The impact of fleece characteristics on insulation and heat exchange, and the consequential effect on vitamin D of alpacas in southern Australia

by

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“The process of scientific discovery is, in effect, a continual flight of wonder.”

- Albert Einstein -
Alpacas are a fleeced mammal, originating from the high altitudes of the Andes in South America, and imported into southern Australia as part of a niche alpaca-fibre industry. The climatic conditions in Australia where alpacas are raised are much hotter, but with lower (and seasonal) ultraviolet (UV) radiation levels, than what alpacas were adapted to in the Andes. Although the industry breeding objectives are to achieve higher quality and quantity of fleece, the knowledge on how these parameters are affecting animal health and welfare in Australia is limited. In particular, it is unknown if the insulation of the fleece would protect alpacas from radiant heat (and heat stress) during an Australian summer. Additionally, it is unknown if certain 'desirable' fleece types (combinations of fleece characteristics) affect the potential of alpacas to sweat, and/or to block out UV radiation penetration to the skin for the synthesis of vitamin D. The purpose of the research in this thesis is to investigate the influence of the fleece characteristics on insulation from radiant heat, and its consequential effect on potential heat stress and vitamin D synthesis of alpacas in southern Australia.

First, the potential for heat loss via sweating was tested by quantifying the density of total, primary, and secondary follicles and sweat gland ducts in the skin of Huacaya alpacas varying in fibre thickness. Second, to measure the impact of the fleece characteristics (diameter, density, length and colour) on the insulation and radiant heat load, I tested alpaca fleeces (half with light colouring and half with dark) which had a range of fibre diameters and densities and were cut to three different fleece lengths. Fibre diameter and fibre/follicle density
were correlated in all circumstances. Third, because of the insulating effects of the fleece, the effect of fleece structure has on the level of vitamin D$_3$ was tested for two groups of alpacas, selected for their fibre quality (fine and dense or thick and sparse fibre), in both winter and summer, and also pre- and post-shearing in spring. Lastly, I investigated the importance of fleece distribution, particularly around the face, and measured the effect of face-wool cover and fleece colour (light vs dark) on vitamin D production during winter when the UV intensity was low.

It is indicative from these results that the fleece is an efficient barrier against solar and UV radiation and should help to prevent heat stress on alpacas if managed correctly, but may hinder vitamin D synthesis. With increased primary follicle density and sweat gland duct density parallel to total follicle density, sweating potential is not limited. While fleece structure had little impact on the insulation, radiant heat load, or vitamin D$_3$ synthesis, fleece length was an important factor, with reduced fleece length being favourable for vitamin D$_3$ synthesis but a longer fleece more favourable for insulation from radiant heat. Additionally, alpacas with more face-wool, or those that are dark-coloured, are at higher risk of vitamin D deficiency in winter than alpacas with lighter-coloured fleeces or less face-wool, and therefore these animals need to be managed during winter by additional supplementation or clipping around the face to expose a larger area to UV radiation. It has been demonstrated that longer fleece will reduce the radiant heat load in summer but shorter fleece is beneficial for vitamin D$_3$ synthesis when levels are low at the end of winter. While vitamin D deficiency remains as an issue for the alpaca fibre industry, overall, breeding selection towards higher quality and quantity of fleece should not be detrimental to the health of alpacas in Australia.
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DECLARATION OF AUTHORSHIP FOR PUBLISHED AND UNPUBLISHED WORK

This thesis has been structured as a series of papers, with the main experimental chapters being prepared for publication in peer reviewed journals, under the supervision of Prof Shane Maloney and Assoc/Prof Dominique Blache. All co-authors have agreed to the inclusion of the work in the thesis.

Chapter 3: High follicle density does not decrease sweat gland density in Huacaya alpacas (Published – details outlined in Chapter 3)

The concept for this chapter was designed in consultation with my supervisors with the samples provided by an alpaca producer in Western Australia. I was responsible for all the histology, under the guidance of the staff at CellCentral, and for acquisition of data from the samples. I then analysed the data and wrote the paper. Prof Maloney contributed by editing the manuscript. Assoc/Prof Blache reviewed the statistical methods, contributed to edit the manuscript and submitted the manuscript for publication.

