs

Parameter	dermatophilosis index			
Week	0	9	15	24
Horse group				
Controls	0.1	0.2	0.1	0.1
EFAs	0.2	0.5	0.0	0.0

Parameter	dorsum distribution				hindlimb distribution			
Week	0	9	15	24	0	9	15	24
Horse group								
Controls	0	0	1.3	1.3	0.2	0.3	0	0
EFAs	0	0.2	0	0	1.0	0.7	0	0

A full set of measurements of dermatophilosis indices is found in Tables I to III in Appendix III.

one horse in the control group had hindlimb lesions at week 0, and another two animals later developed lesions. In the EFA group, hindlimb lesions were noticed on two horses at the beginning of the study and on a further four animals later in the study. No significant differences were detected between groups for severity or distribution of dermatophilosis lesions. Examination of the data revealed no differences in the severity or the extent of distribution of dermatophilosis lesions between EFA and control groups.

The horses were in good body condition, and the coat, mane, tail and hoof conditions were above average. The indices were not significantly higher, however, for the EFA group than for the controls. The clinical indices of coat condition, mane condition, tail condition, hoof condition, and body condition score, are presented in Table 14. For coat, mane, tail and hoof condition, the majority of scores fell within the upper half of the 10 point index scale for both groups, they were above 5, or above average condition. Most animals were awarded condition scores of 3 or above, that is, most animals showed optimal condition score, or tended towards the fat end of the scale.

There were statistically significant time differences for mane, tail and body condition: fluctuations were small, and values for the mane and tail condition improved over the latter half of the study. No definite pattern was detected for body condition score values.

Although differences in time pattern for mane condition values between the two groups was significantly different, no definite pattern was established for either group. No statistically significant difference occurred between groups over time for any of the other parameters.

The tables of statistical analyses by analysis of variance are given in Appendix VII.

Haematology

With the exception of several low platelet counts, three control

Table 14. Mean values at weeks 0, 9, 15 and 24 weeks for clinical parameters of condition of the coat, mane, tail and hooves, general body condition, dermatophilosis index, dorsal and hindlimb lesion distribution for two groups of horses in a prophylactic study; 12 animals received no treatment, and 11 animals received 20g of n-3 and n-6 EFAs daily

Parameter	coat condition				mane condition			
Week	0	9	15	24	0	9	15	24
Horse group								
Controls	7.0	6.8	7.0	7.0	6.8	7.0	7.1	7.8
EFAs	6.8	6.4	7.0	6.9	7.1	6.6	7.2	6.9

Parameter	tail condition				hoof condition			
Week	0	9	15	24	0	9	15	24
Horse group								
Controls	7.3	7.2	7.1	7.5	6.8	6.9	7.0	7.1
EFAs	7.3	6.4	7.2	7.2	7.2	7.0	7.3	7.2

Parameter	body condition			
Week	0	9	15	24
Horse group				
Controls	3.4	3.5	3.3	3.4
EFAs	3.3	3.4	3.1	3.2

A full set of clinical results is found in Tables IV to VIII in Appendix III.

values, and seven values for EFA treated horses, values for haematological parameters were within normal ranges. Tables IX to XVI of Appendix III show the values for haematological parameters. Normal ranges for hot and cold blooded animals, Thoroughbreds and ponies, and the units used in measurement of each parameter are indicated in Appendix IV.

Although a statistically significant difference between groups for time pattern was recorded for mean cell haemoglobin values, trends were not marked. No statistically significant differences in time patterns were found between groups for the other haematological parameters. The tables of statistical analyses by analysis of variance are given in Appendix VII.

Biochemistry

Most values were within normal ranges for biochemical parameters, although a large proportion of animals in both groups showed AP values above the normal ranges at each time point. AST values for two animals in each group were elevated. At the week 3 time point four control animals and three treated animals demonstrated reduced triglyceride values. Elevated triglycerides values were for one control and three treated horses at week 0; for two controls and one treated horse at week 8; and for two controls and one treated horse at week 16.

Tables XVII to XXXII of Appendix III show values for biochemical parameters. Normal ranges and the units used in measurement of each parameter are indicated in Appendix IV.

No significant difference existed between groups over time for any biochemical parameter. Although statistically significant differences over time were recorded for several parameters, both treated and untreated animals behaved in the same way, so these patterns of change could not be attributed to EFAs. Calcium, magnesium and AST values, increased over time for both groups; and potassium and AP values fell over time for both groups. The tables of statistical analyses by analysis of variance are given in Appendix VII.

EFA Estimations

There were no significant increases in EFA values in circulatory fractions over time for horses which received n-3 and n-6 EFAs as compared to the control, untreated animals. Means values at weeks 0, 8, 16 and 24 for n-6 EFAs LA, and its metabolites DGLA and AA, and n-3 EPA in red cell phospholipids and plasma phospholipids are contained in Tables 15 and 16, respectively.

Although statistically significant differences were seen over time for LA, AA and EPA values in red cell phospholipids, definite trends for values for AA and EPA were not seen. LA values for both groups showed a pattern of increase between week 0 and week 8, then fell towards week 24. A statistically different pattern over time was recorded between groups for AA values, but trends were not marked for either group. Values for DGLA were too low for analyses to be performed.

No definite trends over time were found for LA, AA or EPA values in plasma phospholipids, although the difference was statistically significant. Examination of the time difference for DGLA revealed that values increased for both groups between weeks 0 and 16, then values dropped until week 24. There was a statistically significant difference between groups over time for AA values, but once again, no definite trends were seen for either group.

ANOVA tables used in statistical analyses are demonstrated in Appendix VII.

Discussion

EFAs were not found to be an effective prophylactic in equine dermatophilosis in this study. There was an unusually low incidence of dermatophilosis infection in the horse herd at the time of the study owing to a very dry autumn prior to the study. Nevetheless, the supplementation of horses with 20g daily of 80% EPO and 20% fish oil and vitamin E (Efamol Marine) did not prevent development of dermatophilosis lesions, nor did it reduce the severity or extent of distribution of lesions in this

Table 15. Mean values at weeks 0, 8, 16 and 24 for linoleic acid (LA), dihomogammalinolenic acid (DGLA), arachidonic acid (AA) and eicosapentanoic acid (EPA) in red cell phospholipids of two groups of horses during a study of the prophylaxis of EFAs in dermatophilosis. Twelve animals received no treatment and 11 received 20g n-3 and n-6 EFAs daily

	LA			
Week	0	8	16	24
Control	9.09	10.17	6.61	6.29
	(2.16)	(1.89)	(2.06)	(4.45)
EFAs	10.06	10.45	6.19	5.54
	(1.20)	(2.17)	(1.49)	(1.27)

	DGLA			
Week	0	8	16	24
Control	0.01	0	0	0.01
	(0)	(0)	(0)	(0.03)
EFAs	0	0	0	0
	(0)	(0)	(0)	(0)

	AA			
Week	0	8	16	24
Control	0.62	0.64	0.63	0.52
	(0.25)	(0.25)	(0.27)	(0.33)
EFAs	0.62	0.44	0.82	0.67
	(0.26)	(0.41)	(0.12)	(0.14)

	EPA			
Week	0	8	16	24
Control	0.09	0.02	0.02	0.03
	(0.11)	(0.06)	(0.06)	(0.06)
EFAs	0.14	0	0.02	0
	(0.01)	(0)	0.05	(0)

Values are given as % of total EFAs
Values in brackets are standard deviations

Table 16. Mean values at weeks 0, 8, 16 and 24 for linoleic acid (LA), dihomogammalinolenic acid (DGLA), arachidonic acid (AA) and eicosapentanoic acid (EPA) in plasma phospholipids of two groups of horses during a study of prophylaxis of EFAs in dermatophilosis. Twelve animals received no treatment and 11 received 20g n-3 and n-6 EFAs daily

	LA			
Week	0	8	16	24
Control	47.79	47.55	45.71	46.42
	(4.93)	(4.71)	(2.86)	(4.30)
EFAs	49.45	49.20	44.71	47.64
	(3.39)	(2.37)	(3.48)	(4.64)

	DGLA			
Week	0	8	16	24
Control	0.56	0.82	0.87	0.67
	(0.14)	(0.10)	(0.10)	(0.06)
EFAs	0.55	0.64	0.78	0.55
	(0.08)	(0.06)	(0.07)	(0.10)

	AA			
Week	0	8	16	24
Control	1.61	1.89	1.95	1.88
	(0.30)	(0.32)	(0.29)	(0.30)
EFAs	1.81	2.04	1.93	1.72
	(0.31)	(0.37)	(0.36)	(0.32)

	EPA			
Week	0	8	16	24
Control	0.49	0.60	0.60	0.52
	(0.19)	(0.13)	(0.31)	(0.16)
EFAs	0.56	0.60	0.55	0.56
	(0.15)	(0.17)	(0.21)	(0.15)

Values are given as % of total EFAs
Values in brackets are standard deviations

controlled study. EFAs were administered over a time period when the risk of dermatophilosis infection was highest, prior to the wet autumn months in the West of Scotland. As in the previous studies, no significant improvement in the condition of the haircoat, mane, tail or hooves was recorded for horses which received n-3 and n-6 EFAs as compared to untreated controls. Changes in haematological or biochemical parameters were attributed to factors such as nutrition rather than to EFA treatment. EFAs were not harmful to these parameters.

EFA values in circulatory fractions of animals which received EFA supplementation did not increase significantly more during the study than those values for animals which received no treatment. Statistically significant differences were recorded over time and between groups for EFAs in red cell phospholipids and in plasma phospholipids. Examination of these differences, however, revealed no specific trends for EFA values between treated and control groups.

SECTION 2. THE CHARACTERISTICS OF *DERMATOPHILUS CONGOLENSIS* IN RELATION TO SITE AND SEVERITY OF LESIONS, LEADING TO INVESTIGATION OF AN EXTRACELLULAR PROTEASE

Introduction

The lifecycle of *Dermatophilus congolensis* begins when a motile zoospore which germinates, settles, loses motility, then swells to form several germ tubes which in turn elongate to form hyphae (Roberts, 1961). As elongation progresses, transverse divisions are established in the oldest part of the hyphae, and there is branching growth. Consequently, areas are divided by new septae, then hyphae divide into cubic arrangements of cocci, often eight cocci wide, by development of longitudinal septae in vertical and horizontal directions. The cocci are thought to move off from the hyphae to form new hyphae or zoospores.

Scanning electron microscopy (SEM) studies of individual colonies of *D. congolensis* isolated from different animal species revealed that more than one form of the organism could be present in any one colony after 48h culture (Abu-Samra, 1977). Germinating zoospores were developed within certain hyphae and were subsequently released, whilst coccoid forms were produced by budding action from a different type of hyphae. Variation in temperature, nutrition or air supply did not influence germination of coccoid forms, contrary to earlier beliefs. No evidence was found that the coccoid form itself could germinate.

Little is currently known regarding the characteristics of *D. congolensis* which are relevant to the production and severity of skin lesion in the horse or in other species. A close relationship was found to exist for example amongst equine and bovine isolates with regard to culture characteristics, morphology and fermentation reactions; but experimental lesions induced in rabbits were found to differ (Stableforth, 1937). *Dermatophilus* lesions in the horse have been considered merely to be of acute or chronic type and of varying severity (Scarnell, 1961).

Serological classification, antibiotic profile and biochemical typing of bovine isolates has proven difficult or impractical (El-Nageh, 1971). Biochemical characters of bovine and donkey strains and their antibiotic sensitivities showed little or no variation (El-Nageh, 1971; Lloyd and Ojo, 1975); and between

fifty-three strains, isolated from lesions in cattle, sheep, goats and horses, only trivial variations were discovered, and these were confined to fermentation tests (Abu-Samra, 1977). In another study, variability existed in colonial morphology, and within a strain variations were evident which were independent of the original host (Gordon, 1964). Antigenic relationship has been proven in bovine strains by direct tube agglutination and by fluorescent antibody test (El-Nageh, 1971) and clear antigenic differences were recorded between strains infecting the domestic donkey: five different serological groups were identified (Lloyd and Ojo, 1975). The most obvious variations between *D. congolensis* strains have been claimed to occur in pigmentation of colonies and in proteolytic ability, but these differences could not be correlated with host species and were sometimes noted among variants of a given isolate (Gordon, 1964).

The subtyping of strains using molecular techniques such as investigation of protein profiles by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) has not, as far as can be determined, been attempted for *D. congolensis*. It was thought that such techniques might allow discrimination between strains isolated from separate lesions on the same or different animals. By application of these methods, correlation was thought possible between subgroups of *D. congolensis* with size, type, severity, distribution and persistence of lesions in the horse.

The bacterial characteristics of *D. congolensis* relating to virulence are not currently understood, and the factors underlying severity of infection in certain regions are particularly obscure (Davis, 1984).

Similarly, the properties of *D. congolensis* responsible for invasion of the epidermis are not known. Penetration of the stratum corneum and epidermal invasion by the organism induces an acute inflammatory response, considered by some to be nonspecific (Roberts, 1965), with neutrophil accumulation, accelerated keratinisation and epidermal proliferation immediately below the inflamed region. *Dermatophilus* is not considered likely to penetrate the barrier formed by neutrophils but, in the early

stages of infection the newly formed epidermis is colonised by extension from adjacent infected sites. Repetition of the process results in the formation of a laminated scab composed of alternate layers of infected epidermis and cellular exudate (Roberts, 1965; Oduye, 1976). Invasion stops and healing commences over a varying time span, which is dependent on previous exposure to *Dermatophilus* (Roberts, 1966). It has been hypothesised that *D. congolensis* can be eliminated from normal skin but if the elimination processes are inhibited, then the lesion becomes chronic (Davis and Philpott, 1980). Elimination of *D. congolensis* from skin may, for example, be inhibited at the site of a delayed type hypersensitivity reaction, induced by hapten either introduced in arthropod saliva at the site of an insect or tick bite or by experimental application of contact sensitizing agents. Moisture and skin injuries have been proposed to predispose to dermatophilosis (Roberts, 1967a), but the severe, generalised field condition has not been produced experimentally, even by introducing large inocula and causing simultaneous mechanical skin damage. This is thought by some authors to indicate the secondary importance of transmission and inoculation factors (Lloyd, 1984), and to highlight the importance of factors that reduce skin resistance or augment infective challenge in pathogenesis of the disease (Davis, 1984).

Few microorganisms are able to penetrate the intact epidermal barrier. The virulence of those that are capable of invading the skin, the dermatophyte fungi, is influenced by the production of proteolytic enzymes, keratinase, collagenase and elastase (Biberstein, 1990). Most *D. congolensis* isolates from cattle were found to be strongly proteolytic *in vitro*, with proteolytic properties varying between strains (Gordon, 1964). Intense necrosis of the skin, induced when rabbits were experimentally infected with *D. congolensis* (Makinde, 1979), suggested the presence of a responsible factor contained intracellularly and/or within crude whole cell fractions of the organism. The presence of such a factor, if it exists, would contest previous belief that *D. congolensis* produces no toxin (Roberts, 1967a), and that the inflammatory response was a consequence of products of cellular damage diffusing from the infected epidermis (Roberts, 1967a).

The production of such a factor, toxin or enzyme would be of great importance to the understanding of pathogenesis of the disease. Purification of the various antigenic components of *D. congolensis* was described as the method most likely to extend knowledge of immune responses to the crude *D. congolensis* antigens and would contribute to the understanding of the pathogenesis and control of the disease in horses and in other species. It might also highlight the most appropriate direction for diagnostic serology for herds and for vaccination (Makinde and Wilkie, 1979). Failure to demonstrate such a factor would be an equally significant finding, as it would suggest that the disease is seen as a result of host reaction, such as hypersensitivity or inflammation, to the presence of the bacterium itself.

In the present study, *D. congolensis* lesions in the horse were examined clinically and bacteriologically. The characteristics of the lesions were recorded, together with bacteriological characters of the strain isolated. Bacteria were examined using SDS-PAGE. The results of clinical examination were correlated with the analytical data in an effort to determine whether different strains were responsible for different types of lesion.

In addition to differentiation of *D. congolensis* isolates, the other aim of the study was to examine *D. congolensis* for production of keratinase and other protease activity which may be involved in the determination of virulence of the organism.

Materials and Methods

Isolation and Growth of Bacteria

Scab material from clinical lesions of *D. congolensis* was soaked in physiological saline overnight at 4°C and inoculated on to selective medium, composed of blood agar base No.2 (Oxoid) with 7% horse blood and 1,000 IU/ml polymixin B sulphate added, according to the method of Abu-Samra (1977), and incubated aerobically for 48h at 37°C. Individual colonies of isolates were subcultured from selective agar onto 5% sheep blood agar and incubated aerobically

for 48h at 37°C. Isolates were preserved by freeze-drying, by inoculation onto Dorset Egg slopes, and by weekly subculture on sheep blood agar. Confirmation of the organism's presence was by examination of smears of soaked scab or paintbrush lesions stained by Giemsa. Subcultured colonies were suspended in saline and stained by the method of Gram for examination.

Preparation of Culture Supernatant

A single colony of *D. congolensis* was inoculated from sheep blood agar into 5 ml Brain Heart Infusion (BHI) broth (Oxoid) in a culture tube. Broths were slope incubated aerobically at 37°C in a Gallenkamp Orbital Incubator at 150 r.p.m. for 48h, centrifuged in a microfuge (3 x 10 sec spins) at high speed, rotating between spins to ensure complete pelletisation, and supernatant drawn off.

Determination and correlation of clinical and bacteriological characteristics of *D. congolensis* isolates obtained from field material

Scab and paintbrush lesion material was collected from field cases of *D. congolensis* infection. Where lesions were swabbed, material was collected in Ames Transport medium. *D. congolensis* organisms were isolated as described above. A clinical index was assigned to each infected animal on a 0 to 10 scale, detailed in the general Materials and Methods section. The index described the severity of the dermatophilosis lesions. A score of 0 indicated the absence of infection and a score of 10 indicated the most severe infection. Bacteriological characteristics of growth of isolates on polymixin B sulphate selective medium, sheep blood (5%) agar, horse blood (5%) agar, chocolate agar and in BHI broth, including presence of haemolysis and pitting of agar by colonies, were recorded after standard incubation for 48h at 37°C.

Enzyme Assays Using Protein Substrates

Keratinase

Keratin degradation was assayed initially by the method of Das and Banerjee (1982). The reaction mixture contained 0.056 M phosphate buffer, pH 7.2, 2.6 ml; hair fragments, 10 mg; and culture filtrate, 0.4 ml. It was incubated at 37°C for 1h and the reaction terminated by the addition of 3 ml of 0.612 M trichloroacetic acid

(TCA) and immediate chilling to 0°C for 30 mins. The solution was then filtered using Whatman filter paper (size 3, 9 cm). The optical density of the clear filtrate was measured at 280 nm, against water as the colorimetric blank, on a Beckman DU-64 spectrophotometer. For the reaction blank, TCA was added before the enzyme source. The assay was run in duplicate, and each absorbance measurement was the average of three readings.

Keratin degradation was then assayed by an alternative method (Apodaca and McKerrow, 1990) using keratin azure (Sigma), a dyed wool product, as substrate. 900 μl purified culture supernatant [prepared by polyethylene glycol (PEG) (20,000 Carbowax) concentration followed by ion exchange chromatography as described below] were incubated with 5 mg of keratin azure in 100 μl of 1 M glycine-NaOH, pH 7.0, 10 mM $CaCl_2$ buffer for 48 h at 37°C. Final reaction volume was 1 ml. A duplicate set of samples were prepared, with 5 mM mercaptoethanol added as a reducing agent. Following incubation the reaction was spun in a microfuge for 5 minutes. The degradation of keratin azure by enzyme present in culture supernatant was measured by determining, by spectrophotometer, the change in A_{595} of the supernatant pre- and post-incubation. Results were compared to controls, containing uninoculated broth.

Collagenase, Elastase, Gelatinase and Caseinase

Assays were performed by the method of Conlan, Baskerville and Ashworth (1986). Collagenase and elastase activities were assayed by incubation at 37°C of 100 μl test solution with 20 mg of the appropriate dye-impregnated substrate in 5 ml 0.05 M-phosphate buffer pH 7.0. Azocoll (Sigma) was used as substrate for collagenase and Elastin-Congo Red (Sigma) was used for elastase activity. Gelatinase activity was detected using gelatin incorporated into agar [0.25% (w/v) gelatin (Difco), 1.0% (w/v) agar, 0.001% NaN_3 in 0.2 M-Tris/HCl pH 7.2]. Molten gelatin-agar was poured into 12 cm Petri dishes, test materials (50 μl volumes) were placed in wells (7 mm diameter) cut in the solidified medium and the dishes were incubated for 16 h at 37°C. The gels were then treated with a solution of 15% (w/v) $HgCl_2$ in 2 M-HCl, causing the gelatin-agar to become milky white except for clear zones where

proteolysis had occurred. Caseinate-precipitating activity (caseinase) was measured by a similar method using a medium containing 1.0% sodium caseinate (Difco), 1.0% agar and 0.001% NaN_3 in 50 mM-phosphate buffer pH 6.2. 40 μl of culture supernatant was inoculated into each test well. After incubation caseinase activity, evident as a zone of precipitate around positive wells without any treatment of the medium, was quantified by measuring the precipitation zone area in mm^2.

Stability of Protease to Heat and Cold

Aliquots of *D. congolensis* broth culture supernatants, 0.5ml volume, were held at 56°C in a waterbath, at 100°C in a steamer, and at 0°C on ice for 15 mins. 0.5ml aliquots of broth culture supernatants were stored at 4°C for one month and at -20°C for 3 months.

Neutralisation of Protease Activity

Broth culture supernatants from *D. congolensis* isolates were incubated with one tenth volume of test serum (180 μl supernatant and 20 μl serum) and held on ice at 0°C for 1h. Test sera were obtained from a calf showing very severe, generalised dermatophilosis of dermatophilosis index (DI) of 10; and from two *Dermatophilus* infected horses, DI of 4. Sera were diluted by two-fold serial dilution to 1:256. Caseinase activity was examined immediately after incubation and compared to control neonatal calf serum.

The IgG fraction of serum from *Dermatophilus* infected animals was separated by the method of Harlow and Lane (1988). One volume of serum was diluted in 2 volumes 0.06M sodium acetate buffer, pH 4.0, and mixed vigorously. 7.5ml N-octanoic acid (BDH 41021) were added per 100ml serum volume slowly and left to mix for 30 mins at room temperature, then filtered through grade 4 filter paper and dialysed overnight against 0.154M NaCl.

Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE)

Preparation of Samples

One ml aliquots of broth culture were centrifuged to provide

pellets. Ten mg lysozyme were mixed with 1 ml 100 mM Tris-HCl buffer, pH 8.0, and a 1:10 dilution made. 2.5 µl of this solution was mixed with pellet resuspended in 100 µl 100 mM Tris-HCl buffer, pH 8.0, and incubated at room temperature for 15 mins. One half volume sample buffer was added and the mixture was heated to 100°C for 15 mins prior to examination by SDS-PAGE.

SDS-PAGE

SDS-Page was performed using the BRL V16 Vertical Gel Electrophoresis Apparatus for discontinuous buffer system, by the method of Rycroft and Taylor (1987). The separating gel consisted of 12.5% (w/v) acrylamide (acrylamide/bisacrylamide ratio, 75:1) in 0.37 M Tris hydrochloride (pH 8.7), 0.1% w/v SDS. The stacking gel consisted of 5% w/v acrylamide (acrylamide/bisacrylamide ratio, 36:1) in 125 mM Tris hydrochloride (pH 6.9), 0.1% SDS. Supernatant was added to one half volume sample buffer, which was 60 mM Tris hydrochloride, 10% v/v glycerol, 2% w/v SDS, 5% v/v mercaptoethanol and electrophoresed at 25 mA for approx. 200 mins in 25 mM Tris-192 mM glycine (pH 8.3). 35 µl samples of culture supernatant were loaded per well.

Proteins were visualised by fixing and staining with 0.25% w/v Coomassie Brilliant Blue stain (Sigma) in 50% water-40% methanol-10% acetic acid overnight at 37°C followed by destaining in the same solvent for 24h at 37°C, or overnight, agitated, at 4°C.

Molecular Weight Markers

High molecular weight markers (Sigma) were dissolved in 192 mM Tris Glycine buffer, pH 8.3, according to the manufacturer's instructions. One ul lysozyme (5 mg/ml) and 4 µl trypsin inhibitor (5 mg/ml) were added to 5 µl markers to increase the range of the markers. The marker mixture was then dissolved in sample buffer to 30 µl volume.

SDS-Gelatin PAGE

Preparation of Samples

One ml aliquots of *D.congolensis* broth culture fluid were centrifuged to provide pellets, which were subjected to 3

freeze-thaw cycles at -70°C, and mixed with 100 µl sample buffer [1.8 ml 1 M Tris-HCl buffer pH 6.8; 3 ml 20% SDS; 3 ml glycerol; 1.5 ml mercaptoethanol; 0.001% BP; 0.7 ml water]. Culture supernatants were mixed with half volume (200 µl supernatant: 100 µl sample buffer) sample buffer.

SDS-PAGE

For separation and analysis of gelatinase activity two systems were used, the BioRad Minigel Electrophoresis Apparatus and BRL V16 Vertical Gel Electrophoresis Apparatus for discontinuous buffer system.

Minigel Apparatus

Electrophoresis when using the Minigel apparatus was performed by the method of Hames (1981). Stacking gel consisted of 2.5% (w/v) acrylamide, (acrylamide-bisacrylamide ratio, 30:0.8) in 0.5 M Tris-HCl buffer, pH 6.8, 5 ml stacking gel buffer stock; 0.2 ml 10% SDS; 1 ml 1.5% ammonium persulphate; 11.3 ml distilled water; 0.015 ml TEMED. Separating gel consisted of 7.5% (w/v) acrylamide, (acrylamide-bisacrylamide ratio, 30:0.8) in 3 M Tris-HCl buffer, pH 8.8, 3.75 ml resolving gel buffer stock; 0.3 ml 10% SDS; 1.5 ml 1.5% ammonium persulphate; 13.95 ml distilled water; 3 ml 2% gelatin solution and 0.015 ml TEMED. Culture supernatant was added to one half volume sample buffer and electrophoresis was performed at 150 V for 45 mins in 0.25 M Tris, 1.92 M glycine buffer, pH 8.3, with 1% SDS added.

V16 Vertical Gel Electrophoresis Apparatus

When using BRL V16 apparatus SDS-PAGE was performed as described above with modification in the form of 10% separating gel with incorporation of 2% gelatin.

After SDS-gelatin PAGE, gels were washed in 2.5% Triton X-100 solution in 0.1M Tris-HCl buffer, pH 7.0, for 1h at 37°C in a waterbath, then incubated for 1h at 37°C in 0.1M sodium acetate buffer, pH 6.0. Proteins were visualised by staining as previously described.

SDS-PAGE Analysis of Caseinase Activity

Culture supernatant of *D. congolensis* isolate, Dc1, was concentrated, by dialysis against polyethylene glycol (PEG) (20,000 Carbowax) for 6h at 4°C, and purified by DEAE-Sepharose ion exchange chromatography (detailed below). Eluted caseinase-active fractions were separated by SDS-gelatin PAGE and compared to unconcentrated samples of culture supernatant of the same isolate. Samples were not heated prior to SDS-PAGE. The resultant gel was washed in 0.1M phosphate buffer, pH 6.0, for 30 mins, agitated, and overlaid with 100ml of test caseinate agar and incubated overnight at 37°C.

Western or Immunoblotting

Immunoblotting was carried out by the method of Rycroft and Taylor (1987). Gels were washed in 25 mM Tris, 192 mM glycine, pH 8.3, for 20 mins at room temperature. Proteins were transferred to nitrocellulose membrane in a Trans Blot apparatus (Bio-Rad) in 25 mM Tris, 192 mM glycine overnight at 0.15A, 22V at 4°C. Membrane was then agitated gently in 10% skimmed milk powder and 0.02% sodium azide in TNT buffer: 10mM Tris-HCl; 0.15M NaCl; 0.05% Tween 20, pH 8.0, for 1h at 4°C. Washing (3 x 5 mins) in TNT buffer was carried out before detection of antigen by incubation with 1:200 dilution of serum from a *Dermatophilus* infected calf in TNT buffer with 5% skimmed milk powder overnight at 4°C. The membrane was then washed (3 x 5 mins in TNT buffer) before antigen was detected by enzyme linked immunosorbent assay (ELISA) involving incubation for 1h at 4°C with 1:2000 solution of rabbit anti-bovine IgG horseradish peroxidase conjugate (HRP), (Sigma), in TNT buffer with 5% skimmed milk powder added. Washing (5 mins in TNT buffer, then 2 x 5 mins in TNT buffer without Tween 20) preceded localization and visualization of bound conjugate using 0.05% w/v 4-chloro-1-naphthol, 4.4 mM H_2O_2 in 16% methanol, 10 mM Tris hydrochloride (pH 8.0), 150 mM NaCl.

The procedure was repeated using a 1:100 dilution of serum from a *Dermatophilus* infected horse and detection of antigen was by a 1:100 solution of rabbit anti-horse IgG horseradish peroxidase (Sigma). The gel was blotted at 100V for 90 mins at 4°C.

Protease Inhibition Experiments

The following inhibitors were used at concentrations given below.

Antipain (Sigma); stock solution 10 mM in water; effective concentration 100 μM.

Bestatin (Sigma); stock solution 1 mM in methanol; effective concentration 10 μM.

Chymostatin (Sigma); stock solution 10 mM in dimethyl sulphoxide (DMSO); effective concentration 100 μM.

3,4-dichloroisocoumarin (3,4-DCI), (Sigma); stock solution 10 mM in DMSO; effective concentration 100 μM.

Ethylenediaminetetraacetic acid (EDTA); stock solution 0.5 M in water, effective concentration 10 mM solution made up in 0.1 M phosphate buffer, pH 7.0.

Iodoacetic Acid (IAA) (Sigma); Stock solution 10 mM in water; effective concentration 100 uM in 0.1 M phosphate buffer, pH 7.0.

Leupeptin (Sigma); Stock solution 10 mM in water; effective concentration 100 μM in 0.1 M phosphate buffer, pH 7.0.

Pepstatin A (Sigma); stock solution 1 mM in methanol; effective concentration 1 μM solution made up in 0.1 M phosphate buffer, pH 7.0.

1,10-Phenanthroline (Sigma); stock solution 200 mM in methanol; effective concentration 10 mM.

Phenylmethanesulphonyl fluoride (PMSF), (Sigma); stock solution 200 mM in methanol; effective concentration 1 mM.

Tosyl lysyl chloromethyl ketone (TLCK), (Sigma); stock solution 10 mM in 1 mM HCl, pH 3.0. Effective concentration 100 μM.

Tosyl phenylalanyl chloromethyl ketone (TPCK), (Sigma); stock

solution 10 mM in methanol, effective concentration 100 μM.

Control was equivalent volume of the respective solvent, e.g. water, methanol or DMSO, without inhibitor added. Stock solutions were made up according to the method of Beynon and Salvesen (1989).

SDS-gelatin PAGE was routinely performed using the Minigel apparatus. A pooled sample of *D. congolensis* isolate culture pellets was examined. The gel was washed in 2.5% Triton X-100 solution in 0.1 M Tris-glycine buffer, pH 7.0, for 1h, then cut into five identical sections, each incubated with a specific protease inhibitor for 4h in a waterbath at 37°C alongside a control, containing no inhibitor. Gel sections were stained and destained as previously described. Gel sections treated with inhibitors were compared to the untreated control. Positive inhibition was indicated by loss of the negatively-staining band of gelatinase activity as compared to the untreated control. Dithiothreitol (DTT), a reducing agent which activates cysteine proteases, was added to inhibitors at 1mM concentration in one experiment.

Inhibitors above were added to culture supernatants of *D. congolensis* at working concentrations according to Beynon and Salvesen (1989). Duplicate experiments were performed, one where the supernatant-inhibitor solution was loaded into wells in caseinase test agar incubated to 37°C, and a second where the agar was at room temperature. 40 μl of sample were loaded into each well. Controls contained equivalent volume of appropriate inhibitor solvent, with no inhibitor present.

Molecular Exclusion Chromatography

D. congolensis culture fluid was centrifuged at 12,000 rpm for 20 mins using a JA 20 rotor, and supernatant filtered (0.45 μm filter, Sartorius). Approx. 75 ml culture filtrate was transferred into visking tubing and dialysed against PEG. A Sephacryl S300 column (16mm internal diameter, 40 cm long) was poured and equilibrated with Tris:NaCl:EDTA buffer, (10:150:0.1), pH 7.2. One ml concentrated supernatant was loaded onto the column and eluted

with the same buffer, and one ml fractions were collected.

Ion Exchange Chromatography

A DEAE-Sepharose CL6B (Pharmacia) column (16mm internal diameter, 20cm long) was poured and equilibrated with Tris-EDTA buffer (10mM:1mM), pH 7.2. The sample was loaded on to the column in Tris-EDTA buffer and was eluted with 40 ml of a 0 to 500 mM NaCl linear gradient using a BRL Gradient Former GA 1080.

Results and Discussion

Isolation of Bacteria

D. congolensis isolates were identified on selective polymixin B sulphate medium after 48h aerobic incubation at 37°C as beta-haemolytic, approximately 2 mm diameter, grey-white to yellowish colonies which often embedded deeply into the agar. Colonial morphology varied between isolates, as described by other authors (Gordon, 1964; El-Nageh, 1971; Lloyd and Ojo, 1975; Abu-Samra, 1977). The appearance of successive colonies altered. After repeated subculture on solid medium the colonies became smoother and smoother, confirming previous work (Gordon, 1964). Diverse forms of the organism had also been reported on different media, under varying conditions, and from one time to another, and even on the same agar plate (Gordon, 1964). Growth was evident after inoculation from selective medium on to sheep blood agar as beta-haemolytic grey-fawn coloured colonies approx. 2mm diameter, after 48h aerobic incubation at 37°C. In contrast to results of Abu-Samra (1977), greater expression of haemolysis was seen on sheep blood agar than on horse blood agar, so this medium was adopted for subcultures.

Optimisation of Growth Conditions

Optimal liquid medium for growth; optimal incubation type, static or orbiting, which provided aeration; and optimal length of incubation were sought for *D. congolensis* isolates. The isolates examined in this area part of the study and their sources are

described in Table 15. Isolates examined were isolate 2, which originated from a swab of a dorsal lesion; isolate 15, which originated from a dorsal scab lesion; and isolate Dur, recovered from a paintbrush lesion of the lower hindlimbs. Three months after isolate 15 was found, isolate Dur was identified from a lesion at a different site on the same horse.

Growth characters of isolates were recorded in broths of varying nutrition, which in general order of increasing nutritional value were L-broth (SOB); Nutrient Broth (MIB); Tryptone Soya Broth (TSB); Blood Agar Broth (BAB) and Brain Heart Infusion (BHI) Broth. Characters were recorded at different stages in the growth cycle, at 3.5h, 20h and 44h.

No visible growth, turbidity of the broth or enlargement of inoculated colonies, were found for any isolate after 3.5 h static or orbiting incubation in any medium. Growth was evident after 20 h for isolate 15 but was sparse for isolate Dur and isolate 2. Most profuse growth was seen for each isolate after 44h, and growth was not affected by aeration.

The type of medium affected growth of isolates. Poor growth for all isolates on nutrient broth, was discovered in contrast to findings of Abu-Samra (1977), but confirmed those of Roberts (1961). The latter author believed that in nutritionally poor medium zoospores did not germinate or that mycelia failed to complete the lengthy, complex growth cycle, and died before new zoospores were produced. No growth of any isolate was seen on TSB at 20 h, and colonies which grew by 44 h were hard and leathery, as found by Abu-Samra (1977). Growth was more prolific in SOB and BAB, and was optimal in BHI broth. Isolate 15 grew more profusely than other isolates, while growth of isolate 2 was sparse, and adherent to the inoculated colony.

Table 15. Clinical details of dermatophilosis lesions and differential bacteriological growth characteristics of D. congolensis isolates

Isolate	Animal	Species	Derm Index	Lesion site	Clinical description	Growth in BHI broth	Haemolysis PM	Haemolysis SB	Haemolysis HB	Split CA	Embedding PM	Embedding SB	Embedding HB
Dc1	Pollux	E	1	HL	Paintbrush	T,F	+	+	-	-	+	+	+
Dc2	Elspeth	E	1	HL	Scab	T,F	+	+	-	-	+	+	+
1	Jill	E	2	D	Scab, pus	T	-	++	+++	-	-	-	-
2	Jill	E	2	D (sw)	Scab, pus	T,F	+	++	-	-	+	+	+
7	Lily	E	2	D	Scab, pus	F	+	++	-	+	+	+	+
8	Lily	E	2	D (sw)	Scab, pus	T,F	+	++	-	-	+	+	+
15	Durando	E	4	D	Scab, pus, pain	T,F	+	+	-	-	+	+	+
16	Durando	E	4	HL	Scab	F	-	+	-	-	-	-	+
24	Choppy	E	1	D	Paintbrush	T++	-	-	-	-	+	+	+
29	Jeeves	E	1	D	Paintbrush	T++	+	-	-	-	+	+P	+
30	Fella	E	1	D	Paintbrush	T++	-	-	-	+++	+P	+P	+
34	Rose	E	1	HL	Scab	T	+	++	+++	+++	+	+	+
110578	Equine	E	1	HL	Paintbrush	T,F	-	-	-	-	-	-	+
Dur	Durando	E	1	HL	Paintbrush	T,F	++	-	-	+	+	+	+
110058	Calf	B	10	G	Scab, pain, cellulitis	T	+	++	-	-	+P	+P	+

Key:
E = Equine HL = Lower Hindlimb (sw) = swab Derm Index = dermatophilosis index
B = Bovine D = Dorsum G = generalised PM = Polymixin B sulphate selective medium
HB = Horse blood agar SB = Sheep blood agar CA = Chocolate agar
T = Turbid growth F = Flocular growth T,F = Turbid growth with flocules
+ = Mild ++ = Moderate +++ = Strong P = Piling of colonies on agar.

Differential Characteristics of *D. congolensis* Isolates Obtained From Field Material

Infective material was collected from field cases of *D. congolensis* infection. The severity of infection varied from animal to animal, and several cases were sampled from different sites on the body. Correlation of bacteriological and clinical characteristics of isolates with site and pathogenicity of lesions was attempted; a bovine isolate was compared to 14 equine isolates. Relationships between protein profiles of isolates, obtained by SDS-PAGE, were sought.

The presence of *D. congolensis* isolates in infective material was confirmed by fixing smears of saline-soaked scab or paintbrush material and staining by Giemsa. Characteristic "railroad tracks" were seen in positive smears, budding hyphal forms and packets of Gram-positive cocci, often 4 to 6 wide, dividing transversely and longitudinally. Subcultured colonies were confirmed positive by the presence of budding hyphae and transversely- and longitudinally-dividing groups of cocci, visualised by Gram stain. Correlation between clinical dermatophilosis indices and differential bacteriological growth characters of isolates was attempted. It was seen from the results presented in Table 15 that no two field isolates showed identical bacteriological characteristics, although clinical lesions were similar. Differential bacterial growth characters of isolates were not species-specific. Clinical severity of infection could not be correlated with bacteriological growth characteristics.

Protein profiles of *D. congolensis* whole solubilised bacteria by SDS-PAGE were found to be very diverse, Figure 15. Although relationships could be detected between isolates, definite correlations could not be made.

Search for Protease Activity in *D. congolensis* Isolates

D. congolensis strains examined by Gordon (1964) were found to be strongly proteolytic *in vitro*, and proteolytic properties varied among strains and could not be correlated with host species. In this study protease activity, in the forms of keratinase, collagenase, elastase, caseinase and gelatinase, was sought.

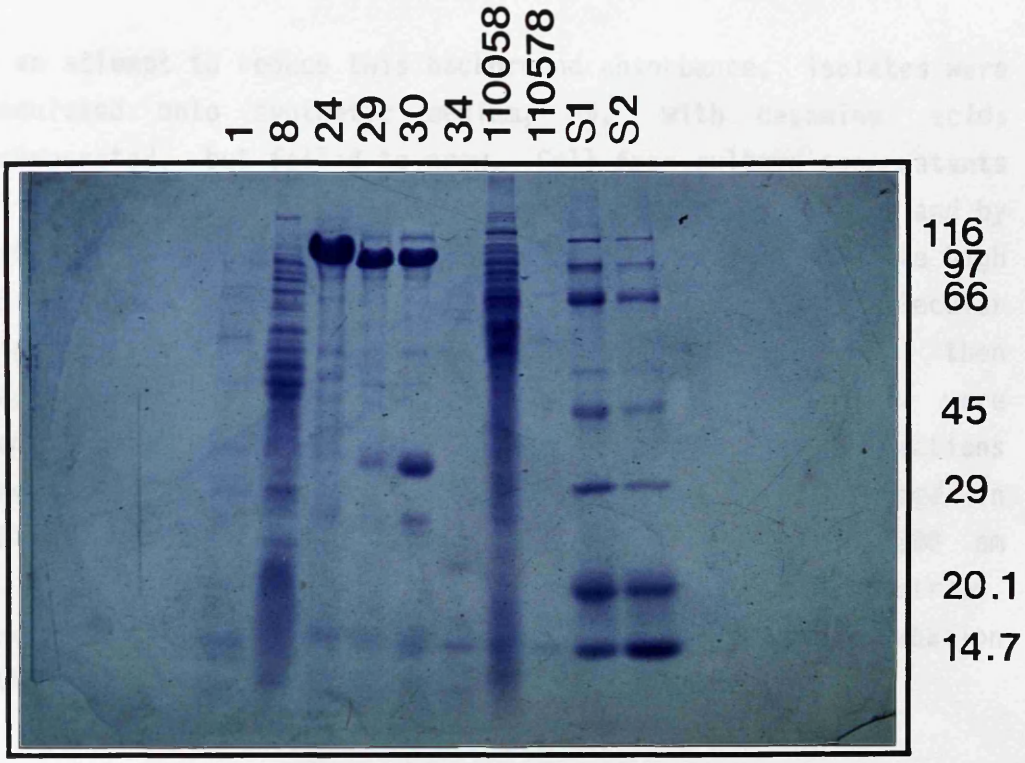

Figure 15. Whole cell protein profiles of *D. congolensis* by SDS-PAGE. From left to right, isolates 1, 8, 24, 29, 30, 34, 110058 (bovine isolate), 110578, S1 and S2 (molecular weight standards)

Culture supernatants of isolates Dur and Dc2 were examined for ability to degrade keratin, using hair fragments as substrate. Keratinase activity was measured by recording absorbance at 280 nm before and after incubation of culture supernatant with hair. Background absorbance at 280 nm, which was initially too high to allow interpretation of results, originated from 280 nm absorbing components in the BHI broth.

In an attempt to reduce this background absorbance, isolates were inoculated onto synthetic medium, M9, with casamino acids incorporated, but failed to grow. Cell free culture supernatants of isolates were then concentrated by dialysis against PEG, and by Lyphogel (Gelman), (polyacrylamide beads used to concentrate high molecular weight components of solutions) to remove low molecular weight 280 nm absorbing material. Supernatants were then fractionated by Sephadex chromatography. Eluted fractions were examined for caseinase activity and caseinase-positive fractions were assayed for keratinase activity; results are presented in Table 16. Although reduction in background absorbance at 280 nm was achieved, allowing distinction of samples from controls, absorbance readings did not increase measureably after incubation with substrate.

Hair substrate was washed six times in acetone prior to the assay in order to remove lipids. Washing in detergent, and a combination of washing in detergent followed by washing in acetone was also tried, but the method of treatment of hair did not influence results.

Different buffers were tried for the assay, 75 mM sodium acetate buffer, pH 4.0; and 50 mM Tris buffer, pH 7.2, were substituted for phosphate buffer, but buffer type did not influence results.

When this initial assay and attempted modifications failed to detect keratinase activity, isolates Dur and Dc1 were examined by an alternative method, using keratin azure as substrate. The effect of the presence of the reducing agent mercaptoethanol, included to facilitate hydrolysis of disulphide bonds, was also

Table 16. Change in absorbance values at 280 nm of culture supernatants of *D. congolensis* isolates after incubation with hair substrate in a search for keratinase activity.

		Absorbance at 280 nm			
Stage of experiment		t_0	t_{60}	t_{TCA}	Change
Sample					
Isolate Dur	PEG	0.129	0.132	0.130	0.001
	Lyphogel	0.099	0.112	0.147	0.048
Isolate Dc2	PEG	0.171	0.179	0.171	0
	Lyphogel	0.110	0.108	0.139	0.029
Control		0.068	0.069	0.079	0.011

Key:

t_0 = preincubation
t_{60} = after 1h incubation with substrate
t_{TCA} = termination of experiment by trichloroacetic acid
PEG = Culture supernatant concentrated by polyethylene glycol
Lyphogel = Culture supernatant concentrated by Lyphogel
Control contained no enzyme source

investigated. Results of absorbance readings, at 595nm, of reaction mixtures, pre- and post-incubation, are presented in Table 17.

Post incubation absorbance values were not significantly higher than pre-incubation values for either isolate when compared with control which contained no enzyme source. The presence of mercaptoethanol did not influence results.

When keratinase activity was not detected, alternative protease assays were attempted. No collagenase or elastase activity was detected for the isolates examined.

The rate of gelatin liquefaction varied among isolates, as found in previous work by Gordon (1964). Liquefaction was seen after 2 days for isolate F2, after 5 days for isolates 15, Dc1, Dc2, F1 and F2, after 7 days for isolates Dur and 7915; and gelatin was not liquefied by isolate 5175.

Expression of caseinase and gelatinase activity was investigated after growth of isolates in different media. The caseinate-precipitating activity detected by the test caseinate agar was equated with, and is therefore described as, caseinase in this work. Caseinase activity was greater when isolates were grown in BHI broth, than when grown in BAB or SOB; and no caseinase activity was detected after growth in TSB. Activity was greater after 44 h than after 20 h. Static and orbiting (aerated) incubation of isolates led to production of similar amounts of caseinase activity. No caseinase activity was detected for isolate 2, and no gelatinase activity was detected for any isolate. The caseinase assay is shown in Figure 16.

D. congolensis isolate cultures were monitored over a period of 8 days as they were incubating. Culture fluid was repeatedly examined microscopically, and at each time point was tested for caseinase production. Similar patterns of growth were found for the isolates examined. Growth of isolates was first detected in culture fluid after 4 h incubation, as ensheathed cocci, and caseinase activity was first detected from isolates after hyphal

Table 17. Change in absorbance values at 595nm of culture supernatants of *D. congolensis* isolates after incubation with keratin azure substrate in a search for keratinase activity.

	Absorbance at 595 nm		
Stage of experiment	t_0	t_{24h}	change
Sample			
Dur	0.176	0.887	0.711
Dcl	0.266	0.545	0.284
Control	0.110	0.574	0.464
Dur + M	0.188	0.548	0.360
Dcl + M	0.194	0.408	0.214
Control + M	0.080	0.749	0.669

Key:
t_0 = preincubation
t_{24h} = post 24-hour incubation with substrate
Control = uninoculated BHI broth, buffer and substrate
M = 5 mM mercaptoethanol present in sample

Samples contained 900 µl culture supernatant, 100 µl 1 M glycine-NaOH buffer, pH 9.0, and 10 mM $CaCl_2$, and 5 mg keratin azure substrate

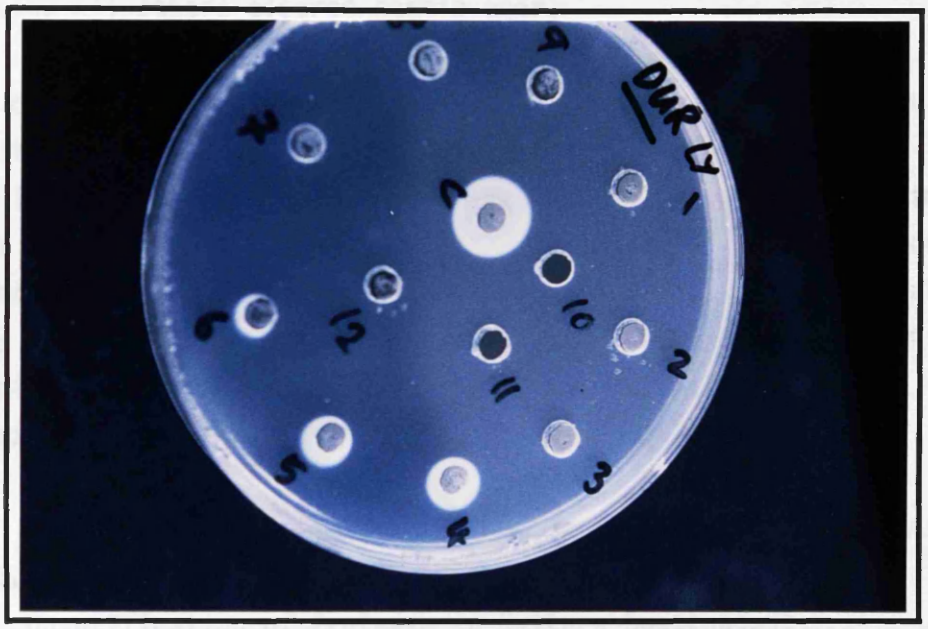

Figure 16. The caseinase assay, with demonstration of positive activity, a milky zone of caseinate precipitation around the test well, e.g., well C, upper right of centre.

elements were seen in the culture fluid, at 22 h. Caseinase production did not intensify beyond 46h for isolate Dur or beyond day 4 for isolate 15.

In a previous study (Lloyd and Ojo, 1975), an equine *D. congolensis* isolate, five bovine isolates, an ovine isolate, and seven donkey isolates, all from Nigeria, decomposed casein, when tested on casein agar. Gordon (1964) examined 17 strains of *D. congolensis* originating in skin lesions of cattle, sheep, horses, deer and man, and found that only two strains failed to hydrolyze casein, all others cleared casein agar.

No correlation existed between lesion severity and the amount of caseinase activity produced by the causative organism, as the results in Table 18 demonstrate. Isolates recovered from animals with the highest dermatophilosis indices, i.e., the most severe infections, did not produce the greatest caseinate precipitation zone areas. Isolates Dc2 and 15, for example, produced identical precipitation zones, but clinical dermatophilosis indices of the animals of origin were very different, 1 and 4, respectively.

Characterisation of Caseinase Activity of *D. congolensis* Culture Supernatants

Quantification of Protease in Culture Supernatant

Culture supernatants of *D. congolensis* isolates were diluted using two-fold serial dilutions and caseinase activity of the dilutions was measured. An inverse relationship was found to exist between caseinase activity (measured by area of the zone of caseinase precipitation in the test agar) and dilution of supernatant. This allowed relation of precipitation zone size to the quantity of the protease enzyme.

Stability of the Protease to Heat and Cold

The stability of caseinase activity of *D. congolensis* isolates was investigated under conditions of extreme heat and cold. Determination of the protease stability was thought necessary to ensure that enzyme activity was not lost under laboratory conditions.

Table 18. Comparison of clinical dermatophilosis index with ability of the causative isolate to produce caseinase activity *in vitro*

Isolate	Date of isolation	Clinical description of lesion of origin	Derm Index	Caseinase Activity
Lower hindlimb lesions				
Dc1	5.9.88	Paintbrush lesions	2	67
Dc2	5.9.88	Paintbrush lesions	1	149
Dorsum lesions				
2	23.2.89	Swab of dorsum lesion Scabs, pus	2	-
15	23.2.89	Scab from dorsum lesion Scabs, pus, pain	4	149
Dur	15.5.89	Scab from dorsum lesion Paintbrush lesions	1	67
F1	20.11.89	Scab from dorsum lesion Scab, paintbrushes, pain	3	105
F2	20.11.89	Scab from dorsum lesion Scab, paintbrushes, pain	3	105

Key:

Derm Index = Clinical Dermatophilosis Index, on a 0 to 10 scale, based on assessment of the severity and extent of distribution of lesions of dermatophilosis. 0 denotes absence of infection, and 10 is the most severe infection

Caseinase activity is given as the area, in mm^2, of caseinate precipitation

Results shown in Table 19 show that the protease was stable to heating of culture supernatant to 56°C for 15 mins, but was destroyed by heating culture supernatant to 100°C for 15 mins. Excepting isolate F2, where activity was reduced, activity was preserved after storage of culture supernatant at 4°C for one month. All isolates except isolate 15 appeared to show increased caseinase activity after storage at -20°C for 3 months.

Search for Antibody to Protease Activity in Serum from *D. congolensis* Infected Animals

Serum from *D. congolensis* infected animals was examined for the presence of antibody to the caseinase activity found in several *D. congolensis* culture supernatants. Neutralisation of caseinase activity was attempted by incubation of serum from a *Dermatophilus* infected calf and from two infected horses with *D. congolensis* culture supernatant, and comparison of caseinase activity to a control, serum from a newborn calf (Gibco BRL), not exposed to *D. congolensis* infection. Sera from the infected animals reduced the caseinase activity of culture supernatant, but this effect was also seen for the control.

As neutralisation of caseinase activity may have been due to non-specific effects of serum, the experiment was repeated, incubating culture supernatant with IgG fraction of serum from a *Dermatophilus* infected calf, and results were compared to a control which was incubated with neonatal calf serum. Although incubation with the IgG serum fraction did reduce caseinase activity, whole serum and control neonatal serum also had the same effect.

Inactivation of serum from *Dermatophilus* infected animals, by heat treatment of culture supernatant to 56°C for 15 mins, did not alter the ability of serum to reduce caseinase production.

Serum was thus found to have a neutralising effect on the caseinase activity, but this effect was not confined to serum from *D. congolensis* infected animals. These findings were supported by later work in this study, when immunoblotting using serum from *Dermatophilus* infected animals failed to detect specific

Table 19. Effect of heat and cold treatment of culture supernatants on ability of D. congolensis isolates to produce caseinase activity in vitro.

	Caseinase activity (area of zone of precipitation in mm^2)							
					4°C		-20°C	
Equine field isolates	Prior to treatment	Treatment A	Treatment B	Prior to storage	1 week	1 month	1 week	1 month
15	173	173	absent	173	173	173	199	199
Dur	173	149	absent	126	126	126	126	149
Dc1	149	149	absent	85	85	85	105	149
Dc2	173	126	absent	173	173	173	199	199
F1	149	149	absent	126	126	126	126	173
F2	126	126	absent	149	126	126	149	199

Notes:

Treatment A = Heating of supernatant to 56°C for 15 minutes

Treatment B = Heating of supernatant to 100°C for 15 minutes

antibodies to the protease.

Investigation of Gelatinase Activity of *D. congolensis* by SDS Gelatin-PAGE

In this study, field equine *D. congolensis* isolates 2, 15, Dur, Dc1, Dc2, F1 and F2 previously showed caseinase activity *in vitro*. Isolates 2, and NCTC laboratory isolates 5175 (equine) and 7915 (ovine), had repeatedly failed to demonstrate this activity. Supernatant and solubilised whole cell fractions of isolates were examined for gelatinase activity using SDS gelatin-PAGE, and bands of gelatinase activity of whole cell and supernatant fractions were visualised as negatively-staining areas on the gel, illustrated in Figures 17 and 18.

Gelatinase activity was developed at varying pH: 0.1M sodium acetate buffer of pH 4.0 and of pH 6.0, and 0.1M Tris-HCl buffer of pH 8.0 were used with equally good results. The pH of development buffer was not therefore influential on protein visualisation, and 0.1M sodium acetate buffer of pH 6.0 was adopted for further work.

In Figures 17 and 18 no gelatinase band was seen for isolate 2 or for NCTC equine isolate, 5175, or ovine isolate, 7915. Several bands were seen in whole cell fractions, but the most distinct one corresponded to that of the supernatant fraction. Where the band was detected, its position was identical for each isolate, at approximately 110 kD when compared to molecular weight markers on a standard SDS-PAGE gel.

Purification, Separation and Identification of the Protease

D. congolensis culture supernatants were concentrated by PEG dialysis and fractionated by Sephacryl chromatography. Eluted fractions were tested for caseinase activity, and active fractions were examined by SDS-PAGE, and compared to molecular weight markers. There was inadequate resolution of proteins by electrophoresis and no specific polypeptide band could be correlated to caseinase activity.

Ion exchange chromatography was used in a further attempt to

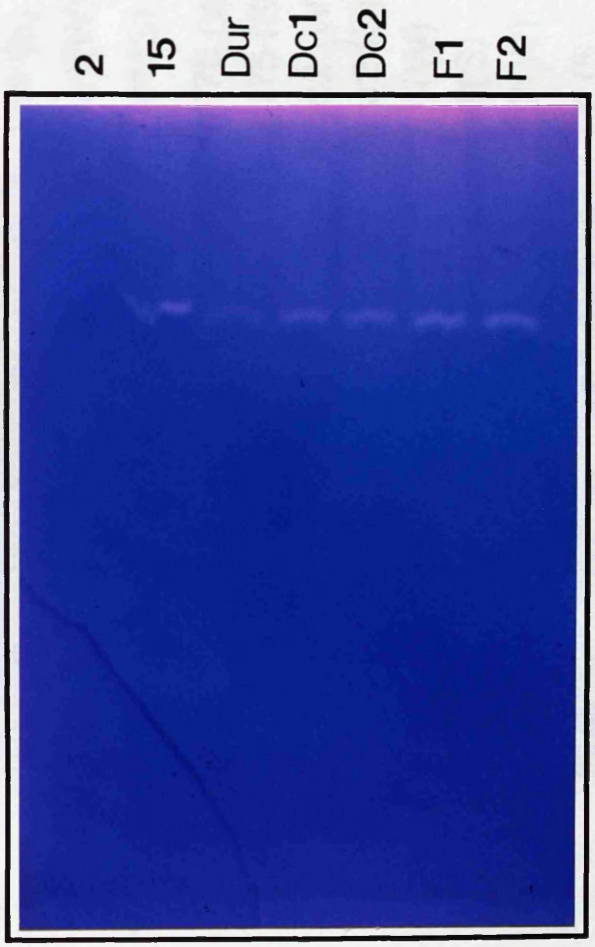

Figure 17. SDS gelatin-PAGE of culture supernatant fractions of *D. congolensis*. From left to right, culture supernatants of isolates 2, 15, Dur, Dc1, Dc2, F1, and F2.

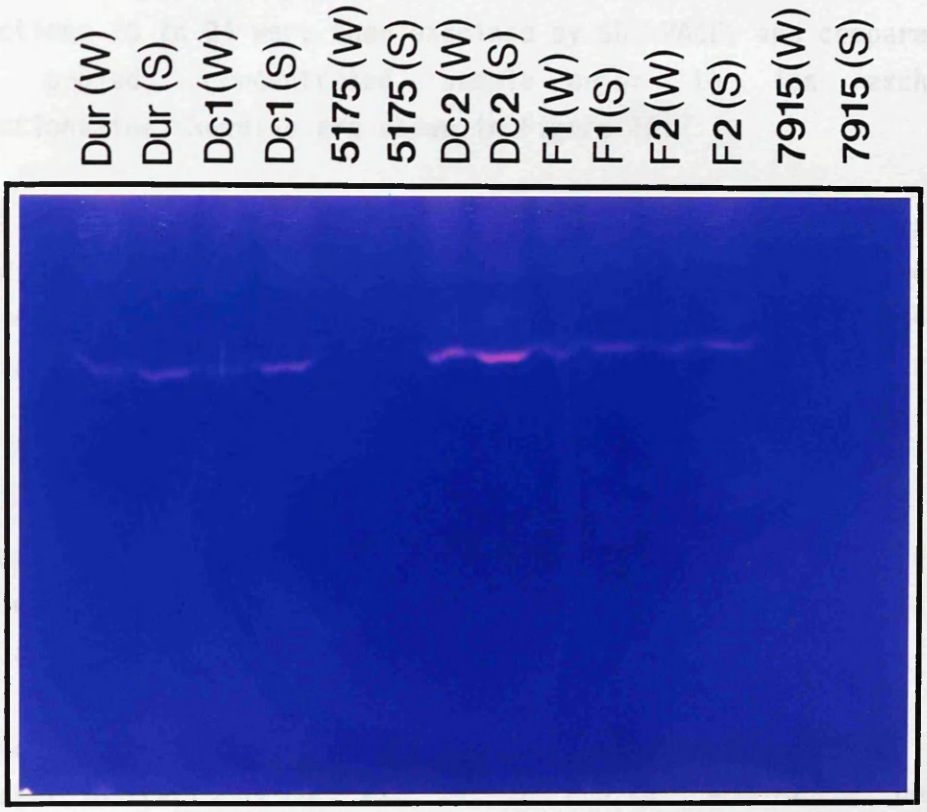

Figure 18. SDS gelatin-PAGE of supernatant and whole cell fractions of *D. congolensis*. From left to right, isolate Dur(W), Dur(S), Dc1(W), Dc1(S), 5175(W), 5175(S), Dc2(W), Dc2(S), F1(W), F1(S), F2(W), F2(S), 7915(W), 7915(S). (W) denotes whole cell fraction, and (S) denotes supernatant fraction.

fractionate and identify the protease. Isolate Dur culture supernatant was again separated by molecular exclusion chromatography, and caseinase-producing fractions were pooled and concentrated prior to ion exchange chromatography. Eluted fractions were assayed for caseinase activity, which was found in fractions 20, 21, 22, 23 and 24, with a peak (measured by the area of caseinate precipitation zone) seen in fraction 22. Fractions 20 to 24 were then examined by SDS-PAGE, and compared to the pooled, concentrated sample prior to ion exchange fractionation. Results are shown in Figure 19.

In Figure 19 two distinct, but very faintly-staining polypeptide bands were separated by ion exchange chromatography: one at approximately 55 kD, in fractions 18, 19, 20, 21, 22, 23, 24 and 25; and the other at around 28 kD, evident in fractions 21, 22 and 23.

The same caseinase-active fractions were also examined for gelatinase activity by SDS gelatin-PAGE, shown in Figure 20. A pooled sample of all caseinase-active chromatography fractions was run alongside the individual fractions.

Figure 20 shows the single, distinct band of gelatinase activity present in fractions 20, 21, 22, 23 and 24, and in the pooled sample. The peak of gelatinase activity, the brightest band on the gel, in fraction 22, corresponded with peak caseinase activity. No protein of 110 kD could be found in the ion exchange fractions.

Protease Inhibition Experiments

Protease activity had been detected for several *D. congolensis* isolates. In order to equate gelatinase and caseinase activities, the gelatinase band of approximately 110 kD was cut out of the gel and implanted into caseinate test agar. Caseinase activity was detected.

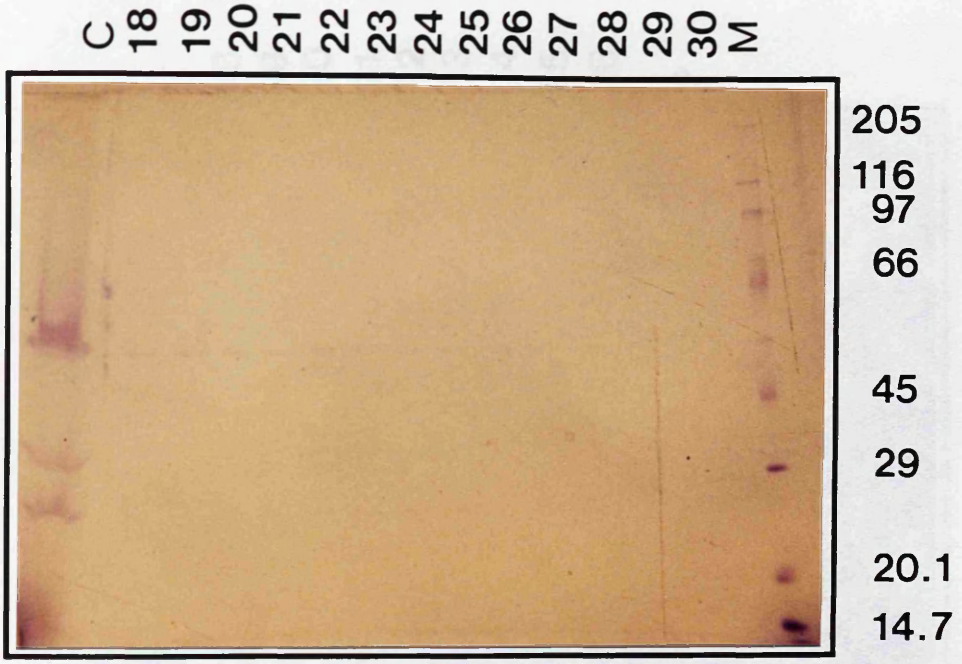

Figure 19. SDS-PAGE of caseinase-active fractions obtained by ion exchange fractionation of *D. congolensis* culture supernatant. From left to right, concentrated supernatant prior to ion exchange chromatography, (C); fraction number 18; 19; 20; 21; 22; 23; 24; 25; 26; 27; 28; 29; 30; molecular weight marker, (M).

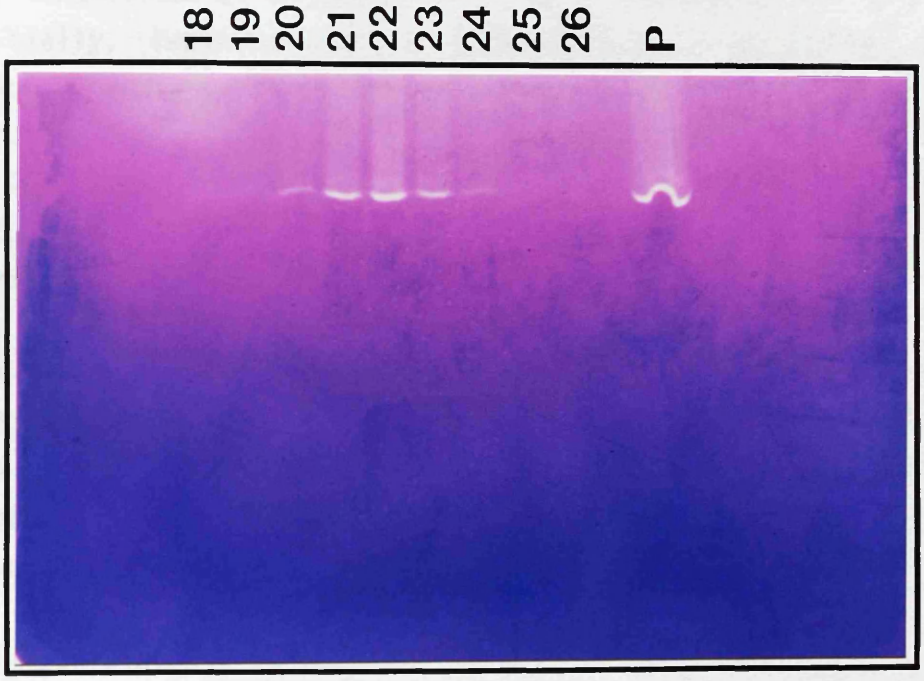

Figure 20. SDS gelatin-PAGE of caseinase-active fractions obtained by ion exchange fractionation of *D. congolensis* culture supernatant. From left to right, fraction number 18; 19; 20; 21; 22; 23; 24; 25; 26; and a pooled, concentrated sample of caseinase-active fractions, (P).

In general, classification of protease activity is made on the basis of the amino acid type involved in the active site of the enzyme. Proteases fall into four mechanistic families: the serine, cysteine, aspartic and metallo proteases (Neurath, 1989). Classification has been based on susceptibility to a group of inhibitors; and a recommended set for the initial classification of a newly discovered protease (Dunn, 1989) was employed to try to categorise the protease activity of *D. congolensis*.

Inhibition of Gelatinase Activity on Polyacrylamide Gels

Initially, Pepstatin A which inhibits aspartic proteases; EDTA, which inhibits metallo proteases; iodoacetic acid (cysteine proteases) and leupeptin (specific for serine and cysteine proteases), were each incubated with a pooled sample of whole, solubilised bacteria. No inhibition of gelatinase occurred with inhibitors at standard concentrations.

The experiment was repeated using sonicated culture fluid (3 x 20 sec periods of ultrasound) to further solubilise protein material, and reducing agent dithiothreitol (DTT), an activator of cysteine proteases, was added to reaction mixtures. Still no inhibition occurred.

Inhibition of Caseinase Activity

Protease inhibitors, EDTA, iodoacetic acid, leupeptin and Pepstatin A, and antipain, specific for trypsin-like serine and some cysteine proteases; bestatin, specific for aminopeptidases; chymostatin, specific for chymotrypsin-like serine proteases and some cysteine proteases; 3,4-dichloroisocoumarin (3,4-DCI) (serine proteases); 1,10-Phenanthroline (metallo-proteases, metal-activated proteases); phenylmethanesulphonyl fluoride (PMSF) (all serine proteases); tosyl lysyl chloromethyl ketone (TLCK) (trypsin-like serine proteases) and tosyl phenylalanyl chloromethyl ketone (TPCK) (chymotrypsin-like serine proteases) were incubated with supernatants of isolates Dur, 15 and Dc1. The influence of temperature on the reaction was investigated. Results presented in Table 20 show that no inhibition of protease occurred for any isolate with antipain, bestatin, chymostatin,

Table 20. Inhibition of caseinase production by *D. congolensis* isolates using specific protease inhibitors

	% of control area of caseinate precipitation					
	Isolate Dur		Isolate 15		Isolate Dc1	
	RT	37°C	RT	37°C	RT	37°C
Inhibitor						
Antipain	100	100	100	100	100	100
Bestatin	100	100	100	100	100	100
Chymostatin	100	100	100	100	100	100
3,4-DCI	0	0	81	67	60	0
EDTA	100	100	100	100	100	100
IAA	100	100	100	100	100	100
Leupeptin	100	100	100	100	100	100
Pepstatin	100	100	100	100	100	100
1,10 Phe	100	100	100	100	100	100
PMSF	0	0	40	53	0	0
TLCK	100	100	100	100	100	100
TPCK	100	100	100	100	100	100

Key:

3,4-DCI = 3,4-dichloroisocoumarin
EDTA = Ethylenediaminetetraacetic acid
IAA = Iodoacetic acid
1,10-Phe = 1,10-Phenanthroline
PMSF = Phenylmethanesulphonyl fluoride
TLCK = Tosyl lysyl chloromethyl ketone
TPCK = Tosyl phenylalanyl chloromethyl ketone

EDTA, IAA, leupeptin, pepstatin A, 1,10-Phenanthroline, TLCK or TPCK. 3,4-DCI inhibited protease activity of isolate Dur at both temperatures, and of isolate Dcl at 37°C only. Reduction of caseinase activity of isolate Dcl by 3,4-DCI occurred at room temperature, and isolate 15 activity was reduced though not abolished at both temperatures. Activity of isolates Dur and Dcl were inhibited by PMSF; and activity of isolate 15 was reduced. Temperature did not influence results significantly.

It was concluded from these results that the protease activity was of the serine classification. The serine proteases form covalent enzyme complexes, and are the most thoroughly studied class of protease enzymes (Dunn, 1989). Examples include pancreatic trypsin, chymotrypsin, elastase and kallikrein (Neurath, 1989).

SDS-PAGE Analysis of Caseinase Activity

PEG concentrated, unheated, culture supernatant of *D. congolensis* isolate, Dcl, was fractionated by ion exchange chromatography. The caseinase-active fractions obtained were examined by SDS-PAGE alongside unconcentrated culture supernatant. The SDS-PAGE gel was run and overlaid with test caseinate agar. Two distinct zones of caseinase protease activity were found on the gel. The larger, predominant zone of activity was seen between approximately 70 and 120 kD. The second, less active zone was easily detected between 20 and 40 kD for the concentrated sample, and was just visible in this position for the unconcentrated sample. These zones of caseinase precipitation are demonstrated on the gel in Figure 21.

Two proteases which showed caseinase activity were thus thought to be produced by *D. congolensis*. One protease, of approximately 110 kD, also demonstrated gelatinase activity. The second caseinase producing protease was of lower molecular weight.

The finding of two zones of caseinase precipitation by SDS-PAGE was thought to invalidate the earlier protease inhibition results, because if two proteases were present, one could mask specific inhibition of the other.

Figure 21. SDS-PAGE analysis of caseinase activity of *D. congolensis*. On the left hand side, concentrated culture supernatant of isolate Dc1, (Dc1 Conc); on the right hand side, unconcentrated culture supernatant of isolate Dc1, (Dc1).

Immunoblotting Using Serum From *D.congolensis* Infected Animals

Equine field isolates 2, 15, Dur, Dc1, Dc2, F1 and F2 and laboratory isolates 5175 (equine) and 7915 (ovine) were immunoblotted using serum from a calf suffering from a very severe, generalised dermatophilosis infection, of dermatophilosis index 10. Neonatal calf serum was used as a control. The isolates examined produced similar patterns, with numerous protein bands detected for whole solubilised bacteria and supernatant fractions. Blotting with serum from infected animals failed to highlight particular bands of activity for fractions of isolates examined.

The experiment was modified by PEG concentration and ion exchange fractionation of supernatants of overnight culture of isolates Dur and Dc1 prior to blotting. Eluted fractions were examined for caseinase activity, and the peak fraction for each isolate was selected for immunoblotting: fraction 25 for Dur and fraction 30 for Dc1. The fractions were divided into two aliquots, one was heated to 100°C for 5 minutes, and the other was untreated. Overnight broth culture supernatants were used as positive controls. The gel was loaded in duplicate, and each identical half was blotted using serum from a *Dermatophilus* infected horse. The horses were both suffering from moderate dermatophilosis infections, of dermatophilosis index of 4 in each case. Results of blotting were similar for the isolates examined, no particular band of activity was visualised by the addition of the serum from the infected horses. One of these immunoblots is shown in Figure 22.

Antibody to the protease was not detected in serum from either infected horse in this study. This result was in agreement with earlier work in this study, where it was found that although serum had a neutralising effect on the protease activity, this effect was not specific for serum from *Dermatophilus* infected animals.

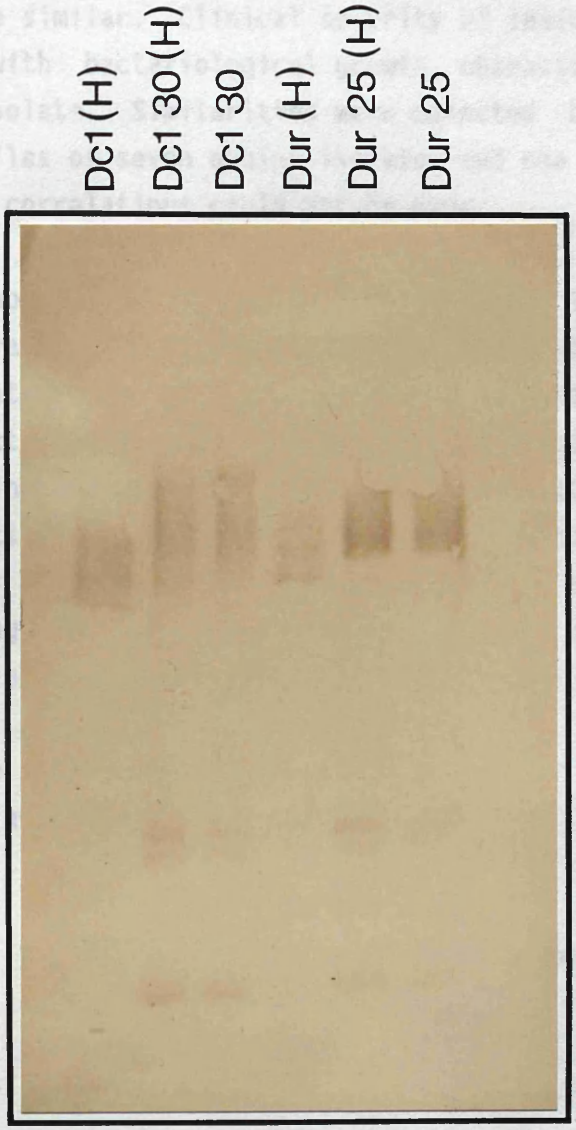

Figure 22. Immunoblot of concentrated supernatant from *D. congolensis* isolates using serum from a *Dermatophilus* infected horse. From left to right, isolate Dc1 [heated to 100°C for 5 mins before loading,(H)], Dc1 30 (H), Dc1 30, Dur (H), Dur 25 (H), Dur 25.

Conclusion

No two *D. congolensis* isolates examined showed identical bacteriological characteristics although lesions induced by all isolates were similar. Clinical severity of lesions could not be correlated with bacteriological growth characteristics of the causative isolate. Similarities were detected between SDS-PAGE protein profiles of seven equine isolates and one bovine isolate, but definite correlations could not be made.

Several but not all of the equine *D. congolensis* isolates examined produced extracellular protease activity. One protein estimated to be approximately 110 kD by SDS-PAGE produced caseinase and gelatinase activities, and a second, of lower molecular weight, showed caseinase activity. The caseinase activity was stable to cold. The activity was non-specifically inhibited, by serum from *Dermatophilus* infected horses, and by serum from an infected calf. Immunoblotting studies failed to demonstrate antibody to any protein species in serum from infected animals.

No keratinase activity was detected using hair or keratin azure as substrate, and no collagenase or elastase production was discovered.

In summary, although similarites existed between the *D. congolensis* isolates examined in this thesis, with regard to differential bacteriological growth characteristics and SDS-PAGE protein profiles, the isolates were all different. Protease activity was produced by some but not all of the isolates, but could not be used as a marker of virulence.

SECTION 3. THE CLINICAL AND HAEMATOLOGICAL CONSEQUENCES OF BLEEDING HORSES AT REGULAR INTERVALS

Introduction

The consequences of bleeding horses in terms of frequency and quantity are a major animal welfare consideration. A situation which offers the opportunity to address this question is where horses are managed for production of blood products. Such an opportunity arose on a farm in the West of Scotland where 200 mature horses of different breeds were maintained and bled at recorded regular intervals thus creating a major database for evaluating the management of this operation in terms of clinical condition, as well as haematological consequences.

The practice of regular, repeated blood harvest arises in horses managed for blood production in a commercial situation. It is therefore not surprising that examination of current and historic literature reveals that details of current practices, and figures regarding incidence and extent to which horses are managed for regular, repeated blood harvest for blood products in the U.K. and elsewhere are difficult to obtain. Blood harvesting has probably been undertaken "in house" in research institutes and in Veterinary Investigation Centres and Universities, for microbiological culture material for their own use (Home Office Veterinary Inspector, personal communication). Prior to the new Animals (Scientific Procedures) Act of 1986 there was no national requirement to disclose such activities.

As far as can be determined, substantiated guidelines for harvest procedures do not exist, and so the area was considered worthy of detailed monitoring studies. Clinical and haematological data were collected and analysed in an attempt to provide background for guidelines for Home Office regulations on regular, repeated blood collection in horses.

An initial study was designed to obtain an overview of the clinical and haematological effects of repeated, regular blood harvest on a group of animals in the herd, under the existing management regime. This was to be followed by a shorter, more detailed haematological investigation using a smaller number of the horse group. Blood samples were to be collected between two

harvest points in order to follow more closely the recovery pattern of the haematological parameters of these animals. Influence of sex, breed and the length of time in blood production on the recovery pattern were to be monitored.

Materials and Methods

Horses

For the purpose of the initial study a group of 48 horses was monitored. The group comprised 21 mares and 27 geldings. Eighteen animals were Thoroughbreds, 13 were cobs or heavy types, and 17 were crosses of the two, i.e., 30 animals were non-Thoroughbreds. The age of the group ranged from six to 22 years old. Twelve animals were younger than 10 years, 27 were between 10 and 15 years old, and nine were over 15 years of age. Height of animals varied from 15 to 17 hands. Twenty horses were between 15 and 15.3 hands, 21 were between 16 and 16.3 hands, and two animals stood 17 hands tall.

For the subsequent study, 10 of the horses were randomly selected from the group above. Four mares and six geldings were involved, and three animals were Thoroughbreds. Age ranged from nine to 20 years of age. Two animals were younger then 10 years, six were between 10 and 15 years, and two were older than 20 years.

Details of the management of the group, including the harvesting procedure, are provided in the general Materials and Methods section.

Experimental Designs

Experiment 1

In the initial study regular clinical and haematological monitoring was carried out over a period of 16 weeks, which spanned five harvest cycles. Clinically, body condition was assessed, as detailed in general Materials and Methods, at monthly intervals in the first study. Blood was collected and haematological examinations were performed according to the details given in the general Materials and Methods section. Total

estimation and electrophoresis of proteins was performed at these times using a Beckman Appraise Junior Densitometer in the Medicine Laboratory of the University of Glasgow Veterinary School. Full haematological examination was carried out at weekly intervals for each horse during the first study. On harvest days, samples were collected immediately prior to harvest.

Experiment 2

Haematological monitoring was performed in this study over a 21 day period, between two harvest points. Blood samples were collected and full haematological examination was performed as described in the general Materials and Methods section on samples collected immediately prior to harvest, t0, and at 30 mins, 60 mins, 90 mins, 2 h, 6h, 12h and 24h after harvest. Thereafter, samples were collected daily until day 21 of the cycle, before the next harvest took place. The aim was to investigate the recovery pattern for the haematological parameters between harvests.

Statistical analyses were undertaken in both studies, to examine influence of sex, breed and the length of time in blood production, on the haematological parameters of packed cell volume (PCV), red cell count (RCC) and haemoglobin (Hb). Influence of body condition was also evaluated in the initial overview study. Details of statistical analyses are provided in the general Materials and Methods section.

Results

Experiment 1. Monitoring over Five Harvest Cycles

Body Condition

There was no evidence that body condition was affected by the five harvest cycles. Body condition scores are presented in Figure 23. Animals were scored on a 1 to 5 point scale, with 0.5 point increments. The horses were in good body condition at each of the monthly measurements, most animals scored 2.5 or above, indicating moderate to good condition. Changes in body condition scores are demonstrated in Figure 24. In the period April to May 87.5% of the

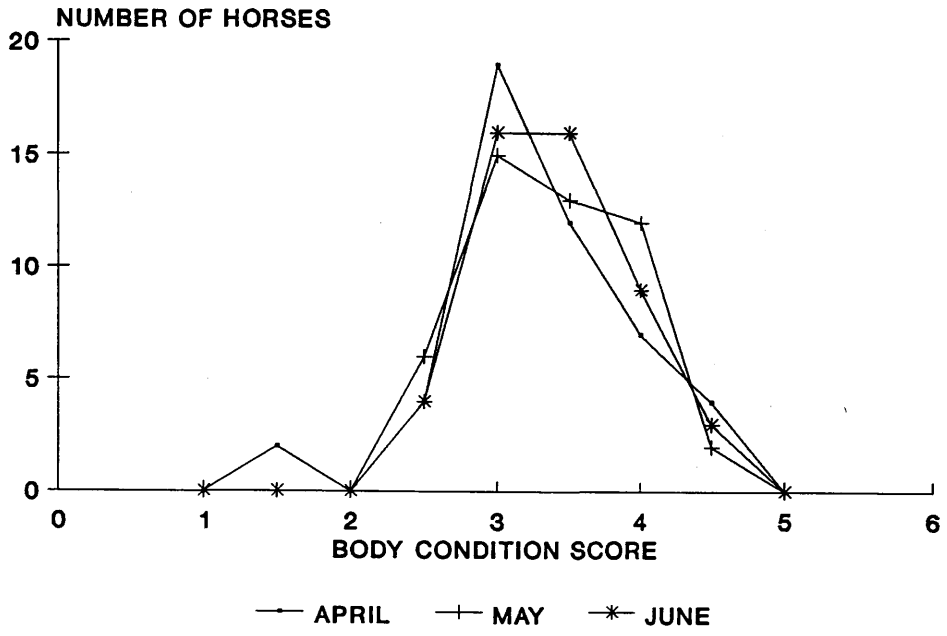

FIGURE 23. BODY CONDITION SCORES

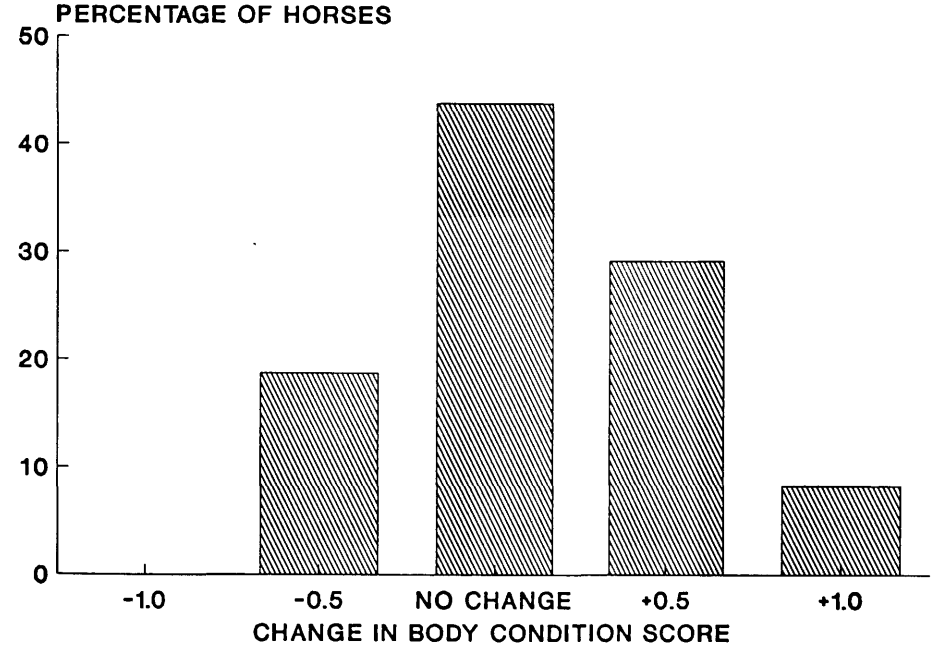

FIGURE 24. CHANGES IN BODY CONDITION (APRIL TO JUNE)

horses either maintained or gained condition. In the period May to June this percentage increased to 89.4% of the horses. Over the duration of the study, between April and June, 39 of the 48 horses (81.3%) maintained or gained condition.

Haematology

Figures 25, 26, and 27 illustrate the values for group mean PCV, RCC and Hb values, respectively. Normal ranges and units used in measurement of haematological parameters in the horse are given in Appendix IV.

A cyclic pattern was found for the group mean PCV values, shown in Figure 25. Values fell to their lowest point the first week post harvest, with gradual restoration over the second and third weeks post harvest. This pattern was repeated uniformly for the five harvest cycles studied. Values were within normal ranges at all points recorded.

Group mean RCC values, demonstrated in Figure 26, showed a similar cyclic pattern to PCV in Figure 25. Values were lowest one week post harvest, and showed gradual increase over the second and third weeks, and peaked temporarily before the next harvest. As with PCV, mean values for RCC were within normal ranges throughout the 16 week study.

The group mean Hb pattern of Figure 27 followed closely the cyclical trends for PCV and RCC. Values lay within normal ranges throughout.

Protein Profiles

Protein profiles, illustrated in Figure 28, include values for mean total plasma proteins, which were within normal range at the three time points monitored. Mean albumin to globulin ratios were above the normal value of 1 at each time point studied. Profiles demonstrate the relative percentages of albumin, alpha 1-, alpha 2-, beta- and gamma- globulins.

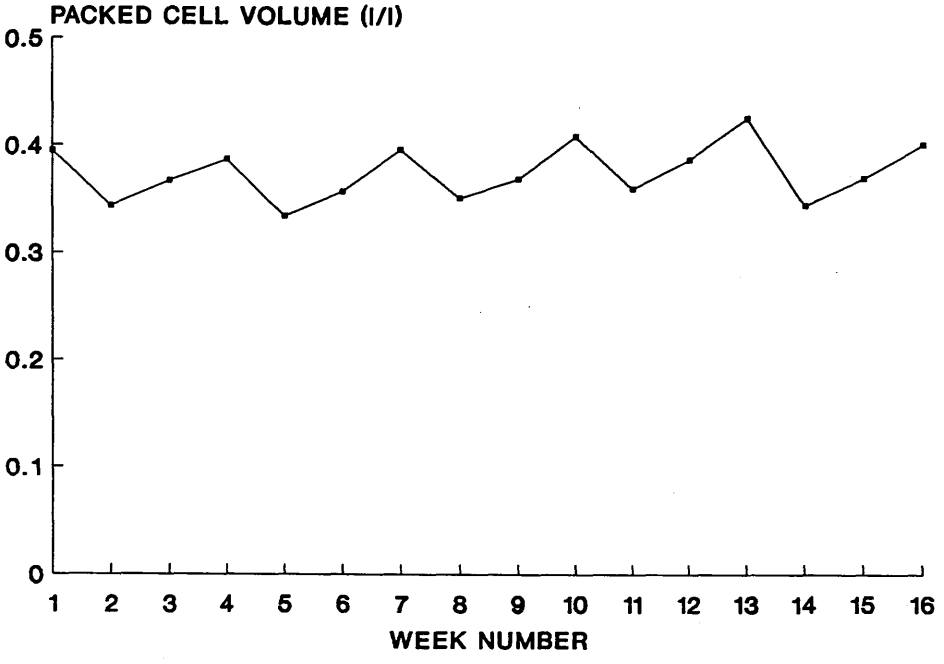

FIGURE 25.GROUP MEAN PCV OF HORSES BLED WEEKS 1,4,7,10,13,16

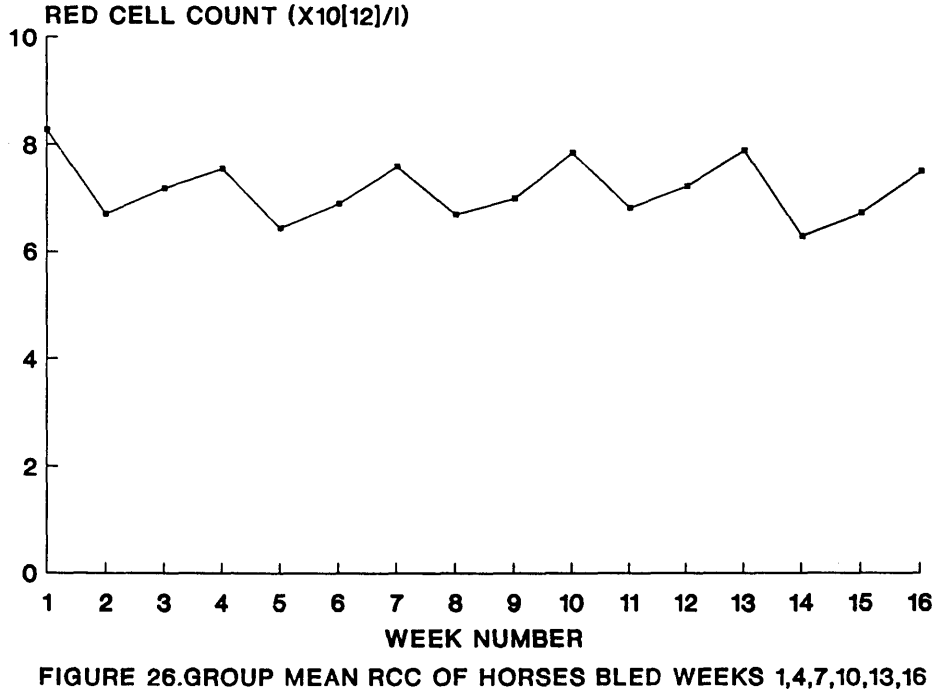

FIGURE 26.GROUP MEAN RCC OF HORSES BLED WEEKS 1,4,7,10,13,16

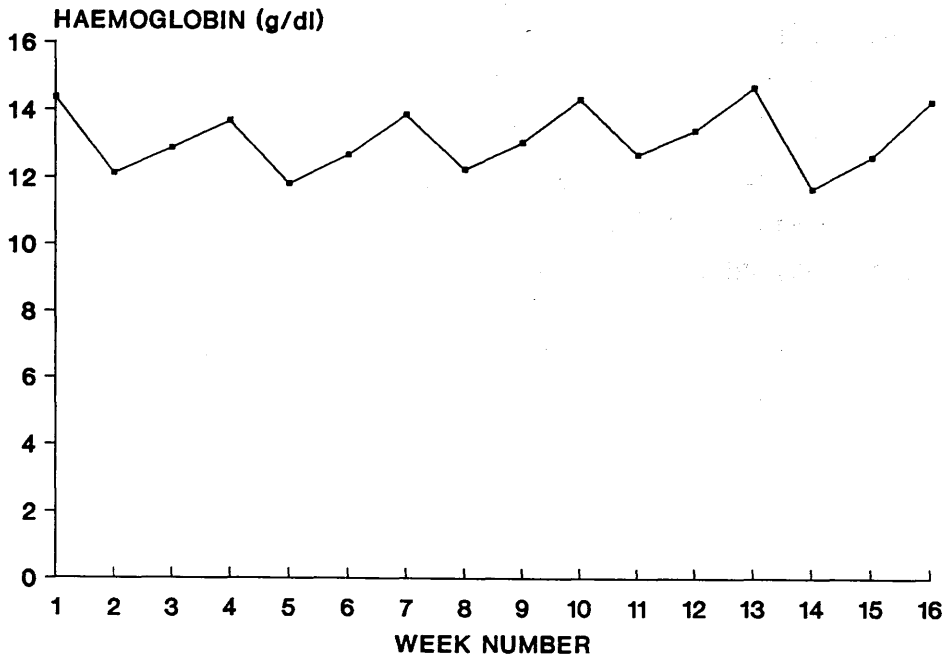

FIGURE 27.GROUP MEAN HB OF HORSES BLED WEEKS 1,4,7,10,13,16

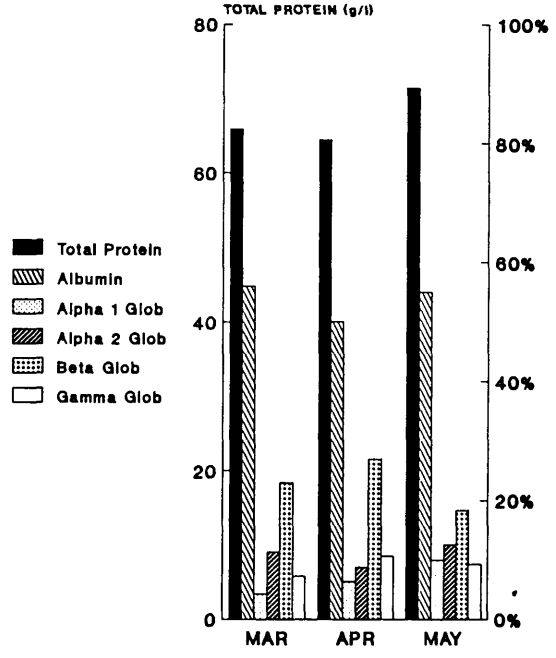

FIGURE 28. PROTEIN PROFILES

Statistical Analyses

In the statistical analyses the performance of geldings was compared to mares, Thoroughbreds to non-Thoroughbreds (halfbreds and heavier types), and the performance of animals used for less than 5 years was compared to those used for longer than 5 years. Thin animals (condition score of 2.5 points or less) were compared to those of moderate condition (condition score of 3 to 3.5 points) and those which were fat (condition score of 4 points or more). Analyses of group mean PCV, RCC and Hb are presented in Table 21.

Results indicated no significant influence of condition score, sex or length of time in blood production on any of the parameters. Significant differences existed between breeds for PCV, RCC and Hb. Values were always higher for Thoroughbreds than for non-Thoroughbreds.

Experiment 2. Monitoring Between Two Harvest Points

Values for the haematological parameters were maintained within normal ranges for the 21 day period between the two harvest points. Normal ranges and units used in measurement of haematological parameters are given in Appendix IV.

For the mean values for PCV, illustrated in Figure 29, a sharp drop in values was evident 30 mins post harvest, then the value rose again at 1 h post harvest and remained relatively constant between 90 mins and 12 h post harvest. PCV was lowest at 4 days post harvest, after which time there was an increase over the remainder of the cycle, but it did not, however, attain the preharvest value.