Chapter 4: Fleece length is the most important characteristic for the reduction of the radiant heat load on Huacaya alpacas

The concept for this chapter stemmed from my honours project (published 2011) and was designed in consultation with Prof Maloney. Samples were
provided by West Australian alpaca producers and I prepared these raw samples for analysis. With some assistance from Prof Maloney I then conducted the experiment and collected the data. Once the data had been obtained I then analysed the data and wrote the paper. Prof Maloney further contributed by providing advice on the physiological aspects of the analysis and edited the chapter. Assoc/Prof Blache reviewed the statistical methods and contributed to edit the chapter.

Chapter 5: Fleece length but not fleece structure influences plasma vitamin D$_3$ levels of Huacaya alpacas in southern Australia

The concept for this chapter was designed in consultation with my supervisors, for which I then designed the experiment with their input. I then collected the samples, with the assistance of other post graduate students and my supervisors. I then prepared the samples to be sent off for analysis. Once the data had been obtained I then analysed the data and wrote the paper. Prof Maloney and Assoc/Prof Blache both contributed by reviewing the statistical methods used and editing the chapter. Prof John Milton assisted by reviewing the nutritional aspects for the experiment and providing feedback on the specifics of vitamin D synthesis.

Chapter 6: Vitamin D$_3$ concentrations of light-coloured alpacas increase with reduced face-wool cover, but not in dark-coloured alpacas in southern Australia

The concept for this chapter stemmed from results obtained from Chapter 5 and after consultation with my supervisors I designed the experiment and collected
the samples, with the assistance from other post graduate students. I then prepared the samples to be sent off for analysis. Once the data had been obtained I then analysed the data and wrote the paper. Prof Maloney and Assoc/Prof Blache both contributed by editing the chapter.

I am in the process of amalgamating and amending Chapter 5 and Chapter 6 into one paper for publication.
CHAPTER ONE: GENERAL INTRODUCTION

Alpaca fibre is a soft and desirable animal fibre, which is used in a niche market for high-end luxury fabrics, knitting wools and outdoor sporting clothing, either on its own or as a blend with wool, cashmere, cotton or silk (IYNF 2009). The alpaca fibre industry in Australia is relatively small, producing 120,000 kg of fleece in comparison to 370,000,000 kg of sheep wool nationwide (Australian Alpaca Fleece Ltd 2008; Australian Wool Innovation Ltd 2008), and the quality and quantity of fibre produced over the national flock is highly variable. Breeding selection to improve the fleece among and within flocks has been a slow process, as alpacas have an 11 month gestation (and therefore it takes nearly a whole year to create a new generation of a breeding line) and also a large number of alpaca owners and breeders in Australia are hobbyists and breed simply for the pleasure of producing young and not strengthening genetically superior bloodlines. However, the number of large commercial farms is growing and they are starting to achieve high quality fine wool and high fibre density, to increase the quantity produced per animal per shearing, among their animals through intensive selective breeding and artificial insemination programs (to increase the output of genetically superior stud animals). Although alpaca production in Australia has now been operating for more than 25 years, there has been very little research undertaken about how an alpaca’s physiology allows them to cope with the Australian environment and how breeding selection for specific fibre types may affect their physiological health (the health of the body and bodily functions) under Australian conditions.
One such concern is how well the alpaca's fleece allows the animal to cope with heat stress. Alpacas are native to the dry tundra of the Andean plateaus of South America where the climate is cool, reaching an average maximum temperature in summer of around 20°C (Thomas and Winterhalder 1976; Bryant and Farfan 1984). In Australia, the regions where alpacas are raised regularly peak above 30-35°C on an average Australian summer's day. Alpacas thereby need to effectively remove heat from their bodies to avoid being heat stressed. Despite this, there has not been an extensive study to record the density of sweat glands in the skin of alpacas or how they relate to the fleece structure.

The fleece structure consists of two types of fibres, thicker primary fibres and finer secondary fibres, and the density thereof. Traditionally, there is one sweat gland per primary fibre and one primary fibre surrounded by secondary fibres per ‘bundle’ of fibres (Antonini et al. 2004; Ferguson et al. 2012). The Australian alpaca industry aims to increase the quality of fibre produced by increasing the number of secondary fibres per primary fibre whilst also reducing the thickness of the fibres. If the number of secondary fibres is increased there may be fewer primary fibres, and therefore speculatively fewer sweat glands, over a given area. Therefore, selective breeding for higher quality and quantity of fibre may also be potentially reducing an alpaca’s ability to dissipate heat from its body as sweat. If, however, alpacas are unable to effectively dissipate heat from their bodies (given that they rarely pant) then they will need to rely solely on the insulative ability of the fleece retarding heat flow into the fleece.