The pattern for mean red cell count, Figure 30, followed the PCV pattern: a sharp fall was seen 30 mins post harvest, with a further drop for 1 h more then the value rose slightly and remained constant till 12 h post harvest, when there was a decline

Table 21. Influence of body condition, sex, breed and the length of time regular bleeding has occurred, on the group mean values for packed cell volume (PCV), red cell count (RCC) and haemoglobin (Hb) of horses bled regularly, in a study which monitored five harvest cycles

Body Condition

	Poor (score<3)	Good (score 3-3.5)	Fat (score>3.5)
Mean PCV	0.427	0.409	0.387
Mean RCC	8.25	7.99	7.21
Mean Hb	14.58	14.31	13.64
Number of horses	6	31	11

Sex

	Geldings	Mares
Mean PCV	0.392	0.418
Mean RCC	7.70	8.03
Mean Hb	13.93	14.53
Number of horses	27	21

Breed

	Thoroughbreds	Non-Thoroughbreds	
Mean PCV	0.431	0.374	*
Mean RCC	8.49	7.03	*
Mean Hb	14.88	13.07	*
Number of horses	13	13	

Length of time regular bleeding has occurred

	>5 years	<5 years
Mean PCV	0.387	0.399
Mean RCC	7.39	7.57
Mean Hb	13.60	13.90
Number of horses	12	12

* indicates a significant difference

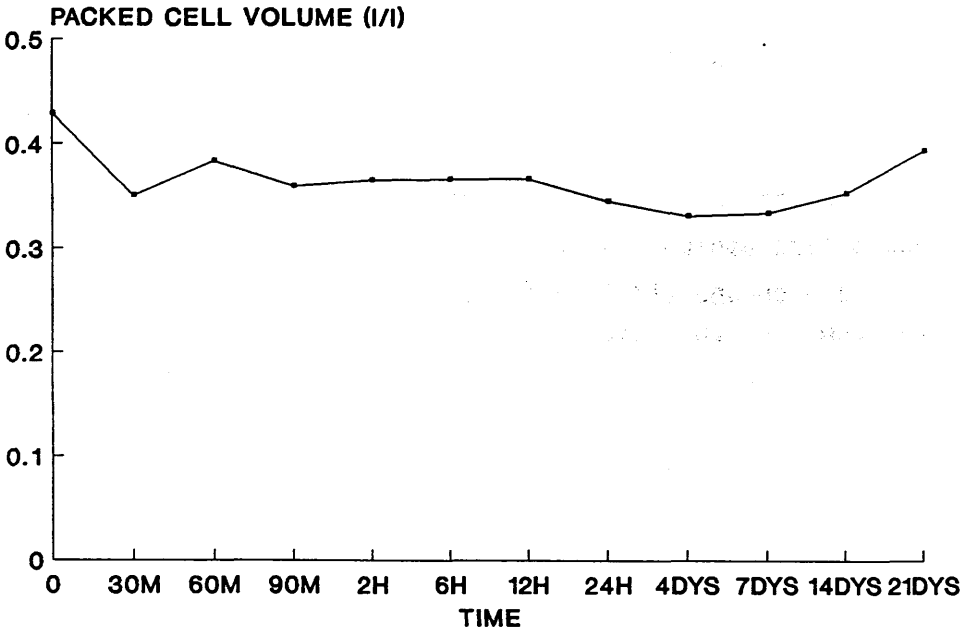

FIGURE 29. GROUP MEAN PCV OF HORSES BETWEEN TWO HARVESTS

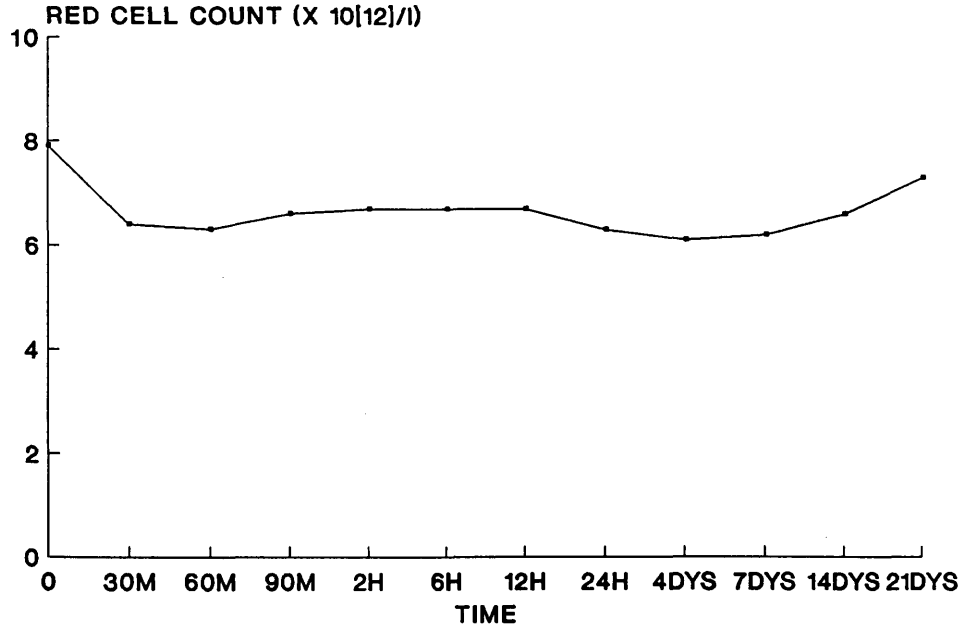

FIGURE 30. GROUP MEAN RCC FOR HORSES BETWEEN TWO HARVESTS

to a trough at 4 days post harvest. RCC climbed over the remainder of the cycle but, as for PCV, it never reached the preharvest figure.

The pattern for mean haemoglobin values, Figure 31, echoed closely those of PCV and RCC. The value preharvest was not attained during the cycle.

Minimal fluctuations were seen in the results for group mean for mean cell volume, Figure 32. The value remained static towards the upper limit of normal range. Very little change was evident in mean cell haemoglobin values until after day 7. MCH had risen again by day 21. Figure 33 illustrates that mean values never regained the preharvest figure. As for MCV, values tended towards the upper limit of normal range. This pattern was followed by group mean values for mean cell haemoglobin concentration, shown in Figure 34.

The pattern which was seen for values of group mean for platelet counts in Figure 35 resembles the pattern shown in Figures 29 to 31: a sharp fall by 30 mins post harvest, temporary recovery then decline by 2 h, a second temporary recovery then further decline by 4 days post harvest. The preharvest value was gradually reached by day 21 post harvest.

Group means for WCC in Figure 36 showed a slight decline for 90 mins after harvest, then an increase was seen until 12 h post harvest, after which values returned to the preharvest level by 4 days, and remained static thereafter.

Statistical analysis of PCV, RCC and Hb, presented in Table 22, indicated no significant difference between the groups for sex, breed or the length of time in blood production.

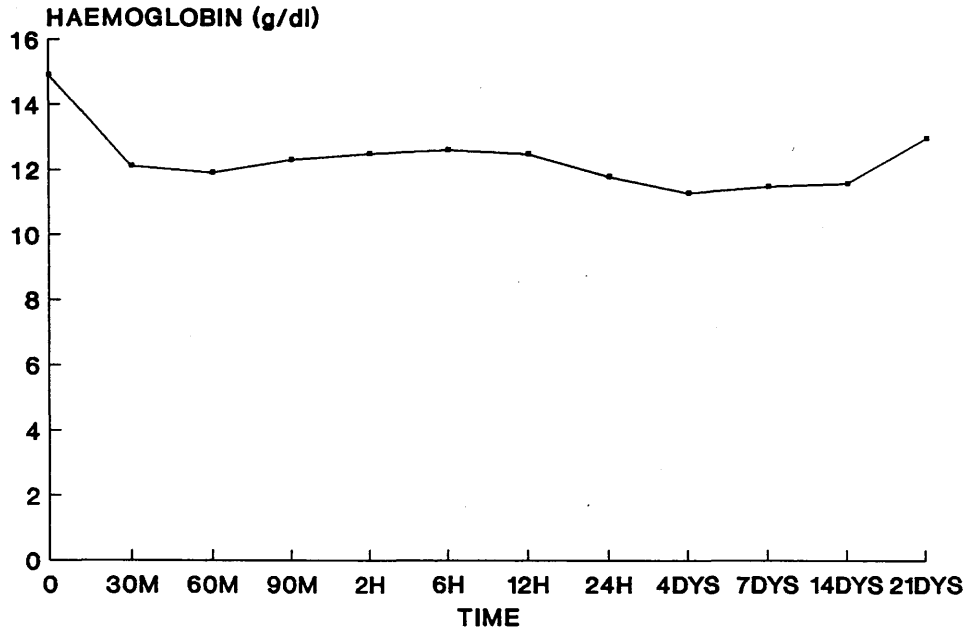

FIGURE 31. GROUP MEAN HB OF HORSES BETWEEN TWO HARVESTS

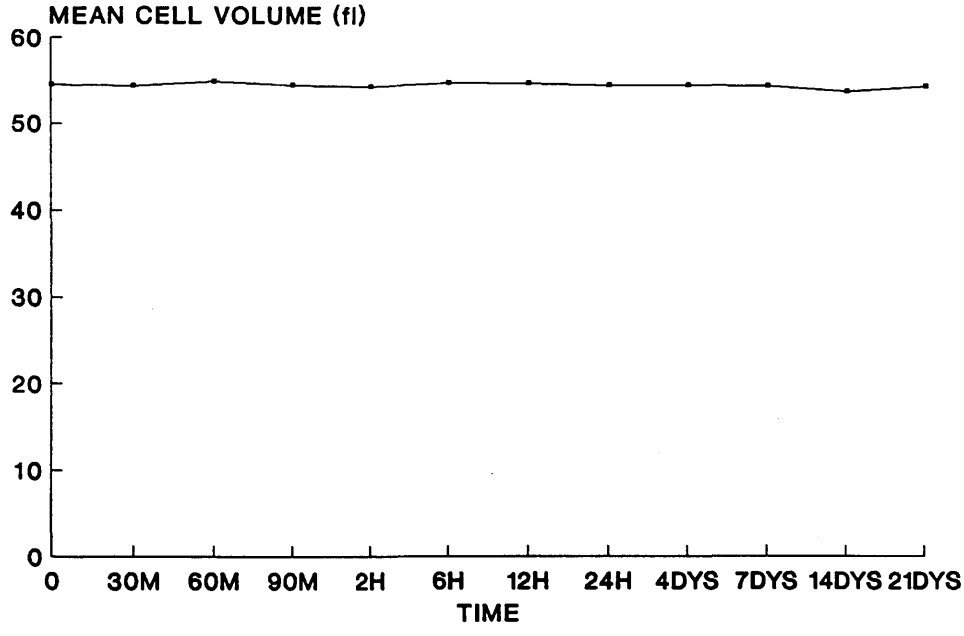

FIGURE 32. GROUP MEAN MCV OF HORSES BETWEEN TWO HARVESTS

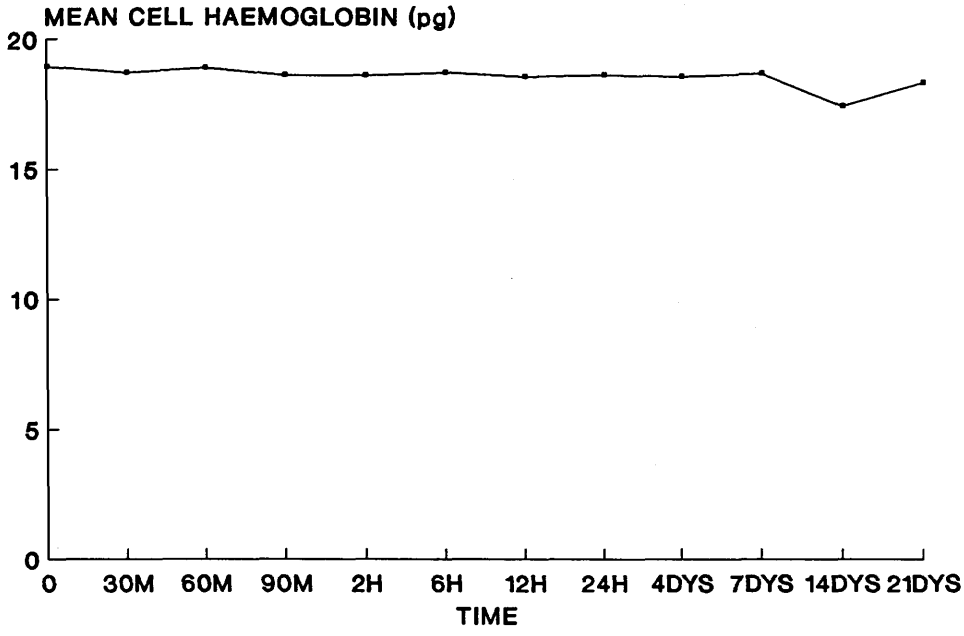

FIGURE 33. GROUP MEAN MCH OH HORSES BETWEEN TWO HARVESTS

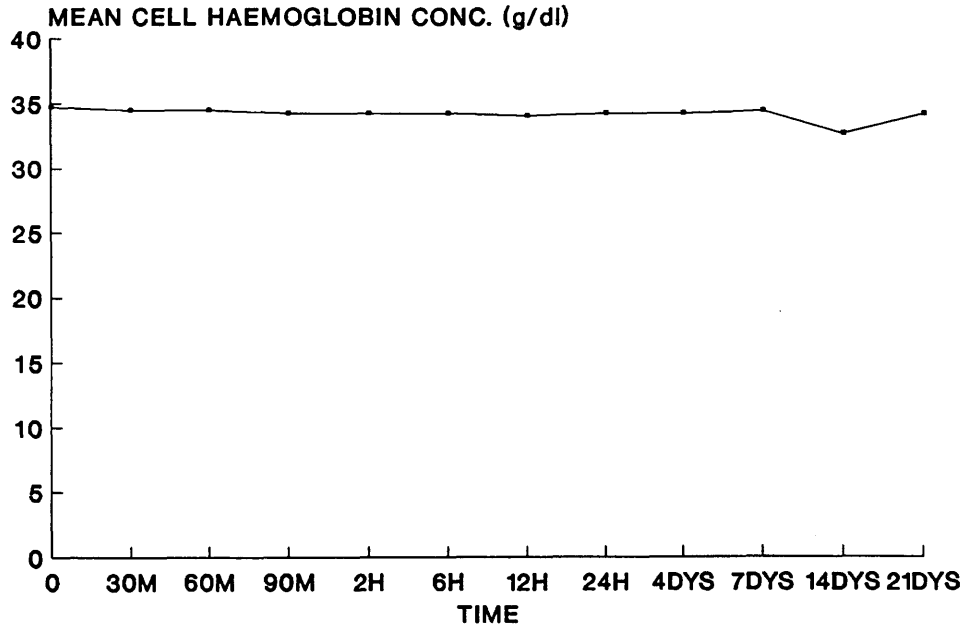

FIGURE 34. GROUP MEAN MCHC OF HORSES BETWEEN TWO HARVESTS

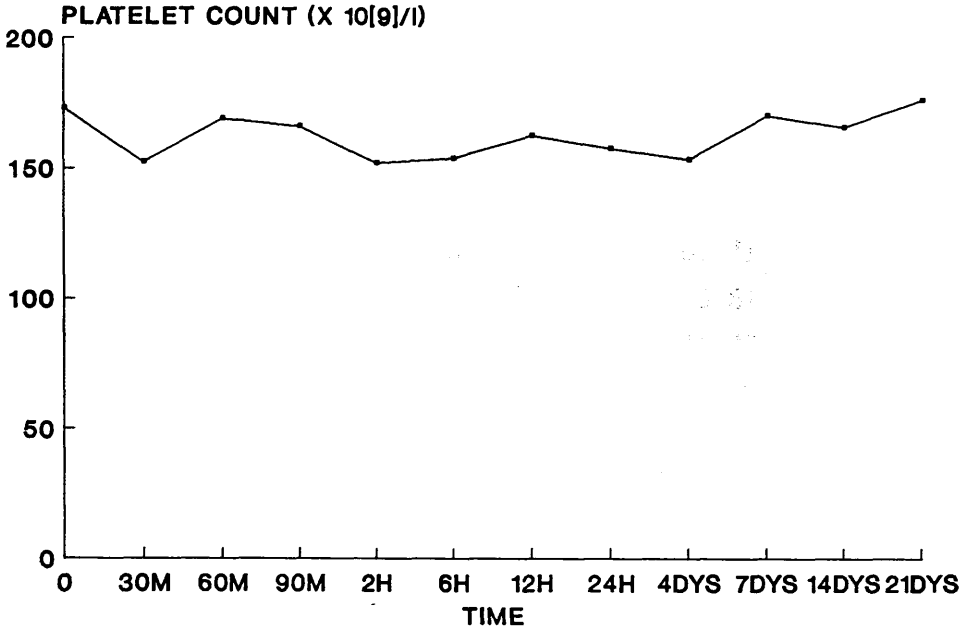

FIGURE 35. GROUP MEAN PL OF HORSES BETWEEN TWO HARVESTS

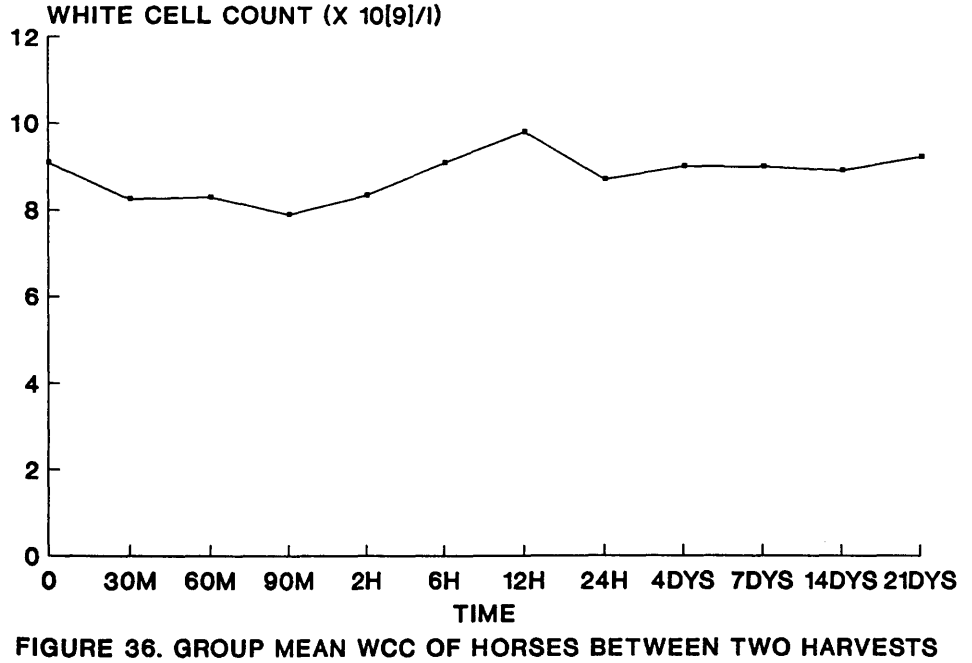

FIGURE 36. GROUP MEAN WCC OF HORSES BETWEEN TWO HARVESTS

Table 22. Influence of sex, breed and the length of time regular bleeding has occurred on the pattern of recovery between two harvest points of group means for packed cell volume (PCV), red cell count (RCC) and haemoglobin (Hb) of horses bled regularly

Sex

	Geldings	Mares
Mean PCV	0.363	0.371
Mean RCC	6.77	6.81
Mean Hb	12.48	12.73
Number of horses	7	3

Breed

	Thoroughbreds	Non-Thoroughbreds
Mean PCV	0.383	0.363
Mean RCC	7.12	6.64
Mean Hb	13.08	12.34
Number of horses	3	7

Length of time regular bleeding has occurred

	> 5 years	< 5 years
Mean PCV	0.312	0.370
Mean RCC	6.69	6.85
Mean Hb	12.55	12.57
Number of horses	6	4

No significant difference was recorded for any parameter

Discussion

The observation that body condition scores were generally good, most horses maintained or gained condition over the period of study, probably reflected the improving plane of nutrition of the horses with seasonal improvement of pasture during the studies, between April and June. The good dietary management and parasite control programme in operation under the current management on this farm support the consistently good body condition scores in the horses.

Total plasma protein values, which were all within normal ranges here, reflect plasma water balance more accurately than do PCV, as the normal TPP range is narrower than that of PCV and is not influenced by pain, fear or excitement (Becht, 1986). The fact that these values are within normal ranges indicates adequate hydration and assures that regular, repeated bleeding in this herd does not induce chronic protein loss. This is not surprising if one considers that it has been calculated, using radioisotope studies, that in a normal 500kg horse of the type in this herd, one litre of plasma is lost into the gut daily (Love, 1990).

The haematological parameters studied remained within the normal ranges during the five harvest cycles studied. The herd comprises predominantly Thoroughbreds and Thoroughbred types, which statistics indicate to have significantly higher erythrocyte-related parameters than heavier, halfbred types. These Thoroughbred types are less influenced by the current harvesting regime than are non-Thoroughbreds. This is not unexpected if one considers the published data which show Thoroughbreds to have higher blood volumes (88-110 ml/kg) than cold blooded or heavier, cob and pony type (62 - 66 ml/kg) animals (Schalm, 1986).

Several of the unique aspects of blood volume and erythropoiesis of horses are exemplified in the studies. Up to half of the red cell volume in the horse may be stored in the spleen; an important adaptive process for exercise which results in a very unstable circulating blood volume (Becht, 1986; Jeffcott, 1977). Splenic

contraction is a normal haematological response, specific to the horse, which is stimulated within seconds in response to haemorrhage, as well as to exercise or excitation. In these studies this response was observed repeatedly, as red cell counts did not fall dramatically even after repeated, sizeable haemorrhage. In healthy Thoroughbreds, splenic contraction can increase PCV by up to 50% above resting levels (Jeffcott, 1977).

According to Torten and Schalm (1964), PCV and other red cell parameters may rise owing to splenic contraction, making determination of the magnitude of blood loss during the first 4 or 5 h after haemorrhage almost impossible. The degree of blood loss may not become clinically evident until up to 48 h after the initiation of haemorrhage, owing to shifts of fluid and the capacity of the spleen (Lumsden, Valli and McSherry, 1975). The results of this study supported this: lowest values for PCV, RCC and Hb were not recorded until 7 days after harvest. Jeffcott (1977) believes that reduction in red cell parameters or anaemia is appreciated by 12 to 24 hours after acute blood loss.

Acute haemorrhage, of up to one-third of circulating blood volume (around 12 litres in a 500kg animal), can be withstood by the horse without exhibition of serious distress (Jeffcott, 1977). Under this management system approximately 8 litres of blood were collected at any one harvest from animals of around 500kg. This blood collection is equivalent to less than one fifth of total blood volume for horses of the Thoroughbred type involved in this herd. Under the current management system, horses on occasion simply appear drowsy for the first hour or so after harvest.

Approximately four days are reported to be required after the onset of haemorrhage for the bone marrow to release increased numbers of mature red blood cells into the circulation in the horse and other animals (Valli, Lumsden, Carter and McSherry, 1975, cited by Becht, 1986). Results obtained in this study revealed that for these horses after day 4 post harvest group means for PCV, RCC and Hb and, to a lesser extent, platelets and WCC showed gradual increases towards the original, preharvest values.

Eight out of the 10 horses monitored during the interharvest study never regained preharvest values for PCV, RCC or Hb prior to the subsequent harvest. Values for these parameters were, however, maintained within normal ranges. It was thought that the PCV and RCC values would probably be elevated for these horses at the harvest point owing to splenic contraction as a result of excitement and apprehension. PCV is the parameter currently monitored at harvest under the routine management system. It must be borne in mind that any excitement prior to or during blood harvest will influence red blood cell parameters, and low values could easily be masked. Ideally, therefore, a full haematological examination would be carried out, but at present only PCV is measured, as this can be quickly performed in the farm laboratory. Adequate haematological monitoring is being provided by PCV assessment under the current management.

It has been reported that between 30 and 60 days are often required after severe anaemia from blood loss for red cell parameters to return to normal in the horse (Becht, 1986). For these horses the degree of blood loss which occurs regularly does not appear to induce anaemia. Fluctuations were small for the red blood cell indices: MCV, MCH and MCHC over the 21 days, but these are claimed to be of little value in equine clinical medicine (Becht, 1986). Values for MCV lay towards the upper range of normal values, suggesting immaturity of red blood cells, although these values did not give grounds for concern.

Sex, breed and length of time in blood production did not influence patterns of recovery between the two harvest points for PCV, RCC or Hb.

In summary, the studies have been reassuring in that the clinical condition of these animals, which are managed for regular, repeated blood harvest, is consistently good. The haematological profiles show regular, cyclic changes between harvest points, but these fluctuations are safely within normal ranges for all haematological parameters when one considers the range of types of horse within the herd. The collection of 8 litres of blood from these horses on a regular three week cycle did not therefore

adversely affect their welfare, clinically or haematologically. It was evident from statistical analyses of values for PCV, RCC and Hb over a period of five harvest cycles, however, that values were consistently higher for the Thoroughbred animals in the herd than for non-Thoroughbreds.

The recovery patterns found for PCV, RCC and Hb over five harvest cycles, and the statistically significant influence of breed on these patterns, provided valuable guidelines for commercial blood production. It was thought that 8 litres of blood could probably be removed on a more frequent basis from the Thoroughbred animals without adverse clinical or haematological effect, perhaps every two weeks rather than every three weeks. Taking into account the PCV, RCC and Hb recovery patterns, and the literature on differences in blood volume between breeds, the possibility of increased frequency of regular blood harvest was not considered for the non-Thoroughbred horses. The results of the studies therefore also supported the belief of the farm owner that, in general, the Thoroughbred animal is more suited to commercial blood production than are the heavier types of horses in the herd.

SECTION 4. THE CREATION OF A MANAGEMENT EQUINE DATABASE

Introduction

The Requirement for a Database

This group of horses is surely unusual for several reasons. It is uncommon in the U.K., and especially in Scotland, to encounter a herd of this size; and less common still to discover horses managed as a group throughout grazing and housing periods, as are these animals. The feeding and the stabling regimes are atypical of the way horses and ponies are generally kept in Britain, they more resemble the routines employed for cattle. Equally, the purpose for which these animals are managed, that is for the production of blood products, is not a common industry. We are unaware of any other commercial blood producers in Scotland, and there are but a few in the U.K. as a whole (personal communication from the owner of the herd).

The management of a horse group of this size is a daunting task, from the point of view of the herd size, but perhaps even more so from a welfare point of view. The business of production of blood products is reliant on a source of horses for purchase, but also relies on the loaning of horses from the public. The people involved, the owner and the staff on the farms, are aware of the necessity of efficient management to protect the health and wellbeing of the horses. The premises are licensed by the Home Office and are regularly visited by appointed Veterinary Inspectors.

The volume of data which requires to be collected to monitor a group of animals of this size is enormous. Approximately 45 to 50 horses are harvested every week, and so large amounts of information are generated very quickly. Reference to historic data can be very time-consuming under these circumstances, and space for storage may become problematic.

In order to monitor the efficiency of blood production there must be regular accurate recording of information for individuals. This is essential, to build up a picture for the herd as a whole. Effective collection and storage of such information aids the selection of animals for the herd, and it identifies which animals

are performing best. It also identifies early those horses which are not suited to the group system of management and to the practice of regular, repeated blood harvest.

The owner of the herd required a simple, efficient system for storage of data and one which would allow analyses of information to further improve the existing management system. The aim was thus to create an equine management database, which was to be tailored to the needs of the herd and the owner, and which took into account the unique features of the existing operations.

It was intended to reach a point where, by collecting specific information for each horse regularly, data for that individual could then be extracted and analysed to provide a performance profile which could be compared to profiles of others in the herd. Groupings for performance could then be made, and management tailored accordingly.

Analyses were then planned on collected data to investigate influence, of breed or type, sex, age and length of time in blood production, for examples, on the performance figures. Seasonal and dietary influences were to be examined; as well as the farm on which the horse was grazed, and where it was harvested were to be compared.

Established Databases

A database is a computerized record-keeping system, the purpose of which is to maintain information and to make it available on request (Date, 1986). Databases may be designed to provide in-depth examination of data, and to facilitate updating when more information is collected (Kock, Clark and Jessup, 1989). The system user may add new data to, or retrieve data from, existing files; and data may be updated or deleted in existing files. New files can be added to the system and existing files may be permanently removed. The components of a database system are the data, the hardware, the software and the users. Advantages of such a system are compactness, speed, reduction in drudgery, and currency (Date, 1986). The operation of databases by menu allows rapid access to data without the need for extensive computer

knowledge or experience (Kock, Clark and Jessup, 1989). Microcomputer spreadsheet packages can be utilised to allow analysis and graphics programs allow visual display of data (Kock, Clark and Jessup, 1989).

Databases are employed in several aspects of veterinary medicine. They have been used, for instance, to develop animal disease data banks (Jamaluddin, Chang, Johar and Yaacob, 1988). The CONSULTANT database, for example, contains information on over 6,000 diseases of dogs, cats, horses, cattle, sheep, goats and swine; and is used by hundreds of veterinary practices and institutions across North America (White, 1988).

Databases also allow interpretation of biological data from wildlife (Kock, Clark and Jessup, 1989). Provision of sources of information in the fields of animal health and production have been achieved (Giovannetti and Meissonnier, 1987). Systematic data storage and retrieval programmes were developed for toxicological and hazardous material case information (Hyde, Schlotfeldt, Schmidt and Stahr, 1983); and a food-animal residue avoidance databank (FARAD) was created by research workers at five U.S. veterinary schools in 1986.

BOVID, Bovine Information and Diagnosis System, is a veterinary computer program which is designed to help the practising veterinary surgeon in the diagnosis of diseases of cattle (Blood, Brightling and Larcombe, 1989). A collection of possible diagnoses are produced in response to the input by the vet of clinical findings, epidemiological patterns and clinico-pathological findings in individuals or in groups of cattle. The diagnoses are listed in order of diagnostic probability, with a numerical probability estimated for each. The primary advantages are that the information provided is current and comprehensive, based on Australian data, but there are future plans to include inputs from the United Kingdom and North America (Blood, Brightling and Larcombe, 1989).

The BENCHMARK database system was developed as the result of a three year study designed to monitor the health and production of

the beef cow industry in Ontario, as a component of efficient food production in Canada (Martin, Lissemore and Kelton, 1990). The system involved cooperation of farmers, private veterinarians, government personnel and university veterinarians. The Veterinary Services of the United States Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) created the National Animal Health Monitoring System (NAHMS). This database is founded on the collection of data and biological specimens, from a statistical sample of United States herds and animals, by government veterinarians and animal health technicians (Pointon and Hueston, 1990). The aim of the system is to protect and improve animal and human health, while guaranteeing quality and quantity of animal-derived food and fibre, and making the U.S. agriculture more competitive (Pointon and Hueston, 1990).

A clinical database was constructed as a consequence of a nationwide survey carried out by the British Cattle Veterinary Association (BCVA) to investigate the circumstances under which Caesarian sections were performed on cattle (Gettinby, Thorpe and Anderson, 1989). The database was developed from two sections of a questionnaire, one section involved basic animal details and details of the operation, including outcome; while the second concerned subsequent fertility. Analyses are underway on the information contained in this database (Gettinby, Thorpe and Anderson, 1989).

Examples of management databases include COSREEL (Computer system for Recording Events affecting Economically important Livestock) in the U.K., which has been designed for the management of cattle, sheep and pigs at the Agricultural Research Council Institute for Research on Animal Diseases (Russell and Rowlands, 1983). In Africa, the International Livestock Centre for Africa (ILCA) developed, between 1984 and 1986, the ILCA Data Entry and Analysis System (IDEAS) to record and analyse all the important performance traits for a wide range of livestock species, and store information on health, nutrition, climate and management aspects (Wissocq, Durkin, Trail, Gettinby, Bell, Berhane and Light, 1989). Equine management databases in the literature are concerned with stud work. The use of a microcomputer was tested for improving the

stud management and raising conception rates by Rohn and Reinhard (1985). The microcomputer evaluated the management and calculated target dates for mating or insemination, for teasing and gynaecological examination. Plans overviewed the stallions and mares with their relations, and dates of each mare, plan of foaling, overview of pregnant mares and plans for various vaccinations. Conception rate increased by 28% over two years of operation of the database system (Rohn and Reinhard, 1985).

The Benefits of Management Databases

The benefits of using management databases are diverse. Relationships between variables that affect performance and results are identified, and variables can be made and monitored on a continuous basis (Bywater and Goodger, 1985). The use of such systems may make the manager into a data user, rather than a data recorder, and the focus of management could be changed. Interest in relating control variables to production will probably increase; and the facility for calculation may provide qualitative answers to performance questions, rather than generalisations. A multidisciplinary approach to management and decision-making may be promoted by the use of a database (Bywater and Goodger, 1985).

Materials and Methods

Herd Details

The horses are part of a large herd, of between 150 and 200 animals, which is managed for blood products in the West of Scotland. The animals are harvested on a regular cycle, of three weeks duration. The details of the horses and their management system are given in the general Materials and Methods section.

Data Currently Recorded

At present on the farm there is a microcomputer, situated at Burnhouse Farm. Data recorded for general management include the name and identification number of each horse, its age, breed, sex, and height. The source of acquisition of the animals are stored on file, along with record of whether the horse is on loan from a member of the horse owning public, or whether it has been

purchased by the owner of the farms. Any other relevant details of health or temperament of horses are noted.

With respect to management for blood products, at each harvest point the PCV for each individual is recorded, in addition to the volume of blood collected, the percentage lost in clotting (waste), and the volume dispensed from each harvest. The average packed cell volume (PCV) for the herd for the week is recorded to allow monthly and annual production figures to be calculated. The date on which the next harvest is due for each horse is projected.

Recording System and Analyses Performed

The details for each horse were stored in the microcomputer using a simple database system. Several simple calculations were undertaken longhand by the owner, for example weekly and monthly blood volumes for the herd as a whole; and retrospective comparisons were made of these totals to totals for previous years.

Details taken at the time of blood harvests were collected in a notebook, and preprinted daily sheets were used to record information once the blood was transferred to the dispensing laboratory.

Databases

A database is a structured collection of data, stored in a consistent fashion within records which have a common format (Gettinby, Thorpe and Anderson, 1989). In a relational database the data are contained in two-dimensional tables, in which entries are not repeated and must all be of the same form, with every column and row in the table being unique (Thrusfield, 1986). Relationships between the records are not determined until they become operational, and so they may be constantly altered, which makes this type of database very simple and flexible (Thrusfield, 1986).

Several important features are afforded by an electronic database, namely the facilities of range checks, lookup, query and transaction (Gettinby, Thorpe and Anderson, 1989). Normal ranges

for values for PCV were built into this database, to emphasise any abnormality to the manager, and to ensure accuracy of information.

The lookup facility ensures that the data entry is valid by comparing it to a limited collection of legal entries. Mistakes and errors in typing, which could result in the failure of accurate extraction of information, are thus prevented. Lookup was used in the static records within this database, for example, in the field for gender, where only f, or female, and g, or gelding, were legal; and in the breed field, where cob, TB (Thoroughbred) and mid (crosses between cobs and Thoroughbreds) were acceptable entries.

The query facility allows rapid, accurate extraction of data using a query language, and is the most valuable asset of the database. The query command [gender] = f and [height] > 16.0 hands could be entered, for example, in this database to identify all the female horses over 16.0 hands in height.

Different databases may share information within a relational database, and the transaction facility permits linkage of information by a common field. In this database the common field was the animal identification number, which allowed the manager to move between the three databases, the static records, the bloodroom records and the laboratory records.

Software Tools

There are several proprietary software packages available which provide data management options to simplify the construction and analysis of electronic databases (Gettinby, Thorpe and Anderson, 1989). The software package used for the database in this thesis was the Smart system, Version 3.1 from Informix Software, which was selected because it is integrated to allow the use of spreadsheet, wordprocessing and graphics facilities. The earliest packages, such as dBASE by Ashton Tate, for example, do not offer this integration.

Results

Overall Structure of the Equine Management Database

The equine management database was designed so that information was initially entered into one of three databases. Horse identification details were entered into the Static Record in the first database. Each horse was treated as a separate record, and each record consisted of nine fields. These fields were horse number and name; its gender (female/gelding); its age, in years; its type, which was subdivided into cob, Thoroughbred, or crosses between the two (cob/TB/mid); whether it was on loan or purchased (O/P); its colour; its height, in hands (< 15h/ 15 - 15.3h / >16h), and the date on which it was acquired.

The second database contained information from the blood harvest room, with eight fields, with one each for the animal number and name; on which farm blood was harvested from the animal (Burnhouse or Gartmorn Hill Farms; B/G); the date of blood harvest; the volume taken; the flow of the blood during harvest (good/poor); and the fraction of blood to be dispensed (defibrinated blood, D, or serum, S). There was also a field for comments, such as the appearance of the blood, or the temperament of the horse during the harvest.

Information from the farm laboratory was collected in the third database. The eight fields contained within this database took account of the laboratory batch number; animal number and name; the volume of blood collected (in litres); the volume of blood dispensed (in litres); the PCV of the blood (as %); and the date of the end of the week of the blood collection. The amount of blood which was lost during clotting (% waste) for each animal was automatically calculated by the computer, from the fields for volume of blood collected and the volume dispensed.

The screen layouts, containing sample data, for the static record, bloodroom and laboratory databases are shown in Figures 37, 38 and 39, respectively.

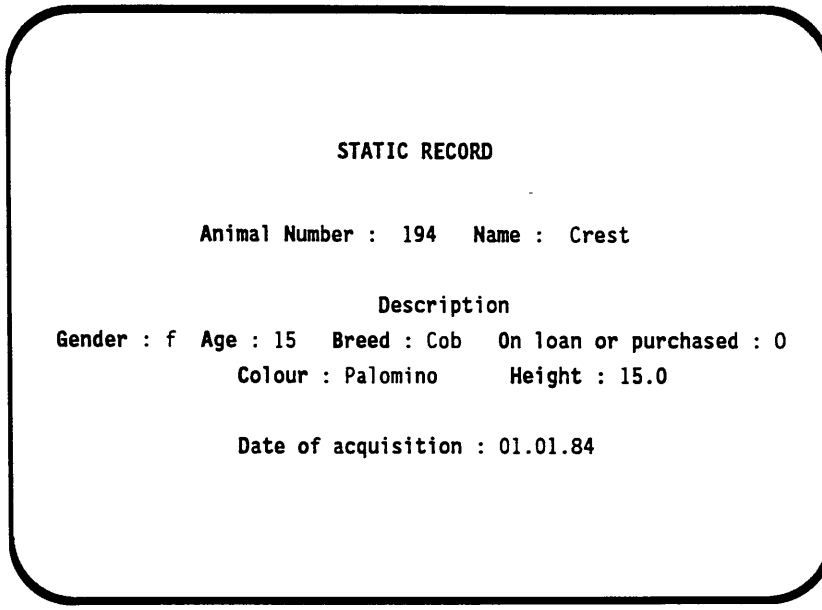

Figure 37. Screen layout for the static record database

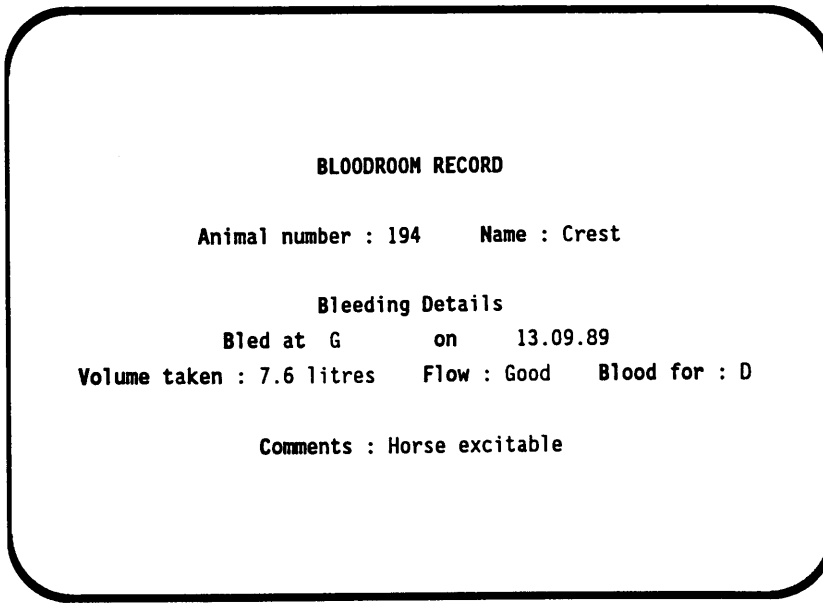

Figure 38. The screen layout for the bloodroom record database

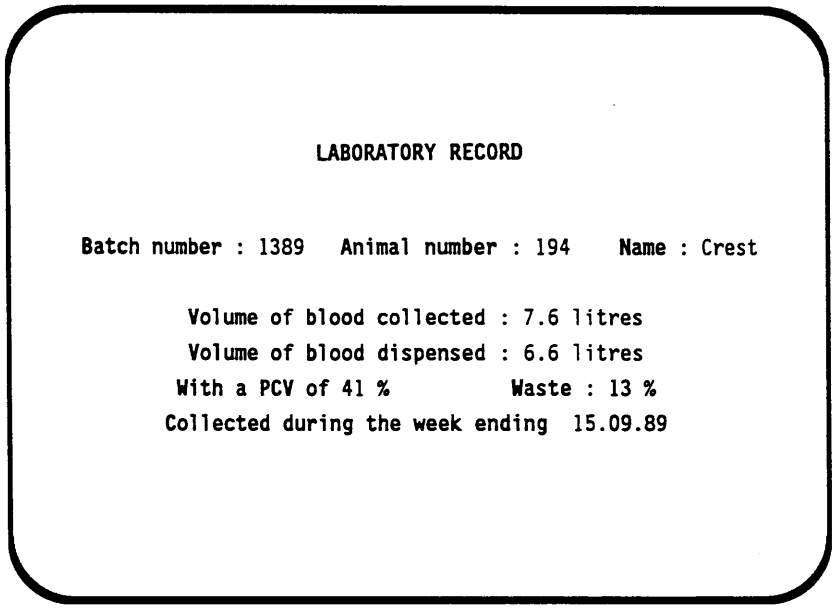

Figure 39. The screen layout for the laboratory record database

The database was operated by a menu system which allowed rapid access to the information without great knowledge or experience of the computer. When the database program was loaded, the operator used the "EXECUTE" command, then was given the options of "ENTER", to add, modify or delete records; or "ANALYSE", which allowed analysis of the information contained within the databases. The third option was the "FINISH" command, which was used to escape from the program.

On selection of either the "ENTER" or "ANALYSE" commands, the user was then asked to select which of the three databases were required: "STATIC" for the animal details; "BLOODROOM" for the bloodroom record database; or "LABORATORY", to access the laboratory record database.

There were two options available within the analysis facility of this database. In the menu the operator was offered the option of analysing "STOCK", the data for the herd as a whole, or analysing "ANIMAL", the analysis of data for a particular animal which was selected by the input of its animal number. The information displayed on the screen in response to the "STOCK" and "ANIMAL" commands are demonstrated in Figures 40 and 41, respectively.

The stock balance analysis record produces three fields of data output for the horse herd as a whole, namely the total volume of blood collected to date, the total volume dispensed to date, and the average waste.

The fields for total volume of blood collected, total volume dispensed, and average waste are also produced in response to the "ANIMAL" analysis command, and in addition, the number of blood collections made and the number of quality control failures are calculated for the individual horse.

The overall structure of the equine management database is presented diagrammatically in Figure 42.

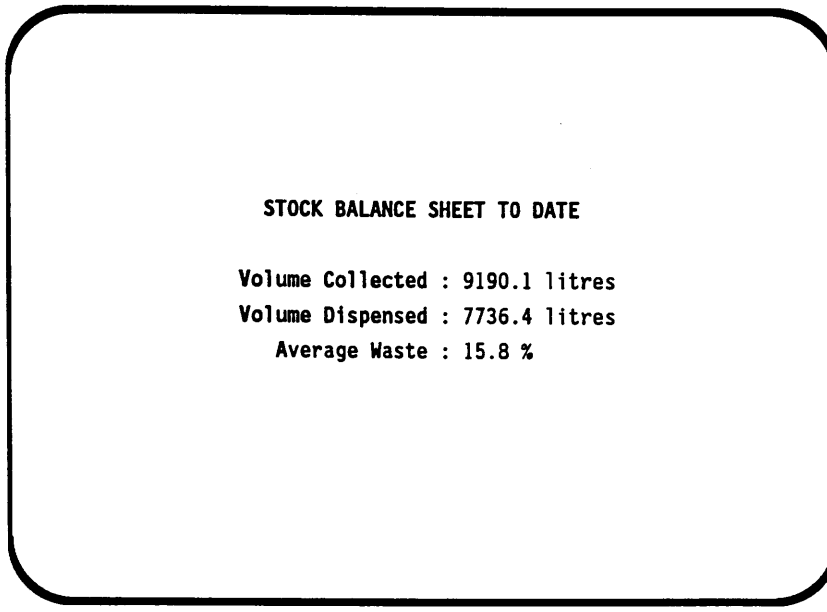

Figure 40. The screen display obtained by selecting the "STOCK" command in analysis within the equine management database

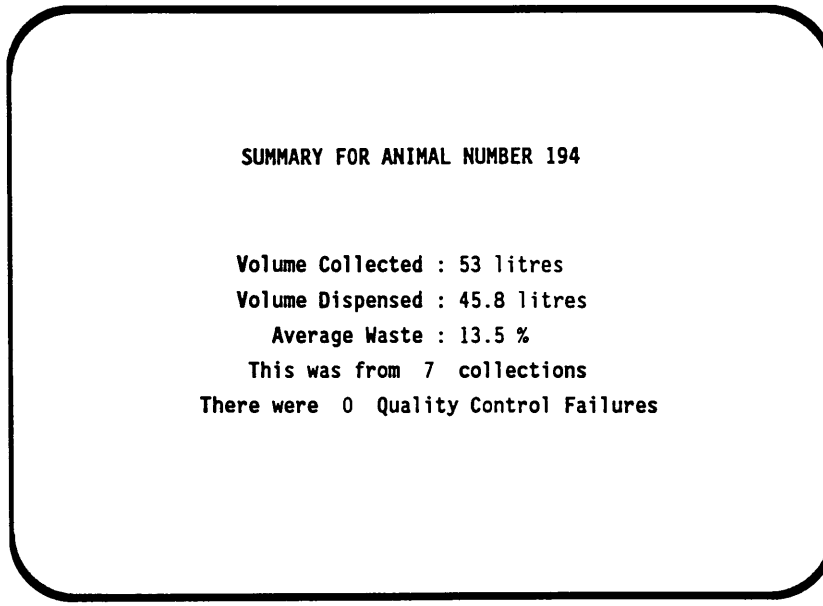

Figure 41. The screen display obtained in response to selection of the "ANIMAL" command in analysis within the equine management database

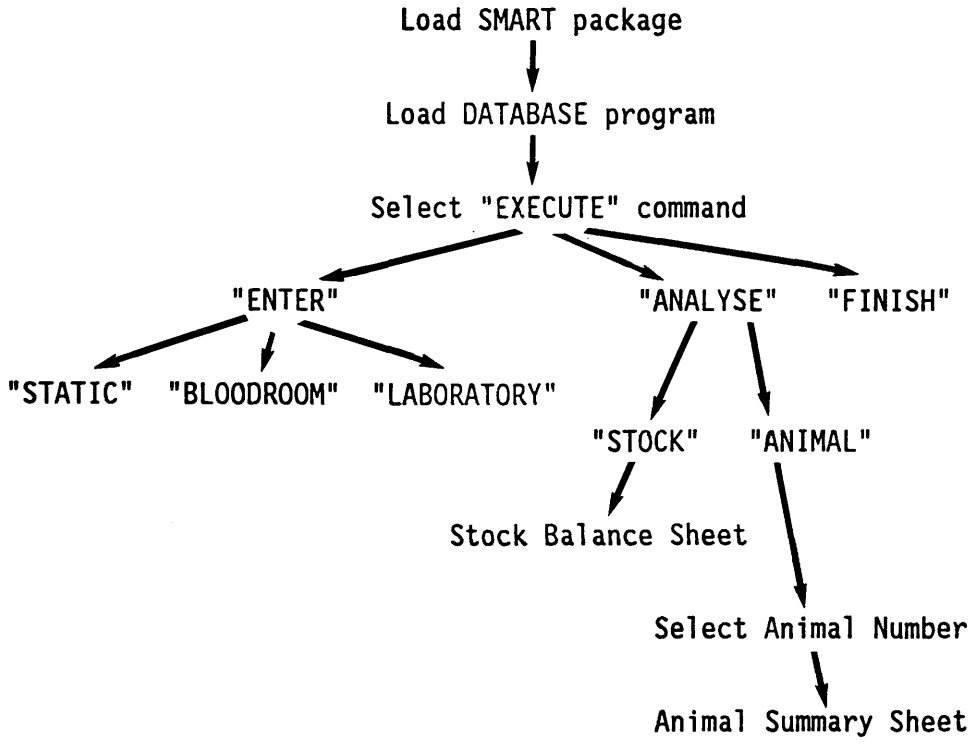

Figure 42. The overall structure of the equine management database

Status Implemented

At the time of writing this thesis, the database contains 1272 records. The data from all blood harvests on the two farms between July, 1989, when the farm laboratory was set up, and April 1990, are recorded.

After entering information into the database for several months, and as a result of discussion with the manager of the farms during this time, a requirement for several working modifications was identified. The aim was to keep the system as simple as possible, and it was thought that the bloodroom and laboratory records could be combined. The modified database was to contain the fields of the laboratory record, namely laboratory batch number, animal name and number, volume of blood collected, volume dispensed, PCV, and waste; and fields for the location of blood harvest, flow of blood at harvest, and blood fraction dispensed, were to be added. The format of the date of harvest was to be altered, from the day, month and year layout currently adopted to the week number, from one to 52, with week one as the first week in January. This modification was aimed to make the comparison of performance figures within the database to those of previous years easier for the manager. Weeks had been numbered in this fashion, from one to 52, to record information collected in previous years.

The lookup facility had been introduced for the breed and height fields on the static records as a modification of an earlier database, to simplify entry of these data. Similarly, the addition of a field for animal name as well as for animal number was the result of modification of an earlier record. The change was instigated because the farm staff found identification of the animal in the blood harvest room easier by horse name than by number. In the original static record there were fields for horse weight, and for the source and reason for acquisition. The weight field was considered unnecessary and was therefore abandoned. The manager prefers to record the information on acquisition of animals in a separate logbook, because the information is often required at locations where there is no microcomputer terminal. These fields were also deleted.

The recording of information for the database by the manager is consistent now that the records have been tailored to her needs, and any irrelevant fields on records have been removed. The data recording is thus kept brief and simple, which is essential in this farm situation, as different members of staff will be involved in recording and in the input of information to the database.

Discussion

The database as developed is a management tool. It was not designed specifically for scientific investigation although suitable information is contained within the database. After consideration of analysis it has become clear that there is a need for a new way of linking or transacting the records. In analysis of performance data, the fields of breed, blood volumes collected and dispensed, and PCV, should be recorded within the same record to allow statistical correlation using the spreadsheet within the Smart software package. At the moment, while the animal number is the only field which permits transaction, this is not possible. The field for breed must be incorporated into the combined bloodroom/laboratory record, also become a linking field for these analyses to become possible.

The manager of the horses has identified several areas for future development of the database. An additional "out-of-circulation temporarily" record is requested. This would contain data for young or brood animals, animals which are used for riding or driving, and horses which are temporarily on loan from the farm for any reason. Information from these horses, which would be removed temporarily from the blood producing herd, could then be kept separate from that for the blood horses.

A facility for slotting individual animals into established seven, 14 or 21 day patterns for bleeding is required by the manager.

Ideally, summary herd statistics and summary animal statistics would be able to be called up in different ways for analyses. At

present, summary herd data analyses are calculated using every entry in the database. Subsequently there is no facility to extract and analyse data for a particular week, or for a particular month for the herd. If information could be analysed according to week number, then comparisons could be drawn between herd performance in successive weeks, months, or between housing and grazing. Performance might be assessed on volume of blood collected and dispensed, or on the percentage average waste recorded. Targets might then be introduced for these parameters, to attempt to improve herd production.

If data could be extracted and analysed for individuals according to key fields, then widespread statistical examinations would be possible within the herd. The influence of breed, sex, age, and length of time in blood production on performance could be investigated, for instance. It would be interesting to compare blood volumes collected over a period of two months, or over six collections, from geldings and from mares. The percentage waste from animals which had been bled for less than five years might be analysed, in contrast to waste from those which had been used for commercial blood production for longer than five years. Volumes of blood collected from horses grazing at Burnhouse Farm over the summer months might be analysed to see if they were more or less productive than those which were at grass at Gartmorn Hill Farm. The permutations for analyses would be numerous.

It might be possible to use results of summary animal data to modify herd management. The herd could perhaps be divided into smaller groups, according to level of performance. Bracketing average blood collection volumes as high, medium or low would permit subgrouping of the herd; and the feeding regime, for example, might be altered to supplement those animals for which poor collection volumes were recorded.

Horses which are consistently performing poorly under this management system might be readily identified using the range check facility within this database system. Limits for action could be decided by the manager and inbuilt into the system for parameters such as volume of blood collected, or PCV value. If

values for an individual for these parameters were repeatedly below this limit, then corrective action would be required. The horse might have to be removed from the harvest group and rested for several weeks, for example.

In this respect the database system could be regarded as a tool to address any queries regarding the welfare of this herd, in addition to being a management tool.

GENERAL DISCUSSION AND CONCLUSIONS

Skin disease, and in particular bacterial skin disease, was highlighted in this thesis as an area which has received little attention in recent years. As such, it gives grounds for concern with respect to the welfare of horses in the U.K.. Bacterial skin disease is often misdiagnosed and difficult to manage, resulting in undue animal suffering and the prevention of useful work. Attention was focused on dermatophilosis, which is one of the commoner bacterial skin conditions of horses and ponies in the West of Scotland. It is a condition to which outwintered animals, therefore the majority of the equine population in Scotland, are particularly vulnerable. Prolonged exposure to excessive wetting predisposes to infection, which may then be manifest in recurrent annual outbreaks and which may be transmitted to other animals and to man. These circumstances warranted investigation of an alternative approach to management of dermatophilosis in horses.

Essential fatty acids (EFAs) were investigated in this respect. The role of EFAs, in the form of evening primrose oil (EPO) which contains n-6 series linoleic acid (LA) and gamma-linolenic acid (GLA), with added fish oil containing n-3 series eicosapentanoic acid (EPA) and vitamin E, and the success of their use in the management of skin disorders in man and in companion animals were reviewed in this thesis. LA and GLA are known to be essential in the maintenance of the integrity of the impermeability barrier formed by the skin to water. EFAs also modulate the immune response, and the supplementation of LA and GLA increases formation of anti-inflammatory metabolites while inhibiting the formation of pro-inflammatory metabolites. EFAs have been shown to be free from adverse events, and are non-carcinogenic. The pharmacokinetics of EFAs were studied in the horse for the first time, and the possible role of EFAs in the management of equine dermatophilosis was investigated.

Baseline information on the metabolism of EFAs in horses was obtained in a pharmacokinetic study. LA must be converted to its metabolites to exert the full range of biological actions of EFAs (Horrobin, 1990b). The conversion of LA to GLA is the first and the rate limiting step in the EFA metabolic cascade, and is controlled by the 6-desaturase enzyme. Considerable species

variation is known to exist in the rate of this conversion, as indicated by the ration of LA to its metabolites. The reaction rate is known to be fastest in the rat, and slowest in the rabbit and the guinea pig (Horrobin, 1990b). It was found in the horse to be very slow, comparable to that in other herbivores such as the rabbit or guinea pig, and was very much slower than the reaction in dogs, cats or in man. This was attributed to slow 6-desaturation of the LA by the 6-desaturase enzyme.

A daily dose regime of 20g of 80% EPO and 20% fish oil and vitamin E (Efamol Marine), providing n-6 and n-3 series EFAs, was adopted from the results of the pharmacokinetic study, to allow maximum opportunity for EFA uptake. This dose was used in consequent controlled studies to investigate the use of EFAs in the treatment, then in the prophylaxis, of dermatophilosis in horses.

During the pharmacokinetic study no influence was recorded on the condition of the coat, mane, tail or hooves, or on general body condition which could be attributed to EFA treatments. No adverse influence was recorded on haematological and biochemical parameters by the various doses of n-6 and n-3 EFAs administered to the horses.

In a placebo-controlled, double blind treatment study no significant effect was seen on the severity or on the extent of distribution of lesions of dermatophilosis when horses received 20g oral n-3 and n-6 EFAs daily. No significant improvement was afforded by EFAs on the condition of the coat, mane, tail or hooves, nor on general body condition. No influence, adverse or beneficial, was exerted by EFAs on haematological or biochemical parameters.

When the use of EFAs in treatment of dermatophilosis in horses was found to be unsuccessful, a controlled experiment was designed to investigate the role of EFAs in the prophylaxis of the disease. The study spanned the period of high rainfall in the autumn and winter months when the risk of developing the disease is highest in horses in the U.K.. The period of supplementation of EFAs was begun before the horses were exposed to the prolonged wetting

which is thought to damage the epidermis, predisposing to dermatophilosis. The treatment was thus provided before dermatophilosis lesions developed, while the epidermis was intact. EFAs did not prevent the development of lesions, nor did they reduce the incidence of infection. As in the pharmacokinetic and treatment studies, no improvement in the condition of the coat, mane, tail and hooves or general body condition was seen; and no significant influence was exerted on haematological or biochemical parameters by EFAs.

Two possibilities were considered to account for the failure of EFAs in the management of equine dermatophilosis, and the failure to improve clinical condition in the horse. Firstly, the dose rate may not have been sufficient. A higher dose rate, however, would be impractical to administer orally and would prove a very expensive treatment. The horses which are at greatest risk from dermatophilosis infection are usually those which are poorly managed, and where little financial consideration is given to the animals. Under these circumstances expensive treatment is unlikely to be viable.

Alternatively, EFAs may not be as important in the horse as they have been shown to be in other species. It may be that this is reflected by the slow 6-desaturase reaction rate found in this species.

EFAs were not found to be harmful in the horse. No adverse effect was recorded on any parameter for any horse by EFA or placebo treatments administered during the studies. Although no influence was believed to be exerted on haematological or biochemical parameters by EFAs during the studies, the monitoring of such parameters has provided a useful database of haematological and biochemical information for healthy horses.

The characteristics of *D. congolensis* were examined in relation to the site and severity of lesions of dermatophilosis. No correlation was found in this respect. Although similarities between isolates were identified, by examination of differential bacteriological growth characteristics and by sodium dodecyl

sulphate polyacrylamide gel electrophoresis (SDS-PAGE), all the isolates of *D. congolensis* investigated were different.

Proteolytic enzyme production by *D. congolensis* was investigated with regard to the virulence of the organism. No keratinase, collagenase or elastase activities were detected. Several isolates did, however, demonstrate extracellular protease activity. The results of the study suggested that two proteases were produced. One, estimated to be of 110 kD, demonstrated caseinase activity which was evidenced as precipitation of caseinate on agar, and gelatinase activity, detected using SDS gelatin-PAGE. The other, a protease of lower molecular weight, demonstrated caseinase activity. In this work, the production of protease activity could not be used as a marker of virulence because it was not produced by every isolate which induced clinical lesions of dermatophilosis. Further investigation is required into the possible implication of protease activity in the disease process of dermatophilosis.

The horses under study in this thesis were kept for commercial blood production and as such were subjected to regular, repeated blood harvest. The clinical and haematological consequences of the practice of bleeding horses regularly have been particularly neglected and this herd provided an unique opportunity to monitor the welfare of animals which are involved in such practices. The clinical, protein and haematological profiles of a group of animals within the herd were followed over five harvest cycles, leading to a more detailed analysis of haematological data obtained from a smaller number of horses between two harvest points. Cyclic patterns of recovery were established for haematological parameters, but values remained within normal ranges at all times. The animals were carefully attended and well fed, and consequently the horses were in excellent body condition. No adverse effect was recorded on clinical, protein or haematological profiles when 8 litres of blood were removed from the horses every three weeks. Reassurance was thus provided that there were no adverse clinical and haematological consequences of regular, repeated bleeding in these horses under their current management system.

Statistical analyses revealed that Thoroughbred animals, which have higher blood volumes, supported repeated bleeding better than non-Thoroughbred animals: red cell parameters were always significantly higher. Based on this information, and the recovery patterns for the haematological parameters, it was thought that 8 litres of blood could be removed from Thoroughbreds more frequently without adverse effect. A harvest frequency of two weeks was therefore suggested for the Thoroughbreds under this management system, but not for the other animals in the herd.

A relational management database system was created for a microcomputer, as a management tool for the manager of the horse herd. An integrated computer software package provided the facilities of spreadsheet, wordprocessor and graphics.

The overall structure of the system comprised three databases which contained information on horse details, bloodroom records and laboratory records, respectively. The manager was afforded flexibility to move between these databases, which were linked by the animal identification number. Information contained within the fields of these records could be constantly updated, and there was provision for analyses of data, either for the horse herd as a group, or for an individual animal.

At the time of writing the database contained 1272 records, and analyses of these data had begun. In working with the system, areas which required modification were identified and relevant alterations were made. The database as developed is a management tool. It was not designed for scientific investigation although within it suitable information is contained for such examinations.

The potential merits of the database as a management tool are highlighted for this horse herd. The widespread benefits afforded by such a system could be appreciated by others, as the database may be adapted to numerous aspects of the management of groups of horses. From the welfare aspect, the database could be adapted to detect quickly any animals which were performing poorly and any which became anaemic. This would allow corrective action to be instigated promptly, thus avoiding any undue animal suffering.

While a cure was not found for dermatophilosis, it is hoped that by highlighting the problem and developing management tools for monitoring equine health and wellbeing that this thesis has contributed to the welfare of horses in the U.K..

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APPENDIX I

TABLES OF RESULTS OF THE STUDY ON THE PHARMACOKINETICS OF EFAs IN HORSES

Clinical Indices, Haematological and Biochemical Parameters

Table I

Coat condition scores

Scored on a 1 to 10 scale, 1 is poorest, 10 is optimal

Horse		Week 0	3	6	
5g EPO daily					
Annabelle		7	7	7	
Donald		4	6	6	
Myrtle		4	6	6	
Calumn		6	8	8	
Giraffe		7	4	6	
Gunsmoke		3	4	7	
Soyereign		4	4	7	
Orlando		5	6	7	
Jack		6	8	8	
Nicholas		4	5	8	
Suzie		4	4	6	Group
Hermione		6	7	8	mean
	Mean	5.0	5.8	7.0	5.9
	S.D.	1.3	1.5	0.9	
10g EPO alternate day					
Charlotte		5	5	5	
Marigold		6	6	6	
Jasper		5	5	5	
Ding Dong		6	6	6	
Flora		4	4	4	
Chocolate		5	5	5	
Goldie		4	4	4	
Durando		6	6	6	
BeBe		6	6	6	
Samuel		5	5	5	
Jordan		5	6	6	Group
Dick		6	8	8	mean
	Mean	5.3	5.5	5.5	5.4
	S.D.	0.8	1.1	1.1	
20g EPO daily					
Foxy		3	5	6	
Wallace		5	5	8	
Gold Loch		5	5	6	
Bonny		4	5	6	
Skippy		6	7	7	
Flynn		5	4	6	
Rocky		5	7	7	
Charlie		6	7	6	
Geno		5	7	7	
Biggles		5	5	7	
Smiler		4	5	6	Group
Aird		6	7	7	mean
	Mean	4.9	5.8	6.6	5.8
	S.D.	0.9	1.1	0.7	
40g EPO alternate day					
Karlops		4	8	8	
Big Ben		5	7	7	
Walter		5	8	8	
Pollux		5	6	7	
Armpit		4	5	6	
Sandy Lad		4	6	6	
Sabrian		6	7	8	
Ollie		4	7	7	
Poppett		3	5	6	
Tessa		5	5	5	
Alfred		5	6	7	Group
Darkie		3	7	8	mean
	Mean	4.4	6.4	6.9	5.9
	S.D.	0.9	1.1	1.0	

Table II

Mane condition scores

Scored on a 1 to 10 scale, 1 is poorest, 10 is optimal

Horse		Week 0	3	6	
5g EPO daily					
Annabelle		6	6	6	
Donald		5	7	6	
Myrtle		5	7	6	
Calumn		4	7	7	
Giraffe		4	4	5	
Gunsmoke		4	6	6	
Soyereign		4	6	6	
Orlando		5	6	6	
Jack		5	6	7	
Nicholas		5	6	6	
Suzie		4	4	4	Group
Hermione		5	6	7	mean
	Mean	4.7	5.9	6.0	5.5
	S.D.	0.7	1.0	0.9	
10g EPO alternate day					
Charlotte		5	6	7	
Marigold		6	6	7	
Jasper		5	6	6	
Ding Dong		6	7	6	
Flora		4	4	6	
Chocolate		5	6	6	
Goldie		5	6	6	
Durando		5	6	6	
BeBe		6	7	6	
Samuel		5	6	7	
Jordan		5	6	6	Group
Dick		5	7	8	mean
	Mean	5.2	6.1	6.4	5.9
	S.D.	0.6	0.8	0.7	
20g EPO daily					
Foxy		5	6	6	
Wallace		3	5	7	
Gold Loch		5	5	6	
Bonny		3	6	7	
Skippy		5	7	6	
Flynn		5	5	7	
Rocky		5	6	6	
Charlie		5	7	6	
Geno		5	7	6	
Biggles		5	5	5	
Smiler		5	6	7	Group
Aird		6	6	6	mean
	Mean	4.8	5.9	6.3	5.6
	S.D.	0.9	0.8	0.6	
40g EPO alternate day					
Karlops		5	6	7	
Big Ben		5	6	6	
Walter		5	7	7	
Pollux		4	5	7	
Armpit		4	4	6	
Sandy Lad		3	4	5	
Sabrian		5	6	6	
Ollie		5	7	8	
Poppett		3	4	6	
Tessa		5	6	6	
Alfred		5	6	6	Group
Darkie		4	7	7	mean
	Mean	4.4	5.7	6.4	5.5
	S.D.	0.8	1.2	0.8	

Table III

Tail condition scores

Scored on a 1 to 10 scale, 1 is poorest, 10 is optimal

Horse		Week 0	3	6	
5g EPO daily					
Annabelle		6	6	7	
Donald		5	6	6	
Myrtle		5	7	7	
Calumn		5	7	7	
Giraffe		4	4	6	
Gunsmoke		4	6	7	
Soyereign		4	4	7	
Orlando		5	6	7	
Jack		5	7	7	
Nicholas		5	6	7	
Suzie		4	5	5	Group
Hermione		5	6	7	mean
	Mean	4.8	5.8	6.7	5.8
	S.D.	0.6	1.0	0.7	
10g EPO alternate day					
Charlotte		5	6	7	
Marigold		6	6	7	
Jasper		5	6	6	
Ding Dong		6	7	6	
Flora		4	6	7	
Chocolate		5	6	7	
Goldie		5	6	7	
Durando		5	6	7	
BeBe		6	7	7	
Samuel		5	6	7	
Jordan		5	6	6	Group
Dick		5	7	8	mean
	Mean	5.2	6.3	6.8	6.1
	S.D.	0.6	0.5	0.6	
20g EPO daily					
Foxy		5	6	7	
Wallace		3	5	8	
Gold Loch		5	5	6	
Bonny		3	6	7	
Skippy		5	7	7	
Flynn		5	5	7	
Rocky		5	6	6	
Charlie		5	7	7	
Geno		5	7	7	
Biggles		5	5	6	
Smiler		5	6	7	Group
Aird		6	6	6	mean
	Mean	4.8	5.9	6.8	5.8
	S.D.	0.9	0.8	0.6	
40g EPO alternate day					
Karlops		5	7	8	
Big Ben		5	7	7	
Walter		5	8	8	
Pollux		5	6	7	
Armpit		4	4	6	
Sandy Lad		3	4	5	
Sabrian		5	6	7	
Ollie		5	7	8	
Poppett		3	5	6	
Tessa		5	6	7	
Alfred		5	7	7	Group
Darkie		4	7	7	mean
	Mean	4.5	6.2	6.9	5.9
	S.D.	0.8	1.3	0.9	

Table IV

Hoof condition scores

Scored on a 1 to 10 scale, 1 is poorest, 10 is optimal

Horse		Week 0	3	6	
5g EPO daily					
Annabelle		5	6	6	
Donald		4	6	6	
Myrtle		3	5	6	
Calumn		5	6	8	
Giraffe		5	5	7	
Gunsmoke		5	7	7	
Sovereign		5	5	7	
Orlando		4	4	6	
Jack		6	7	8	
Nicholas		4	4	7	
Suzie		5	5	5	Group
Hermione		4	5	7	mean
	Mean	4.6	5.4	6.7	5.6
	S.D.	0.8	1.0	0.9	
10g EPO alternate day					
Charlotte		4	6	7	
Marigold		6	7	7	
Jasper		5	7	8	
Ding Dong		6	7	6	
Flora		4	6	8	
Chocolate		5	6	8	
Goldie		5	6	6	
Durando		4	6	8	
BeBe		4	5	6	
Samuel		5	6	7	
Jordan		4	6	6	Group
Dick		5	8	8	mean
	Mean	4.8	6.3	7.1	6.1
	S.D.	0.8	0.8	0.9	
20g EPO daily					
Foxy		4	6	5	
Wallace		4	5	7	
Gold Loch		4	5	6	
Bonny		2	4	6	
Skippy		5	5	6	
Flynn		4	4	8	
Rocky		5	6	7	
Charlie		5	7	8	
Geno		5	6	7	
Biggles		4	5	6	
Smiler		5	5	6	Group
Aird		6	7	7	mean
	Mean	4.4	5.4	6.6	5.5
	S.D.	1.0	1.0	0.9	
40g EPO alternate day					
Karlops		6	7	8	
Big Ben		4	6	7	
Walter		5	6	7	
Pollux		3	6	6	
Armpit		5	4	6	
Sandy Lad		4	6	6	
Sabrian		6	7	7	
Ollie		5	7	7	
Poppett		5	5	6	
Tessa		5	5	7	
Alfred		4	5	6	Group
Darkie		4	5	7	mean
	Mean	4.7	5.8	6.7	5.7
	S.D.	0.9	1.0	0.7	

Table V

Red cell counts Values $x10^{12}/l$

Horse	Week 0	Week 3	Week 6		
5g EPO daily					
Annabelle	7.6	7.2	7.1		
Donald	6.6	6.6	7.4		
Myrtle	6.4	6.8	6.7		
Calumn	5.9	6.2	6.7		
Giraffe	6.7	6.0	7.5		
Gunsmoke	6.7	7.2	7.3		
Sovereign	5.3	5.6	7.3		
Orlando	7.8	6.1	8.1		
Jack	8.2	6.0	8.8		
Nicholas	6.1	6.6	7.5		
Suzie	7.6	6.8	8.0	Group	
Hermione	6.9	6.0	7.1	mean	
Mean	6.82	6.43	7.46		6.90
S.D.	0.85	0.52	0.60		
10g EPO alternate day					
Charlotte	7.2	5.4	8.5		
Marigold	7.2	6.4	8.0		
Jasper	6.8	5.4	8.6		
Ding Dong	6.8	5.3	6.3		
Flora	7.7	8.3	9.9		
Chocolate	8.6	7.5	8.6		
Goldie	7.7	6.2	8.4		
Durando	6.6	5.1	7.3		
BeBe	5.5	5.4	6.8		
Samuel	7.0	7.9	8.2		
Jordan	7.4	5.9	6.3	Group	
Dick	8.9	8.8	9.9	mean	
Mean	7.28	6.47	8.07		7.27
S.D.	0.90	1.31	1.21		
20g EPO daily					
Foxy	7.5	6.6	8.4		
Wallace	7.0	6.4	7.9		
Gold Loch	7.3	6.5	7.5		
Bonny	7.0	6.4	7.7		
Skippy	7.0	6.6	7.4		
Flynn	6.0	5.9	5.9		
Rocky	6.5	6.1	7.9		
Charlie	6.8	6.3	6.8		
Geno	6.3	5.3	7.6		
Biggles	7.3	5.9	7.9		
Smiler	8.5	7.5	8.5	Group	
Aird	6.2	7.3	7.5	mean	
Mean	6.95	6.40	7.58		6.98
S.D.	0.68	0.60	0.70		
40g EPO alternate day					
Karlops	7.2	8.1	8.7		
Big Ben	6.4	6.1	7.1		
Walter	5.4	6.5	7.3		
Pollux	5.1	5.5	7.5		
Armpit	6.9	6.9	7.9		
Sandy Lad	5.7	8.3	9.0		
Sabrian	4.5	6.3	7.5		
Ollie	6.6	6.6	7.4		
Poppett	7.4	8.1	9.2		
Tessa	6.2	6.1	6.6		
Alfred	6.2	5.9	8.0	Group	
Darkie	5.7	6.8	7.0	mean	
Mean	6.11	6.77	7.77		6.88
S.D.	0.87	0.93	0.82		

Table VI

Haemoglobin values Values in g/dl

Horse	Week 0	Week 3	Week 6		
5g EPO daily					
Annabelle	15.3	14.0	12.7		
Donald	13.0	12.8	13.1		
Myrtle	12.7	13.3	11.7		
Calumn	11.7	12.5	12.3		
Giraffe	12.5	11.3	12.4		
Gunsmoke	12.3	13.5	12.3		
Sovereign	10.0	11.0	12.8		
Orlando	14.8	11.1	13.6		
Jack	14.9	10.9	14.1		
Nicholas	11.7	12.6	13.2		
Suzie	13.5	12.3	12.8	Group	
Hermione	12.0	10.6	11.2	mean	
Mean	12.87	12.16	12.68		12.57
S.D.	1.55	1.15	0.79		
10g EPO alternate day					
Charlotte	13.3	9.3	13.8		
Marigold	12.7	12.1	13.3		
Jasper	11.2	8.9	12.3		
Ding Dong	12.4	10.0	10.4		
Flora	13.5	13.9	15.1		
Chocolate	14.7	12.9	13.2		
Goldie	15.1	11.8	13.9		
Durando	12.0	9.4	12.0		
BeBe	10.6	10.5	12.0		
Samuel	13.0	14.2	13.5		
Jordan	14.6	11.5	11.0	Group	
Dick	16.6	16.6	16.9	mean	
Mean	13.31	11.76	13.12		12.73
S.D.	1.72	2.33	1.77		
20g EPO daily					
Foxy	12.5	11.8	13.1		
Wallace	13.5	12.3	13.7		
Gold Loch	13.4	11.8	12.2		
Bonny	14.4	12.9	14.5		
Skippy	12.0	10.9	11.5		
Flynn	11.3	11.2	10.3		
Rocky	12.4	11.7	13.4		
Charlie	12.6	11.5	11.6		
Geno	12.2	10.1	13.0		
Biggles	13.5	10.8	12.9		
Smiler	14.6	12.8	11.6	Group	
Aird	10.6	12.8	12.0	mean	
Mean	12.75	11.72	12.48		12.32
S.D.	1.19	0.88	1.16		
40g EPO alternate day					
Karlops	12.6	14.3	14.0		
Big Ben	12.8	12.0	13.1		
Walter	10.3	11.9	12.3		
Pollux	9.6	10.8	13.1		
Armpit	12.3	12.5	12.8		
Sandy Lad	10.6	14.9	14.3		
Sabrian	8.4	11.4	12.0		
Ollie	12.8	12.5	13.1		
Poppett	13.1	14.0	14.3		
Tessa	11.9	11.7	11.5		
Alfred	12.1	11.2	13.9	Group	
Darkie	10.9	13.0	11.6	mean	
Mean	11.45	12.52	13.00		12.32
S.D.	1.48	1.30	1.00		

Table VII

Packed cell volumes Values in l/l

Horse	Week 0	Week 3	Week 6	
5g EPO daily				
Annabelle	0.440	0.408	0.373	
Donald	0.379	0.376	0.383	
Myrtle	0.367	0.388	0.358	
Calumn	0.336	0.360	0.360	
Giraffe	0.371	0.327	0.377	
Gunsmoke	0.364	0.390	0.361	
Sovereign	0.297	0.317	0.375	
Orlando	0.434	0.336	0.412	
Jack	0.430	0.313	0.428	
Nicholas	0.343	0.368	0.425	
Suzie	0.401	0.356	0.379	Group
Hermione	0.358	0.314	0.341	mean
Mean	0.3767	0.3544	0.3810	0.3707
S.D.	0.0432	0.0327	0.0273	
10g EPO alternate day				
Charlotte	0.390	0.289	0.432	
Marigold	0.376	0.348	0.404	
Jasper	0.339	0.258	0.383	
Ding Dong	0.369	0.287	0.305	
Flora	0.397	0.413	0.466	
Chocolate	0.445	0.377	0.406	
Goldie	0.434	0.343	0.431	
Durando	0.353	0.269	0.360	
BeBe	0.314	0.301	0.365	
Samuel	0.374	0.411	0.393	
Jordan	0.425	0.331	0.329	Group
Dick	0.491	0.477	0.537	mean
Mean	0.3923	0.3420	0.4009	0.3784
S.D.	0.0496	0.0670	0.0621	
20g EPO daily				
Foxy	0.362	0.333	0.391	
Wallace	0.391	0.355	0.397	
Gold Loch	0.393	0.344	0.364	
Bonny	0.429	0.381	0.447	
Skippy	0.347	0.323	0.363	
Flynn	0.329	0.323	0.307	
Rocky	0.363	0.339	0.400	
Charlie	0.375	0.343	0.357	
Geno	0.354	0.296	0.398	
Biggles	0.392	0.316	0.398	
Smiler	0.435	0.384	0.362	Group
Aird	0.325	0.382	0.359	mean
Mean	0.3746	0.3433	0.3786	0.3655
S.D.	0.0351	0.0280	0.0345	
40g EPO alternate day				
Karlops	0.369	0.415	0.425	
Big Ben	0.370	0.354	0.387	
Walter	0.296	0.346	0.360	
Pollux	0.288	0.304	0.391	
Armpit	0.372	0.365	0.388	
Sandy Lad	0.309	0.441	0.447	
Sabrian	0.244	0.332	0.367	
Ollie	0.367	0.364	0.421	
Poppett	0.394	0.418	0.453	
Tessa	0.353	0.341	0.328	
Alfred	0.353	0.330	0.410	Group
Darkie	0.322	0.373	0.352	mean
Mean	0.3364	0.3653	0.3941	0.3653
S.D.	0.0445	0.0407	0.0385	

Table VIII

Mean cell volume values, in fl

Horse	Week 0	Week 3	Week 6	
5g EPO daily				
Annabelle	57.9	56.7	52.5	
Donald	57.4	56.9	52.1	
Myrtle	57.4	57.1	53.8	
Calumn	56.9	58.0	53.6	
Giraffe	55.3	54.5	50.1	
Gunsmoke	54.3	54.2	49.2	
Sovereign	56.0	56.6	51.2	
Orlando	55.6	55.1	51.2	
Jack	52.4	52.1	48.6	
Nicholas	56.2	55.7	56.1	
Suzie	52.8	52.4	47.5	Group
Hermione	51.9	52.3	48.1	mean
Mean	55.34	55.13	51.17	53.88
S.D.	2.06	2.05	2.60	
10g EPO alternate day				
Charlotte	54.2	53.6	51.0	
Marigold	52.6	54.4	50.8	
Jasper	49.8	47.8	44.6	
Ding Dong	54.3	54.2	48.6	
Flora	51.6	49.8	47.0	
Chocolate	51.8	50.2	47.3	
Goldie	56.3	55.3	51.6	
Durando	53.5	52.7	49.0	
BeBe	57.1	55.7	53.5	
Samuel	53.4	52.0	48.2	
Jordan	57.5	56.1	52.2	Group
Dick	55.2	54.2	53.8	mean
Mean	53.94	53.00	49.80	52.25
S.D.	2.33	2.59	2.81	
20g EPO daily				
Foxy	48.4	50.5	46.4	
Wallace	55.9	55.5	50.3	
Gold Loch	53.8	52.9	43.8	
Bonny	61.3	59.6	57.8	
Skippy	49.6	49.0	48.9	
Flynn	54.8	54.7	52.0	
Rocky	55.9	55.6	50.8	
Charlie	55.2	54.5	52.6	
Geno	56.2	55.8	52.2	
Biggles	53.7	53.5	50.5	
Smiler	51.2	51.2	48.5	Group
Aird	52.4	52.3	48.1	mean
Mean	54.03	53.76	50.16	52.65
S.D.	3.42	2.84	3.51	
40g EPO alternate day				
Karlops	51.3	51.2	48.9	
Big Ben	57.8	58.1	54.7	
Walter	54.8	53.3	49.2	
Pollux	56.4	55.2	51.9	
Armpit	53.9	52.9	49.4	
Sandy Lad	54.2	53.1	49.8	
Sabrian	54.3	52.7	48.9	
Ollie	55.7	55.2	52.9	
Poppett	53.2	51.6	49.2	
Tessa	57.0	55.9	49.6	
Alfred	56.9	55.9	51.6	Group
Darkie	56.5	54.9	50.1	mean
Mean	55.17	54.17	50.52	53.28
S.D.	1.89	2.03	1.85	

Table IX

Mean cell haemoglobin values, in pg

Horse	Week 0	Week 3	Week 6	
5g EPO daily				
Annabelle	20.1	19.4	17.9	
Donald	19.7	19.4	17.8	
Myrtle	19.8	19.6	17.6	
Calumn	19.8	20.2	18.3	
Giraffe	18.7	18.8	16.5	
Gunsmoke	18.4	18.8	16.8	
Sovereign	18.9	19.6	17.5	
Orlando	19.0	18.2	16.9	
Jack	18.2	18.2	16.0	
Nicholas	19.2	19.1	17.4	
Suzie	17.8	18.1	16.0	Group
Hermione	17.4	17.7	15.8	mean
Mean	18.92	18.93	17.04	18.29
S.D.	0.85	0.76	0.83	
10g EPO alternate day				
Charlotte	18.5	17.2	16.3	
Marigold	17.8	18.9	16.7	
Jasper	16.5	16.5	14.3	
Ding Dong	18.2	18.9	16.6	
Flora	17.5	16.7	15.2	
Chocolate	17.1	17.2	15.4	
Goldie	19.6	19.0	16.6	
Durando	18.2	18.4	16.3	
BeBe	19.3	19.4	17.6	
Samuel	18.6	18.0	16.5	
Jordan	19.7	19.5	17.5	Group
Dick	18.7	18.9	16.9	mean
Mean	18.31	18.22	16.33	17.62
S.D.	0.98	1.06	0.95	
20g EPO daily				
Foxy	16.8	17.9	15.6	
Wallace	19.3	19.2	17.3	
Gold Loch	18.4	18.2	16.2	
Bonny	20.6	20.2	18.8	
Skippy	17.1	16.5	15.5	
Flynn	18.8	19.0	17.5	
Rocky	19.1	19.2	17.0	
Charlie	18.5	18.3	17.1	
Geno	19.4	19.1	17.1	
Biggles	18.5	18.3	16.4	
Smiler	17.2	17.1	15.5	Group
Aird	17.1	17.5	16.1	mean
Mean	18.40	18.38	16.68	17.82
S.D.	1.16	1.03	0.98	
40g EPO alternate day				
Karlops	17.5	17.7	16.1	
Big Ben	20.0	19.7	18.5	
Walter	19.1	18.3	16.8	
Pollux	18.8	19.6	17.4	
Armpit	17.8	18.1	16.3	
Sandy Lad	18.6	18.0	15.9	
Sabrian	18.7	18.1	16.0	
Ollie	19.4	18.9	16.5	
Poppett	17.7	17.3	15.5	
Tessa	19.2	19.2	17.4	
Alfred	19.5	19.0	17.5	Group
Darkie	19.1	19.1	16.5	mean
Mean	18.78	18.58	16.70	18.02
S.D.	0.77	0.77	0.85	

Table X

Mean cell haemoglobin concentration values, in g/dl

Horse	Week 0	Week 3	Week 6	
5g EPO daily				
Annabelle	34.8	34.3	34.0	
Donald	34.3	34.1	34.2	
Myrtle	34.6	34.3	32.7	
Calumn	34.9	34.8	34.2	
Giraffe	33.7	34.6	32.9	
Gunsmoke	33.8	34.6	34.1	
Sovereign	33.7	34.7	34.1	
Orlando	34.1	33.0	33.0	
Jack	34.7	34.9	32.9	
Nicholas	34.1	34.3	31.1	
Suzie	33.6	34.5	33.8	Group
Hermione	33.5	33.8	32.8	mean
Mean	34.15	34.33	33.32	33.93
S.D.	0.50	0.52	0.93	
10g EPO alternate day				
Charlotte	34.7	32.1	31.9	
Marigold	33.9	34.8	32.9	
Jasper	33.1	34.5	32.1	
Ding Dong	33.6	34.8	34.1	
Flora	34.0	33.6	32.4	
Chocolate	33.0	34.3	32.5	
Goldie	34.8	34.4	32.3	
Durando	34.0	35.0	33.3	
BeBe	33.8	34.9	32.9	
Samuel	34.8	34.6	34.4	
Jordan	34.3	34.7	33.4	Group
Dick	33.8	34.8	31.5	mean
Mean	33.98	34.38	32.81	33.72
S.D.	0.60	0.81	0.87	
20g EPO daily				
Foxy	34.5	35.4	33.5	
Wallace	34.5	34.6	34.5	
Gold Loch	34.1	34.3	33.5	
Bonny	33.6	33.8	32.8	
Skippy	34.6	33.7	31.7	
Flynn	34.4	34.7	33.6	
Rocky	34.1	34.5	33.5	
Charlie	33.6	33.5	32.5	
Geno	34.5	34.2	32.7	
Biggles	34.4	34.2	32.4	
Smiler	33.5	33.3	32.0	Group
Aird	32.6	33.5	33.4	mean
Mean	34.03	34.14	33.01	33.73
S.D.	0.60	0.61	0.79	
40g EPO alternate day				
Karlops	34.1	34.5	32.9	
Big Ben	34.6	33.9	33.9	
Walter	34.8	34.3	34.2	
Pollux	33.4	35.6	33.5	
Armpit	33.1	34.2	33.0	
Sandy Lad	34.3	33.8	32.0	
Sabrian	34.4	34.3	32.7	
Ollie	34.8	34.3	31.1	
Poppett	33.3	33.5	31.6	
Tessa	33.7	34.3	35.1	
Alfred	34.3	34.0	33.9	Group
Darkie	33.8	34.8	33.0	mean
Mean	34.05	34.29	33.08	33.81
S.D.	0.58	0.53	1.14	

Table XI

Platelet values Values x10^9/l

Horse	Week 0	Week 3	Week 6	
5g EPO daily				
Annabelle	180	210	183	
Donald	180	130	113	
Myrtle	100	60	40	
Calumn	170	140	169	
Giraffe	198	130	233	
Gunsmoke	240	190	219	
Sovereign	130	170	129	
Orlando	130	130	118	
Jack	150	220	174	
Nicholas	170	190	160	
Suzie	130	150	205	Group
Hermione	170	170	240	mean
Mean	162.3	157.5	165.3	161.7
S.D.	37.3	43.9	58.0	
10g EPO alternate day				
Charlotte	210	180	222	
Marigold	200	160	240	
Jasper	160	180	97	
Ding Dong	210	170	198	
Flora	180	200	103	
Chocolate	200	120	167	
Goldie	120	210	183	
Durando	210	210	204	
BeBe	170	220	164	
Samuel	120	190	114	
Jordan	150	120	117	Group
Dick	188	150	96	mean
Mean	176.5	175.8	158.8	170.4
S.D.	33.1	33.4	51.8	
20g EPO daily				
Foxy	185	190	181	
Wallace	250	110	138	
Gold Loch	150	210	162	
Bonny	219	160	124	
Skippy	140	120	123	
Flynn	160	190	157	
Rocky	160	180	94	
Charlie	170	140	105	
Geno	180	190	208	
Biggles	180	200	180	
Smiler	160	130	131	Group
Aird	120	110	54	mean
Mean	172.8	160.8	138.1	157.3
S.D.	34.7	37.0	42.6	
40g EPO alternate day				
Karlops	210	220	207	
Big Ben	170	180	190	
Walter	210	160	128	
Pollux	180	200	117	
Armpit	150	160	162	
Sandy Lad	150	110	84	
Sabrian	210	260	182	
Ollie	150	140	142	
Poppett	140	120	107	
Tessa	140	170	191	
Alfred	170	200	155	Group
Darkie	112	150	126	mean
Mean	166.0	172.5	149.3	162.6
S.D.	31.7	42.7	38.3	

Table XII

White cell counts Values x10^9/l

Horse	Week 0	Week 3	Week 6	
5g EPO daily				
Annabelle	12.2	14.3	9.2	
Donald	11.0	10.2	11.6	
Myrtle	8.5	8.5	7.9	
Calumn	6.9	7.3	8.4	
Giraffe	6.8	5.5	7.3	
Gunsmoke	7.5	8.6	6.6	
Sovereign	6.9	5.6	6.2	
Orlando	6.9	8.1	7.8	
Jack	8.2	14.9	9.7	
Nicholas	9.3	9.2	9.3	
Suzie	8.4	12.5	11.0	Group
Hermione	8.6	11.1	6.4	mean
Mean	8.43	9.65	8.45	8.84
S.D.	1.71	3.07	1.76	
10g EPO alternate day				
Charlotte	8.2	15.3	11.3	
Marigold	10.5	8.8	12.3	
Jasper	9.0	19.3	14.2	
Ding Dong	8.0	11.8	8.5	
Flora	18.3	14.5	8.6	
Chocolate	8.7	10.4	8.7	
Goldie	9.3	17.9	15.1	
Durando	6.9	11.2	6.8	
BeBe	8.0	9.2	7.8	
Samuel	17.1	14.1	9.1	
Jordan	8.7	8.0	6.8	Group
Dick	15.1	11.8	9.1	mean
Mean	10.65	12.69	9.86	11.07
S.D.	3.89	3.58	2.75	
20g EPO daily				
Foxy	9.0	9.3	8.8	
Wallace	10.2	7.6	13.0	
Gold Loch	5.9	12.9	6.4	
Bonny	13.3	9.0	8.7	
Skippy	8.9	7.4	9.5	
Flynn	9.0	8.2	8.5	
Rocky	7.1	6.9	13.3	
Charlie	9.3	6.9	13.3	
Geno	8.2	9.4	8.2	
Biggles	9.8	14.2	12.4	
Smiler	12.7	9.5	8.5	Group
Aird	9.4	8.5	7.4	mean
Mean	9.40	9.15	9.83	9.46
S.D.	2.06	2.27	2.47	
40g EPO alternate day				
Karlops	9.0	11.1	8.8	
Big Ben	7.3	7.8	7.4	
Walter	16.1	12.5	7.6	
Pollux	8.3	9.9	7.5	
Armpit	7.0	7.7	9.9	
Sandy Lad	11.8	13.5	10.1	
Sabrian	13.9	10.0	9.7	
Ollie	7.6	8.0	7.4	
Poppett	8.7	11.8	8.1	
Tessa	7.8	7.0	7.3	
Alfred	9.4	9.7	8.1	Group
Darkie	7.5	14.3	8.9	mean
Mean	9.53	10.28	8.40	9.40
S.D.	2.89	2.41	1.05	

Table XIII

Urea values Values in mmol/l

Horse	Week 0	Week 3	Week 6	
5g EPO daily				
Annabelle	6.8	6.2	6.7	
Donald	5.1	4.0	4.2	
Myrtle	5.7	5.5	5.7	
Calumn	7.2	6.0	7.1	
Giraffe	6.5	2.3	2.9	
Gunsmoke	6.7	6.2	5.9	
Sovereign	6.9	6.1	6.5	
Orlando	6.3	4.1	6.8	
Jack	5.7	2.5	5.1	
Nicholas	6.8	5.5	7.2	
Suzie	6.1	3.5	6.3	Group
Hermione	6.5	4.3	5.7	mean
Mean	6.36	4.68	5.84	5.63
S.D.	0.61	1.43	1.27	
10g EPO alternate day				
Charlotte	7.3	2.9	5.5	
Marigold	7.1	6.2	6.3	
Jasper	6.5	2.8	3.4	
Ding Dong	4.7	2.2	3.9	
Flora	4.2	5.0	7.8	
Chocolate	6.8	2.3	5.9	
Goldie	7.0	3.1	6.0	
Durando	7.2	2.8	5.0	
BeBe	4.2	4.5	5.9	
Samuel	3.8	4.9	6.7	
Jordan	5.9	5.5	6.4	Group
Dick	6.0	6.1	7.3	mean
Mean	5.89	4.03	5.84	5.25
S.D.	1.32	1.49	1.27	
20g EPO daily				
Foxy	7.5	3.6	7.4	
Wallace	5.1	4.0	4.5	
Gold Loch	5.8	2.6	6.0	
Bonny	5.6	5.9	8.2	
Skippy	6.3	5.5	7.1	
Flynn	6.6	6.0	6.7	
Rocky	6.2	4.8	5.0	
Charlie	6.8	4.8	5.7	
Geno	6.8	4.5	6.7	
Biggles	6.8	2.9	6.1	
Smiler	8.7	5.8	6.2	Group
Aird	6.6	5.9	6.8	mean
Mean	6.57	4.69	6.37	5.88
S.D.	0.93	1.20	1.02	
40g EPO alternate day				
Karlops	7.2	6.2	7.9	
Big Ben	7.6	6.2	6.8	
Walter	4.2	5.7	6.5	
Pollux	7.6	6.9	8.1	
Armpit	6.3	5.0	5.8	
Sandy Lad	5.7	6.5	8.1	
Sabrian	4.0	6.1	6.7	
Ollie	7.1	6.7	6.8	
Poppett	5.5	4.7	6.2	
Tessa	7.6	7.2	8.5	
Alfred	5.7	5.0	7.7	Group
Darkie	5.5	4.7	6.1	mean
Mean	6.17	5.91	7.10	6.39
S.D.	1.28	0.88	0.91	

Table XIV

Sodium values Values in mmol/l

Horse	Week 0	Week 3	Week 6	
5g EPO daily				
Annabelle	140	138	136	
Donald	136	138	136	
Myrtle	136	138	134	
Calumn	138	135	138	
Giraffe	137	127	135	
Gunsmoke	136	137	137	
Sovereign	137	138	136	
Orlando	135	129	134	
Jack	136	135	135	
Nicholas	138	140	140	
Suzie	135	130	134	Group
Hermione	137	138	136	mean
Mean	136.8	135.3	135.9	136.0
S.D.	1.4	4.2	1.8	
10g EPO alternate day				
Charlotte	138	132	136	
Marigold	135	139	133	
Jasper	138	128	134	
Ding Dong	136	134	133	
Flora	132	137	135	
Chocolate	137	128	131	
Goldie	136	131	133	
Durando	136	133	132	
BeBe	134	137	134	
Samuel	133	137	136	
Jordan	139	139	134	Group
Dick	136	137	135	mean
Mean	135.8	134.3	133.8	134.7
S.D.	2.1	3.9	1.5	
20g EPO daily				
Foxy	136	133	131	
Wallace	135	138	137	
Gold Loch	136	130	134	
Bonny	138	136	134	
Skippy	138	139	136	
Flynn	133	138	134	
Rocky	135	137	135	
Charlie	135	137	136	
Geno	136	134	133	
Biggles	136	132	132	
Smiler	136	138	129	Group
Aird	133	133	132	mean
Mean	135.6	135.4	133.6	134.9
S.D.	1.6	2.9	2.3	
40g EPO alternate day				
Karlops	138	137	133	
Big Ben	137	137	135	
Walter	130	128	135	
Pollux	134	138	133	
Armpit	135	139	134	
Sandy Lad	136	136	133	
Sabrian	131	152	128	
Ollie	138	135	136	
Poppett	136	135	134	
Tessa	136	138	133	
Alfred	134	134	135	Group
Darkie	133	137	133	mean
Mean	134.8	137.2	133.5	135.2
S.D.	2.6	5.5	2.0	