The fleece is an insulative barrier against radiant heat as it hinders solar radiation from penetrating to the skin level, increasing the body temperature and activating evaporative cooling (Hammel 1955; Cena and Monteith 1975; Walsberg 1988a, 1988b). However, if the fleece structure is effective as good
insulation, then it can prevent solar radiation from increasing the heat load on the animal’s body (Walsberg 1988b). I was therefore aiming to identify which fleece characteristics have the most influence on the insulation of an alpacas fleece and would reduce the penetration of radiant heat into the fleece towards the body and the subsequent radiant heat load. Therefore, producers can be aware which fleece types of alpacas are more prone to heat stress and can manage their alpacas accordingly.

Another major health concern prominent in alpacas in Australia is the deficiency of vitamin D (Judson et al. 2008). Vitamin D is essential for bone health and general wellbeing and a lack of it can cause debilitating bone diseases, a reduction in production (fibre and muscle growth), discomfort, and even disfigurement leading to death, particularly in the young (Dittmer and Thompson 2011). Vitamin D is synthesised by a photochemical reaction between ultraviolet B radiation, a component of solar radiation, and a sterol precursor in animal skin (Webb and Holick 1988). A vitamin D deficiency in Australia may be surprising; due to the intensity of solar radiation and the number of cloudless and sunny days, but the altitude in alpaca production regions of Australia is much lower than it is in their native environment and the fleece is also a thick insulative layer which may only be penetrated by a small percentage of incident UV radiation. It is unknown how the fleece structure affects the amount of UV radiation penetrating through the fleece for the synthesis of vitamin D, and if these changes in fleece structure would be beneficial or detrimental.

There has been very little research conducted on alpacas in Australia, particularly in regards to physiological health. As the fleece is the sole purpose of alpaca production in Australia, it is crucial to determine how changes to the
fleece structure will affect their wellbeing. The purpose of this thesis is to investigate the influence of fleece characteristics on insulation from radiant heat, and its consequential effect on potential heat stress and vitamin D synthesis of alpacas in southern Australia.
2.1. Introduction

The alpaca is a fleeced South American camelid (SAC), that was introduced to Australia in the late 1980's from Peru/Chile (via New Zealand) to establish a breeding flock for a niche fibre market (Hack et al. 1999; McGregor 2002; McGregor and Butler 2004). Alpaca fibre is used for high-end luxury fabrics, suits, scarves and outdoor sporting clothing as either on its own or as a blend with wool, cashmere, cotton and silk (FAO 2009).

The SAC family consists of four species; (i) alpacas, (ii) llama, (ii) guanaco and (iv) vicuña, the latter two are undomesticated species (Figure 2.1). The alpaca are one of the smaller SACs, generally standing less than one metre at the withers, and are a docile, hardy and low maintenance livestock (Fysh 2008). The alpaca has been valued as a fibre production animal since pre-hispanic times when its soft, light and warm fibre was considered the ‘fibre of the Gods’ by the native Incan peoples (Reiner and Bryant 1983). It was not until the 1980’s that the alpaca was recognised outside of South America as a potential fibre production animal (ACIL Consulting 2001; Fysh 2008).

A potential issue with the establishment of an alpaca fibre industry in southern Australia is that environmental temperatures are higher than those of the alpaca’s native range in the Andes of western South America. At the high altitudes and low latitudes of the Andes, the alpaca fleece would have evolved to insulate the body from the cold as well as the high intensity of solar, including ultraviolet (UV), radiation (Bryant and Farfan 1984; Hill et al. 1994; Cabrera et
al. 1995). As a result, health issues like vitamin D deficiency have been identified in flocks established at lower altitudes and higher latitudes that have a hotter climate, and less intense radiation, possibly stemming from the insulative ability (Judson et al. 2008) and fibre structure of the fleece.