Table XV

Potassium values Values in mmol/l

Horse	Week 0	Week 3	Week 6		
5g EPO daily					
Annabelle	4.7	4.0	3.5		
Donald	4.9	4.2	3.7		
Myrtle	4.8	4.0	3.6		
Calumn	5.3	4.0	3.8		
Giraffe	5.1	3.9	3.2		
Gunsmoke	4.6	3.8	3.8		
Sovereign	4.6	3.8	3.8		
Orlando	5.0	4.2	4.2		
Jack	4.0	3.9	3.7		
Nicholas	4.3	3.9	3.4		
Suzie	5.0	3.8	3.5	Group	
Hermione	4.3	3.6	3.9	mean	
Mean	4.72	3.93	3.68		4.11
S.D.	0.38	0.17	0.26		
10g EPO alternate day					
Charlotte	4.5	2.7	3.8		
Marigold	6.0	3.8	3.7		
Jasper	4.0	4.2	3.5		
Ding Dong	4.7	4.4	3.4		
Flora	4.2	4.2	3.4		
Chocolate	4.2	3.5	3.8		
Goldie	4.8	3.5	4.0		
Durando	4.5	3.6	3.4		
BeBe	3.8	4.5	3.7		
Samuel	4.3	4.5	3.6		
Jordan	4.0	4.1	3.2	Group	
Dick	4.7	4.9	3.6	mean	
Mean	4.48	3.99	3.59		4.02
S.D.	0.57	0.60	0.22		
20g EPO daily					
Foxy	4.1	3.8	3.5		
Wallace	4.2	4.2	4.2		
Gold Loch	4.7	4.0	4.5		
Bonny	4.2	4.0	3.5		
Skippy	5.2	4.7	3.3		
Flynn	5.4	4.6	4.7		
Rocky	4.8	4.4	3.5		
Charlie	5.2	4.8	3.8		
Geno	4.5	4.4	3.6		
Biggles	4.9	3.9	3.5		
Smiler	5.5	4.1	3.2	Group	
Aird	3.8	3.8	3.3	mean	
Mean	4.71	4.23	3.72		4.22
S.D.	0.56	0.35	0.49		
40g EPO alternate day					
Karlops	3.6	3.9	3.8		
Big Ben	4.6	4.7	4.0		
Walter	4.4	4.1	3.2		
Pollux	4.2	4.5	3.9		
Armpit	4.7	4.7	4.2		
Sandy Lad	4.7	4.0	4.2		
Sabrian	4.8	4.3	3.4		
Ollie	5.2	4.5	4.3		
Poppett	4.4	3.7	3.5		
Tessa	4.5	3.6	3.8		
Alfred	5.1	3.7	3.8	Group	
Darkie	4.4	4.0	3.6	mean	
Mean	4.55	4.14	3.81		4.17
S.D.	0.42	0.39	0.34		

Table XVI

Calcium values Values in mmol/l

Horse	Week 0	Week 3	Week 6	
5g EPO daily				
Annabelle	2.94	2.71	3.08	
Donald	2.96	2.96	2.92	
Myrtle	3.03	2.89	2.76	
Calumn	3.03	2.86	2.87	
Giraffe	2.85	2.63	2.86	
Gunsmoke	3.02	3.15	3.29	
Sovereign	2.93	2.88	2.83	
Orlando	2.98	2.98	2.99	
Jack	3.09	2.82	3.04	
Nicholas	3.02	2.87	2.92	
Suzie	3.07	2.77	2.81	Group
Hermione	3.02	2.97	2.93	mean
Mean	2.995	2.874	2.942	2.937
S.D.	0.067	0.136	0.144	
10g EPO alternate day				
Charlotte	3.05	2.86	2.94	
Marigold	2.96	2.98	3.01	
Jasper	3.08	3.07	3.06	
Ding Dong	2.96	2.77	2.84	
Flora	3.04	2.87	2.99	
Chocolate	3.00	2.75	2.92	
Goldie	3.05	2.88	3.08	
Durando	3.10	2.69	2.96	
BeBe	2.72	2.84	2.96	
Samuel	2.69	2.88	3.07	
Jordan	2.89	2.90	2.73	Group
Dick	2.87	3.08	3.30	mean
Mean	2.951	2.881	2.988	2.940
S.D.	0.135	0.118	0.140	
20g EPO daily				
Foxy	3.10	2.82	2.92	
Wallace	3.05	3.05	3.05	
Gold Loch	3.03	2.77	2.74	
Bonny	2.63	2.79	3.14	
Skippy	2.91	2.81	3.08	
Flynn	2.84	2.83	2.82	
Rocky	3.03	2.97	2.92	
Charlie	3.06	3.07	3.02	
Geno	2.93	2.76	2.77	
Biggles	2.96	2.99	3.03	
Smiler	2.99	2.90	2.81	Group
Aird	2.90	3.01	3.03	mean
Mean	2.953	2.898	2.944	2.931
S.D.	0.127	0.115	0.133	
40g EPO alternate day				
Karlops	2.76	2.93	3.10	
Big Ben	2.77	2.84	2.92	
Walter	2.76	2.55	2.97	
Pollux	3.09	3.04	3.14	
Armpit	3.08	3.07	3.07	
Sandy Lad	3.02	2.90	3.12	
Sabrian	2.81	2.58	2.91	
Ollie	3.00	3.07	3.14	
Poppett	2.94	2.96	2.99	
Tessa	3.00	2.86	3.06	
Alfred	2.99	2.89	3.20	Group
Darkie	2.90	2.97	2.99	mean
Mean	2.927	2.888	3.051	2.955
S.D.	0.124	0.169	0.094	

Table XVII

Chloride values Values in mmol/l

Horse	Week 0	Week 3	Week 6		
5g EPO daily					
Annabelle	100	97	96		
Donald	97	96	96		
Myrtle	95	101	97		
Calumn	96	94	96		
Giraffe	100	91	96		
Gunsmoke	98	94	99		
Sovereign	99	100	97		
Orlando	98	97	95		
Jack	96	92	95		
Nicholas	98	99	98		
Suzie	94	92	95	Group	
Hermione	96	95	95	mean	
Mean	97.3	95.7	96.3		96.4
S.D.	1.9	3.3	1.3		
10g EPO alternate day					
Charlotte	98	95	97		
Marigold	95	96	95		
Jasper	94	84	94		
Ding Dong	98	93	93		
Flora	91	96	96		
Chocolate	99	93	95		
Goldie	96	90	92		
Durando	97	92	91		
BeBe	94	94	97		
Samuel	95	97	96		
Jordan	97	99	92	Group	
Dick	99	95	97	mean	
Mean	96.1	93.7	94.6		94.8
S.D.	2.4	3.9	2.2		
20g EPO daily					
Foxy	95	94	92		
Wallace	96	91	94		
Gold Loch	97	88	98		
Bonny	97	97	95		
Skippy	99	99	92		
Flynn	95	99	101		
Rocky	98	94	97		
Charlie	98	97	94		
Geno	96	98	96		
Biggles	102	94	93		
Smiler	97	98	95	Group	
Aird	92	93	86	mean	
Mean	96.8	95.2	94.4		95.5
S.D.	2.4	3.4	3.7		
40g EPO alternate day					
Karlops	94	97	93		
Big Ben	93	103	94		
Walter	91	92	95		
Pollux	96	97	97		
Armpit	92	99	95		
Sandy Lad	100	95	92		
Sabrian	94	89	95		
Ollie	99	96	96		
Poppett	94	97	95		
Tessa	95	97	97		
Alfred	94	99	95	Group	
Darkie	94	97	97	mean	
Mean	94.7	96.5	95.1		95.4
S.D.	2.6	3.5	1.6		

Table XVIII

Magnesium values Values in mmol/l

Horse	Week 0	Week 3	Week 6	
5g EPO daily				
Annabelle	0.63	0.63	0.73	
Donald	0.55	0.56	0.59	
Myrtle	0.69	0.72	0.75	
Calumn	0.66	0.64	0.76	
Giraffe	0.63	0.49	0.60	
Gunsmoke	0.60	0.60	0.60	
Sovereign	0.66	0.61	0.66	
Orlando	0.63	0.65	0.66	
Jack	0.62	0.50	0.71	
Nicholas	0.65	0.65	0.71	
Suzie	0.63	0.53	0.64	Group
Hermione	0.60	0.60	0.61	mean
Mean	0.629	0.598	0.668	0.632
S.D.	0.036	0.068	0.062	
10g EPO alternate day				
Charlotte	0.66	0.50	0.68	
Marigold	0.63	0.67	0.71	
Jasper	0.64	0.66	0.68	
Ding Dong	0.65	0.55	0.65	
Flora	0.50	0.62	0.74	
Chocolate	0.68	0.49	0.69	
Goldie	0.59	0.42	0.66	
Durando	0.67	0.44	0.73	
BeBe	0.59	0.66	0.73	
Samuel	0.52	0.63	0.75	
Jordan	0.68	0.65	0.65	Group
Dick	0.62	0.61	0.77	mean
Mean	0.619	0.575	0.703	0.633
S.D.	0.059	0.091	0.041	
20g EPO daily				
Foxy	0.66	0.58	0.72	
Wallace	0.23	0.23	0.23	
Gold Loch	0.64	0.55	0.67	
Bonny	0.56	0.63	0.77	
Skippy	0.74	0.69	0.69	
Flynn	0.67	0.70	0.74	
Rocky	0.68	0.70	0.71	
Charlie	0.64	0.69	0.81	
Geno	0.65	0.61	0.77	
Biggles	0.66	0.68	0.70	
Smiler	0.70	0.69	0.68	Group
Aird	0.63	0.67	0.65	mean
Mean	0.622	0.618	0.678	0.639
S.D.	0.131	0.132	0.149	
40g EPO alternate day				
Karlops	0.66	0.72	0.79	
Big Ben	0.65	0.67	0.69	
Walter	0.50	0.57	0.68	
Pollux	0.62	0.69	0.72	
Armpit	0.61	0.64	0.68	
Sandy Lad	0.60	0.74	0.68	
Sabrian	0.53	0.62	0.69	
Ollie	0.63	0.68	0.73	
Poppett	0.63	0.65	0.66	
Tessa	0.65	0.67	0.72	
Alfred	0.59	0.71	0.70	Group
Darkie	0.63	0.72	0.78	mean
Mean	0.608	0.674	0.710	0.664
S.D.	0.049	0.048	0.040	

Table XIX

Inorganic phosphate values, in mmol/l

Horse	Week 0	Week 3	Week 6		
5g EPO daily					
Annabelle	1.16	0.72	0.95		
Donald	1.10	1.47	0.94		
Myrtle	1.30	1.41	1.06		
Calumn	1.14	0.88	0.97		
Giraffe	1.02	1.32	0.75		
Gunsmoke	0.84	0.68	0.88		
Sovereign	1.17	1.18	0.88		
Orlando	1.21	1.18	1.11		
Jack	1.26	1.33	0.87		
Nicholas	1.07	1.09	0.95		
Suzie	1.13	1.17	1.09	Group	
Hermione	1.00	0.91	1.16	mean	
Mean	1.117	1.112	0.968		1.065
S.D.	0.124	0.262	0.118		
10g EPO alternate day					
Charlotte	1.06	1.03	0.84		
Marigold	1.11	1.11	0.65		
Jasper	1.60	1.62	1.55		
Ding Dong	1.13	1.23	0.80		
Flora	1.05	1.12	1.01		
Chocolate	1.10	1.30	0.82		
Goldie	1.31	1.09	0.91		
Durando	1.09	0.91	0.99		
BeBe	0.81	1.27	0.91		
Samuel	1.17	1.20	0.88		
Jordan	1.17	1.13	1.19	Group	
Dick	1.14	1.11	0.88	mean	
Mean	1.145	1.177	0.953		1.091
S.D.	0.184	0.175	0.229		
20g EPO daily					
Foxy	1.33	1.41	0.84		
Wallace	1.20	1.24	1.22		
Gold Loch	1.17	1.38	0.85		
Bonny	1.18	1.17	0.85		
Skippy	1.03	1.08	0.77		
Flynn	1.07	1.22	0.87		
Rocky	1.37	1.22	0.85		
Charlie	1.12	1.20	0.86		
Geno	1.23	1.10	0.88		
Biggles	1.04	1.12	0.76		
Smiler	1.06	0.94	0.86	Group	
Aird	1.45	0.82	0.98	mean	
Mean	1.188	1.158	0.883		1.076
S.D.	0.137	0.166	0.120		
40g EPO alternate day					
Karlops	1.51	1.04	1.18		
Big Ben	1.33	1.22	0.81		
Walter	0.98	0.78	1.04		
Pollux	1.00	0.77	0.81		
Armpit	1.03	1.43	0.90		
Sandy Lad	1.05	0.86	0.95		
Sabrian	1.18	0.91	0.86		
Ollie	0.99	0.70	0.94		
Poppett	1.05	0.80	1.13		
Tessa	0.95	0.78	1.70		
Alfred	0.89	0.80	1.22	Group	
Darkie	1.31	1.08	0.93	mean	
Mean	1.106	0.931	1.039		1.025
S.D.	0.187	0.220	0.249		

Table XX

Alkaline phosphatase values, in I.U.

Horse	Week 0	Week 3	Week 6	
5g EPO daily				
Annabelle	418	484	359	
Donald	456	375	488	
Myrtle	312	284	263	
Calumn	359	337	303	
Giraffe	500	349	346	
Gunsmoke	316	384	328	
Sovereign	345	242	249	
Orlando	361	345	419	
Jack	558	346	368	
Nicholas	379	319	328	
Suzie	343	231	283	Group
Hermione	253	238	219	mean
Mean	383.3	327.8	329.4	346.9
S.D.	86.0	72.4	74.9	
10g EPO alternate day				
Charlotte	303	302	302	
Marigold	241	230	194	
Jasper	525	341	486	
Ding Dong	353	266	377	
Flora	297	421	346	
Chocolate	362	310	354	
Goldie	471	351	500	
Durando	211	168	255	
BeBe	419	349	341	
Samuel	352	469	392	
Jordan	289	430	396	Group
Dick	297	320	321	mean
Mean	343.3	329.8	355.3	342.8
S.D.	91.7	85.4	86.3	
20g EPO daily				
Foxy	352	230	274	
Wallace	268	268	268	
Gold Loch	283	218	310	
Bonny	454	409	408	
Skippy	274	294	283	
Flynn	463	350	340	
Rocky	381	361	287	
Charlie	389	392	387	
Geno	298	343	268	
Biggles	362	240	340	
Smiler	499	641	515	Group
Aird	418	449	358	mean
Mean	370.1	349.6	336.5	352.1
S.D.	78.6	118.2	73.4	
40g EPO alternate day				
Karlops	343	406	330	
Big Ben	328	406	322	
Walter	246	350	314	
Pollux	409	322	329	
Armpit	444	428	362	
Sandy Lad	273	314	270	
Sabrian	264	256	279	
Ollie	383	346	310	
Poppett	386	397	360	
Tessa	329	308	299	
Alfred	423	396	374	Group
Darkie	303	327	329	mean
Mean	344.3	354.7	323.2	340.7
S.D.	65.3	51.9	31.9	

Table XXI

Aspartate aminotransaminase values, in I.U.

Horse	Week 0	Week 3	Week 6	
5g EPO daily				
Annabelle	265	389	336	
Donald	383	373	399	
Myrtle	263	310	278	
Calumn	341	367	313	
Giraffe	267	171	106	
Gunsmoke	301	313	301	
Sovereign	385	362	334	
Orlando	278	310	271	
Jack	299	186	237	
Nicholas	248	285	301	
Suzie	274	253	166	Group
Hermione	253	273	198	mean
Mean	296.4	299.3	270.0	288.6
S.D.	48.1	70.5	81.4	
10g EPO alternate day				
Charlotte	257	212	235	
Marigold	207	249	183	
Jasper	283	191	220	
Ding Dong	307	255	197	
Flora	369	235	325	
Chocolate	265	182	232	
Goldie	331	261	248	
Durando	280	242	195	
BeBe	907	321	349	
Samuel	314	241	239	
Jordan	241	292	319	Group
Dick	284	273	281	mean
Mean	337.1	246.2	251.9	278.4
S.D.	184.4	39.6	54.8	
20g EPO daily				
Foxy	305	235	254	
Wallace	285	319	302	
Gold Loch	276	228	212	
Bonny	379	325	314	
Skippy	348	367	324	
Flynn	321	356	317	
Rocky	262	307	197	
Charlie	315	379	335	
Geno	267	282	266	
Biggles	289	352	248	
Smiler	337	379	312	Group
Aird	220	314	306	mean
Mean	300.3	320.3	282.3	300.9
S.D.	43.1	51.2	45.7	
40g EPO alternate day				
Karlops	263	384	324	
Big Ben	412	384	342	
Walter	115	225	278	
Pollux	266	325	280	
Armpit	286	384	224	
Sandy Lad	186	234	245	
Sabrian	124	213	260	
Ollie	420	358	329	
Poppett	206	258	286	
Tessa	337	476	347	
Alfred	396	434	423	Group
Darkie	169	266	273	mean
Mean	265.0	328.4	300.9	298.1
S.D.	108.6	87.8	54.5	

Table XXII

Bilirubin values Values in umol/l

Horse	Week 0	Week 3	Week 6	
5g EPO daily				
Annabelle	5	6	9	
Donald	20	2	9	
Myrtle	10	11	12	
Calumn	5	8	9	
Giraffe	13	52	12	
Gunsmoke	9	11	10	
Sovereign	8	6	10	
Orlando	7	11	8	
Jack	8	19	10	
Nicholas	9	6	13	
Suzie	7	26	7	Group
Hermione	6	10	7	mean
Mean	8.9	14.0	9.7	10.9
S.D.	4.1	13.6	1.9	
10g EPO alternate day				
Charlotte	7	19	13	
Marigold	10	10	12	
Jasper	8	22	10	
Ding Dong	8	32	12	
Flora	44	8	16	
Chocolate	7	23	8	
Goldie	7	19	8	
Durando	6	15	9	
BeBe	19	7	11	
Samuel	25	8	11	
Jordan	4	8	8	Group
Dick	14	6	13	mean
Mean	13.3	14.8	10.9	13.0
S.D.	11.4	8.3	2.5	
20g EPO daily				
Foxy	10	15	13	
Wallace	9	9	9	
Gold Loch	10	25	11	
Bonny	15	6	12	
Skippy	6	8	8	
Flynn	6	7	9	
Rocky	8	10	12	
Charlie	5	4	8	
Geno	7	9	10	
Biggles	8	38	9	
Smiler	12	7	10	Group
Aird	7	7	13	mean
Mean	8.6	12.1	10.3	10.3
S.D.	2.8	9.8	1.8	
40g EPO alternate day				
Karlops	10	10	9	
Big Ben	9	10	10	
Walter	8	5	7	
Pollux	6	7	10	
Armpit	11	14	14	
Sandy Lad	9	9	11	
Sabrian	15	7	10	
Ollie	9	7	19	
Poppett	8	16	10	
Tessa	5	6	8	
Alfred	6	7	9	Group
Darkie	14	17	26	mean
Mean	9.2	9.6	11.9	10.2
S.D.	3.0	4.0	5.4	

Table XXIII

Total plasma protein values Values in g/l

Horse	Week 0	Week 3	Week 6		
5g EPO daily					
Annabelle	75	76	69		
Donald	74	74	70		
Myrtle	70	66	61		
Calumn	70	74	70		
Giraffe	68	102	98		
Gunsmoke	76	74	70		
Sovereign	63	60	63		
Orlando	70	76	70		
Jack	70	91	73		
Nicholas	69	85	70		
Suzie	74	90	102	Group	
Hermione	63	68	77	mean	
Mean	70.2	78.0	74.4		74.2
S.D.	4.2	11.9	12.7		
10g EPO alternate day					
Charlotte	66	85	75		
Marigold	74	74	87		
Jasper	71	89	95		
Ding Dong	66	84	82		
Flora	77	93	76		
Chocolate	63	96	79		
Goldie	73	90	89		
Durando	65	75	81		
BeBe	79	81	76		
Samuel	81	76	67		
Jordan	68	75	65	Group	
Dick	73	81	77	mean	
Mean	71.3	83.3	79.1		77.9
S.D.	5.8	7.5	8.6		
20g EPO daily					
Foxy	71	91	69		
Wallace	69	68	68		
Gold Loch	67	98	70		
Bonny	81	86	76		
Skippy	76	77	74		
Flynn	66	64	62		
Rocky	67	64	79		
Charlie	64	65	65		
Geno	65	73	69		
Biggles	70	86	77		
Smiler	70	73	63	Group	
Aird	69	70	66	mean	
Mean	69.6	76.3	69.8		71.9
S.D.	4.8	11.4	5.6		
40g EPO alternate day					
Karlops	65	74	72		
Big Ben	65	74	72		
Walter	103	78	76		
Pollux	66	69	65		
Armpit	68	66	80		
Sandy Lad	71	90	73		
Sabrian	100	117	74		
Ollie	74	71	71		
Poppett	87	75	68		
Tessa	79	70	72		
Alfred	72	75	72	Group	
Darkie	84	80	70	mean	
Mean	77.8	78.3	72.1		76.1
S.D.	13.2	13.7	3.8		

Table XXIV

Albumin values Values in g/l

Horse	Week 0	Week 3	Week 6	
5g EPO daily				
Annabelle	31	33	32	
Donald	34	33	35	
Myrtle	31	34	19	
Calumn	30	27	31	
Giraffe	31	25	31	
Gunsmoke	29	33	36	
Sovereign	28	29	29	
Orlando	33	35	30	
Jack	35	35	27	
Nicholas	30	29	30	
Suzie	34	36	22	Group
Hermione	33	34	36	mean
Mean	31.6	31.9	29.8	31.1
S.D.	2.2	3.5	5.2	
10g EPO alternate day				
Charlotte	31	33	32	
Marigold	30	33	29	
Jasper	31	35	28	
Ding Dong	33	34	25	
Flora	32	23	31	
Chocolate	33	31	22	
Goldie	33	29	25	
Durando	33	28	30	
BeBe	31	31	29	
Samuel	31	32	31	
Jordan	27	32	32	Group
Dick	28	28	31	mean
Mean	31.1	30.8	28.8	30.2
S.D.	2.0	3.3	3.2	
20g EPO daily				
Foxy	32	24	28	
Wallace	36	37	36	
Gold Loch	33	29	31	
Bonny	31	26	30	
Skippy	29	26	31	
Flynn	31	36	28	
Rocky	33	36	28	
Charlie	31	30	33	
Geno	31	22	27	
Biggles	30	33	23	
Smiler	29	34	30	Group
Aird	27	27	32	mean
Mean	31.1	30.0	29.8	30.3
S.D.	2.3	5.1	3.3	
40g EPO alternate day				
Karlops	28	37	36	
Big Ben	29	37	34	
Walter	24	25	32	
Pollux	33	33	35	
Armpit	33	38	32	
Sandy Lad	30	27	33	
Sabrian	26	22	30	
Ollie	35	38	36	
Poppett	30	35	35	
Tessa	31	29	33	
Alfred	32	27	35	Group
Darkie	27	31	29	mean
Mean	29.8	31.6	33.3	31.6
S.D.	3.2	5.6	2.3	

Table XXV

Globulin values Values in g/l

Horse	Week 0	Week 3	Week 6	
5g EPO daily				
Annabelle	44	44	37	
Donald	40	41	35	
Myrtle	39	32	42	
Calumn	40	47	39	
Giraffe	37	77	67	
Gunsmoke	47	31	34	
Sovereign	35	31	34	
Orlando	37	31	40	
Jack	35	56	46	
Nicholas	39	56	40	
Suzie	40	54	80	Group
Hermione	30	34	41	mean
Mean	38.6	44.5	44.6	42.6
S.D.	4.4	14.3	14.2	
10g EPO alternate day				
Charlotte	35	52	43	
Marigold	44	41	58	
Jasper	40	54	67	
Ding Dong	33	50	57	
Flora	45	70	45	
Chocolate	30	65	57	
Goldie	40	61	64	
Durando	32	47	51	
BeBe	48	50	47	
Samuel	50	44	36	
Jordan	41	43	33	Group
Dick	45	53	46	mean
Mean	40.3	52.5	50.3	47.7
S.D.	6.5	8.9	10.6	
20g EPO daily				
Foxy	39	67	41	
Wallace	33	31	32	
Gold Loch	34	69	39	
Bonny	50	60	46	
Skippy	47	51	43	
Flynn	35	28	34	
Rocky	34	28	51	
Charlie	33	25	22	
Geno	34	51	42	
Biggles	40	53	54	
Smiler	41	39	33	Group
Aird	42	43	34	mean
Mean	38.5	45.4	39.3	41.1
S.D.	5.7	15.5	8.9	
40g EPO alternate day				
Karlops	37	37	36	
Big Ben	36	37	38	
Walter	79	53	44	
Pollux	33	36	30	
Armpit	35	28	48	
Sandy Lad	41	63	40	
Sabrian	74	95	44	
Ollie	39	33	35	
Poppett	57	40	33	
Tessa	48	41	39	
Alfred	40	48	37	Group
Darkie	57	49	41	mean
Mean	48.0	46.7	38.8	44.5
S.D.	15.5	18.0	5.1	

Table XXVI

Triglyceride values Values in mmol/l

Horse	Week 0	Week 3	Week 6	
5g EPO daily				
Annabelle	0.68	0.45	0.37	
Donald	0.15	0.59	0.65	
Myrtle	0.47	0.34	0.32	
Calumn	0.56	0.20	0.25	
Giraffe	0.33	0.29	0.17	
Gunsmoke	0.51	0.37	0.41	
Sovereign	0.48	0.27	0.36	
Orlando	0.41	0.36	0.38	
Jack	0.54	0.19	0.38	
Nicholas	0.53	0.30	0.56	
Suzie	0.53	0.16	0.24	Group
Hermione	0.34	0.23	0.56	mean
Mean	0.461	0.313	0.388	0.387
S.D.	0.137	0.122	0.142	
10g EPO alternate day				
Charlotte	0.49	0.21	0.50	
Marigold	0.51	0.30	0.42	
Jasper	0.47	0.33	0.31	
Ding Dong	0.31	0.19	0.17	
Flora	0.19	0.53	0.43	
Chocolate	0.38	0.20	0.28	
Goldie	0.57	0.23	0.58	
Durando	0.39	0.25	0.35	
BeBe	0.24	0.29	0.41	
Samuel	0.44	0.33	0.47	
Jordan	0.55	0.60	0.61	Group
Dick	0.38	0.32	0.51	mean
Mean	0.410	0.315	0.420	0.382
S.D.	0.119	0.128	0.127	
20g EPO daily				
Foxy	0.53	0.21	0.39	
Wallace	0.44	0.46	0.45	
Gold Loch	0.50	0.26	0.33	
Bonny	0.33	0.23	0.26	
Skippy	0.37	0.34	0.29	
Flynn	0.53	0.35	0.38	
Rocky	0.34	0.32	0.25	
Charlie	0.53	1.77	0.47	
Geno	0.37	0.34	0.48	
Biggles	0.56	0.25	0.40	
Smiler	0.18	0.32	0.30	Group
Aird	0.51	0.73	0.32	mean
Mean	0.433	0.465	0.360	0.419
S.D.	0.115	0.434	0.080	
40g EPO alternate day				
Karlops	0.24	0.48	0.37	
Big Ben	0.25	0.28	0.55	
Walter	0.40	0.25	0.24	
Pollux	0.34	0.38	0.23	
Armpit	0.28	0.32	0.44	
Sandy Lad	0.46	0.41	0.37	
Sabrian	0.46	0.49	0.34	
Ollie	0.50	0.35	0.29	
Poppett	0.37	0.29	0.29	
Tessa	0.29	0.14	0.27	
Alfred	0.51	0.38	0.31	Group
Darkie	0.32	0.31	0.30	mean
Mean	0.368	0.340	0.333	0.347
S.D.	0.097	0.098	0.090	

Table XXVII

Cholesterol values Values in mmol/l

Horse	Week 0	Week 3	Week 6		
5g EPO daily					
Annabelle	3.03	3.25	3.24		
Donald	1.61	2.93	3.06		
Myrtle	2.57	2.64	2.40		
Calumn	2.77	2.66	2.68		
Giraffe	2.03	2.26	1.57		
Gunsmoke	1.84	1.91	2.43		
Sovereign	2.62	2.40	2.32		
Orlando	2.73	2.56	2.49		
Jack	2.71	2.42	2.35		
Nicholas	2.21	2.13	2.19		
Suzie	2.16	2.46	1.63	Group	
Hermione	2.68	2.40	1.61	mean	
Mean	2.413	2.502	2.331		2.415
S.D.	0.433	0.352	0.533		
10g EPO alternate day					
Charlotte	2.27	2.19	1.39		
Marigold	2.29	2.28	1.78		
Jasper	2.12	2.26	1.47		
Ding Dong	1.96	1.86	1.56		
Flora	2.04	1.65	2.17		
Chocolate	2.04	2.19	1.58		
Goldie	2.81	2.12	1.93		
Durando	2.28	1.94	1.43		
BeBe	1.99	1.55	2.04		
Samuel	2.14	1.76	2.41		
Jordan	1.72	2.08	2.39	Group	
Dick	1.64	1.72	2.18	mean	
Mean	2.108	1.967	1.861		1.979
S.D.	0.301	0.254	0.376		
20g EPO daily					
Foxy	2.56	2.34	1.95		
Wallace	2.79	2.83	2.81		
Gold Loch	2.19	2.31	1.73		
Bonny	1.85	1.79	2.07		
Skippy	2.59	2.70	2.88		
Flynn	2.64	2.74	2.36		
Rocky	2.07	1.91	1.43		
Charlie	2.47	2.34	2.38		
Geno	2.52	2.39	2.15		
Biggles	2.64	2.73	1.74		
Smiler	2.12	2.36	2.30	Group	
Aird	1.56	2.31	2.78	mean	
Mean	2.333	2.396	2.215		2.315
S.D.	0.373	0.321	0.462		
40g EPO alternate day					
Karlops	1.42	2.04	2.05		
Big Ben	2.49	2.04	3.24		
Walter	1.41	1.54	2.45		
Pollux	2.46	2.56	2.69		
Armpit	2.14	2.17	1.67		
Sandy Lad	1.59	1.77	2.12		
Sabrian	1.91	1.85	2.18		
Ollie	2.24	2.38	2.60		
Poppett	1.86	2.13	2.61		
Tessa	2.27	2.57	2.19		
Alfred	2.27	2.44	2.69	Group	
Darkie	1.62	2.18	2.33	mean	
Mean	1.973	2.139	2.402		2.171
S.D.	0.392	0.318	0.404		

APPENDIX II

TABLES OF RESULTS OF THE STUDY ON THE USE OF EFAs IN THE TREATMENT OF DERMATOPHILOSIS IN HORSES

Clinical Indices, Haematological and Biochemical Parameters

Clinical Indices

Table I

Indices of dermatophilosis lesion severity

Scored on a 1 to 10 scale, 0 is absence of infection, 10 is most severe infection

Horse	Week 0	2	4	6	8	10	12	14	16	
Controls										
Felfit	1	0	0	0	0	0	0	0	1	
Jill	8	6	6	5	3	4	3	2	2	
Fred	1	0	2	1	0	0	1	0	0	
Temple	1	3	2	1	0	0	1	1	1	
Delta	2	0	0	1	0	0	1	1	1	
Beau	3	4	1	0	0	0	0	0	0	
Fella	5	4	4	2	2	2	2	1	1	
Sprig	1	0	0	1	0	0	0	0	1	
Dick	0	0	0	0	0	0	0	0	0	
Noddy	5	4	4	3	3	3	2	3	1	
Durando	4	3	4	3	3	4	5	3	4	
Natasha	7	6	4	3	4	3	3	1	1	Group mean
Mean	3.2	2.5	2.3	1.7	1.3	1.3	1.5	1.0	1.1	1.8
S.D.	2.6	2.4	2.1	1.6	1.6	1.7	1.6	1.1	1.1	
Placebo										
Charlotte	3	2	3	2	2	1	2	1	1	
Charlie	0	0	0	0	0	0	0	0	0	
Jack	2	0	0	1	1	0	0	2	1	
Rose	4	4	2	1	2	2	1	1	1	
North Star	7	6	6	4	4	3	3	2	5	
Lord Muckle	8	8	8	4	4	5	4	2	2	
Big Ben	5	0	0	0	0	0	0	2	2	
Mike	6	4	4	2	3	2	2	3	1	
Rainbeam	4	2	2	2	2	2	1	2	2	
Choppy	6	8	4	2	3	3	3	2	2	
Spring	0	0	0	0	0	0	0	0	1	
Officer	6	6	6	6	4	4	5	2	2	Group mean
Mean	4.2	3.3	2.9	2.0	2.1	1.8	1.8	1.6	1.7	2.4
S.D.	2.6	3.1	2.7	1.9	1.6	1.7	1.7	0.9	1.2	
EFAs										
Veronica	5	6	6	6	4	2	4	3	2	
Dolly	1	2	4	1	1	1	0	1	0	
Danny	5	6	4	2	3	3	2	2	2	
Prudence	1	1	0	1	1	0	0	0	0	
Jeeves	6	6	5	3	4	3	2	3	1	
Heidi	3	3	3	2	2	2	1	1	1	
Elspeth	6	4	5	3	1	0	2	1	1	
Anne	4	2	2	3	2	1	1	1	1	
Lily	4	5	8	6	5	3	4	4	2	
Polly	3	6	6	4	2	3	3	2	2	
Henry	9	10	9	7	7	5	6	5	5	Group mean
Mean	4.3	4.6	4.7	3.5	2.9	2.1	2.3	2.1	1.5	3.1
S.D.	2.3	2.6	2.6	2.1	1.9	1.5	1.8	1.5	1.4	

Table II

Extent of distribution of dorsal dermatophilosis lesions

Scored by % of body surface area affected

Horse	Week 0	2	4	6	8	10	12	14	16	
Controls										
Felfit	2.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	
Jill	55.0	25.0	30.0	20.0	10.0	10.0	1.0	1.0	2.5	
Fred	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Temple	0.0	2.5	0.0	0.0	0.0	0.0	1.0	3.6	2.5	
Delta	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Beau	20.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Fella	30.0	40.0	30.0	20.0	25.0	15.0	20.0	20.0	10.0	
Sprig	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Dick	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Noddy	45.0	25.0	25.0	30.0	10.0	10.0	10.0	10.0	10.0	
Durando	15.0	20.0	20.0	15.0	10.0	10.0	5.0	5.0	1.0	
Natasha	40.0	45.0	20.0	25.0	30.0	15.0	20.0	3.6	2.5	Group mean
Mean	18.1	13.5	10.4	9.2	7.1	5.0	4.8	3.6	2.5	8.2
S.D.	19.7	16.8	13.2	11.8	10.5	6.4	7.7	6.0	3.7	
Placebo										
Charlotte	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Charlie	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Jack	2.5	0.0	0.0	1.0	2.5	0.0	0.0	7.3	5.5	
Rose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
North Star	30.0	35.0	25.0	30.0	10.0	20.0	15.0	15.0	15.0	
Lord Muckle	50.0	55.0	40.0	20.0	20.0	20.0	20.0	5.0	5.0	
Big Ben	25.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	6.9	
Mike	45.0	45.0	35.0	30.0	25.0	25.0	20.0	20.0	15.0	
Rainbeam	15.0	20.0	20.0	15.0	20.0	10.0	10.0	10.0	6.9	
Choppy	40.0	30.0	30.0	20.0	10.0	20.0	20.0	10.0	5.0	
Spring	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Officer	30.0	45.0	35.0	25.0	25.0	30.0	25.0	10.0	6.9	Group mean
Mean	19.8	19.2	15.4	11.8	9.4	10.4	9.2	7.3	5.5	12.0
S.D.	19.3	21.7	16.8	12.8	10.5	11.8	10.2	6.5	5.3	
EFAs										
Veronica	20.0	20.0	20.0	15.0	10.0	10.0	20.0	20.0	2.5	
Dolly	5.0	2.5	2.5	2.5	0.0	1.0	0.0	0.0	0.0	
Danny	30.0	35.0	25.0	20.0	20.0	15.0	10.0	10.0	5.0	
Prudence	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Jeeves	30.0	25.0	20.0	20.0	5.0	10.0	5.0	1.0	1.0	
Heidi	0.0	2.5	0.0	0.0	0.0	0.0	0.0	5.2	5.2	
Elspeth	25.0	10.0	5.0	2.5	0.0	0.0	1.0	1.0	1.0	
Anne	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Lily	25.0	30.0	15.0	15.0	10.0	10.0	15.0	10.0	10.0	
Polly	25.0	35.0	30.0	30.0	20.0	25.0	25.0	25.0	35.0	
Henry	60.0	55.0	45.0	40.0	30.0	25.0	30.0	20.0	15.0	Group mean
Mean	20.5	19.5	14.8	13.2	8.6	8.7	9.6	8.4	6.8	12.2
S.D.	17.7	18.2	14.9	13.6	10.5	9.7	11.2	9.4	10.5	

Table III

Extent of distribution of hindlimb dermatophilosis lesions
Scored by % of body surface area affected

Horse	Week 0	2	4	6	8	10	12	14	16
Controls									
Felfit	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Jill	40.0	40.0	50.0	15.0	10.0	10.0	1.0	1.0	2.5
Fred	0.0	0.0	5.0	1.0	0.0	0.0	1.0	0.0	0.0
Temple	20.0	20.0	10.0	1.0	0.0	0.0	0.0	0.4	2.7
Delta	20.0	0.0	0.0	1.0	0.0	0.0	1.0	1.0	1.0
Beau	20.0	20.0	2.5	0.0	0.0	0.0	0.0	0.0	0.0
Fella	20.0	10.0	15.0	1.0	0.0	0.0	0.0	0.0	10.0
Sprig	15.0	0.0	0.0	2.5	0.0	0.0	0.0	0.0	1.0
Dick	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Noddy	50.0	20.0	35.0	20.0	2.5	2.5	1.0	1.0	0.0
Durando	5.0	5.0	0.0	2.5	2.5	2.5	2.5	1.0	10.0
Natasha	20.0	20.0	10.0	2.5	10.0	0.0	2.5	0.4	2.7
Mean	17.50	11.25	10.63	3.88	2.08	1.25	0.75	0.40	2.49
S.D.	15.59	12.81	16.03	6.52	3.82	2.92	0.94	0.47	3.67
							Group mean 5.58		
Placebo									
Charlotte	10.0	25.0	20.0	10.0	5.0	5.0	10.0	5.0	1.0
Charlie	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Jack	5.0	0.0	0.0	0.0	0.0	0.0	0.0	6.6	8.5
Rose	60.0	15.0	10.0	10.0	20.0	20.0	20.0	20.0	20.0
North Star	30.0	20.0	30.0	0.0	0.0	0.0	0.0	0.0	1.0
Lord Muckle	60.0	60.0	60.0	50.0	20.0	40.0	50.0	30.0	30.0
Big Ben	30.5	0.0	0.0	0.0	0.0	0.0	0.0	6.8	9.6
Mike	25.0	20.0	0.0	0.0	2.5	0.0	0.0	2.5	0.0
Rainbeam	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.6
Choppy	30.0	20.0	20.0	2.5	2.5	2.5	2.5	1.0	2.5
Spring	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0
Officer	40.0	30.0	40.0	30.0	0.0	2.5	2.5	6.8	9.6
Mean	25.04	15.83	15.00	8.54	4.17	5.83	7.08	6.56	8.48
S.D.	21.01	17.94	19.77	15.76	7.56	12.17	14.80	9.30	9.01
							Group mean 10.73		
EFAs									
Veronica	20.0	20.0	10.0	20.0	10.0	0.0	5.0	5.0	2.5
Dolly	0.0	0.0	10.0	0.0	2.5	0.0	0.0	1.0	0.0
Danny	20.0	30.0	20.0	0.0	2.5	0.0	0.0	1.0	0.0
Prudence	10.0	5.0	0.0	2.5	2.5	0.0	0.0	0.0	0.0
Jeeves	20.0	20.0	20.0	1.0	2.5	0.0	5.0	5.0	0.0
Heidi	40.0	40.0	40.0	30.0	25.0	20.0	20.0	1.7	0.9
Elspeth	20.0	5.0	2.5	2.5	2.5	0.0	1.0	0.0	0.0
Anne	30.0	25.0	20.0	5.0	2.5	2.5	2.5	2.5	2.5
Lily	30.0	30.0	20.0	10.0	2.5	2.5	2.5	5.0	20.0
Polly	0.0	20.0	15.0	2.5	0.0	2.5	2.5	0.0	2.5
Henry	60.0	50.0	30.0	10.0	10.0	20.0	20.0	5.0	2.5
Mean	19.00	19.50	15.75	7.35	5.25	2.75	3.85	2.12	2.84
S.D.	17.37	15.23	11.56	9.54	7.17	7.83	7.47	2.21	5.82
							Group mean 8.70		

Table IV

Coat condition scores

Scored on a 1 to 10 scale, 1 is poorest, 10 is optimal

Horse	Week 0	2	4	6	8	10	12	14	16	
Controls										
Felfit	5	5	7	7	8	8	8	8	8	
Jill	5	4	5	6	5	6	6	7	7	
Fred	4	5	7	8	8	8	8	8	8	
Temple	6	5	7	8	8	8	8	8	7	
Delta	6	4	4	7	7	6	7	8	8	
Beau	7	6	6	8	8	8	8	8	8	
Fella	5	4	5	6	6	6	7	7	6	
Sprig	6	4	4	6	7	6	7	7	7	
Dick	6	7	7	8	8	8	8	8	8	
Noddy	4	5	4	6	6	7	7	7	7	
Durando	5	4	5	6	7	7	8	8	6	
Natasha	5	4	4	6	5	5	5	8	7	Group mean
Mean	5.3	4.8	5.4	6.8	6.9	6.9	7.3	7.6	7.3	6.5
S.D.	0.9	1.0	1.3	0.9	1.2	1.1	1.0	0.5	0.7	
Placebo										
Charlotte	6	6	7	8	8	8	8	7	8	
Charlie	6	6	6	7	8	8	8	8	8	
Jack	5	7	7	8	8	8	8	7	7	
Rose	5	4	5	6	8	7	7	6	6	
North Star	6	5	5	6	7	5	7	8	6	
Lord Muckle	3	2	3	7	5	5	7	7	5	
Big Ben	5	6	6	7	7	8	8	7	7	
Mike	5	4	4	8	8	7	8	7	7	
Rainbeam	4	5	7	7	8	8	8	7	7	
Choppy	5	5	4	7	7	7	7	8	9	
Spring	6	4	6	5	7	7	8	7	7	
Officer	8	3	3	6	7	6	6	7	7	Group mean
Mean	5.4	4.8	5.3	6.8	7.3	7.0	7.5	7.2	6.9	6.5
S.D.	1.2	1.4	1.5	0.9	0.9	1.1	0.7	0.6	1.1	
EFAs										
Veronica	5	4	4	6	7	7	8	7	7	
Dolly	6	6	6	7	8	7	7	8	8	
Danny	6	6	6	7	7	6	8	8	7	
Prudence	6	6	6	7	8	7	7	7	7	
Jeeves	5	5	4	8	7	8	7	8	8	
Heidi	6	4	5	6	7	7	8	8	8	
Elspeth	5	5	4	6	8	8	7	8	8	
Anne	6	6	6	7	7	7	7	8	7	
Lily	6	4	5	6	6	7	6	7	6	
Polly	5	4	5	6	7	7	8	6	7	
Henry	4	3	6	7	6	6	6	7	7	Group mean
Mean	5.5	4.8	5.2	6.6	7.1	7.0	7.2	7.4	7.2	6.4
S.D.	0.7	1.1	0.9	0.7	0.7	0.6	0.8	0.7	0.6	

Table V

Mane condition scores

Scored on a 1 to 10 scale, 1 is poorest, 10 is optimal

Horse	Week 0	2	4	6	8	10	12	14	16	
Controls										
Felfit	6	5	6	6	8	8	8	8	8	
Jill	5	5	5	7	7	8	8	8	7	
Fred	7	6	6	7	8	8	8	8	8	
Temple	6	6	6	7	7	8	8	8	8	
Delta	6	6	6	7	7	7	7	8	8	
Beau	7	5	6	8	8	8	8	8	8	
Fella	5	4	6	6	6	7	7	8	6	
Sprig	5	6	6	6	7	7	8	8	8	
Dick	6	7	7	7	8	8	8	8	8	
Noddy	4	5	4	7	5	8	8	8	8	
Durando	6	5	6	7	7	8	8	8	8	
Natasha	4	4	5	6	5	6	7	8	8	Group mean
Mean	5.6	5.3	5.8	6.8	6.9	7.6	7.8	8.0	7.7	6.8
S.D.	1.0	0.9	0.8	0.6	1.1	0.7	0.5	0.0	0.6	
Placebo										
Charlotte	5	5	6	8	7	8	8	8	8	
Charlie	6	6	6	7	8	8	8	8	8	
Jack	5	7	6	8	7	8	8	8	8	
Rose	5	5	6	6	7	7	7	7	8	
North Star	6	6	5	6	7	7	8	8	8	
Lord Muckle	5	4	5	7	7	6	7	8	7	
Big Ben	5	5	6	7	7	8	8	8	8	
Mike	4	5	6	7	8	7	8	8	8	
Rainbeam	4	4	6	6	7	7	8	8	8	
Choppy	5	6	6	7	8	8	8	8	9	
Spring	6	6	6	6	8	8	8	8	8	
Officer	5	4	3	6	7	6	7	8	8	Group mean
Mean	5.1	5.3	5.6	6.8	7.3	7.3	7.8	7.9	7.9	6.8
S.D.	0.7	1.0	0.9	0.8	0.5	0.8	0.5	0.3	0.4	
EFAs										
Veronica	5	6	4	7	7	7	8	7	7	
Dolly	6	6	6	7	8	7	7	8	8	
Danny	6	6	6	7	7	7	8	8	8	
Prudence	6	6	6	7	8	8	7	8	8	
Jeeves	5	5	5	8	6	7	8	8	8	
Heidi	6	5	6	6	7	8	8	8	8	
Elspeth	5	6	5	7	7	8	7	8	7	
Anne	6	6	6	7	6	6	7	7	7	
Lily	6	5	5	7	7	6	7	8	8	
Polly	5	5	5	6	6	7	8	7	8	
Henry	5	6	6	7	6	7	8	8	8	Group mean
Mean	5.5	5.6	5.5	6.9	6.8	7.1	7.5	7.7	7.7	6.7
S.D.	0.5	0.5	0.7	0.5	0.8	0.7	0.5	0.5	0.5	

Table VI

Tail condition scores

Scored on a 1 to 10 scale, 1 is poorest, 10 is optimal

Horse	Week 0	2	4	6	8	10	12	14	16	
Controls										
Felfit	6	5	6	7	8	8	8	8	8	
Jill	5	5	5	7	7	8	8	8	7	
Fred	5	6	6	7	8	8	8	8	8	
Temple	6	6	6	7	8	8	8	8	7	
Delta	6	6	6	7	7	7	7	8	8	
Beau	7	5	6	8	7	7	8	8	7	
Fella	5	4	6	7	6	7	7	7	7	
Sprig	5	6	6	6	7	7	8	7	7	
Dick	6	7	7	8	8	8	8	8	0	
Noddy	4	5	6	7	6	7	7	8	8	
Durando	6	6	6	7	7	8	8	8	8	
Natasha	4	5	6	6	6	7	7	8	7	Group mean
Mean	5.4	5.5	6.0	7.0	7.1	7.5	7.7	7.8	6.8	6.8
S.D.	0.9	0.8	0.4	0.6	0.8	0.5	0.5	0.4	2.2	
Placebo										
Charlotte	6	5	6	8	7	8	8	8	8	
Charlie	7	6	6	7	8	8	8	8	8	
Jack	5	7	7	7	7	8	8	8	8	
Rose	5	5	6	6	8	7	8	7	8	
North Star	6	6	6	6	7	7	7	8	8	
Lord Muckle	5	4	6	7	7	6	7	8	7	
Big Ben	5	6	6	8	7	8	8	8	8	
Mike	4	5	4	8	7	7	8	8	8	
Rainbeam	5	6	6	7	7	8	8	8	8	
Choppy	5	6	6	8	8	8	8	8	9	
Spring	5	6	6	6	7	7	7	8	7	
Officer	6	5	6	6	7	7	7	8	8	Group mean
Mean	5.3	5.6	5.9	7.0	7.3	7.4	7.7	7.9	7.9	6.9
S.D.	0.8	0.8	0.7	0.9	0.5	0.7	0.5	0.3	0.5	
EFAs										
Veronica	6	6	4	7	7	7	8	8	8	
Dolly	6	6	6	7	8	7	7	8	7	
Danny	6	6	6	7	7	7	8	8	7	
Prudence	6	6	6	7	8	8	7	8	8	
Jeeves	5	5	6	8	7	7	8	8	7	
Heidi	7	5	6	7	7	7	8	8	8	
Elspeth	5	6	5	7	7	8	7	8	8	
Anne	6	6	6	7	7	7	7	8	8	
Lily	4	5	5	7	6	6	7	8	7	
Polly	6	5	5	6	6	7	8	7	8	
Henry	5	6	6	7	7	7	8	8	7	Group mean
Mean	5.6	5.6	5.5	7.0	7.0	7.1	7.5	7.9	7.5	6.8
S.D.	0.8	0.5	0.7	0.4	0.6	0.5	0.5	0.3	0.5	

Table VII

Hoof condition scores

Scored on a 1 to 10 scale, 1 is poorest, 10 is optimal

Horse	Week 0	2	4	6	8	10	12	14	16	
Controls										
Felfit	7	7	6	7	8	8	8	7	7	
Jill	6	5	6	7	7	7	7	7	7	
Fred	7	7	7	7	8	8	8	7	7	
Temple	7	7	6	7	7	7	7	7	7	
Delta	6	6	6	7	7	7	7	7	6	
Beau	7	8	7	7	7	8	8	8	8	
Fella	6	6	6	7	7	7	7	6	7	
Sprig	7	6	6	6	7	7	7	7	7	
Dick	7	8	7	7	8	8	8	8	8	
Noddy	6	6	6	7	7	7	7	7	7	
Durando	7	6	7	6	7	7	7	7	7	
Natasha	7	7	7	7	8	7	8	7	7	Group mean
Mean	6.7	6.6	6.4	6.8	7.3	7.3	7.4	7.1	7.1	7.0
S.D.	0.5	0.9	0.5	0.4	0.5	0.5	0.5	0.5	0.5	
Placebo										
Charlotte	7	7	7	7	8	7	8	7	7	
Charlie	7	7	7	6	7	7	7	7	7	
Jack	7	8	7	7	8	8	8	7	7	
Rose	6	6	6	5	7	7	7	6	6	
North Star	6	6	6	7	7	7	7	8	7	
Lord Muckle	5	6	6	6	7	7	6	6	7	
Big Ben	6	6	6	6	7	7	7	7	7	
Mike	6	6	7	7	7	7	8	6	8	
Rainbeam	6	6	7	7	8	7	8	7	7	
Choppy	7	7	6	7	7	8	8	8	8	
Spring	7	7	6	6	8	7	8	7	7	
Officer	7	7	6	6	7	7	7	7	7	Group mean
Mean	6.4	6.6	6.4	6.4	7.3	7.2	7.4	6.9	7.1	6.9
S.D.	0.7	0.7	0.5	0.7	0.5	0.4	0.7	0.7	0.5	
EFAs										
Veronica	6	4	6	7	7	7	8	7	7	
Dolly	5	5	6	6	7	6	6	6	6	
Danny	7	7	6	7	7	7	7	7	7	
Prudence	7	6	7	7	7	7	7	7	7	
Jeeves	7	6	6	7	6	7	7	7	7	
Heidi	7	6	6	6	7	7	7	7	7	
Elspeth	6	6	6	7	7	7	7	7	7	
Anne	7	7	6	7	8	8	7	8	7	
Lily	6	6	7	7	7	7	7	7	7	
Polly	6	6	6	6	6	7	6	7	7	
Henry	7	6	6	7	7	7	7	7	7	Group mean
Mean	6.5	5.9	6.2	6.7	6.9	7.0	6.9	7.0	6.9	6.7
S.D.	0.7	0.8	0.4	0.5	0.5	0.4	0.5	0.4	0.3	

Table VIII

Body condition scores

Scored on a 1 to 5 scale, 1 is emaciated, 5 is obese.

Horse	Week 0	2	4	6	8	10	12	14	16	
Controls										
Felfit	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	
Jill	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	
Fred	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	
Temple	3.5	3.0	3.0	3.0	3.5	3.5	3.5	3.5	3.5	
Delta	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	
Beau	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	
Fella	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	
Sprig	3.5	3.5	3.5	3.0	4.0	3.0	3.5	3.5	3.0	
Dick	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	
Noddy	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	
Durando	3.0	3.0	3.0	3.5	3.5	3.0	3.0	3.0	3.0	Group
Natasha	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	mean
Mean	3.08	3.04	3.04	3.04	3.17	3.04	3.08	3.08	3.04	3.07
S.D.	0.19	0.14	0.14	0.14	0.33	0.14	0.19	0.19	0.14	
Placebo										
Charlotte	3.0	3.0	3.0	3.5	3.5	3.5	3.5	4.0	4.0	
Charlie	4.0	3.5	4.0	3.5	4.0	4.0	4.0	4.0	4.0	
Jack	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	
Rose	3.0	3.5	3.0	3.5	3.5	3.0	3.5	3.5	3.0	
North Star	3.0	3.0	3.0	3.0	2.5	3.0	3.0	3.0	3.0	
L.Muckle	2.0	1.5	2.0	2.5	2.5	2.5	2.5	2.5	1.5	
Big Ben	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	
Mike	3.0	3.0	3.0	2.5	3.0	2.5	3.0	3.0	3.0	
Rainbeam	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	
Choppy	3.0	3.0	3.0	3.0	3.0	3.5	3.5	3.0	3.0	
Spring	3.0	3.0	3.0	3.5	3.5	3.5	3.5	3.0	3.5	Group
Officer	3.0	3.5	3.0	3.0	3.0	3.0	3.0	3.0	3.0	mean
Mean	3.04	3.04	3.04	3.13	3.17	3.17	3.25	3.21	3.13	3.13
S.D.	0.45	0.54	0.45	0.38	0.44	0.44	0.40	0.45	0.64	
EFAs										
Veronica	3.0	3.0	3.0	2.5	3.0	3.0	3.0	3.0	3.0	
Dolly	3.5	3.5	3.5	3.5	4.0	3.5	4.0	3.5	4.0	
Danny	3.0	3.0	3.0	3.5	3.5	3.0	3.0	3.0	3.0	
Prudence	3.5	3.5	4.0	4.0	4.0	4.0	4.0	4.0	4.0	
Jeeves	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	
Heidi	3.0	3.0	3.5	3.0	3.5	3.5	3.5	3.5	3.0	
Elspeth	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	
Anne	3.0	3.0	3.0	3.0	3.5	3.0	3.0	3.0	3.0	
Lily	3.0	3.0	3.0	3.0	3.5	3.5	3.0	3.0	3.0	
Polly	3.5	3.5	3.5	3.5	3.0	3.5	3.0	3.0	3.0	Group
Henry	2.5	1.5	2.0	2.5	2.5	2.5	2.5	2.0	2.5	mean
Mean	3.09	3.00	3.14	3.14	3.32	3.23	3.18	3.09	3.14	3.15
S.D.	0.30	0.55	0.50	0.45	0.46	0.41	0.46	0.49	0.45	

Haematological Parameters

Table IX

Red cell counts Values $x10^{12}/l$

Horse	Week 0	4	8	12	16	
Controls						
Felfit	9.0	7.5	8.6	7.1	6.0	
Jill	7.7	7.3	6.5	6.8	6.2	
Fred	5.5	6.3	5.2	5.5	5.3	
Temple	6.9	6.3	6.5	6.0	6.3	
Delta	6.3	5.4	6.2	5.6	5.4	
Beau	7.0	7.1	7.2	7.2	7.1	
Fella	7.4	8.1	6.7	7.2	7.4	
Sprig	7.4	7.1	6.8	7.1	8.1	
Dick	6.8	6.9	6.4	6.0	5.8	
Noddy	6.4	5.9	5.9	6.5	5.9	
Durando	6.1	6.7	7.8	5.7	6.1	
Natasha	6.6	7.6	7.0	6.2	6.3	Group mean
Mean	6.93	6.85	6.73	6.41	6.33	6.65
S.D.	0.90	0.77	0.88	0.66	0.82	
Placebo						
Charlotte	7.4	6.7	7.0	6.8	6.3	
Charlie	6.5	6.0	6.0	6.3	5.8	
Jack	8.0	7.1	7.2	7.0	6.6	
Rose	7.1	5.9	5.6	5.9	6.9	
North Star	6.6	6.5	6.4	7.5	6.9	
Lord Muckle	7.2	8.2	6.1	7.8	7.3	
Big Ben	6.7	6.2	5.2	5.7	6.5	
Mike	6.6	6.5	6.6	6.4	5.7	
Rainbeam	6.6	7.2	6.2	6.7	6.5	
Choppy	7.8	8.2	8.8	8.6	8.4	
Spring	6.1	6.4	6.1	6.7	6.0	
Officer	7.0	7.2	6.4	7.0	6.5	Group mean
Mean	6.97	6.84	6.47	6.87	6.62	6.75
S.D.	0.56	0.77	0.91	0.81	0.73	
EFAs						
Veronica	6.4	5.8	6.4	6.4	7.4	
Dolly	6.7	5.6	6.6	8.0	6.2	
Danny	7.7	7.8	9.9	6.6	7.1	
Prudence	5.2	6.0	5.1	5.0	5.2	
Jeeves	6.4	6.2	6.2	6.6	6.1	
Heidi	7.3	6.5	5.2	6.8	6.5	
Elspeth	6.9	5.2	5.7	5.5	5.7	
Anne	6.7	8.5	6.7	6.2	6.9	
Lily	6.2	6.2	6.1	6.6	5.6	
Polly	7.7	7.2	5.3	6.2	7.0	
Henry	7.6	6.6	6.8	6.6	6.6	Group mean
Mean	6.80	6.51	6.36	6.41	6.39	6.49
S.D.	0.76	0.98	1.32	0.76	0.70	

Table X

Haemoglobin values Values given in g/dl

Horse	Week 0	4	8	12	16	
Controls						
Felfit	16.0	12.9	15.5	12.5	10.2	
Jill	13.5	12.3	11.0	11.7	10.5	
Fred	12.7	11.6	9.2	10.1	9.4	
Temple	12.7	11.4	11.7	10.9	11.2	
Delta	12.7	10.4	12.1	11.0	10.5	
Beau	12.3	12.2	12.4	12.3	12.0	
Fella	12.8	14.1	11.3	12.3	12.6	
Sprig	13.9	13.3	12.6	12.9	15.0	
Dick	12.2	12.4	11.4	10.6	10.2	
Noddy	12.3	10.7	11.0	12.0	10.9	
Durando	10.5	12.0	13.5	9.7	10.3	
Natasha	12.7	14.2	12.7	11.3	11.2	Group mean
Mean	12.86	12.29	12.03	11.44	11.17	11.96
S.D.	1.28	1.20	1.55	1.01	1.48	
Placebo						
Charlotte	13.3	11.7	12.5	12.3	11.4	
Charlie	11.9	10.9	11.1	11.2	10.3	
Jack	14.2	12.2	12.6	12.6	11.6	
Rose	13.5	10.9	10.1	10.5	12.4	
North Star	11.8	11.2	10.9	12.8	11.7	
Lord Muckle	12.1	13.4	10.6	12.5	12.0	
Big Ben	12.2	12.2	10.1	11.0	11.6	
Mike	12.2	11.7	12.1	11.7	10.3	
Rainbeam	12.0	12.7	11.1	11.8	11.6	
Choppy	13.4	14.0	15.3	14.6	14.1	
Spring	10.8	11.5	11.1	11.8	10.8	
Officer	12.1	12.4	11.0	12.1	11.6	Group mean
Mean	12.46	12.07	11.54	12.08	11.62	11.95
S.D.	0.94	0.96	1.44	1.05	1.01	
EFAs						
Veronica	12.7	10.8	12.0	11.8	14.2	
Dolly	12.8	10.2	12.0	14.9	11.0	
Danny	13.9	13.8	17.8	11.6	12.4	
Prudence	10.0	12.2	9.8	10.2	10.2	
Jeeves	11.9	10.8	11.4	12.3	11.2	
Heidi	13.8	12.4	9.8	12.7	11.8	
Elspeth	12.8	9.3	10.7	10.5	10.5	
Anne	12.3	15.4	11.8	10.9	12.4	
Lily	11.7	11.7	11.4	12.3	10.7	
Polly	14.8	13.2	9.7	11.5	13.0	
Henry	13.9	11.7	11.9	11.6	11.6	Group mean
Mean	12.78	11.95	11.66	11.85	11.73	11.99
S.D.	1.32	1.73	2.23	1.27	1.20	

Table XI

Packed cell volumes Values given as l/l

Horse	Week 0	4	8	12	16	
Controls						
Felfit	0.472	0.392	0.460	0.371	0.309	
Jill	0.405	0.371	0.332	0.346	0.316	
Fred	0.304	0.352	0.283	0.299	0.281	
Temple	0.383	0.348	0.352	0.323	0.334	
Delta	0.356	0.312	0.363	0.324	0.313	
Beau	0.360	0.367	0.373	0.369	0.361	
Fella	0.377	0.411	0.339	0.364	0.374	
Sprig	0.405	0.388	0.370	0.381	0.434	
Dick	0.364	0.376	0.347	0.322	0.309	
Noddy	0.358	0.332	0.329	0.360	0.329	
Durando	0.308	0.346	0.399	0.294	0.312	
Natasha	0.360	0.414	0.386	0.337	0.334	Group mean
Mean	0.3710	0.3674	0.3611	0.3418	0.3338	0.3548
S.D.	0.0446	0.0310	0.0435	0.0289	0.0401	
Placebo						
Charlotte	0.393	0.358	0.382	0.370	0.343	
Charlie	0.355	0.334	0.331	0.343	0.310	
Jack	0.418	0.377	0.378	0.365	0.000	
Rose	0.393	0.323	0.305	0.315	0.371	
North Star	0.345	0.338	0.330	0.381	0.351	
Lord Muckle	0.359	0.400	0.296	0.372	0.358	
Big Ben	0.361	0.362	0.301	0.326	0.345	
Mike	0.363	0.361	0.370	0.354	0.316	
Rainbeam	0.349	0.383	0.334	0.352	0.345	
Lily	0.351	0.353	0.353	0.368	0.314	
Spring	0.324	0.344	0.328	0.362	0.323	
Officer	0.368	0.375	0.337	0.364	0.345	Group mean
Mean	0.3649	0.3590	0.3371	0.3560	0.3101	0.3454
S.D.	0.0254	0.0223	0.0290	0.0195	0.0994	
EFAs						
Veronica	0.360	0.328	0.356	0.353	0.411	
Dolly	0.379	0.314	0.364	0.438	0.340	
Danny	0.407	0.415	0.524	0.345	0.370	
Prudence	0.297	0.349	0.298	0.286	0.301	
Jeeves	0.353	0.341	0.345	0.368	0.339	
Heidi	0.402	0.364	0.288	0.377	0.351	
Elspeth	0.377	0.289	0.319	0.310	0.317	
Anne	0.358	0.455	0.352	0.327	0.364	
Lily	0.351	0.353	0.353	0.368	0.314	
Polly	0.430	0.397	0.286	0.335	0.374	
Henry	0.407	0.357	0.365	0.352	0.351	Group mean
Mean	0.3746	0.3602	0.3500	0.3508	0.3484	0.3568
S.D.	0.367	0.471	0.651	0.395	0.314	

Table XII

Mean cell volumes Values given in fl

Horse	Week 0	4	8	12	16	
Controls						
Felfit	52.4	52.3	53.5	52.3	51.5	
Jill	52.6	50.8	51.1	50.9	50.9	
Fred	55.3	55.8	54.5	54.3	53.0	
Temple	55.5	55.3	54.2	53.8	52.9	
Delta	56.5	57.8	58.6	57.8	58.0	
Beau	51.4	51.7	51.8	51.2	50.9	
Fella	50.9	50.7	50.6	50.6	50.6	
Sprig	54.7	54.7	54.4	53.7	53.6	
Dick	53.5	54.5	54.2	53.7	53.2	
Noddy	56.0	56.2	55.8	55.4	55.7	
Durando	50.5	51.7	51.1	51.5	51.2	
Natasha	54.5	54.5	55.2	54.3	52.9	Group mean
Mean	53.65	53.83	53.75	53.29	52.87	53.48
S.D.	2.06	2.33	2.32	2.11	2.19	
Placebo						
Charlotte	53.1	53.4	54.5	54.4	54.4	
Charlie	54.6	55.7	55.2	54.5	53.4	
Jack	52.3	53.1	52.5	52.2	52.7	
Rose	55.4	54.8	54.4	53.4	53.7	
North Star	52.3	52.0	51.6	50.8	50.9	
Lord Muckle	49.9	48.8	48.5	47.7	49.0	
Big Ben	58.4	57.8	57.2	59.0	53.4	
Mike	55.0	55.6	56.0	55.3	55.4	
Rainbeam	52.9	53.2	54.2	52.5	53.4	
Choppy	51.0	51.6	50.0	50.4	48.9	
Spring	53.1	53.7	53.8	54.1	53.8	
Officer	52.5	52.1	52.7	52.0	53.4	Group mean
Mean	53.59	53.48	53.38	53.03	52.70	53.24
S.D.	2.20	2.33	2.48	2.84	2.04	
EFAs						
Veronica	56.3	56.6	55.6	55.2	55.5	
Dolly	56.5	56.1	55.1	54.7	54.9	
Danny	52.9	53.2	52.9	52.3	52.1	
Prudence	57.2	58.2	58.4	57.3	57.8	
Jeeves	55.1	55.0	55.7	55.8	55.6	
Heidi	55.0	56.0	55.4	55.4	54.0	
Elspeth	54.7	55.6	55.9	56.3	55.7	
Anne	53.5	53.5	52.5	52.7	52.8	
Lily	56.6	56.9	57.9	55.8	56.1	
Polly	55.9	55.1	54.0	54.0	53.4	
Henry	53.6	54.1	53.7	53.3	53.2	Group mean
Mean	55.21	55.48	55.19	54.80	54.65	55.07
S.D.	1.43	1.51	1.86	1.57	1.70	

Table XIII

Mean cell haemoglobin values in pg

Horse	Week 0	4	8	12	16	
Controls						
Felfit	17.8	17.2	18.0	17.6	17.0	
Jill	17.5	16.8	16.9	17.2	16.9	
Fred	18.4	18.4	17.7	18.4	17.7	
Temple	18.4	18.1	18.0	18.2	17.6	
Delta	20.2	19.3	19.5	19.6	19.4	
Beau	17.6	17.2	17.2	17.1	16.9	
Fella	17.3	17.4	16.9	17.1	17.0	
Sprig	18.8	18.7	18.5	18.2	18.5	
Dick	17.9	18.0	17.8	17.7	17.6	
Noddy	19.2	18.1	18.6	18.5	18.5	
Durando	17.2	17.9	17.3	17.0	16.9	
Natasha	19.2	18.7	18.1	18.2	17.6	Group mean
Mean	18.29	17.98	17.88	17.90	17.63	17.94
S.D.	0.92	0.73	0.76	0.77	0.80	
Placebo						
Charlotte	18.0	17.5	17.9	18.1	18.1	
Charlie	18.3	18.2	18.5	17.8	17.8	
Jack	17.8	17.2	17.5	18.0	17.6	
Rose	19.0	18.5	18.0	17.8	18.0	
North Star	17.9	17.2	17.0	17.1	17.0	
Lord Muckle	16.8	16.3	17.4	16.0	16.4	
Big Ben	18.2	19.7	19.4	19.3	17.9	
Mike	18.5	18.0	18.3	18.3	18.1	
Rainbeam	18.2	17.6	18.1	17.6	17.9	
Choppy	17.2	17.1	17.4	17.0	16.8	
Spring	17.7	18.0	18.2	17.6	18.0	
Officer	17.3	17.2	17.2	17.3	17.9	Group mean
Mean	17.91	17.71	17.91	17.66	17.63	17.76
S.D.	0.61	0.86	0.67	0.80	0.57	
EFAs						
Veronica	19.8	18.6	18.8	18.4	19.2	
Dolly	19.1	18.2	18.2	18.6	17.7	
Danny	18.1	17.7	18.0	17.6	17.5	
Prudence	19.2	20.3	19.2	20.4	19.6	
Jeeves	18.6	17.4	18.4	18.6	18.4	
Heidi	18.9	19.1	18.8	18.7	18.1	
Elspeth	18.6	17.9	18.8	19.1	18.4	
Anne	18.4	18.1	17.6	17.6	18.0	
Lily	18.9	18.9	18.7	18.6	19.1	
Polly	19.2	18.3	18.3	18.5	18.6	
Henry	18.3	17.7	17.5	17.6	17.6	Group mean
Mean	18.83	18.38	18.39	18.52	18.38	18.50
S.D.	0.49	0.82	0.54	0.80	0.69	

Table XIV

Mean cell haemoglobin concentration values (g/dl)

Horse	Week 0	4	8	12	16	
Controls						
Felfit	33.9	32.9	33.7	33.7	33.0	
Jill	33.3	33.2	33.1	33.8	33.3	
Fred	33.2	33.0	32.5	33.8	33.5	
Temple	33.2	32.7	33.2	33.8	33.4	
Delta	35.7	33.3	33.3	34.0	33.5	
Beau	34.2	33.2	33.2	33.4	33.2	
Fella	34.0	34.3	33.3	33.8	33.7	
Sprig	34.3	34.2	34.1	33.8	34.5	
Dick	33.5	33.0	32.9	32.9	33.1	
Noddy	34.3	32.3	33.4	33.3	33.2	
Durando	34.1	34.6	33.9	33.0	33.0	
Natasha	35.3	34.3	32.9	33.6	33.4	Group mean
Mean	34.08	33.42	33.29	33.58	33.40	33.55
S.D.	0.78	0.74	0.45	0.35	0.41	
Placebo						
Charlotte	33.8	32.7	32.8	33.3	33.3	
Charlie	33.5	32.6	33.5	32.6	33.3	
Jack	33.9	32.4	33.3	34.5	33.4	
Rose	34.3	33.7	33.2	33.3	33.5	
North Star	34.2	33.1	33.0	33.6	33.3	
Lord Muckle	33.7	33.5	35.8	33.6	33.5	
Big Ben	33.9	33.7	33.6	33.7	33.5	
Mike	33.6	32.4	32.7	33.1	32.6	
Rainbeam	34.4	33.2	33.3	33.5	33.5	
Choppy	33.7	33.1	34.8	33.7	34.3	
Spring	33.3	33.5	33.8	32.6	33.5	
Officer	32.9	33.1	32.6	33.2	33.5	Group mean
Mean	33.77	33.08	33.53	33.39	33.43	33.44
S.D.	0.42	0.47	0.93	0.51	0.37	
EFAs						
Veronica	35.2	32.9	33.7	33.4	34.6	
Dolly	33.8	32.5	33.0	34.0	32.3	
Danny	34.1	33.3	34.0	33.6	33.5	
Prudence	33.6	34.9	32.9	35.6	33.9	
Jeeves	33.7	31.7	33.0	33.4	33.0	
Heidi	34.4	34.1	34.0	33.7	33.6	
Elspeth	33.9	32.2	33.6	33.9	33.1	
Anne	34.3	33.9	33.5	33.4	34.0	
Lily	33.3	33.2	32.3	33.4	34.1	
Polly	34.4	33.3	33.9	34.3	34.8	
Henry	34.1	32.8	32.6	33.0	33.0	Group mean
Mean	34.07	33.16	33.32	33.79	33.63	33.59
S.D.	0.51	0.90	0.59	0.70	0.75	

Table XV

Platelet counts Values x10^9/l

Horse	Week 0	4	8	12	16	
Controls						
Felfit	150	130	140	150	130	
Jill	130	110	150	130	170	
Fred	160	140	150	150	140	
Temple	110	100	130	100	145	
Delta	220	160	230	160	200	
Beau	160	150	120	120	140	
Fella	190	110	150	170	160	
Sprig	160	130	170	160	150	
Dick	140	130	160	140	120	
Noddy	170	130	140	150	110	
Durando	180	180	140	160	130	
Natasha	210	200	220	190	145	Group mean
Mean	165.0	139.2	158.3	148.3	145.0	151.2
S.D.	31.8	29.4	33.8	23.7	23.8	
Placebo						
Charlotte	170	130	140	170	140	
Charlie	146	122	130	100	142	
Jack	180	180	170	170	146	
Rose	190	180	150	180	240	
North Star	110	30	60	30	142	
Lord Muckle	200	180	170	200	140	
Big Ben	146	140	150	140	142	
Mike	60	80	80	60	142	
Rainbeam	130	80	130	50	142	
Choppy	130	110	120	120	120	
Spring	150	130	130	110	110	
Officer	170	140	140	180	142	Group mean
Mean	148.5	125.2	130.8	125.8	145.7	135.2
S.D.	38.5	45.6	32.6	57.0	31.6	
EFAs						
Veronica	160	120	120	100	100	
Dolly	120	140	160	120	80	
Danny	170	140	140	170	160	
Prudence	220	200	200	180	140	
Jeeves	160	120	120	120	110	
Heidi	10	152	158	100	141	
Elspeth	230	210	210	210	180	
Anne	170	140	150	150	170	
Lily	130	120	130	120	130	
Polly	220	150	170	160	170	
Henry	160	140	170	130	130	Group mean
Mean	159.1	148.4	157.1	141.8	137.4	148.7
S.D.	61.1	30.3	29.7	35.2	31.7	

Table XVI

White cell counts Values x10^9/l

Horse	Week 0	4	8	12	16	
Controls						
Felfit	5.9	4.9	5.2	6.0	5.3	
Jill	7.8	7.1	6.1	9.0	7.1	
Fred	5.5	5.4	5.2	4.7	5.6	
Temple	6.7	5.4	4.7	6.9	5.8	
Delta	7.9	7.1	8.0	7.1	6.6	
Beau	9.4	6.2	6.7	6.0	6.0	
Fella	7.2	5.5	4.6	4.6	5.2	
Sprig	6.7	6.8	6.1	6.7	6.4	
Dick	7.0	6.3	5.8	5.7	5.6	
Noddy	8.0	5.9	6.2	5.5	6.0	
Durando	5.0	4.5	5.2	4.5	4.4	
Natasha	10.6	8.1	9.5	8.3	5.8	Group mean
Mean	7.31	6.10	6.11	6.25	5.82	6.32
S.D.	1.59	1.04	1.42	1.42	0.70	
Placebo						
Charlotte	6.8	6.4	7.1	6.9	7.1	
Charlie	6.3	5.6	6.6	5.4	5.5	
Jack	6.0	7.9	5.4	6.1	6.4	
Rose	7.4	6.4	4.9	5.0	6.3	
North Star	9.0	5.0	7.0	8.7	7.9	
Lord Muckle	8.0	8.6	16.0	7.8	9.1	
Big Ben	7.1	5.5	5.7	4.7	6.5	
Mike	6.6	5.2	6.1	5.0	4.7	
Rainbeam	5.4	6.5	6.9	5.5	6.5	
Choppy	6.2	6.5	7.4	8.5	7.1	
Spring	4.0	4.9	4.7	4.7	3.6	
Officer	7.3	5.7	5.5	7.2	6.5	Group mean
Mean	6.68	6.18	6.94	6.29	6.43	6.51
S.D.	1.28	1.13	2.99	1.48	1.42	
EFAs						
Veronica	11.4	7.3	8.4	7.6	9.9	
Dolly	5.9	5.7	6.2	5.3	5.8	
Danny	9.0	9.4	8.4	7.5	7.6	
Prudence	6.8	6.3	5.6	6.3	4.9	
Jeeves	7.1	5.3	5.9	7.1	5.7	
Heidi	6.9	5.7	5.4	6.3	6.5	
Elspeth	8.3	5.7	5.8	9.3	6.3	
Anne	9.5	10.1	7.3	7.0	8.2	
Lily	6.8	6.0	6.0	7.1	5.6	
Polly	8.2	6.0	5.2	4.6	5.7	
Henry	6.4	6.0	5.2	7.2	5.6	Group mean
Mean	7.85	6.68	6.31	6.85	6.53	6.84
S.D.	1.63	1.61	1.19	1.24	1.47	

Biochemical Parameters

Table XVII

Urea values Values in mmol/l

Horse	Week 0	4	8	12	16	
Horse						
Controls						
Felfit	5.6	5.5	4.7	4.7	4.4	
Jill	5.7	5.0	3.9	4.6	4.6	
Fred	6.6	6.8	5.8	5.6	5.5	
Temple	4.6	4.1	4.1	3.8	5.4	
Delta	6.4	5.7	4.4	4.4	5.4	
Beau	5.2	4.9	4.5	4.9	5.1	
Fella	5.5	5.3	4.4	5.0	5.7	
Sprig	4.7	5.0	3.8	4.1	5.0	
Dick	5.8	5.6	4.8	5.7	5.4	
Noddy	4.8	5.9	4.7	5.2	5.4	
Durando	4.3	4.9	4.1	4.5	4.4	
Natasha	5.3	5.0	4.5	5.6	5.4	Group mean
Mean	5.38	5.31	4.48	4.84	5.14	5.03
S.D.	0.71	0.67	0.53	0.61	0.45	
Placebo						
Charlotte	5.9	5.4	4.2	4.3	5.4	
Charlie	4.3	4.9	4.0	4.3	4.4	
Jack	5.3	5.4	4.5	4.4	4.7	
Rose	4.6	5.2	4.5	4.9	5.3	
North Star	5.3	5.6	5.2	5.2	4.8	
Lord Muckle	7.7	5.4	4.4	6.7	7.0	
Big Ben	5.2	6.9	5.4	5.5	5.1	
Mike	5.4	5.4	4.7	5.2	4.7	
Rainbeam	4.3	4.9	4.2	4.9	5.1	
Choppy	4.9	5.4	4.5	5.9	4.2	
Spring	3.9	3.6	3.2	3.0	3.9	
Officer	4.2	4.5	4.4	4.2	5.1	Group mean
Mean	5.08	5.22	4.43	4.88	4.98	4.92
S.D.	1.02	0.77	0.56	0.95	0.78	
EFAs						
Veronica	6.2	5.9	6.3	6.1	5.8	
Dolly	4.9	4.7	4.2	4.2	4.8	
Danny	5.7	4.8	4.6	5.3	5.5	
Prudence	5.7	5.6	5.0	5.4	4.7	
Jeeves	5.6	5.5	4.2	5.0	5.0	
Heidi	4.9	5.0	4.8	4.5	4.9	
Elspeth	4.9	4.6	3.5	4.4	3.9	
Anne	6.4	6.5	4.8	5.5	5.8	
Lily	5.4	5.9	4.6	5.2	5.4	
Polly	5.2	6.1	4.7	4.6	4.7	
Henry	5.5	4.8	5.3	5.3	5.0	Group mean
Mean	5.49	5.40	4.73	5.05	5.05	5.14
S.D.	0.51	0.65	0.71	0.57	0.56	

Table XVIII

Sodium values Values in mmol/l

Horse	Week 0	4	8	12	16	
Controls						
Felfit	137	140	139	135	138	
Jill	136	136	133	136	137	
Fred	136	136	135	138	134	
Temple	137	139	138	136	135	
Delta	137	135	135	138	132	
Beau	139	135	136	138	135	
Fella	136	136	136	132	133	
Sprig	132	136	132	133	136	
Dick	139	137	138	130	133	
Noddy	139	137	136	138	133	
Durando	141	143	136	130	134	
Natasha	139	137	132	136	135	Group mean
Mean	137.3	137.3	135.5	135.0	134.6	135.9
S.D.	2.3	2.3	2.3	3.0	1.8	
Placebo						
Charlotte	136	139	135	133	134	
Charlie	141	144	133	138	133	
Rose	138	137	133	138	138	
North Star	139	137	135	136	135	
Lord Muckle	134	137	131	138	131	
Big Ben	138	139	137	129	135	
Mike	138	138	135	135	138	
Rainbeam	139	135	136	132	135	
Choppy	141	138	137	135	136	
Spring	136	136	134	134	133	
Officer	137	134	135	135	135	Group mean
Mean	137.9	137.6	134.6	134.8	134.8	136.0
S.D.	2.1	2.6	1.8	2.8	2.1	
EFAs						
Veronica	136	136	136	133	134	
Dolly	136	139	136	141	134	
Danny	138	143	137	137	138	
Prudence	141	140	139	134	136	
Jeeves	141	140	139	134	136	
Heidi	138	136	136	133	135	
Elspeth	141	140	138	134	134	
Anne	138	139	136	131	133	
Lily	138	138	134	136	136	
Polly	140	139	133	130	132	
Henry	138	146	135	134	138	Group mean
Mean	138.6	139.6	136.3	134.3	135.1	136.8
S.D.	1.9	2.9	1.9	3.0	1.9	

Table XIX

Potassium values Values in mmol/l

Horse	Week 0	4	8	12	16	
Controls						
Felfit	4.2	4.5	2.7	3.9	3.9	
Jill	4.2	4.8	4.3	4.6	3.5	
Fred	4.6	4.5	4.3	4.0	3.7	
Temple	4.6	4.4	2.7	4.0	3.8	
Delta	4.4	4.4	4.5	4.2	3.3	
Beau	3.8	2.6	4.1	3.9	4.3	
Fella	3.4	3.6	4.6	3.7	4.2	
Sprig	5.0	4.5	3.6	4.0	2.5	
Dick	5.2	4.7	4.4	3.9	4.0	
Noddy	3.7	3.1	3.8	3.7	4.2	
Durando	4.3	3.0	3.8	3.9	4.2	
Natasha	4.2	4.7	4.5	4.3	3.8	Group mean
Mean	4.30	4.07	3.94	4.01	3.78	4.02
S.D.	0.52	0.77	0.66	0.25	0.51	
Placebo						
Charlotte	5.9	3.7	4.2	4.3	5.4	
Charlie	4.7	4.2	4.0	3.7	3.9	
Jack	4.1	3.7	2.8	4.1	3.0	
Rose	3.8	3.3	4.3	4.1	4.3	
North Star	4.3	3.2	4.3	3.0	4.5	
Lord Muckle	4.0	4.4	3.0	4.1	4.3	
Big Ben	3.8	3.8	3.5	3.7	4.0	
Mike	4.0	3.9	4.5	4.0	3.8	
Rainbeam	3.0	4.2	4.5	4.2	4.0	
Choppy	3.7	3.7	4.0	4.4	3.9	
Spring	3.6	4.2	4.1	4.2	3.6	
Officer	3.7	2.5	4.2	3.1	4.4	Group mean
Mean	4.05	3.73	3.95	3.91	4.09	3.95
S.D.	0.71	0.53	0.56	0.45	0.58	
EFAs						
Veronica	3.2	3.9	3.3	3.1	4.0	
Dolly	5.5	3.8	3.9	3.9	4.0	
Danny	5.0	3.4	4.7	4.0	4.1	
Prudence	3.7	3.1	2.6	5.7	3.4	
Jeeves	4.5	3.4	4.1	2.6	3.7	
Heidi	3.9	4.8	3.8	3.9	3.9	
Elspeth	3.7	3.2	3.6	3.7	3.5	
Anne	4.1	4.3	2.1	3.9	4.1	
Lily	4.2	4.0	3.4	3.3	3.6	
Polly	5.2	3.7	3.8	3.6	4.0	
Henry	3.5	4.6	3.5	3.2	3.9	Group mean
Mean	4.23	3.84	3.53	3.72	3.84	3.83
S.D.	0.74	0.56	0.70	0.79	0.25	

Table XX

Calcium values Values in mmol/l

Horse	Week 0	4	8	12	16	
Controls						
Felfit	3.18	3.35	2.66	2.92	2.74	
Jill	2.97	3.11	2.96	3.10	2.92	
Fred	3.20	3.17	2.93	2.96	2.96	
Temple	3.04	3.17	2.59	2.74	2.93	
Delta	3.10	2.84	2.97	2.93	2.89	
Beau	3.18	3.00	3.02	3.13	3.05	
Fella	3.19	3.05	3.03	3.11	2.94	
Sprig	2.95	3.12	2.78	3.10	2.96	
Dick	3.12	3.21	2.90	3.01	2.88	
Noddy	3.11	2.93	3.25	2.97	2.97	
Durando	2.91	3.08	2.90	3.01	3.00	
Natasha	3.24	3.12	2.98	2.97	2.93	Group mean
Mean	3.099	3.096	2.914	2.996	2.931	3.007
S.D.	0.109	0.133	0.175	0.109	0.076	
Placebo						
Charlotte	3.15	3.10	2.97	3.04	2.89	
Charlie	3.16	3.22	2.84	2.75	2.99	
Jack	3.08	3.10	3.02	2.93	3.00	
Rose	2.90	3.15	2.82	2.89	3.00	
North Star	3.13	2.81	2.92	2.95	2.93	
Lord Muckle	3.14	3.12	2.82	2.99	3.03	
Big Ben	3.09	3.09	2.55	3.06	2.97	
Mike	3.10	3.12	2.95	2.97	3.04	
Rainbeam	3.20	3.10	2.89	2.97	2.97	
Choppy	2.88	3.06	2.78	3.00	2.96	
Spring	3.17	3.07	3.00	3.14	2.98	
Officer	3.00	2.96	2.88	3.04	2.97	Group mean
Mean	3.083	3.075	2.870	2.978	2.978	2.997
S.D.	0.104	0.103	0.126	0.097	0.041	
EFAs						
Veronica	2.92	3.04	2.95	2.88	2.70	
Dolly	3.08	3.01	2.80	2.50	3.10	
Danny	3.13	3.16	3.02	3.04	2.99	
Prudence	2.88	3.10	2.89	2.94	2.82	
Jeeves	3.12	3.09	3.01	3.01	3.05	
Heidi	3.08	3.36	2.82	3.03	3.00	
Elspeth	3.08	2.99	3.08	3.00	3.07	
Anne	3.16	3.17	2.71	2.89	3.10	
Lily	3.21	3.06	3.00	3.14	3.08	
Polly	2.99	2.97	2.81	3.17	3.05	
Henry	3.10	3.08	3.08	3.09	3.02	Group mean
Mean	3.068	3.094	2.925	2.972	2.998	3.011
S.D.	0.100	0.109	0.126	0.182	0.126	

Table XXI

Chloride values Values in mmol/l

Horse	Week 0	4	8	12	16	
Controls						
Felfit	103	102	92	99	96	
Jill	98	97	94	95	95	
Fred	103	98	97	98	95	
Temple	102	100	94	100	98	
Delta	104	98	98	96	97	
Beau	102	97	93	97	92	
Fella	100	97	94	100	94	
Sprig	96	99	97	97	96	
Dick	105	99	96	98	96	
Noddy	99	101	93	99	97	
Durando	97	101	96	102	97	
Natasha	101	101	95	100	96	Group mean
Mean	100.8	99.2	94.9	98.4	95.8	97.8
S.D.	2.9	1.8	1.9	2.0	1.6	
Placebo						
Charlotte	102	99	97	99	95	
Charlie	102	100	95	101	96	
Jack	101	99	95	95	95	
Rose	101	100	98	102	97	
North Star	97	95	97	101	97	
Lord Muckle	101	98	93	99	92	
Big Ben	100	98	97	99	96	
Mike	99	99	96	97	100	
Rainbeam	100	97	95	97	96	
Choppy	100	100	93	101	96	
Spring	102	98	97	100	97	
Officer	99	100	98	103	96	Group mean
Mean	100.3	98.6	95.9	99.5	96.1	98.1
S.D.	1.5	1.5	1.7	2.3	1.8	
EFAs						
Veronica	95	96	93	98	93	
Dolly	102	101	94	97	97	
Jack	101	99	95	95	95	
Danny	100	97	93	97	95	
Prudence	100	102	99	101	97	
Jeeves	102	100	98	102	97	
Heidi	102	99	94	100	95	
Elspeth	108	99	99	102	94	
Anne	103	97	93	99	92	
Polly	97	98	95	97	93	
Henry	101	96	95	99	93	Group mean
Mean	101.0	98.5	95.3	98.8	94.6	97.7
S.D.	3.3	2.0	2.3	2.3	1.8	

Table XXII

Magnesium values Values in mmol/l

Horse	Week 0	4	8	12	16	
Controls						
Felfit	0.71	0.61	0.63	0.80	0.74	
Jill	0.70	0.67	0.78	0.90	0.88	
Fred	0.81	0.93	0.82	0.95	0.84	
Temple	0.78	0.79	0.63	0.78	0.76	
Delta	0.72	0.62	0.84	0.84	0.84	
Beau	0.68	0.82	0.71	0.83	0.70	
Fella	0.74	0.50	0.72	0.90	0.69	
Sprig	0.70	0.56	0.76	0.78	0.75	
Dick	0.73	0.56	0.70	0.78	0.72	
Noddy	0.65	0.79	0.71	0.85	0.66	
Durando	0.66	0.54	0.75	0.83	0.74	
Natasha	0.73	0.49	0.72	0.72	0.76	Group mean
Mean	0.718	0.657	0.731	0.830	0.757	0.738
S.D.	0.046	0.143	0.065	0.064	0.066	
Placebo						
Charlotte	0.71	0.76	0.74	0.84	0.70	
Charlie	0.84	0.64	0.79	0.83	0.84	
Jack	0.67	0.76	0.75	0.94	0.71	
Rose	0.82	0.64	0.76	0.80	0.71	
North Star	0.64	0.70	0.77	0.89	0.74	
Lord Muckle	0.59	0.92	0.60	0.80	0.80	
Big Ben	0.71	0.69	0.75	0.84	0.74	
Mike	0.72	0.86	0.70	0.94	0.68	
Rainbeam	0.70	0.71	0.70	0.81	0.74	
Choppy	0.65	0.83	0.68	0.72	0.56	
Spring	0.73	0.72	0.83	0.84	0.73	
Officer	0.60	0.80	0.65	0.72	0.74	Group mean
Mean	0.70	0.753	0.727	0.831	0.724	0.747
S.D.	0.08	0.087	0.064	0.070	0.067	
EFAs						
Veronica	0.67	0.61	0.67	0.61	0.60	
Dolly	0.81	0.61	0.76	0.67	0.88	
Danny	0.66	0.64	0.66	0.66	0.78	
Prudence	0.54	0.78	0.74	0.78	0.68	
Jeves	0.79	0.83	0.77	0.90	0.68	
Heidi	0.72	0.57	0.65	0.75	0.71	
Elspeth	0.75	0.57	0.83	0.75	0.74	
Anne	0.81	0.64	0.68	0.74	0.71	
Lily	0.71	0.68	0.84	0.87	0.76	
Polly	0.66	0.56	0.74	0.69	0.66	
Henry	0.80	0.61	0.79	0.77	0.80	Group mean
Mean	0.720	0.645	0.739	0.745	0.727	0.715
S.D.	0.084	0.087	0.067	0.087	0.076	

Table XXIII

Inorganic phosphate values Values in mmol/l

Horse	Week 0	4	8	12	16	
Controls						
Felfit	0.92	0.78	1.12	1.05	0.82	
Jill	1.01	1.38	1.08	1.04	0.80	
Fred	0.78	0.68	0.69	1.04	0.64	
Temple	0.82	0.80	0.71	0.96	1.14	
Delta	1.02	0.88	1.07	1.31	0.73	
Beau	0.97	1.27	0.90	0.99	1.10	
Fella	0.68	1.23	0.95	0.72	0.68	
Sprig	0.83	0.78	0.78	0.79	0.59	
Dick	0.95	0.68	0.89	0.86	0.77	
Noddy	0.83	1.09	0.98	0.74	0.88	
Durando	0.75	0.88	0.76	0.93	1.04	
Natasha	0.99	1.25	1.03	0.97	0.84	Group mean
Mean	0.879	0.975	0.913	0.950	0.836	0.911
S.D.	0.112	0.253	0.150	0.162	0.177	
Placebo						
Charlotte	0.92	0.97	0.92	1.04	0.72	
Charlie	0.77	0.67	0.87	0.98	1.01	
Jack	0.91	0.97	0.89	1.03	0.98	
Rose	0.96	0.63	0.98	0.80	0.78	
North Star	0.75	1.18	1.04	1.05	0.76	
Lord Muckle	0.82	0.73	0.54	0.88	0.70	
Big Ben	0.88	0.86	0.77	0.73	0.88	
Mike	0.93	0.94	0.84	0.82	1.04	
Rainbeam	0.99	0.70	1.20	1.04	0.88	
Choppy	1.19	1.15	1.00	1.04	0.92	
Spring	0.94	0.87	0.91	1.22	0.92	
Officer	0.91	1.15	0.60	0.73	0.88	Group mean
Mean	0.914	0.902	0.880	0.947	0.873	0.903
S.D.	0.114	0.194	0.182	0.152	0.112	
EFAs						
Veronica	0.88	0.96	0.68	1.05	0.79	
Dolly	0.86	0.91	0.99	1.31	0.74	
Danny	0.96	0.92	1.36	0.90	0.90	
Prudence	0.79	0.96	0.96	1.07	1.21	
Jeeves	1.01	1.17	0.91	0.95	1.08	
Heidi	1.04	0.88	0.78	0.81	0.98	
Elspeth	0.76	0.92	0.99	1.08	1.33	
Anne	0.88	0.70	0.87	0.88	0.84	
Lily	0.82	1.26	0.77	0.86	0.99	
Polly	0.89	1.12	1.02	1.33	1.07	
Henry	0.87	0.68	1.06	0.86	0.95	Group mean
Mean	0.887	0.953	0.945	1.009	0.989	0.957
S.D.	0.087	0.178	0.182	0.179	0.177	

Table XXIV

Alkaline phosphatase values, in I.U.

Horse	Week 0	4	8	12	16	
Controls						
Felfit	141	134	133	117	145	
Jill	374	236	223	236	256	
Fred	216	206	191	263	262	
Temple	241	182	153	173	196	
Delta	209	165	162	154	159	
Beau	312	240	213	211	200	
Fella	283	188	163	187	160	
Sprig	321	252	289	267	320	
Dick	205	244	175	151	155	
Noddy	307	201	189	218	176	
Durando	153	137	179	129	131	
Natasha	262	215	200	191	196	Group mean
Mean	252.0	200.0	189.2	191.4	196.3	205.8
S.D.	70.7	40.2	40.4	49.3	56.3	
Placebo						
Charlotte	211	213	194	179	187	
Charlie	183	216	239	195	234	
Jack	217	213	192	180	202	
Rose	265	195	179	211	245	
North Star	312	234	222	221	245	
Lord Muckle	274	251	219	198	209	
Big Ben	263	193	192	189	201	
Mike	279	233	195	184	181	
Choppy	253	225	232	286	241	
Rainbeam	238	238	207	239	201	
Spring	160	159	137	143	149	
Officer	331	226	181	222	201	Group mean
Mean	248.8	216.3	199.1	203.9	208.0	215.2
S.D.	50.0	24.8	27.6	36.0	29.1	
EFAs						
Veronica	259	205	192	185	203	
Dolly	202	165	179	203	158	
Danny	369	214	249	211	216	
Prudence	242	242	233	204	202	
Jeeves	311	198	177	177	174	
Heidi	251	209	157	182	202	
Elspeth	225	223	189	163	185	
Anne	230	245	244	222	245	
Lily	241	158	167	165	145	
Polly	381	193	228	240	213	
Henry	352	251	189	179	194	Group mean
Mean	278.5	209.4	200.4	193.7	194.3	215.2
S.D.	63.3	30.5	32.3	24.3	28.0	

Table XXV

Aspartate aminotransaminase values, in I.U.