From an industry perspective, specific fibre properties, such as high fibre density and finer fibre, are being selected in breeding programs aimed to improve the quality and quantity of fibre produced over the national flock (Wuliji et al. 2000; McGregor 2006). The selection emphasis for particular fibre properties has altered the structure of the fleece, which potentially could affect its insulative ability (Øritsland and Ronald 1978; Campbell et al. 1980; Walsberg 1988b, 1992) and may be detrimental to the welfare of alpacas. While the production qualities of the fleece may be improved, we may inadvertently be selecting for a fleece type that is not suited to Australian conditions.

The purpose of this doctoral research was to investigate the influence of fleece structure on insulation and radiant heat gain (from solar radiation), its consequential effect on vitamin D synthesis, and potential to dissipate heat as sweat and to establish if selective breeding for specific fibre characteristics would be detrimental to the health of alpacas in southern Australia.

2.2. Natural History
Camelids originated in North America 40-45 million years ago during the Eocene and 30 million years later diverged into the Camelini (camels) and Lamini (SACs) tribes (Stanley et al. 1994; Wheeler et al. 2006). Members of these groups exited North America, with the ancestral Camelini moving west
into Eurasia across the Bering Strait land bridge and the ancestral Lamini
moving south into South America via the Isthmus of Panama (Figure 2.2). All of
the camelids that remained in North America became extinct by the end of the
last ice age, approximately 10,000 – 12,000 years ago (Fowler 1998; Hoffman
2006).

![Figure 2.2](image)

**Figure 2.2** The movement of camelids from North America. Diagram, modified from
Roberts (1982).

After permanently migrating into South America 3 million years ago, the Lamini
evolved into three genera, two surviving to the present day (Figure 2.3, Stahl
2008). Until recently the alpaca was thought to be descended from the guanaco
and had evolved in parallel with the llama (*Lama glama*), classified within the
*Lama* genera and thereby going by the scientific name *Lama pacos*. However
recent genetic studies have shown that the alpaca is descended from the
vicuña (*Vicugna vicugna*) and in 2001 was re-classified as *Vicugna pacos*
(Figure 2.3, Kadwell *et al.* 2001).
2.3. Native Environment & Adaptations

The SACs are native to the rugged altiplano (highland plateaus >3,800 m above sea level) of the Andes mountain range that runs through Peru, Chile and Bolivia and along the western coastal regions of South America (<3,800 m above sea level), with the guanaco being the widest ranging species extending down into other southern regions where the other three SAC species are not found (Figure 2.4). The alpaca has the smallest native range of the SACs, probably due to being restricted by pastoralism and altitude, as they usually are found only between 4,100-4,700 m above sea level. (Figure 2.4, Braga et al. 2007).
Meteorologically, the altiplano has a long dry season and short wet season from November to March when less than 700 mm of rainfall is received on average (San Martin and Bryant 1989). The mean daily ambient temperature is low ranging from 1.5 to 20°C in summer and -11 to 13°C in winter, with diurnal variations sometimes reaching 25-30°C (Thomas and Winterhalder 1976; Bryant and Farfan 1984; Bonacic 2000; Garreaud et al. 2003). The combination of low rainfall and mild temperatures creates a dry tundra environment. The native vegetation on the altiplano is of low nutritive quality, usually puna grasslands consisting of grasses, herbs and woody shrubs (Figure 2.5, San Martin and Bryant 1989). Occasionally, alpacas and llamas graze on the bofedale peatlands in northern Peru, but generally it is wild guanacos and vicuñas that graze this region (Squeo et al. 2006).
At these high altitudes, there is an intense solar (including UV) radiation all year, with the UV index (UVI) ranging from about 11 in winter to 22 in summer (Figure 2.6, Liley and McKenzie 2006). In comparison, a UVI above 11 is considered extreme in Australia and it only ever reaches these levels during summer (Australian Bureau of Meteorology 2013a). The UV intensity increases by 10-12% for every 1,000m increase in altitude and is estimated to be about 20% higher on the altiplano than at sea level on a clear day (Reiner and Bryant 1983; Cabrera et al. 1995; Agusti 2008). The exposure to a cool climate paired with the high intensity of radiation may have provided the selection pressure that has resulted in alpaca fleece being adapted to insulate against the cold and prevent radiation damage on the skin (Gerken 2010).
Figure 2.6  Peak UV intensity over the world, the scale at the top indicates the
colours which represent the increasing UV level (left to right), noting that the Andes in
South America has the highest levels in the world (20-24 UVI) and at its peak intensity
southern Australia can reach 16-17 UVI  (Liley and McKenzie 2006). Black outline
shows the native distribution of alpacas.