Horse	Week 0	4	8	12	16	
Controls						
Felfit	297	314	261	236	287	
Jill	295	327	279	291	360	
Fred	284	379	266	298	353	
Temple	275	333	241	254	310	
Delta	373	342	319	356	389	
Beau	254	292	250	260	337	
Fella	265	322	324	304	317	
Sprig	222	265	222	237	240	
Dick	252	273	221	245	256	
Noddy	225	299	303	293	288	
Durando	235	273	231	223	274	
Natasha	359	373	320	336	310	Group mean
Mean	278.0	316.0	269.8	277.8	310.1	290.3
S.D.	48.1	37.5	38.8	42.0	44.2	
Placebo						
Charlotte	299	336	226	240	234	
Charlie	285	345	316	279	308	
Jack	213	336	251	267	336	
Rose	249	247	189	214	237	
North Star	258	316	284	313	331	
Lord Muckle	370	355	276	279	265	
Big Ben	322	319	287	316	311	
Mike	307	362	318	327	322	
Rainbeam	433	413	403	383	311	
Choppy	342	396	344	398	780	
Spring	400	386	300	336	367	
Officer	284	345	283	296	311	Group mean
Mean	313.5	346.3	289.8	304.0	342.8	319.3
S.D.	64.0	43.2	55.1	53.7	143.2	
EFAs						
Veronica	306	324	304	291	332	
Dolly	359	356	294	373	302	
Danny	287	314	319	328	335	
Prudence	322	353	310	285	307	
Jeeves	258	303	255	274	259	
Heidi	319	341	266	317	336	
Elspeth	283	308	272	285	305	
Anne	279	341	276	271	252	
Lily	272	286	251	264	270	
Polly	332	313	266	253	250	
Henry	376	393	368	361	369	Group mean
Mean	308.5	330.2	289.2	300.2	301.5	305.9
S.D.	37.2	30.2	34.6	39.6	39.7	

Table XXVI

Bilirubin values, in umol/l

Horse	Week 0	4	8	12	16	
Controls						
Felfit	14	13	27	15	12	
Jill	16	16	30	20	19	
Fred	19	11	16	18	16	
Temple	14	15	14	16	16	
Delta	17	7	9	15	10	
Beau	11	13	14	15	14	
Fella	17	19	18	17	12	
Sprig	17	18	20	19	18	
Dick	17	19	16	25	22	
Noddy	17	24	28	25	23	
Durando	16	10	21	17	9	
Natasha	17	11	21	16	16	Group mean
Mean	16.0	14.7	19.5	18.2	15.6	16.8
S.D.	2.1	4.8	6.3	3.6	4.4	
Placebo						
Charlotte	17	17	17	17	17	
Charlie	10	11	13	15	11	
Jack	21	20	18	15	22	
Rose	7	12	10	10	12	
North Star	18	24	15	24	26	
Lord Muckle	29	15	69	31	24	
Big Ben	16	14	16	15	17	
Mike	10	14	13	16	13	
Rainbeam	16	20	36	23	17	
Choppy	20	19	30	60	40	
Spring	23	8	18	16	12	
Officer	9	15	16	17	17	Group mean
Mean	16.3	15.8	22.6	21.6	19.0	19.1
S.D.	6.5	4.5	16.4	13.3	8.2	
EFAs						
Veronica	13	13	13	13	13	
Dolly	18	15	14	14	11	
Danny	17	19	19	14	15	
Prudence	12	5	9	10	8	
Jeeves	12	15	17	16	14	
Heidi	13	9	11	13	22	
Elspeth	22	14	18	16	14	
Lily	15	18	24	20	16	
Anne	23	11	17	23	12	
Polly	11	78	13	111	63	
Henry	24	22	31	32	22	Group mean
Mean	16.4	19.9	16.9	25.6	19.1	19.6
S.D.	4.8	19.8	6.3	29.0	15.2	

Table XXVII

Total plasma protein values, in g/l

Horse	Week 0	4	8	12	16	
Controls						
Felfit	61	63	58	54	55	
Jill	69	65	65	65	65	
Fred	60	78	59	63	63	
Temple	66	63	57	56	61	
Delta	63	58	60	57	58	
Beau	61	60	57	60	59	
Fella	65	66	57	61	55	
Sprig	74	72	67	67	73	
Dick	60	71	60	62	61	
Noddy	67	60	61	63	57	
Durando	68	74	61	60	62	
Natasha	64	69	65	62	61	Group mean
Mean	64.8	66.6	60.6	60.8	60.8	62.7
S.D.	4.2	6.2	3.4	3.7	4.9	
Placebo						
Charlotte	68	68	66	65	62	
Charlie	67	68	63	57	60	
Jack	67	68	58	59	63	
Rose	65	68	57	61	65	
North Star	72	69	67	67	65	
Lord Muckle	61	68	67	67	67	
Big Ben	64	60	60	61	63	
Mike	60	64	65	65	63	
Rainbeam	56	66	58	59	63	
Choppy	72	67	65	71	69	
Spring	64	62	60	62	57	
Officer	66	65	59	63	63	Group mean
Mean	65.2	66.1	62.1	63.1	63.3	64.0
S.D.	4.7	2.8	3.8	4.1	3.1	
EFAs						
Veronica	65	64	63	61	62	
Dolly	65	62	58	66	61	
Danny	71	75	70	62	68	
Prudence	71	69	62	61	61	
Jeeves	63	60	56	58	52	
Heidi	68	71	57	65	63	
Elspeth	64	71	67	66	67	
Anne	67	71	61	63	68	
Lily	61	65	64	66	59	
Polly	70	62	63	64	62	
Henry	67	78	67	65	68	Group mean
Mean	66.5	68.0	62.5	63.4	62.8	64.7
S.D.	3.3	5.8	4.4	2.6	4.9	

Table XXVIII

Albumin values, in g/l

Horse	Week 0	4	8	12	16	
Controls						
Felfit	37	29	35	32	34	
Jill	34	33	33	32	34	
Fred	33	34	28	30	31	
Temple	37	36	31	30	33	
Delta	33	37	31	30	31	
Beau	37	34	34	35	34	
Fella	34	38	33	33	30	
Sprig	35	38	33	34	36	
Dick	33	38	32	32	36	
Noddy	33	33	32	32	33	
Durando	31	33	32	32	32	
Natasha	36	40	31	31	33	Group mean
Mean	34.4	35.3	32.1	31.9	33.1	33.4
S.D.	2.0	3.1	1.8	1.6	1.9	
Placebo						
Charlotte	35	35	30	34	31	
Charlie	39	35	31	31	38	
Jack	36	35	34	39	33	
Rose	33	35	27	28	27	
North Star	32	35	32	34	33	
Lord Muckle	36	34	34	32	31	
Big Ben	35	31	30	32	33	
Mike	35	35	35	35	34	
Officer	34	34	30	33	33	
Rainbeam	31	29	31	31	33	
Spring	39	36	35	35	33	
Officer	34	34	30	33	33	Group mean
Mean	34.9	34.0	31.6	33.1	32.7	33.3
S.D.	2.4	2.0	2.5	2.7	2.5	
EFAs						
Veronica	32	34	34	33	31	
Dolly	36	36	33	35	31	
Danny	39	35	37	33	32	
Prudence	32	35	34	32	35	
Jeeves	36	36	33	35	35	
Heidi	39	32	31	34	33	
Elspeth	34	39	35	33	34	
Anne	36	39	34	34	34	
Lily	35	34	35	36	32	
Polly	33	29	32	33	31	
Henry	39	35	37	35	35	Group mean
Mean	35.5	34.9	34.1	33.9	33.0	34.3
S.D.	2.7	2.8	1.9	1.2	1.7	

Table XXIX

Globulin values, in g/l

Horse	Week 0	4	8	12	16	
Controls						
Felfit	24	34	23	22	21	
Jill	35	32	32	33	31	
Fred	27	44	31	33	32	
Temple	29	27	26	26	28	
Delta	30	21	29	27	27	
Beau	24	26	23	25	25	
Fella	31	28	24	28	25	
Sprig	39	34	34	33	37	
Dick	27	33	28	30	25	
Noddy	34	27	29	31	24	
Durando	37	41	29	28	30	
Natasha	28	29	34	31	28	Group mean
Mean	30.4	31.3	28.5	28.9	27.8	29.4
S.D.	4.9	6.5	3.9	3.5	4.3	
Placebo						
Charlotte	33	33	36	31	31	
Charlie	28	33	32	26	22	
Jack	31	33	24	20	27	
Rose	32	33	30	33	38	
North Star	40	34	35	33	32	
Lord Muckle	25	34	33	35	36	
Big Ben	29	29	30	29	30	
Mike	25	29	30	30	29	
Rainbeam	25	37	27	28	30	
Choppy	38	33	30	33	35	
Spring	25	26	25	27	24	
Officer	32	31	29	30	30	Group mean
Mean	30.3	32.1	30.1	29.6	30.3	30.5
S.D.	5.1	2.9	3.6	4.1	4.7	
EFAs						
Veronica	33	30	29	28	31	
Dolly	29	26	25	31	30	
Danny	32	40	33	29	36	
Prudence	39	34	28	29	26	
Jeeves	27	24	23	23	17	
Heidi	29	39	26	31	27	
Elspeth	30	32	32	33	33	
Anne	31	32	27	29	34	
Lily	26	31	29	30	27	
Polly	37	33	31	31	31	
Henry	28	43	30	30	33	Group mean
Mean	31.0	33.1	28.5	29.5	29.5	30.3
S.D.	4.0	5.8	3.0	2.5	5.2	

Table XXX

Gamma glutamyl transpeptidase values, in I.U./l

Horse	Week 0	4	8	12	16	
Controls						
Felfit	19	18	16	11	22	
Jill	28	13	23	18	16	
Fred	15	25	16	11	10	
Temple	17	23	21	15	19	
Delta	22	19	11	15	14	
Beau	23	18	26	15	19	
Fella	27	18	18	17	19	
Sprig	27	26	20	22	26	
Dick	34	11	30	31	33	
Noddy	24	18	14	15	22	
Durando	15	17	11	16	9	
Natasha	24	39	30	16	19	Group mean
Mean	22.9	20.4	19.7	16.8	19.0	19.8
S.D.	5.7	7.3	6.6	5.3	6.6	
Placebo						
Charlotte	23	23	29	28	16	
Charlie	20	28	34	21	25	
Jack	26	23	16	18	18	
Rose	20	20	14	19	11	
North Star	32	25	16	15	17	
Lord Muckle	23	28	25	15	18	
Big Ben	25	19	16	21	17	
Mike	21	27	29	23	23	
Rainbeam	25	20	14	24	17	
Choppy	28	29	30	26	35	
Spring	27	33	23	31	20	
Officer	31	26	18	30	17	Group mean
Mean	25.1	25.1	22.0	22.6	19.5	22.9
S.D.	4.0	4.2	7.2	5.4	6.0	
EFAs						
Veronica	14	17	16	23	7	
Dolly	24	30	29	23	15	
Danny	23	18	18	16	17	
Prudence	24	27	29	16	22	
Jeeves	24	18	20	22	17	
Heidi	30	9	11	13	18	
Elspeth	17	16	16	18	11	
Anne	26	35	30	19	16	
Lily	24	19	17	24	8	
Polly	35	26	16	28	12	
Henry	33	27	21	22	20	Group mean
Mean	24.9	22.0	20.3	20.4	14.8	20.5
S.D.	6.2	7.5	6.4	4.4	4.8	

Table XXXI

Triglyceride values, in mmol/l

Horse	Week 0	8	12	16	
Controls					
Felfit	0.29	0.08	0.13	0.11	
Jill	0.32	0.48	0.40	0.33	
Fred	0.18	0.48	0.27	0.14	
Temple	0.27	0.38	0.39	0.00	
Delta	0.11	0.45	0.12	0.12	
Beau	0.50	0.30	0.32	0.24	
Fella	0.19	0.13	0.21	0.19	
Sprig	0.47	0.53	0.30	0.29	
Dick	0.17	0.26	0.45	0.32	
Noddy	0.33	0.02	0.17	0.17	
Durando	0.19	0.44	0.25	0.35	
Natasha	0.14	0.18	0.35	0.21	Group mean
Mean	0.263	0.311	0.280	0.206	0.265
S.D.	0.125	0.175	0.108	0.105	
Placebo					
Charlotte	0.32	0.48	0.28	0.28	
Charlie	0.33	0.23	0.26	0.43	
Jack	0.23	0.44	0.23	0.23	
Rose	0.35	0.66	0.37	0.48	
North Star	0.35	0.43	0.13	0.10	
Lord Muckle	0.30	0.45	0.23	0.24	
Big Ben	0.25	0.48	0.42	0.28	
Mike	0.12	0.29	0.24	0.18	
Rainbeam	0.21	0.44	0.16	0.28	
Choppy	0.13	0.20	0.11	0.10	
Spring	0.28	0.52	0.35	0.31	
Officer	0.14	0.43	0.41	0.28	Group mean
Mean	0.251	0.421	0.266	0.266	0.301
S.D.	0.085	0.127	0.104	0.113	
EFAs					
Veronica	0.14	0.34	0.16	0.22	
Dolly	0.08	0.23	0.24	0.30	
Danny	0.31	0.54	0.38	0.22	
Prudence	0.47	0.46	0.55	0.59	
Jeeves	0.51	0.49	0.32	0.24	
Heidi	0.43	0.10	0.24	0.23	
Elspeth	0.33	0.44	0.18	0.18	
Anne	0.38	0.23	0.24	0.26	
Lily	0.16	0.51	0.27	0.33	
Polly	0.29	0.42	0.12	0.14	
Henry	0.39	0.44	0.27	0.22	Group mean
Mean	0.317	0.382	0.270	0.266	0.309
S.D.	0.140	0.140	0.118	0.119	

Table XXXII

Cholesterol values, in mmol/l

Horse	Week 0	8	12	16	
Controls					
Felfit	1.90	2.39	2.26	2.07	
Jill	2.10	2.65	2.58	2.09	
Fred	1.42	1.77	1.99	1.76	
Temple	2.44	2.79	2.72	2.22	
Delta	2.03	2.40	1.92	1.89	
Beau	2.07	2.65	2.90	2.75	
Fella	3.19	3.74	3.70	3.29	
Sprig	2.08	2.23	2.10	2.15	
Dick	1.61	2.14	2.03	1.96	
Noddy	1.84	2.43	2.60	2.25	
Durando	1.72	1.76	2.17	1.98	
Natasha	1.91	2.44	1.87	2.22	Group mean
Mean	2.026	2.449	2.403	2.219	2.274
S.D.	0.451	0.518	0.530	0.417	
Placebo					
Charlotte	1.76	2.17	2.24	2.04	
Charlie	2.13	2.59	2.20	2.59	
Jack	2.33	2.71	2.75	2.46	
Rose	2.05	1.92	2.38	2.38	
North Star	1.60	1.87	1.83	2.01	
Lord Muckle	2.10	2.07	1.78	1.53	
Big Ben	1.77	2.78	3.12	2.19	
Mike	1.21	2.18	2.51	2.09	
Rainbeam	0.44	2.52	2.49	2.19	
Choppy	0.85	2.22	2.37	1.82	
Spring	2.10	2.94	3.11	2.64	
Officer	2.21	2.41	3.00	2.19	Group mean
Mean	1.713	2.365	2.482	2.178	2.184
S.D.	0.592	0.347	0.449	0.317	
EFAs					
Veronica	1.89	2.40	1.92	2.46	
Dolly	2.10	2.81	3.40	3.02	
Danny	2.27	2.62	2.61	2.33	
Prudence	2.52	3.57	3.70	3.46	
Jeeves	1.69	2.46	2.64	2.29	
Heidi	2.11	1.95	1.30	2.46	
Elspeth	2.21	2.15	2.81	2.79	
Anne	1.86	2.47	2.43	1.96	
Lily	1.56	2.13	2.10	1.83	
Polly	2.10	2.54	2.58	2.46	
Henry	2.16	2.77	2.72	2.22	Group mean
Mean	2.043	2.534	2.565	2.480	2.405
S.D.	0.273	0.435	0.658	0.466	

APPENDIX III

TABLES OF RESULTS OF THE STUDY ON THE USE OF EFAs IN THE PROPHYLAXIS OF DERMATOPHILOSIS IN HORSES

Clinical Indices, Haematological and Biochemical Parameters

Table I. Indices of dermatophilosis lesion severity

Scored on a 1 to 10 scale, 1 is mildest, 10 is most severe

Horse	Week 0	3	6	9	12	15	20	24	
Controls									
Polly	0.0	0.0	0.0	0.0	1.0	1.0	1.0	1.0	
Lily	1.0	1.0	1.0	1.0	1.0	0.0	0.0	0.0	
Sprig	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Danny	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Dolly	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Jeeves	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Charlie	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Smiler	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Elspeth	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	
Sean	0.0	0.0	1.0	1.0	0.0	0.0	0.0	0.0	
Bliss	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	Group
LP	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	mean
Mean	0.1	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1
S.D.	0.3	0.4	0.4	0.4	0.4	0.3	0.3	0.3	
EFAs									
Fella	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Spring	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Beau	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Mike	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Dick	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	
Durando	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Henry	1.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0	
Jill	0.0	0.0	1.0	1.0	0.0	0.0	0.0	0.0	
Fred	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	
R. Bridge	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	Group
John	0.0	0.0	0.0	2.0	0.0	0.0	0.0	0.0	mean
Mean	0.2	0.1	0.3	0.5	0.1	0.0	0.0	0.0	0.1
S.D.	0.4	0.3	0.5	0.7	0.3	0.0	0.0	0.0	

Table II. Extent of distribution of dorsal dermatophilosis lesions

% of dorsal body surface area affected by dermatophilosis lesions

Horse	Week 0	3	6	9	12	15	20	24	
Controls									
Polly	0.0	0.0	0.0	0.0	25.0	15.0	15.0	15.0	
Lily	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Sprig	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Danny	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Dolly	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Jeeves	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Charlie	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Smiler	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Elspeth	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Sean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Bliss	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	Group
LP	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	mean
Mean	0.0	0.0	0.0	0.0	2.1	1.3	1.3	1.3	0.7
S.D.	0.0	0.0	0.0	0.0	7.2	4.3	4.3	4.3	
EFAs									
Fella	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Spring	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Beau	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Mike	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Dick	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Durando	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Henry	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Jill	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Fred	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
R. Bridge	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	Group
John	0.0	0.0	0.0	2.5	0.0	0.0	0.0	0.0	mean
Mean	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0
S.D.	0.0	0.0	0.0	0.8	0.0	0.0	0.0	0.0	

Table III. Extent of distribution of hindlimb dermatophilosis lesions

% of dorsal pastern surface area affected

Horse	Week 0	3	6	9	12	15	20	24	
Controls									
Polly	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Lily	2.5	2.5	1.0	1.0	1.0	0.0	0.0	0.0	
Sprig	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Danny	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Dolly	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Jeeves	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Charlie	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Smiler	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Elspeth	0.0	2.5	0.0	0.0	0.0	0.0	0.0	0.0	
Sean	0.0	0.0	1.0	2.5	0.0	0.0	0.0	0.0	
Bliss	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	Group
LP	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	mean
Mean	0.2	0.4	0.2	0.3	0.1	0.0	0.0	0.0	0.1
S.D.	0.7	1.0	0.4	0.8	0.3	0.0	0.0	0.0	
EFAs									
Fella	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Spring	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Beau	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Mike	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Dick	0.0	0.0	0.0	2.5	0.0	0.0	0.0	0.0	
Durando	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Henry	10.0	2.5	2.5	2.5	2.5	0.0	0.0	0.0	
Jill	0.0	0.0	2.5	2.5	0.0	0.0	0.0	0.0	
Fred	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	
R. Bridge	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	Group
John	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	mean
Mean	1.0	0.3	0.5	0.7	0.2	0.0	0.0	0.0	0.3
S.D.	3.0	0.8	1.0	1.2	0.8	0.0	0.0	0.0	

Table IV. Coat condition scores

Scored on a 1 to 10 scale, 1 is poorest, 10 is optimal

Horse	Week 0	3	6	9	12	15	20	24	
Controls									
Polly	6.0	6.0	6.0	8.0	7.0	7.0	6.0	8.0	
Lily	7.0	7.0	6.0	6.0	7.0	7.0	7.0	7.0	
Sprig	7.0	7.0	8.0	6.0	7.0	7.0	7.0	7.0	
Danny	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	
Dolly	7.0	7.0	7.0	6.0	7.0	8.0	8.0	7.0	
Jeeves	6.0	7.0	7.0	6.0	6.0	7.0	8.0	7.0	
Charlie	7.0	7.0	8.0	7.0	7.0	7.0	7.0	7.0	
Smiler	7.0	7.0	7.0	7.0	6.0	6.0	6.0	7.0	
Elspeth	7.0	6.0	8.0	7.0	8.0	7.0	7.0	7.0	
Sean	9.0	9.0	9.0	8.0	7.0	7.0	6.0	6.0	
Bliss	7.0	7.0	7.0	7.0	7.0	7.0	7.0	8.0	Group
LP	7.0	7.0	7.0	6.0	6.0	7.0	6.0	6.0	mean
Mean	7.00	7.00	7.25	6.75	6.83	7.00	6.83	7.00	6.96
S.D.	0.74	0.74	0.87	0.75	0.58	0.43	0.72	0.60	
EFAs									
Fella	7.0	7.0	6.0	7.0	7.0	7.0	6.0	6.5	
Spring	7.0	7.0	7.0	6.0	7.0	7.0	7.0	8.0	
Beau	8.0	8.0	7.0	7.0	7.0	8.0	8.0	8.0	
Mike	7.0	7.0	7.0	7.0	8.0	8.0	7.0	7.0	
Dick	7.0	7.0	8.0	7.0	7.0	8.0	7.0	8.0	
Durando	8.0	7.0	8.0	7.0	7.0	7.0	7.0	7.0	
Henry	6.0	7.0	6.0	6.0	6.0	6.0	7.0	6.5	
Jill	6.0	7.0	6.0	6.0	6.0	6.0	7.0	6.0	
Fred	7.0	7.0	7.0	6.0	7.0	7.0	7.0	7.0	
R. Bridge	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	Group
John	6.0	7.0	6.0	5.0	7.0	7.0	7.0	6.0	mean
Mean	6.82	7.00	6.73	6.36	6.82	7.00	6.91	6.91	6.82
S.D.	0.75	0.45	0.79	0.67	0.60	0.77	0.54	0.80	

Table V. Mane condition scores

Scored on a 1 to 10 scale, 1 is poorest, 10 is optimal

Horse	Week 0	3	6	9	12	15	20	24	
Controls									
Polly	6.0	7.0	6.0	8.0	7.0	7.0	7.0	8.0	
Lily	6.0	7.0	7.0	6.0	7.0	7.0	8.0	8.0	
Sprig	6.0	7.0	7.0	7.0	7.0	7.0	7.0	8.0	
Danny	7.0	7.0	8.0	7.0	6.0	7.0	7.0	7.0	
Dolly	7.0	7.0	8.0	6.0	7.0	7.0	8.0	7.0	
Jeeves	6.0	7.0	7.0	7.0	7.0	7.0	8.0	8.0	
Charlie	6.0	7.0	7.0	7.0	7.0	7.0	8.0	8.0	
Smiler	8.0	6.0	7.0	7.0	6.0	7.0	8.0	8.0	
Elspeth	8.0	7.0	7.0	7.0	8.0	7.0	8.0	8.0	
Sean	8.0	9.0	9.0	8.0	8.0	7.0	8.0	8.0	
Bliss	7.0	7.0	7.0	7.0	7.0	8.0	8.0	8.0	Group
LP	7.0	7.0	8.0	7.0	6.0	7.0	8.0	8.0	mean
Mean	6.83	7.08	7.33	7.00	6.92	7.08	7.75	7.83	7.23
S.D.	0.83	0.67	0.78	0.60	0.67	0.29	0.45	0.39	
EFAs									
Fella	6.0	7.0	5.0	6.0	7.0	6.0	6.0	6.0	
Spring	7.0	8.0	8.0	7.0	7.0	7.0	7.0	8.0	
Beau	8.0	8.0	7.0	7.0	7.0	8.0	8.0	8.0	
Mike	7.0	7.0	8.0	8.0	8.0	8.0	7.0	7.0	
Dick	8.0	7.0	8.0	7.0	8.0	8.0	7.0	8.0	
Durando	8.0	7.0	8.0	7.0	8.0	7.0	7.0	7.0	
Henry	7.0	7.0	6.0	7.0	6.0	7.0	7.0	7.0	
Jill	7.0	6.0	6.0	6.0	6.0	7.0	7.0	6.0	
Fred	7.0	7.0	7.0	6.0	7.0	7.0	7.0	7.0	
R. Bridge	7.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	Group
John	6.0	5.0	6.0	6.0	7.0	8.0	7.0	6.0	mean
Mean	7.09	6.82	6.82	6.64	7.00	7.18	6.91	6.91	6.92
S.D.	0.70	0.87	1.08	0.67	0.77	0.75	0.54	0.83	

Table VI. Tail condition scores

Scored on a 1 to 10 scale, 1 is poorest, 10 is optimal

Horse	Week 0	3	6	9	12	15	20	24	
Controls									
Polly	7.0	7.0	6.0	8.0	7.0	7.0	7.0	8.0	
Lily	6.0	8.0	7.0	7.0	7.0	7.0	8.0	7.0	
Sprig	6.0	7.0	8.0	7.0	7.0	7.0	7.0	8.0	
Danny	7.0	7.0	8.0	7.0	6.0	7.0	7.0	7.0	
Dolly	7.0	7.0	7.0	7.0	6.0	7.0	7.0	7.0	
Jeeves	6.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	
Charlie	8.0	7.0	8.0	7.0	7.0	7.0	7.0	8.0	
Smiler	8.0	7.0	7.0	7.0	6.0	7.0	8.0	8.0	
Elspeth	8.0	7.0	8.0	7.0	8.0	8.0	8.0	7.0	
Sean	9.0	9.0	9.0	8.0	8.0	7.0	7.0	8.0	
Bliss	8.0	7.0	7.0	7.0	7.0	7.0	7.0	8.0	Group
LP	8.0	8.0	7.0	7.0	7.0	7.0	7.0	7.0	mean
Mean	7.33	7.33	7.42	7.17	6.92	7.08	7.27	7.50	7.25
S.D.	0.98	0.65	0.79	0.39	0.67	0.29	0.47	0.52	
EFAs									
Fella	7.0	7.0	6.0	6.0	7.0	7.0	7.0	7.0	
Spring	7.0	8.0	8.0	6.0	6.0	7.0	6.0	7.0	
Beau	8.0	7.0	7.0	6.0	7.0	7.0	8.0	8.0	
Mike	7.0	7.0	7.0	7.0	8.0	8.0	8.0	7.0	
Dick	8.0	7.0	8.0	7.0	8.0	8.0	7.0	8.0	
Durando	8.0	7.0	7.0	7.0	8.0	7.0	8.0	8.0	
Henry	7.0	7.0	7.0	7.0	6.0	7.0	7.0	7.0	
Jill	7.0	7.0	6.0	6.0	6.0	7.0	7.0	6.0	
Fred	7.0	8.0	7.0	6.0	7.0	7.0	7.0	7.0	
R. Bridge	7.0	7.0	6.0	6.0	6.0	6.0	7.0	7.0	Group
John	7.0	7.0	7.0	6.0	7.0	8.0	7.0	7.0	mean
Mean	7.27	7.18	6.91	6.36	6.91	7.18	7.18	7.18	7.02
S.D.	0.47	0.40	0.70	0.50	0.83	0.60	0.60	0.60	

Table VII. Hoof condition scores

Scored on a 1 to 10 scale, 1 is poorest, 10 is optimal

Horse	Week 0	3	6	9	12	15	20	24	
Controls									
Polly	6.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	
Lily	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	
Sprig	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	
Danny	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	
Dolly	6.0	7.0	6.0	7.0	7.0	7.0	7.0	7.0	
Jeeves	6.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	
Charlie	8.0	8.0	7.0	7.0	6.0	7.0	7.0	7.0	
Smiler	6.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	
Elspeth	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	
Sean	8.0	8.0	9.0	7.0	7.0	7.0	8.0	7.0	
Bliss	8.0	7.0	7.0	7.0	7.0	7.0	7.0	8.0	Group
LP	6.0	6.0	7.0	6.0	7.0	7.0	7.0	7.0	mean
Mean	6.83	7.08	7.08	6.92	6.92	7.00	7.08	7.08	7.00
S.D.	0.83	0.51	0.67	0.29	0.29	0.00	0.29	0.29	
EFAs									
Fella	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	
Spring	8.0	8.0	7.0	7.0	7.0	7.0	7.0	7.0	
Beau	7.0	7.0	7.0	7.0	7.0	7.0	8.0	8.0	
Mike	7.0	7.0	7.0	7.0	7.0	8.0	7.0	7.0	
Dick	8.0	7.0	7.0	7.0	7.0	8.0	8.0	8.0	
Durando	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	
Henry	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	
Jill	7.0	7.0	7.0	7.0	6.0	7.0	7.0	7.0	
Fred	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	
R. Bridge	7.0	7.0	7.0	7.0	6.0	7.0	7.0	7.0	Group
John	7.0	7.0	7.0	7.0	7.0	8.0	7.0	7.0	mean
Mean	7.18	7.09	7.00	7.00	6.82	7.27	7.18	7.18	7.09
S.D.	0.40	0.30	0.00	0.00	0.40	0.47	0.40	0.40	

Table VIII. Body condition scores

Scored on a 1 to 5 scale, 1 is emaciated, 5 is obese.

Horse	Week 0	3	6	9	12	15	20	24	
Controls									
Polly	3.5	4.0	4.0	4.0	3.5	3.0	3.5	3.0	
Lily	3.5	3.5	3.0	3.5	3.5	3.0	3.5	3.5	
Sprig	3.5	4.0	3.5	3.5	3.0	3.5	3.5	3.5	
Danny	3.5	4.0	3.5	3.5	3.5	3.5	3.5	3.5	
Dolly	4.0	4.0	4.0	3.5	4.0	4.0	4.5	4.0	
Jeeves	3.5	3.5	3.0	3.5	3.5	3.5	3.5	3.5	
Charlie	4.0	4.5	4.5	4.5	4.5	4.0	4.5	4.0	
Smiler	2.5	3.0	3.0	3.0	3.0	2.5	3.0	3.0	
Elspeth	3.5	3.0	3.0	3.5	3.0	3.0	3.0	3.0	
Sean	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	
Bliss	3.0	3.0	3.0	3.5	3.0	3.0	3.0	3.0	Group
LP	3.0	3.0	3.0	3.0	3.0	3.0	4.5	4.0	mean
Mean	3.38	3.54	3.38	3.50	3.38	3.25	3.58	3.42	3.43
S.D.	0.43	0.54	0.53	0.43	0.48	0.45	0.60	0.42	
EFAs									
Fella	3.5	3.5	3.0	3.5	3.0	3.0	3.0	3.0	
Spring	4.0	4.0	4.0	4.0	4.0	3.5	4.0	4.0	
Beau	3.5	4.0	4.0	4.0	3.5	4.0	3.5	3.5	
Mike	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	
Dick	3.5	3.5	3.5	3.5	3.0	3.0	3.0	3.0	
Durando	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	
Henry	2.5	3.0	3.0	3.0	3.0	2.5	3.0	3.0	
Jill	3.5	3.5	3.5	3.0	3.5	3.0	3.0	3.0	
Fred	3.5	4.0	3.5	4.0	3.5	3.5	4.0	3.5	
R. Bridge	3.0	3.0	3.5	3.5	3.0	3.0	3.0	3.0	Group
John	2.5	3.0	2.0	2.5	2.5	2.5	2.5	2.5	mean
Mean	3.27	3.45	3.32	3.41	3.23	3.14	3.23	3.18	3.28
S.D.	0.47	0.42	0.56	0.49	0.41	0.45	0.47	0.40	

Table IX. Red cell counts Values are $x10^{12}/l$

Horse	Week 0	8	16	
Controls				
Polly	7.0	7.4	7.7	
Lily	7.6	5.9	6.4	
Sprig	6.4	8.1	6.8	
Danny	7.5	7.3	6.6	
Dolly	4.9	6.1	6.9	
Jeeves	6.1	6.2	6.3	
Charlie	6.2	6.8	6.1	
Smiler	5.8	7.0	7.1	
Elspeth	7.4	6.5	7.4	
Sean	8.3	7.1	6.6	
Bliss	6.1	6.8	6.3	Group
LP	6.5	6.5	7.6	mean
Mean	6.65	6.81	6.82	6.76
S.D.	0.94	0.62	0.53	
EFAs				
Fella	8.9	8.3	8.0	
Spring	6.6	6.5	7.4	
Beau	8.1	6.7	7.9	
Mike	7.1	7.2	6.8	
Dick	5.9	6.4	6.3	
Durando	5.7	6.3	7.5	
Henry	6.9	7.0	8.0	
Jill	7.1	7.5	7.2	
Fred	7.9	5.2	5.5	
R.Bridge	7.0	6.3	6.2	Group
John	6.6	6.4	6.9	mean
Mean	7.07	6.71	7.06	6.95
S.D.	0.94	0.80	0.82	

Table X. Haemoglobin values Values are given in g/dl

Horse	Week 0	8	16	
Controls				
Polly	13.5	14.0	14.1	
Lily	14.9	10.9	12.0	
Sprig	12.1	14.8	13.7	
Danny	13.4	13.0	11.7	
Dolly	9.4	11.7	13.3	
Jeeves	11.5	11.3	11.7	
Charlie	11.6	12.4	11.4	
Smiler	10.7	12.5	12.6	
Elspeth	14.4	12.6	14.5	
Sean	13.9	11.8	11.3	
Bliss	10.5	12.4	10.7	Group
LP	11.6	11.6	14.1	mean
Mean	12.29	12.42	12.59	12.43
S.D.	1.71	1.12	1.30	
EFAs				
Fella	15.2	14.6	13.8	
Spring	12.3	12.1	13.6	
Beau	14.0	12.0	13.9	
Mike	12.9	13.4	12.4	
Dick	10.5	11.5	11.2	
Durando	10.0	11.1	13.3	
Henry	11.9	12.4	13.7	
Jill	12.6	12.9	12.5	
Fred	14.0	9.5	10.2	
R.Bridge	11.6	11.1	11.1	Group
John	12.2	11.8	12.8	mean
Mean	12.47	12.04	12.59	12.37
S.D.	1.53	1.34	1.26	

Table XI. Packed cell volumes Values are given as l/l

Horse	Week 0	8	16	
Controls				
Polly	0.356	0.383	0.391	
Lily	0.401	0.307	0.330	
Sprig	0.330	0.415	0.315	
Danny	0.368	0.364	0.324	
Dolly	0.263	0.331	0.366	
Jeeves	0.315	0.315	0.330	
Charlie	0.324	0.348	0.320	
Smiler	0.289	0.354	0.354	
Elspeth	0.394	0.354	0.397	
Sean	0.378	0.327	0.312	
Bliss	0.296	0.348	0.307	Group
LP	0.329	0.329	0.389	mean
Mean	0.337	0.348	0.345	**0.343**
S.D.	0.043	0.030	0.033	
EFAs				
Fella	0.414	0.395	0.384	
Spring	0.339	0.338	0.374	
Beau	0.385	0.338	0.379	
Mike	0.360	0.371	0.352	
Dick	0.299	0.330	0.314	
Durando	0.280	0.312	0.362	
Henry	0.328	0.356	0.376	
Jill	0.349	0.362	0.347	
Fred	0.378	0.268	0.289	
R.Bridge	0.322	0.315	0.312	Group
John	0.329	0.324	0.355	mean
Mean	0.344	0.337	0.349	**0.344**
S.D.	0.039	0.034	0.031	

Table XII. Mean cell volumes Values given in fl

Horse	Week 0	8	16	
Controls				
Polly	50.9	51.7	50.8	
Lily	52.8	52.0	51.6	
Sprig	51.5	51.2	50.9	
Danny	49.0	49.9	49.1	
Dolly	53.7	54.2	53.0	
Jeeves	51.6	50.8	52.4	
Charlie	52.3	51.2	52.4	
Smiler	49.8	50.6	49.9	
Elspeth	53.3	54.4	53.6	
Sean	45.6	46.1	47.3	
Bliss	48.6	51.2	48.8	Group
LP	50.6	50.6	51.2	mean
Mean	50.81	51.15	50.92	**50.96**
S.D.	2.30	2.10	1.87	
EFAs				
Fella	46.5	47.6	48.0	
Spring	51.3	52.0	50.6	
Beau	47.5	50.4	48.0	
Mike	50.7	51.5	51.8	
Dick	50.7	51.5	49.8	
Durando	49.1	49.6	48.3	
Henry	47.6	50.8	47.0	
Jill	49.2	48.3	48.2	
Fred	47.8	51.6	52.5	
R.Bridge	46.0	50.0	50.3	Group
John	49.5	50.7	51.4	mean
Mean	48.72	50.36	49.63	**49.57**
S.D.	1.77	1.40	1.83	

Table XIII. Mean cell haemoglobin values Values in pg

Horse	Week 0	8	16	
Controls				
Polly	18.6	18.9	18.3	
Lily	19.6	18.5	18.8	
Sprig	18.9	18.3	18.6	
Danny	17.9	17.8	17.7	
Dolly	19.2	19.2	19.3	
Jeeves	18.9	18.2	18.6	
Charlie	18.7	18.7	18.7	
Smiler	18.4	17.9	17.7	
Elspeth	19.5	19.4	19.6	
Sean	16.7	16.6	17.1	
Bliss	17.2	17.1	17.0	Group
LP	17.8	17.8	18.6	mean
Mean	18.45	18.20	18.33	18.33
S.D.	0.90	0.82	0.81	
EFAs				
Fella	17.1	17.6	17.2	
Spring	18.6	18.6	18.4	
Beau	17.3	17.9	17.6	
Mike	18.2	18.6	18.2	
Dick	17.8	18.0	17.8	
Durando	17.5	17.6	17.7	
Henry	17.2	17.7	17.1	
Jill	17.7	17.2	17.4	
Fred	17.7	18.3	18.5	
R.Bridge	16.6	17.6	17.9	Group
John	18.5	18.4	18.6	mean
Mean	17.65	17.95	17.85	17.82
S.D.	0.61	0.47	0.52	

Table XIV. Mean cell haemoglobin concentration

Values are given in g/dl

Horse	Week 0	8	16	
Controls				
Polly	36.5	36.6	36.0	
Lily	37.1	35.5	36.3	
Sprig	36.7	35.7	35.9	
Danny	36.5	35.7	36.1	
Dolly	35.7	35.4	36.4	
Jeeves	36.5	35.9	35.4	
Charlie	35.8	35.7	35.7	
Smiler	37.0	35.3	35.6	
Elspeth	36.5	35.6	36.6	
Sean	36.7	36.1	36.2	
Bliss	35.4	35.7	34.8	Group
LP	35.3	35.3	36.2	mean
Mean	36.31	35.71	35.94	35.99
S.D.	0.61	0.37	0.50	
EFAs				
Fella	36.7	37.0	35.9	
Spring	36.3	35.8	36.3	
Charlotte	35.5	34.5	35.0	
Beau	36.4	35.6	36.7	
Mike	35.8	36.1	35.2	
Dick	35.1	34.9	35.7	
Durando	35.7	35.5	36.7	
Henry	36.2	34.9	36.4	
Jill	36.1	35.6	36.0	
Fred	37.1	35.4	35.3	
R.Bridge	36.0	35.2	35.6	Group
John	37.3	36.4	36.1	mean
Mean	36.18	35.57	35.91	35.89
S.D.	0.64	0.69	0.57	

Table XV. Platelet counts Values $x10^9/l$

Horse	Week 0	8	16	
Controls				
Polly	170.0	140.0	140.0	
Lily	120.0	120.0	110.0	
Sprig	180.0	140.0	122.0	
Danny	150.0	140.0	110.0	
Dolly	100.0	70.0	122.0	
Jeeves	160.0	130.0	120.0	
Charlie	210.0	127.0	100.0	
Smiler	140.0	110.0	120.0	
Elspeth	210.0	200.0	160.0	
Sean	130.0	130.0	130.0	
Bliss	170.0	127.0	130.0	Group
LP	90.0	90.0	100.0	mean
Mean	152.50	127.00	122.00	**133.83**
S.D.	38.64	31.35	16.95	
EFAs				
Fella	140.0	140.0	120.0	
Spring	90.0	110.0	130.0	
Beau	140.0	120.0	130.0	
Mike	60.0	80.0	120.0	
Dick	130.0	120.0	90.0	
Durando	170.0	180.0	130.0	
Henry	130.0	130.0	110.0	
Jill	120.0	130.0	120.0	
Fred	170.0	160.0	130.0	
R.Bridge	130.0	90.0	120.0	Group
John	20.0	20.0	120.0	mean
Mean	118.18	116.36	120.00	**118.18**
S.D.	45.35	42.73	11.83	

Table XVI. White cell counts Values $x10^9/l$

Horse	Week 0	8	16	
Controls				
Polly	6.6	6.3	6.1	
Lily	6.6	6.0	5.4	
Sprig	7.3	6.2	5.8	
Danny	7.4	6.8	6.5	
Dolly	8.5	5.8	5.9	
Jeeves	5.7	5.7	5.4	
Charlie	8.2	6.2	4.6	
Smiler	7.7	6.5	6.5	
Elspeth	7.7	7.6	6.7	
Sean	5.9	5.6	7.3	
Bliss	6.3	6.2	4.4	Group
LP	5.8	5.8	6.5	mean
Mean	6.98	6.23	5.93	**6.38**
S.D.	0.96	0.55	0.86	
EFAs				
Fella	6.8	7.0	5.8	
Spring	5.4	5.3	6.2	
Beau	7.6	5.7	6.7	
Mike	5.5	4.8	5.2	
Dick	7.9	5.6	5.9	
Durando	5.7	5.4	4.7	
Henry	7.7	5.9	5.5	
Jill	6.8	6.6	5.8	
Fred	6.2	4.7	5.1	
R. Bridge	6.3	6.3	5.2	Group
John	5.9	4.8	5.6	mean
Mean	6.53	5.65	5.61	**5.93**
S.D.	0.90	0.76	0.56	

Table XVII. Urea values Values are given in mmol/l

Horse	Week 0	8	16	
Controls				
Polly	5.5	5.4	5.4	
Lily	6.0	4.3	6.4	
Sprig	7.7	3.5	5.9	
Danny	6.0	3.6	5.5	
Dolly	4.5	3.5	5.3	
Jeeves	4.9	3.6	5.6	
Charlie	4.8	3.3	5.5	
Smiler	5.1	3.7	6.2	
Elspeth	6.1	3.8	4.5	
Sean	4.6	3.6	5.4	
Bliss	5.4	3.9	6.2	Group
LP	4.6	4.6	6.6	mean
Mean	5.43	3.90	5.71	5.01
S.D.	0.92	0.60	0.58	
EFAs				
Fella	5.0	4.2	5.5	
Spring	3.6	1.6	3.7	
Beau	4.9	3.9	5.4	
Mike	5.4	4.4	4.1	
Dick	6.5	4.4	5.5	
Durando	5.4	3.5	5.1	
Henry	5.9	3.7	6.5	
Jill	7.4	4.1	5.5	
Fred	6.1	3.7	6.1	
R. Bridge	4.4	4.1	5.8	Group
John	6.6	5.2	7.2	mean
Mean	5.56	3.89	5.49	4.98
S.D.	1.08	0.89	0.99	

Table XVIII. Sodium values Values are given in mmol/l

Horse	Week 0	8	16	
Controls				
Polly	140.0	136.0	136.0	
Lily	136.0	137.0	135.0	
Sprig	136.0	138.0	137.0	
Danny	137.0	139.0	135.0	
Dolly	137.0	140.0	138.0	
Jeeves	138.0	141.0	137.0	
Charlie	136.0	137.0	138.0	
Smiler	132.0	138.0	137.0	
Elspeth	139.0	138.0	137.0	
Sean	138.0	139.0	136.0	
Bliss	141.0	140.0	136.0	Group
LP	140.0	140.0	136.0	mean
Mean	137.50	138.58	136.50	137.53
S.D.	2.43	1.51	1.00	
EFAs				
Fella	139.0	137.0	134.0	
Spring	139.0	139.0	138.0	
Beau	137.0	139.0	135.0	
Mike	139.0	139.0	135.0	
Dick	135.0	139.0	135.0	
Durando	140.0	140.0	139.0	
Henry	138.0	138.0	136.0	
Jill	136.0	140.0	137.0	
Fred	139.0	140.0	139.0	
R. Bridge	136.0	138.0	138.0	Group
John	136.0	139.0	135.0	mean
Mean	137.64	138.91	136.45	137.67
S.D.	1.69	0.94	1.81	

Table XIX. Potassium values Values are given in mmol/l

Horse	Week 0	8	16	
Controls				
Polly	3.9	3.0	4.1	
Lily	3.6	3.7	3.5	
Sprig	4.5	3.1	3.0	
Danny	4.0	3.6	3.1	
Dolly	3.9	2.5	3.3	
Jeeves	4.1	3.6	2.9	
Charlie	4.1	4.0	2.2	
Smiler	4.3	3.9	4.1	
Elspeth	4.4	4.1	3.4	
Sean	4.4	3.8	3.6	
Bliss	4.3	3.7	3.2	Group
LP	2.4	2.4	3.9	mean
Mean	3.99	3.45	3.36	3.60
S.D.	0.56	0.57	0.54	
EFAs				
Fella	4.0	3.9	3.9	
Spring	4.4	3.6	3.4	
Beau	3.8	3.4	3.5	
Mike	4.0	3.1	3.7	
Dick	4.6	3.4	3.8	
Durando	4.1	3.1	2.8	
Henry	4.8	4.4	3.1	
Jill	4.5	3.6	3.6	
Fred	4.0	4.2	2.9	
R. Bridge	4.0	3.4	3.0	Group
John	4.1	3.4	2.7	mean
Mean	4.21	3.59	3.31	3.70
S.D.	0.31	0.42	0.43	

Table XX. Calcium values Values are given in mmol/l

Horse	Week 0	8	16	
Controls				
Polly	3.11	2.87	3.06	
Lily	2.96	3.01	3.13	
Sprig	3.12	3.07	3.20	
Danny	2.94	2.87	3.03	
Dolly	2.92	3.81	3.17	
Jeeves	2.92	2.90	3.10	
Charlie	3.17	3.00	3.04	
Smiler	2.94	3.00	3.08	
Elspeth	2.94	2.93	3.15	
Sean	2.95	2.95	2.97	
Bliss	2.98	3.01	3.12	Group
LP	2.92	2.92	3.24	mean
Mean	2.989	3.028	3.108	3.042
S.D.	0.090	0.250	0.080	
EFAs				
Fella	3.05	3.16	3.28	
Spring	2.86	3.09	3.03	
Beau	2.81	3.01	3.16	
Mike	3.01	3.42	3.16	
Dick	3.01	3.05	3.08	
Durando	2.89	2.80	3.09	
Henry	2.89	2.99	2.95	
Jill	2.97	2.66	2.98	
Fred	2.87	2.86	3.06	
R. Bridge	3.10	2.81	2.89	Group
John	3.16	3.07	3.16	mean
Mean	2.965	2.993	3.076	3.012
S.D.	0.110	0.210	0.110	

Table XXI. Chloride values Values are given in mmol/l

Horse	Week 0	8	16	
Controls				
Polly	96.0	97.0	97.0	
Lily	99.0	98.0	97.0	
Spring	95.0	100.0	98.0	
Danny	97.0	95.0	96.0	
Dolly	101.0	101.0	102.0	
Jeeves	97.0	97.0	99.0	
Charlie	98.0	97.0	100.0	
Smiler	98.0	99.0	100.0	
Elspeth	98.0	100.0	95.0	
Sean	99.0	98.0	95.0	
Bliss	98.0	95.0	97.0	Group
LP	99.0	99.0	96.0	mean
Mean	97.92	98.00	97.67	97.86
S.D.	1.56	1.91	2.19	
EFAs				
Fella	97.0	101.0	97.0	
Spring	99.0	99.0	98.0	
Beau	96.0	96.0	97.0	
Mike	96.0	100.0	98.0	
Dick	99.0	95.0	100.0	
Durando	95.0	97.0	99.0	
Henry	95.0	100.0	95.0	
Jill	99.0	97.0	98.0	
Fred	98.0	100.0	97.0	
R. Bridge	96.0	94.0	98.0	Group
John	97.0	96.0	97.0	mean
Mean	97.00	97.73	97.64	97.45
S.D.	1.55	2.37	1.29	

Table XXII. Magnesium values Values are given in mmol/l

Horse	Week 0	8	16	
Controls				
Polly	0.66	0.69	0.62	
Lily	0.69	0.69	0.78	
Sprig	0.69	0.73	0.77	
Danny	0.61	0.58	0.63	
Dolly	0.68	0.71	0.81	
Jeeves	0.65	0.64	0.67	
Charlie	0.66	0.66	0.79	
Smiler	0.61	0.68	0.77	
Elspeth	0.68	0.72	0.72	
Sean	0.71	0.66	0.55	
Bliss	0.73	0.69	0.70	Group
LP	0.61	0.61	0.78	mean
Mean	0.665	0.672	0.716	0.684
S.D.	0.040	0.040	0.080	
EFAs				
Fella	0.74	0.74	0.81	
Spring	0.60	0.69	0.59	
Beau	0.67	0.67	0.74	
Mike	0.63	0.79	0.75	
Dick	0.64	0.69	0.79	
Durando	0.59	0.63	0.76	
Henry	0.69	0.77	0.72	
Jill	0.67	0.70	0.82	
Fred	0.80	0.73	0.83	
R. Bridge	0.65	0.62	0.70	Group
John	0.68	0.70	0.72	mean
Mean	0.669	0.703	0.748	0.707
S.D.	0.060	0.050	0.070	

Table XXIII. Inorganic phosphate values

Values are given in mmol/l

Horse	Week 0	8	16	
Controls				
Polly	0.88	1.25	0.79	
Lily	0.85	1.50	1.02	
Sprig	0.86	1.05	0.88	
Danny	0.98	1.13	0.98	
Dolly	1.13	1.14	0.86	
Jeeves	0.84	1.24	1.17	
Charlie	1.14	1.24	1.19	
Smiler	1.08	1.31	0.97	
Elspeth	0.90	0.98	0.93	
Sean	1.12	1.06	0.69	
Bliss	0.85	1.24	1.11	Group
LP	1.15	1.15	1.03	mean
Mean	0.982	1.191	0.968	1.047
S.D.	0.130	0.140	0.150	
EFAs				
Fella	1.00	1.23	0.81	
Spring	1.37	1.67	1.02	
Beau	0.84	1.04	0.86	
Mike	0.98	1.03	0.82	
Dick	0.75	1.30	0.93	
Durando	1.13	1.21	1.17	
Henry	0.78	1.28	1.05	
Jill	1.20	1.24	1.06	
Fred	1.11	1.01	1.05	
R. Bridge	0.84	1.39	1.08	Group
John	0.62	1.15	0.96	mean
Mean	0.965	1.232	0.983	1.060
S.D.	0.220	0.190	0.120	

Table XXIV. Alkaline phosphatase values

Values are given in IU/l

Horse	Week 0	8	16	
Controls				
Polly	469.0	382.0	234.0	
Lily	248.0	233.0	199.0	
Sprig	424.0	390.0	251.0	
Danny	286.0	294.0	214.0	
Dolly	336.0	279.0	203.0	
Jeeves	286.0	254.0	220.0	
Charlie	448.0	360.0	248.0	
Smiler	434.0	450.0	301.0	
Elspeth	310.0	243.0	169.0	
Sean	212.0	189.0	160.0	
Bliss	223.0	231.0	186.0	Group
LP	255.0	255.0	205.0	mean
Mean	327.58	296.67	215.83	280.03
S.D.	92.86	79.90	38.90	
EFAs				
Fella	347.0	292.0	208.0	
Spring	263.0	237.0	174.0	
Beau	264.0	378.0	275.0	
Mike	265.0	329.0	330.0	
Dick	392.0	370.0	210.0	
Durando	274.0	218.0	178.0	
Henry	424.0	375.0	253.0	
Jill	426.0	467.0	296.0	
Fred	288.0	256.0	171.0	
R.Bridge	234.0	193.0	239.0	Group
John	309.0	220.0	215.0	mean
Mean	316.91	303.18	231.73	283.96
S.D.	69.23	87.05	52.33	

Table XXV. Aspartate aminotransaminase values

Values are given in IU/l

Horse	Week 0	8	16	
Controls				
Polly	278.0	436.0	280.0	
Lily	230.0	217.0	304.0	
Sprig	254.0	358.0	328.0	
Danny	262.0	252.0	318.0	
Dolly	249.0	372.0	421.0	
Jeeves	249.0	238.0	293.0	
Charlie	290.0	359.0	393.0	
Smiler	217.0	296.0	347.0	
Elspeth	262.0	293.0	328.0	
Sean	300.0	239.0	347.0	
Bliss	227.0	351.0	303.0	Group
LP	309.0	309.0	366.0	mean
Mean	260.58	310.00	335.67	302.08
S.D.	29.15	66.63	41.74	
EFAs				
Fella	271.0	361.0	346.0	
Spring	323.0	405.0	519.0	
Beau	235.0	329.0	295.0	
Mike	278.0	349.0	314.0	
Dick	208.0	270.0	292.0	
Durando	245.0	304.0	310.0	
Henry	215.0	357.0	279.0	
Jill	263.0	283.0	352.0	
Fred	232.0	247.0	328.0	
R.Bridge	274.0	283.0	340.0	Group
John	268.0	444.0	456.0	mean
Mean	255.64	330.18	348.27	311.36
S.D.	32.92	60.21	73.96	

Table XXVI. Bilirubin values Values are given in umol/l

Horse	Week 0	8	16	
Controls				
Polly	85.0	79.0	108.0	
Lily	16.0	17.0	20.0	
Sprig	6.0	20.0	19.0	
Danny	27.0	20.0	28.0	
Dolly	12.0	14.0	13.0	
Jeeves	24.0	13.0	21.0	
Charlie	7.0	13.0	13.0	
Smiler	10.0	15.0	13.0	
Elspeth	10.0	28.0	17.0	
Sean	26.0	33.0	36.0	
Bliss	16.0	14.0	14.0	Group
LP	37.0	37.0	23.0	mean
Mean	23.00	25.25	27.08	25.11
S.D.	21.68	18.77	26.40	
EFAs				
Fella	13.0	29.0	17.0	
Spring	9.0	14.0	19.0	
Beau	26.0	21.0	16.0	
Mike	26.0	13.0	31.0	
Dick	15.0	21.0	25.0	
Durando	17.0	11.0	16.0	
Henry	13.0	21.0	23.0	
Jill	10.0	23.0	23.0	
Fred	6.0	19.0	17.0	
R. Bridge	35.0	18.0	24.0	Group
John	27.0	28.0	20.0	mean
Mean	17.91	19.82	21.00	19.58
S.D.	9.20	5.72	4.69	

Table XXVII. Total plasma protein values

Values are given in g/l

Horse	Week 0	8	16	
Controls				
Polly	70.0	73.0	68.0	
Lily	66.0	71.0	62.0	
Sprig	76.0	78.0	73.0	
Danny	70.0	67.0	69.0	
Dolly	62.0	65.0	73.0	
Jeeves	63.0	60.0	64.0	
Charlie	77.0	68.0	68.0	
Smiler	79.0	70.0	66.0	
Elspeth	74.0	64.0	73.0	
Sean	67.0	67.0	68.0	
Bliss	66.0	65.0	68.0	Group
LP	68.0	68.0	72.0	mean
Mean	69.83	68.00	68.67	68.83
S.D.	5.56	4.65	3.60	
EFAs				
Fella	69.0	66.0	63.0	
Spring	66.0	67.0	72.0	
Beau	63.0	73.0	66.0	
Mike	65.0	73.0	62.0	
Dick	69.0	72.0	68.0	
Durando	68.0	71.0	65.0	
Henry	71.0	68.0	73.0	
Jill	73.0	67.0	69.0	
Fred	63.0	61.0	64.0	
R.Bridge	70.0	62.0	71.0	Group
John	70.0	67.0	66.0	mean
Mean	67.91	67.91	67.18	67.67
S.D.	3.27	4.09	3.71	

Table XXVIII. Albumin values Values are given in g/l

Horse	Week 0	8	16	
Controls				
Polly	33.0	33.0	35.0	
Lily	34.0	30.0	37.0	
Sprig	37.0	36.0	32.0	
Danny	37.0	37.0	31.0	
Dolly	30.0	31.0	40.0	
Jeeves	37.0	35.0	36.0	
Charlie	42.0	35.0	32.0	
Smiler	32.0	35.0	31.0	
Elspeth	35.0	33.0	32.0	
Sean	36.0	36.0	36.0	
Bliss	33.0	36.0	36.0	Group
LP	36.0	36.0	35.0	mean
Mean	35.17	34.42	34.42	34.67
S.D.	3.10	2.19	2.81	
EFAs				
Fella	36.0	34.0	34.0	
Spring	43.0	35.0	42.0	
Beau	35.0	42.0	36.0	
Mike	37.0	39.0	33.0	
Dick	32.0	34.0	32.0	
Durando	30.0	33.0	34.0	
Henry	30.0	38.0	35.0	
Jill	39.0	37.0	37.0	
Fred	34.0	31.0	29.0	
R.Bridge	44.0	29.0	30.0	Group
John	39.0	32.0	35.0	mean
Mean	36.27	34.91	34.27	35.15
S.D.	4.73	3.81	3.52	

Table XXIX. Globulin value Values are given in g/l

Horse	Week 0	8	16	
Controls				
Polly	37.0	40.0	33.0	
Lily	32.0	41.0	25.0	
Sprig	39.0	42.0	41.0	
Danny	33.0	30.0	38.0	
Dolly	32.0	34.0	33.0	
Jeeves	26.0	25.0	28.0	
Charlie	35.0	33.0	36.0	
Smiler	47.0	35.0	35.0	
Elspeth	39.0	31.0	41.0	
Sean	31.0	31.0	32.0	
Bliss	33.0	29.0	32.0	Group
LP	32.0	32.0	37.0	mean
Mean	34.67	33.58	34.25	34.17
S.D.	5.31	5.16	4.81	
EFAs				
Fella	33.0	32.0	29.0	
Spring	23.0	32.0	30.0	
Beau	28.0	31.0	30.0	
Mike	28.0	34.0	29.0	
Dick	37.0	38.0	36.0	
Durando	38.0	38.0	31.0	
Henry	41.0	30.0	38.0	
Jill	34.0	30.0	32.0	
Fred	29.0	30.0	35.0	
R.Bridge	26.0	33.0	41.0	Group
John	31.0	35.0	31.0	mean
Mean	31.64	33.00	32.91	32.52
S.D.	5.52	2.97	4.01	

Table XXX. Gamma glutamyl transpeptidase values

Values are given in IU/l

Horse	Week 0	8	16	
Controls				
Polly	31.0	39.0	36.0	
Lily	22.0	24.0	22.0	
Sprig	34.0	30.0	20.0	
Danny	27.0	21.0	25.0	
Dolly	22.0	28.0	28.0	
Jeeves	37.0	26.0	30.0	
Charlie	30.0	40.0	33.0	
Smiler	22.0	16.0	20.0	
Elspeth	26.0	19.0	16.0	
Sean	29.0	19.0	21.0	
Bliss	16.0	14.0	14.0	Group
LP	37.0	37.0	20.0	mean
Mean	27.75	26.08	23.75	25.86
S.D.	6.52	8.93	6.76	
EFAs				
Fella	33.0	22.0	26.0	
Spring	30.0	37.0	57.0	
Beau	21.0	28.0	26.0	
Mike	23.0	26.0	27.0	
Dick	26.0	24.0	31.0	
Durando	21.0	16.0	24.0	
Henry	21.0	20.0	22.0	
Jill	34.0	20.0	26.0	
Fred	31.0	20.0	28.0	
R. Bridge	22.0	14.0	23.0	Group
John	19.0	18.0	22.0	mean
Mean	25.55	22.27	28.36	25.39
S.D.	5.48	6.39	9.87	

Table XXXI. Triglyceride values Values are given in mmol/l

Horse	Week 0	8	16	
Controls				
Polly	0.16	0.33	0.42	
Lily	0.24	0.41	0.28	
Sprig	0.34	0.27	0.25	
Danny	0.38	0.23	0.33	
Dolly	0.24	0.24	0.22	
Jeeves	0.19	0.13	0.35	
Charlie	0.23	0.44	0.56	
Smiler	0.16	0.01	0.21	
Elspeth	0.26	0.06	0.19	
Sean	0.18	0.03	0.35	
Bliss	0.25	0.04	0.20	Group
LP	0.14	0.14	0.18	mean
Mean	0.231	0.194	0.295	0.240
S.D.	0.070	0.150	0.110	
EFAs				
Fella	0.25	0.22	0.20	
Spring	0.22	0.41	0.23	
Beau	0.09	0.05	0.28	
Mike	0.10	0.09	0.22	
Dick	0.45	0.23	0.29	
Durando	0.27	0.15	0.29	
Henry	0.17	0.11	0.29	
Jill	0.36	0.33	0.32	
Fred	0.62	0.28	0.36	
R. Bridge	0.28	0.04	0.24	Group
John	0.15	0.23	0.34	mean
Mean	0.269	0.195	0.278	0.247
S.D.	0.160	0.120	0.050	

Table XXXII. Cholesterol values Values are given in mmol/l

Horse	Week 0	8	16	
Controls				
Polly	2.29	2.11	2.52	
Lily	1.60	1.33	1.82	
Sprig	1.99	2.20	2.19	
Danny	2.56	1.93	2.39	
Dolly	1.82	2.56	2.89	
Jeeves	2.46	2.01	2.37	
Charlie	2.17	2.05	2.02	
Smiler	1.79	1.77	1.92	
Elspeth	2.21	2.21	2.48	
Sean	2.46	2.23	2.30	
Bliss	1.66	2.21	2.40	Group
LP	2.12	2.12	2.15	mean
Mean	2.094	2.061	2.288	2.148
S.D.	0.320	0.300	0.290	
EFAs				
Fella	4.14	4.15	3.99	
Spring	2.33	2.51	2.41	
Beau	2.28	2.57	2.54	
Mike	2.62	2.86	2.44	
Dick	1.70	1.82	1.89	
Durando	1.91	1.88	2.07	
Henry	1.35	2.34	1.73	
Jill	2.15	2.37	2.12	
Fred	2.29	1.49	1.55	
R. Bridge	2.31	1.57	2.05	Group
John	2.21	2.00	2.11	mean
Mean	2.299	2.324	2.264	2.295
S.D.	0.700	0.740	0.650	

APPENDIX IV

NORMAL RANGES FOR HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS AND UNITS USED IN THEIR MEASUREMENT

NORMAL RANGES FOR HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS

Normal Ranges for Haematological Parameters

Parameter	Horse type				
	All	Hot Blooded		Cold Blooded	
	(Ref 1)	(Ref 2)	(Ref 3)	(Ref 2)	(Ref 3)
RBC	7-14	6-12	6.8-12.9	5.5-9.5	5.5-9.5
Hb	10-16.9	11-19	11-19	8-14	8-14
PCV	0.29-0.47	0.32-0.52	0.32-0.53	0.24-0.44	0.24-0.44
MCV	31-43.2	34-58	37-58.5	40-48	-
MCH	12-19.2	-	12.3-19.7	-	-
MCHC	32-36	32-38	31-38.6	32-38	-
PL	120-360	100-350	-	100-350	-
WBC	4.1-10.1	5.5-12.5	5.4-14.3	6-12	6-12

	Horse type		Units
	TB	Ponies	
	(Ref 4)		
RBC	8.8+/-1.09	7.39+/-1.07	$x10^{12}/l$
Hb	14.6+/-1.61	12.81+/-1.65	g/dl
PCV	0.398+/-0.047	0.32+/-0.011	l/l
MCV	45.5+/-2.56	48.2+/-4.0	fl
MCH	16.6+/-0.96	17.4+/-1.3	pg
MCHC	36.5+/-1.36	36.2+/-1.1	g/dl
PL	132-226	223-276	$x10^9/l$
WBC	9.54+/-1.83	8.44+/-1.46	$x10^9/l$

Normal Ranges for Biochemical Parameters

Parameter	Ref 1	Ref 2	Ref 4	Ref 5
Urea	2.5-7.0	3.6-8.6	4.48+/-0.83	3.5-8.0
Sodium	134-150	132-146	144+/-5	134-143
Potassium	2.7-5.5	2.4-4.7	4.2+/-0.6	3.3-5.3
Calcium	2.6-3.3	2.7-3.2	2.95+/-0.08	2.9-3.9
Chloride	98-109	99-109	96+/-3	89-106
Magnesium	0.7-1.2	0.53-1.02	0.79+/-0.08	0.6-0.9
Phosphate	0.8-1.8	0.52-1.45	1.25+/-0.2	0.5-1.6
Alk. Phos.	<131	83-283	80+/-283	50-250
AST	45-150	153-411	107+/-20	60-230
Bilirubin	17-34	1.7-42.8	28.1+/-8.55	13-39
TPP	60-73	59-84	64.6	46-70
Albumin	25-38	28-32	37.4	17-37
Globulins	30-48	31-52	27.2	21-41
GGT	-	11-44	-	10-40
Triglycerides	-	-	-	0.12-0.35
Cholesterol	-	0.8-2.2	3.16+/-0.47	2.4-3.7

Ref 1 (Henston, 1985-86); Ref 2 (Brobst and Parry, 1987); Ref 3 (Schalm, 1986); Ref 4 (Jeffcott, 1977); Ref 5 (Ricketts, 1987)

Parameter	Units	Parameter	Units
Urea	mmol/l	AST	IU/l
Sodium	mmol/l	Bilirubin	umol/l
Potassium	mmol/l	TPP	g/l
Calcium	mmol/l	Albumin	g/l
Chloride	mmol/l	Globulins	g/l
Magnesium	mmol/l	GGT	IU/l
Phosphate	mmol/l	Triglycerides	mmol/l
Alk.Phos	IU/l	Cholesterol	mmol/l

APPENDIX V

TABLES DETAILING STATISTICAL ANALYSES PERFORMED IN THE STUDY OF THE PHARMACOKINETICS OF EFAs IN HORSES

Clinical Indices, Haematological and Biochemical Parameters and EFA Estimations

Clinical Indices

Coat condition

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	6.00	3	2.00	0.88
Error	100.33	44	2.28	
Time	62.54	2	31.27	58.55
Group*Time	21.13	6	3.52	6.59
Error	47.00	88	0.53	

Significant differences over time, and between groups over time

Mane condition

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	3.39	3	1.13	0.98
Error	50.50	44	1.15	
Time	60.26	2	30.13	72.65
Group*Time	2.57	6	0.43	1.03
Error	36.50	88	0.41	

Significant difference over time

Tail condition

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	2.30	3	0.77	0.66
Error	51.44	44	1.17	
Time	98.00	2	49.00	135.93
Group*Time	2.28	6	0.38	1.05
Error	31.72	88	0.36	

Significant difference over time

Hoof condition

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	7.17	3	2.39	1.69
Error	62.06	44	1.41	
Time	110.60	2	55.30	119.83
Group*Time	2.12	6	0.35	0.77
Error	40.61	88	0.46	

Significant difference over time

Haematological Parameters

Red cell count

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	3.55	3	1.18	0.74
Error	70.06	44	1.59	
Time	38.22	2	19.11	59.55
Group*Time	8.82	6	1.47	4.58
Error	28.24	88	0.32	

Significant differences over time, and between groups over time

Haemoglobin

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	4.34	3	1.45	0.37
Error	173.89	44	3.95	
Time	15.58	2	7.79	7.36
Group*Time	26.80	6	4.47	4.22
Error	93.18	88	1.06	

Significant differences over time, and between groups over time

Packed cell volume

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.00	3	0.00	0.36
Error	0.17	44	0.00	
Time	0.00	2	0.00	17.55
Group*Time	0.02	6	0.00	4.27
Error	0.08	88	0.00	

Significant differences over time, and between groups over time

Mean cell volume

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	55.59	3	18.53	1.06
Error	772.50	44	17.56	
Time	497.41	2	248.70	243.94
Group*Time	4.59	6	0.77	0.75
Error	89.72	88	1.02	

Significant difference over time

Mean cell haemoglobin

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	9.08	3	3.03	1.28
Error	103.98	44	2.36	
Time	113.02	2	56.51	548.73
Group*Time	0.48	6	0.08	0.78
Error	9.06	88	0.10	

Significant difference over time

Mean cell haemoglobin concentration

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	1.09	3	0.36	0.51
Error	31.64	44	0.72	
Time	41.27	2	20.63	46.24
Group*Time	1.02	6	0.17	0.38
Error	39.27	88	0.45	

Significant difference over time

Platelets

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	3208.75	3	1069.58	0.34
Error	137008.00	44	3113.82	
Time	7583.00	2	3791.50	3.89
Group*Time	6143.75	6	1023.96	1.05
Error	85880.50	88	975.91	

Significant difference over time

White cell count

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	100.28	3	33.43	3.32
Error	443.01	44	10.07	
Time	50.96	2	25.48	5.11
Group*Time	33.66	6	5.61	1.13
Error	438.44	88	4.98	

Significant differences between groups, and over time

Biochemical Parameters

Urea

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	24.63	3	8.21	3.76
Error	96.18	44	2.19	
Time	66.36	2	33.18	35.67
Group*Time	13.32	6	2.22	2.39
Error	81.86	88	0.93	

Significant differences between groups, over time, and between groups over time

Sodium

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	35.75	3	11.92	1.40
Error	373.50	44	8.49	
Time	67.00	2	33.50	3.93
Group*Time	84.75	6	14.13	1.66
Error	751.00	88	8.53	

Significant difference over time

Potassium

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.78	3	0.26	1.17
Error	9.75	44	0.22	
Time	20.30	2	10.15	67.33
Group*Time	0.70	6	0.12	0.78
Error	13.27	88	0.15	

Significant differences over time

Calcium

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.01	3	0.00	0.15
Error	1.13	44	0.03	
Time	0.24	2	0.12	10.33
Group*Time	0.12	6	0.02	1.67
Error	1.01	88	0.01	

Significant difference over time

Chloride

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	47.50	3	15.83	1.71
Error	407.88	44	9.27	
Time	35.38	2	17.69	2.48
Group*Time	74.63	6	12.44	1.74
Error	628.63	88	7.14	

No significant difference was seen

Magnesium

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.02	3	0.00	0.49
Error	0.73	44	0.02	
Time	0.17	2	0.08	34.02
Group*Time	0.06	6	0.00	3.81
Error	0.21	88	0.00	

Significant difference over time, and between groups over time

Inorganic phosphate

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.09	3	0.03	0.59
Error	2.13	44	0.05	
Time	0.83	2	0.41	14.56
Group*Time	0.56	6	0.09	3.31
Error	2.50	88	0.03	

Significant difference over time, and between groups over time

Alkaline phosphatase

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	2706.00	3	902.00	0.06
Error	624870.00	44	14201.59	
Time	15900.00	2	7950.00	3.51
Group*Time	25048.00	6	4174.67	1.84
Error	199126.00	88	2262.80	

Significant difference over time

Aspartate aminotransaminase

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	11278.00	3	3759.33	0.28
Error	586815.00	44	13336.70	
Time	16746.00	2	8373.00	2.38
Group*Time	84674.00	6	14112.33	4.00
Error	310195.00	88	3524.94	

Significant difference over time, and between groups over time

Bilirubin

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	177.14	3	59.05	1.18
Error	2203.50	44	50.08	
Time	176.26	2	88.13	1.89
Group*Time	220.23	6	36.71	0.79
Error	4105.50	88	46.65	

No significant difference was seen

Total plasma proteins

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	712.31	3	237.44	1.71
Error	6116.00	44	139.00	
Time	1175.75	2	587.88	9.64
Group*Time	698.81	6	116.47	1.91
Error	5366.13	88	60.98	

Significant difference over time

Albumin

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	48.58	3	16.19	0.97
Error	733.84	44	16.68	
Time	10.80	2	5.40	0.47
Group*Time	143.05	6	23.84	2.09
Error	1005.48	88	11.43	

No significant difference was seen

Globulins

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	886.16	3	295.39	1.38
Error	9394.06	44	213.50	
Time	882.94	2	441.47	4.73
Group*Time	1372.63	6	228.77	2.45
Error	8217.78	88	93.38	

Significant difference over time, and between groups over time

Triglycerides

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.09	3	0.03	0.89
Error	1.54	44	0.03	
Time	0.09	2	0.05	1.86
Group*Time	0.20	6	0.03	1.36
Error	2.15	88	0.02	

No significant difference was seen

Cholesterol

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	3.88	3	1.29	5.52
Error	10.31	44	0.23	
Time	0.07	2	0.03	0.33
Group*Time	1.80	6	0.30	2.89
Error	9.11	88	0.10	

Significant differences between groups, and between groups over time

EFA Estimations

Table of Means and One Way Analysis of Variance for Linoleic Acid (LA) Values, Comparing Week 0 to Week 3

Means by Group

Level	Count	Average	Stnd.Error	95% Confidence level	
5g daily	12	5.35	1.11	2.91	7.78
10g eod	12	6.21	1.24	3.77	8.64
20g daily	12	7.50	1.17	5.07	9.94
40g eod	12	4.47	1.58	2.03	6.90

Analysis of Variance

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio	Sig.level
Between groups	60.35	3	20.12	1.01	0.376
Within groups	876.88	44	19.93		

No significant differences were evident

Table of Means and One Way Analysis of Variance for Linoleic Acid (LA) Values, Comparing Week 0 to Week 6

Means by Group

Level	Count	Average	Stnd.Error	95% Confidence Level	
5g daily	12	5.10	1.54	1.83	8.38
10g eod	12	4.16	2.11	0.89	7.43
20g daily	12	6.22	1.35	2.95	9.49
40g eod	12	3.99	1.84	0.71	7.26

Analysis of Variance

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio	Sig.level
Between groups	37.95	3	12.65	0.35	0.79
Within groups	1586.89	44	36.07		

No significant differences were evident

Table of Means and One Way Analysis of Variance for Dihomo-gamma-linolenic Acid (DGLA) Values, Comparing Week 0 to Week 6

Means by Group

Level	Count	Average	Stnd.Error	95% Confidence Level.	
5g daily	12	0.08	0.02	0.03	0.12
10g eod	11	0.04	0.02	-0.01	0.09
20g daily	12	0.11	0.04	0.06	0.15
40g eod	11	0.11	0.02	0.06	0.16

Analysis of Variance

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio	Sig.level
Between groups	0.04	3	0.01	1.63	0.20
Within groups	0.30	42	0.01		

No significant differences were evident

Table of Means, One Way Analysis of Variance and Multiple Range Test for Dihomo-gamma-linolenic Acid (DGLA) Values, Comparing Week 0 to Week 6

Means by Group

Level	Count	Average	Stnd.Error	95% Confidence Level	
5g daily	12	0.08	0.02	0.03	0.12
10g eod	9	0.04	0.03	-0.09	0.01
20g daily	11	0.11	0.02	0.07	0.16
40g eod	11	0.11	0.02	0.07	0.16

Analysis of Variance

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio	Sig.level
Between groups	0.16	3	0.05	8.40	0.00
Within groups	0.25	39	0.01		

Significant difference was seen between groups

Multiple range analysis revealed significantly lower value for 10g eod than for 5g daily, 20g daily and 40g eod

Table of Means and One Way Analysis of Variance for Arachidonic Acid (AA) Values, Comparing Week 0 to Week 3

Means by Group

Level	Count	Average	Stnd.Error	95% Confidence Level	
5g daily	12	0.18	0.08	-0.07	0.42
10g eod	12	0.00	0.11	-0.24	0.25
20g daily	12	0.11	0.15	-0.14	0.36
40g eod	12	-0.11	0.17	-0.36	0.14

Analysis of Variance

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio	Sig.level
Between groups	0.57	3	0.19	0.93	0.44
Within groups	9.03	44	0.21		

No significant differences were evident

Table of Means and One Way Analysis of Variance for Arachidonic Acid (AA) Values, Comparing Week 0 to Week 6

Means by Group

Level	Count	Average	Stnd.Error	95% Confidence Level	
5g daily	12	0.18	0.13	-0.08	0.43
10g eod	12	0.05	0.16	-0.20	0.31
20g daily	12	0.13	0.07	-0.12	0.39
40g eod	12	-0.13	0.16	-0.38	0.13

Analysis of Variance

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio	Sig.level
Between groups	0.65	3	0.22	1.00	0.40
Within groups	9.62	44	0.22		

No significant differences were evident

Table of Means and One Way Analysis of Variance for Eicosapentanoic Acid (EPA) Values, Comparing Week 0 to Week 3

Means by Group

Level	Count	Average	Stnd.Error	95% Confidence Level	
5g daily	12	-0.03	0.02	-0.12	0.05
10g eod	12	-0.04	0.02	-0.12	0.05
20g daily	12	0.01	0.06	-0.08	0.09
40g eod	11	0.00	0.07	-0.09	0.09

Analysis of Variance

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio	Sig.level
Between groups	0.02	3	0.01	0.22	0.88
Within groups	1.06	43	0.02		

No significant differences were evident

Table of Means and One Way Analysis of Variance for Eicosapentanoic Acid (EPA) Values, Comparing Week 0 to Week 6

Means by Group

Level	Count	Average	Stnd.Error	95% Confidence Level	
5g daily	12	-0.12	0.03	-0.20	-0.02
10g eod	12	-0.06	0.03	-0.15	0.03
20g daily	12	0.00	0.03	-0.09	0.09
40g eod	11	-0.12	0.09	-0.21	-0.02

Analysis of Variance

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio	Sig.level
Between groups	0.10	3	0.03	1.22	0.32
Within groups	1.14	42	0.03		

No significant differences were evident

APPENDIX VI

TABLES DETAILING STATISTICAL ANALYSES PERFORMED IN THE STUDY ON THE USE OF EFAs IN THE TREATMENT OF DERMATOPHILOSIS IN HORSES

Clinical Indices, Haematological and Biochemical Parameters and EFA Estimations

Clinical Indices

Dermatophilosis Index - Lesion severity

Source of Variation	Sum of Squares	d.f.	Mean square	F-ratio
Group	96.02	2	48.01	1.82
Error	842.03	32	26.31	
Time	228.45	8	28.50	27.15
Group*Time	30.11	16	1.80	1.78
Error	269.23	256	1.00	

Significant difference over time

Dorsal Distribution of Dermatophilosis Lesions

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	1064.98	2	532.49	0.46
Error	36278.35	32	1133.69	
Time	7088.11	8	886.01	18.81
Group*Time	156.80	16	9.80	0.20
Error	12057.80	256	47.10	

Significant difference over time

Hindlimb Distribution of Dermatophilosis Lesions

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	1665.63	2	832.81	1.10
Error	24180.29	32	755.63	
Time	13101.24	8	1637.65	26.86
Group*Time	755.32	16	47.20	0.77
Error	15607.78	256	60.96	

Significant difference over time

Coat Condition

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.06	2	0.00	0.01
Error	120.33	32	3.76	
Time	290.46	8	36.31	65.76
Group*Time	4.32	16	0.27	0.48
Error	141.32	256	0.55	

Significant difference over time

Mane Condition

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	1.81	2	0.91	0.78
Error	37.16	32	1.16	
Time	0.96	7	0.14	0.91
Group*Time	22.42	14	1.60	1.06
Error	338.11	224	1.51	

No significant difference was seen

Tail Condition

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	1.12	2	0.56	0.60
Error	29.84	32	0.93	
Time	243.24	8	30.40	60.22
Group*Time	9.72	16	0.61	1.20
Error	129.25	256	0.50	

Significant difference over time

Hoof Condition

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	4.96	2	2.48	1.90
Error	41.83	32	1.30	
Time	37.62	8	4.70	24.51
Group*Time	4.93	16	0.30	1.60
Error	49.12	256	0.19	

Significant difference over time

Body condition score

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.34	2	0.17	0.16
Error	34.54	32	1.08	
Time	0.90	8	0.11	2.86
Group*Time	0.54	16	0.03	0.86
Error	10.06	256	0.04	

Significant difference over time

Haematological Parameters

Red Cell Count

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	1.91	2	0.96	0.46
Error	67.29	32	2.10	
Time	4.68	4	1.17	3.38
Group*Time	2.17	8	0.27	0.78
Error	44.35	128	0.35	

Significant difference over time

Haemoglobin

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.06	2	0.03	0.01
Error	128.15	32	4.00	
Time	29.90	4	7.48	5.94
Group*Time	7.80	8	0.97	0.77
Error	161.12	128	1.26	

Significant difference over time

Packed Cell Volume

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	43.38	2	21.69	0.75
Error	919.83	32	28.74	
Time	321.50	4	80.38	4.55
Group*Time	102.40	8	12.81	0.72
Error	2262.90	128	17.68	

Significant difference over time

Mean Cell Volume

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	115.40	2	57.70	2.83
Error	651.30	32	20.35	
Time	17.40	4	4.36	8.87
Group*Time	0.30	8	0.04	0.09
Error	62.94	128	0.49	

Significant difference over time

Mean Cell Haemoglobin

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	16.88	2	8.44	3.86
Error	69.99	32	2.19	
Time	3.99	4	0.99	7.89
Group*Time	1.25	8	0.16	1.24
Error	16.12	128	0.12	

Significant differences between groups and over time

Mean Cell Haemoglobin Concentration

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.78	2	0.39	0.53
Error	23.44	32	0.73	
Time	11.05	4	2.76	9.38
Group*Time	2.38	8	0.29	1.01
Error	37.70	128	0.29	

Significant difference over time

White Cell Count

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	8.07	2	4.04	0.56
Error	229.82	32	7.19	
Time	23.40	4	5.85	5.36
Group*Time	12.60	8	1.57	1.44
Error	139.57	128	1.09	

Significant difference over time

Platelets

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	8814.50	2	4407.25	0.97
Error	145647.00	32	4551.46	
Time	9597.00	4	2399.25	4.09
Group*Time	5421.00	8	677.63	1.15
Error	75139.50	128	587.03	

Significant difference over time

Biochemical Parameters

Urea

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	37.32	2	18.66	1.86
Error	320.25	32	10.00	
Time	177.23	4	44.30	11.70
Group*Time	44.23	8	5.52	1.46
Error	484.53	128	3.78	

Significant difference over time

Sodium

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	45.25	2	22.62	3.32
Error	217.75	32	6.80	
Time	420.50	4	105.12	21.19
Group*Time	46.75	8	5.84	1.17
Error	634.75	128	4.95	

Significant difference between groups, and over time

Potassium

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	1.05	2	0.53	1.46
Error	11.58	32	0.36	
Time	3.04	4	0.76	2.18
Group*Time	2.49	8	0.31	0.89
Error	44.67	128	0.35	

No significant difference was seen

Calcium

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.01	2	0.00	0.17
Error	0.53	32	0.02	
Time	0.89	4	0.22	16.63
Group*Time	0.05	8	0.01	0.50
Error	1.72	128	0.01	

Significant difference over time

Chloride

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	5.38	2	2.69	0.33
Error	262.00	32	8.19	
Time	762.38	4	190.59	55.58
Group*Time	26.75	8	3.34	0.97
Error	438.88	128	3.42	

Significant difference over time

Magnesium

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.03	2	0.01	1.49
Error	0.32	32	0.01	
Time	0.27	4	0.07	12.37
Group*Time	0.12	8	0.01	2.76
Error	0.69	128	0.00	

Significant differences over time, and between groups over time

Inorganic Phosphate

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.09	2	0.05	1.15
Error	1.31	32	0.04	
Time	0.14	4	0.04	1.47
Group*Time	0.14	8	0.02	0.74
Error	3.08	128	0.02	

No significant difference was seen

Alkaline Phosphatase

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	3523.00	2	1761.50	0.30
Error	186970.50	32	5842.80	
Time	1145.50	4	25286.30	30.74
Group*Time	7304.00	8	913.00	1.11
Error	105274.50	128	822.40	

Significant difference over time

Aspartate Aminotransaminase

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	25190.00	2	12595.00	1.30
Error	310968.00	32	9717.70	
Time	52610.00	4	13152.50	8.00
Group*Time	8045.00	8	1005.60	0.61
Error	210225.00	128	1642.30	

Significant difference over time

Bilirubin

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	258.70	2	129.30	0.35
Error	11750.80	32	367.20	
Time	718.80	4	179.70	2.11
Group*Time	516.60	8	64.50	0.75
Error	10898.00	128	85.10	

No significant difference was seen

Total Plasma Proteins

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	109.30	2	54.68	1.10
Error	1590.20	32	49.69	
Time	724.30	4	181.07	17.70
Group*Time	43.10	8	5.39	0.53
Error	1303.30	128	10.18	

Significant difference over time

Albumin

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	37.32	2	18.66	1.86
Error	320.25	32	10.00	
Time	177.23	4	44.30	11.70
Group*Time	44.23	8	5.52	1.46
Error	484.53	128	3.78	

Significant difference over time

Globulins

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	40.78	2	20.39	0.40
Error	1627.65	32	50.86	
Time	242.54	4	60.63	5.26
Group*Time	45.79	8	5.72	0.49
Error	1472.85	128	11.50	

Significant difference over time

Gamma Glutamyl Transpeptidase

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	311.54	2	155.77	2.04
Error	2433.68	32	76.05	
Time	848.89	4	212.20	8.44
Group*Time	244.06	8	30.50	1.21
Error	3215.50	128	25.12	

Significant difference over time

Triglycerides

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.05	2	0.03	0.82
Error	0.93	32	0.03	
Time	0.32	3	0.10	9.91
Group*Time	0.08	6	0.01	1.27
Error	1.02	96	0.01	

Significant difference over time

Cholesterol

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	1.13	2	0.56	0.91
Error	19.85	32	0.62	
Time	6.83	3	2.28	27.37
Group*Time	0.61	6	0.10	1.21
Error	7.98	96	0.08	

Significant difference over time

EFA Estimations

Red Cell Phospholipids

Linoleic acid (18:2n6)

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	4.44	2	2.22	0.13
Error	570.30	33	17.28	
Time	2089.30	4	522.32	44.40
Group*Time	51.27	8	6.41	0.54
Error	1552.71	132	11.76	

Significant difference over time

Arachidonic acid (20:4n6)

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.57	2	0.28	1.39
Error	6.72	33	0.20	
Time	24.83	4	6.21	32.82
Group*Time	1.89	8	0.24	1.25
Error	24.96	132	0.19	

Significant difference over time

Eicosapentanoic acid (20:5n3)

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.22	2	0.11	0.38
Error	9.80	33	0.30	
Time	16.99	4	4.25	14.50
Group*Time	2.66	8	0.33	1.14
Error	38.69	132	0.29	

Significant difference over time

Plasma Phospholipids

Linoleic acid (18:2n6)

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	244.53	2	122.27	2.90
Error	1393.56	33	42.23	
Time	1491.38	4	372.84	16.02
Group*Time	80.75	8	10.09	0.43
Error	3072.59	132	23.28	

Significant difference over time

Dihomogammalinolenic acid (20:3n6)

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.15	2	0.08	1.26
Error	2.02	33	0.06	
Time	2.00	4	0.50	11.34
Group*Time	0.77	8	0.10	2.18
Error	5.83	132	0.04	

Significant difference over time

Arachidonic acid (20:4n6)

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.78	2	0.39	0.66
Error	19.68	33	0.60	
Time	17.39	4	4.35	16.60
Group*Time	1.23	8	0.15	0.59
Error	34.57	132	0.26	

Significant difference over time

Eicosapentanoic acid (20:5n3)

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.71	2	0.36	3.54
Error	3.33	33	0.10	
Time	1.67	4	0.42	5.10
Group*Time	0.94	8	0.12	1.44
Error	10.79	132	0.08	

Significant difference between groups, and over time

Plasma Cholesteryl Esters

Linoleic acid (18:2n6)

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	87.38	2	43.69	1.43
Error	1006.94	33	30.51	
Time	1134.56	4	283.64	8.55
Group*Time	287.88	8	35.98	1.08
Error	4378.38	132	33.17	

Significant difference over time

Dihomogammalinolenic acid (20:3n6)

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.14	2	0.07	0.22
Error	10.24	33	0.31	
Time	8.97	4	2.24	8.85
Group*Time	0.88	8	0.11	0.44
Error	33.46	132	0.25	

Significant time difference

Arachidonic acid (20:4n6)

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	1.77	2	0.88	1.48
Error	19.68	33	0.60	
Time	9.65	4	2.41	3.47
Group*Time	2.44	8	0.31	0.44
Error	91.77	132	0.70	

Significant difference over time

Eicosapentanoic acid (20:5n3)

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.11	2	0.05	1.05
Error	1.69	33	0.05	
Time	0.54	4	0.13	2.25
Group*Time	0.35	8	0.04	0.74
Error	7.86	132	0.06	

No significant difference was seen

APPENDIX VII

TABLES DETAILING STATISTICAL ANALYSES PERFORMED IN THE STUDY ON THE USE OF EFAs IN THE PROPHYLAXIS OF DERMATOPHILOSIS IN HORSES

Clinical Indices, Haematological and Biochemical Parameters and EFA Estimations

Clinical Indices

Coat condition

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.90	1	0.90	0.62
Error	30.74	21	1.46	
Time	3.48	7	0.50	1.51
Group*Time	1.79	7	0.26	0.78
Error	48.42	147	0.33	

No significant differences were seen

Mane condition

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	4.38	1	4.38	2.40
Error	38.28	21	1.82	
Time	6.48	7	0.92	3.03
Group*Time	7.75	7	1.11	3.62
Error	44.90	147	0.31	

Significant differences over time, and between groups over time

Tail condition

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	2.37	1	2.37	2.27
Error	21.96	21	1.04	
Time	6.24	7	0.89	3.11
Group*Time	3.62	7	0.52	1.81
Error	42.14	147	0.29	

Significant difference over time

Hoof condition

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.38	1	0.38	0.93
Error	8.52	21	0.41	
Time	1.48	7	0.21	1.53
Group*Time	0.99	7	0.14	1.03
Error	20.28	147	0.14	

No significant differences were seen

Body condition score

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	1.01	1	1.01	0.73
Error	29.07	21	1.38	
Time	1.52	7	0.22	3.58
Group*Time	0.40	7	0.06	0.94
Error	8.93	147	0.06	

Significant difference over time

Haematological Parameters

Red cell count

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.52	1	0.52	0.56
Error	20.25	22	0.92	
Time	0.32	2	0.16	0.37
Group*Time	0.70	2	0.35	0.81
Error	18.99	44	0.43	

No significant differences were seen

Haemoglobin

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.20	1	0.20	0.07
Error	61.91	22	2.81	
Time	1.42	2	0.71	0.51
Group*Time	0.85	2	0.42	0.31
Error	60.54	44	1.38	

No significant differences were seen

Packed cell volume

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.00	1	0.00	0.00
Error	0.04	22	0.00	
Time	0.00	2	0.00	0.27
Group*Time	0.00	2	0.00	0.48
Error	0.04	44	0.00	

No significant differences were seen

Mean cell volume

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	30.13	1	30.13	3.44
Error	192.89	22	8.77	
Time	10.23	2	5.12	5.34
Group*Time	4.08	2	2.04	2.13
Error	42.19	44	0.96	

Significant difference over time

Mean cell haemoglobin

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	4.72	1	4.71	3.62
Error	28.68	22	1.30	
Time	0.03	2	0.01	0.16
Group*Time	0.70	2	0.35	4.19
Error	3.67	44	0.08	

Significant difference between groups over time

Mean cell haemoglobin concentration

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.17	1	0.17	0.35
Error	10.81	22	0.49	
Time	4.38	2	2.19	9.00
Group*Time	0.03	2	0.02	0.06
Error	10.70	44	0.24	

Significant difference over time

White cell count

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	2.53	1	2.53	2.64
Error	21.13	22	0.96	
Time	12.87	2	6.44	14.36
Group*Time	0.27	2	0.14	0.30
Error	19.72	44	0.45	

Significant difference over time

Platelets

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	2485	1	2485	1.02
Error	53818.5	22	2446.30	
Time	3282.25	2	1641.13	3.16
Group*Time	3227.38	2	1613.69	3.11
Error	22875.75	44	519.49	

No significant differences were seen

Biochemical Parameters

Urea

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.03	1	0.03	0.02
Error	27.52	22	1.25	
Time	43.72	2	21.86	50.21
Group*Time	0.55	2	0.27	0.63
Error	19.16	44	0.44	

Significant difference over time

Sodium

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.75	1	0.75	0.22
Error	76.50	22	3.48	
Time	58.88	2	29.44	12.70
Group*Time	0.50	2	0.25	0.11
Error	102.00	44	2.32	

Significant difference over time

Potassium

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.19	1	0.19	0.77
Error	5.42	22	0.25	
Time	7.53	2	3.77	17.05
Group*Time	0.16	2	0.08	0.37
Error	9.72	44	0.22	

Significant difference over time

Calcium

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.00	1	0.00	0.04
Error	1.34	22	0.06	
Time	0.20	2	0.10	3.46
Group*Time	0.01	2	0.00	0.21
Error	1.27	44	0.03	

Significant difference over time

Chloride

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	3.56	1	3.56	0.89
Error	87.75	22	3.99	
Time	1.69	2	0.84	0.29
Group*Time	2.19	2	1.09	0.37
Error	129.44	44	2.94	

No significant differences were seen

Magnesium

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.00	1	0.00	1.23
Error	0.12	22	0.00	
Time	0.05	2	0.02	9.81
Group*Time	0.00	2	0.00	0.53
Error	0.11	44	0.00	

Significant difference over time

Inorganic phosphate

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.01	1	0.01	0.31
Error	0.82	22	0.04	
Time	0.92	2	0.46	22.43
Group*Time	0.02	2	0.00	0.40
Error	0.90	44	0.02	

Significant difference over time

Alkaline phosphatase

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	1458	1	1458	0.12
Error	265439.5	22	12065.43	
Time	120162	2	60081	32.03
Group*Time	2908.50	2	1454.25	0.78
Error	82532	44	1875.73	

Significant difference over time

Aspartate aminotransaminase

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	760.50	1	760.50	0.15
Error	109581	22	4980.96	
Time	83008	2	41504	23.88
Group*Time	1189.50	2	594.75	0.34
Error	76481	44	1738.21	

Significant difference over time

Bilirubin

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.17	1	0.17	0.00
Error	2207.66	21	105.13	
Time	108.20	2	54.10	1.56
Group*Time	6.93	2	3.47	0.10
Error	1456.20	42	34.67	

No significant differences were seen

Total plasma proteins

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	20.06	1	20.06	0.85
Error	521.88	22	23.72	
Time	17.44	2	8.72	0.62
Group*Time	7.44	2	3.72	0.26
Error	622.47	44	14.15	

No significant differences were seen

Albumin

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	5.01	1	5.01	0.43
Error	253.65	22	11.53	
Time	20.53	2	10.27	0.92
Group*Time	3.03	2	1.52	0.14
Error	490.44	44	11.15	

No significant differences were seen

Globulins

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	43.55	1	43.55	1.32
Error	724.89	22	32.95	
Time	2.19	2	1.10	0.07
Group*Time	11.86	2	5.93	0.37
Error	708.61	44	16.10	

No significant differences were seen

Gamma glutamyl transpeptidase

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	1.13	1	1.13	0.01
Error	2460.86	22	111.86	
Time	83.44	2	41.72	1.66
Group*Time	220.34	2	110.17	4.39
Error	1104.22	44	25.10	

Significant difference between groups over time

Triglycerides

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.00	1	0.00	0.22
Error	0.50	22	0.02	
Time	0.09	2	0.05	5.14
Group*Time	0.00	2	0.00	0.48
Error	0.40	44	0.00	

Significant difference over time

Cholesterol

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.22	1	0.22	0.31
Error	15.67	22	0.71	
Time	0.11	2	0.06	0.93
Group*Time	0.27	2	0.13	2.22
Error	2.63	44	0.06	

No significant differences were seen

EFA Estimations

Red Cell Phospholipids

Linoleic acid (18:2n6)

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.01	1	0.01	0.00
Error	68.43	21	3.26	
Time	333.33	3	111.11	17.94
Group*Time	10.00	3	3.33	0.54
Error	390.25	63	6.19	

Significant difference over time

Arachidonic acid (20:4n6)

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.02	1	0.02	0.24
Error	2.10	21	0.10	
Time	0.37	3	0.12	1.99
Group*Time	0.54	3	0.18	2.91
Error	3.92	63	0.06	

Significant difference between groups over time

Eicosapentanoic acid (20:5n3)

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.00	1	0.00	0.01
Error	0.12	21	0.00	
Time	0.18	3	0.06	15.51
Group*Time	0.03	3	0.00	2.17
Error	0.24	63	0.00	

Significant difference over time

Plasma Phospholipids

Linoleic acid (18:2n6)

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	19.88	1	19.88	0.74
Error	560.33	21	26.69	
Time	158.30	3	52.77	4.42
Group*Time	30.02	3	10.00	0.84
Error	751.58	63	11.93	

Significant difference over time

Dihomogammalinolenic acid (20:3n6)

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.25	1	0.25	3.73
Error	1.42	21	0.07	
Time	1.00	3	0.33	6.04
Group*Time	0.08	3	0.03	0.48
Error	3.48	63	0.05	

Significant difference over time

Arachidonic acid (20:4n6)

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.04	1	0.04	0.14
Error	5.71	21	0.27	
Time	0.99	3	0.33	7.12
Group*Time	0.45	3	0.15	3.26
Error	2.93	63	0.05	

Significant difference over time, and between groups over time

Eicosapentanoic acid (20:5n3)

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio
Group	0.00	1	0.00	0.10
Error	1.02	21	0.05	
Time	0.09	3	0.03	1.02
Group*Time	0.04	3	0.01	0.44
Error	1.88	63	0.03	

No significant differences were seen