2.4. Alpacas in Southern Australia

The alpaca was first introduced into Australia by entrepreneur Charles Ledger in
1858 for the New South Wales government. After several years the government
decided not to set up a new rural industry and the flock of 275 alpacas, llamas
and vicuñas was disbanded and presumably died out (Ledger 1875; Mitchell
2010). Alpacas were next introduced in 1987 by Victorian pastoralist Geoff
Halpin who imported a “small number” (quantity unstated) of animals from
Peru/Chile via New Zealand to establish a breeding stock in Australia (Rankin
2009). Since 1989, the industry has grown considerably, with continued imports
and local breeding. There were 120,000 animals registered in Australia in 2009
(Rankin 2009), with a suggested growth rate of the national herd of around 29% per annum (ACIL Consulting 2001). The majority of these alpacas are farmed in the south of the Australian continent, ranging from Dowerin (northern WA wheatbelt) to Esperance in Western Australia and from Adelaide to north of Sydney on the east coast, as well as in Tasmania.

Southern Australia has a moderate temperate climate with seasonal winter rainfall and hot dry summers. As there is a very large area across Australia that alpacas are farmed, the minimum and maximum daily temperatures vary a lot within seasons. In the cooler lower south east, the average minimum and maximum daily temperatures range from 9 to 30°C in summer and -3 to 15°C in winter. The remainder of the area where alpacas are farmed in Australia is warmer with the average minimum and maximum daily temperatures ranging from 15 to 33°C in summer and 6 to 18°C during winter (Figure 2.7, Australian Bureau of Meteorology 2013b), with the maximum temperature during both summer and winter higher than that on the altiplano. In southern Australia, sheep and goats can be exposed to heat stress conditions (McGregor 1985; Stockman 2006), when the ambient temperature regularly rises above average body temperature (38°C) during summer (Australian Bureau of Meteorology 2013b). If alpacas have similar physiology to sheep and goats, then they too will be at risk of heat stress, remembering that they are adapted to cool conditions.
Figure 2.7  Summer and winter maximum and minimum temperatures in Australia (Australian Bureau of Meteorology 2013b). Black outlines a rough estimate of the areas where alpacas are farmed in Australia.
The fleece of the alpaca forms the interface with the environment and so is important for heat exchange, and therefore also for thermoregulation. In Australia there is a strong emphasis towards selective breeding to identify the genetic potential for high quality fleece characteristics (McGregor and Butler 2004). Across the national flock the quality (fibre fineness) and quantity (fibre density and growth rate) are still quite variable, and to be competitive in the international market, alpacas are being selectively bred to produce larger quantities of fine fleece. However, it is not known if changing these fleece characteristics will affect the alpaca’s ability to thermoregulate and prevent heat stress during the Australian summer.

2.5. The Alpaca’s Fleece

The fleece of the alpaca is a little different to some other fibre production animals in that it does not possess a double-coated structure with a typical guard hair (very thick hairs) and under-wool (very fine hairs) structure as found in fibre production animals like Cashmere goats and is more like Merino sheep-wool which has a single-coated structure (Ryder and Stephenson 1968b; Galbraith 2010). The reason being that Merino sheep and alpacas have a single-coated structure is that there is not a great enough difference between the thickest primary fibres and the finest secondary fibres, creating a relatively uniform fleece without pronounced guard hairs, which the non-domesticated SACs, goats, wild-type sheep and other mammalian species possess (Sumner and Bigham 1993; Galbraith 2010).

There are two breeds of alpacas, the Huacaya and the Suri, differing only in the type of fleece that they produce. The Huacaya produces a crimped fleece
similar to sheep, whereas the Suri produces a straight fibre that forms ringlets and hangs from the body in a way similar to the Angora goat (Figure 2.8). The different fleece in the Huacaya and Suri is due to a difference in the α-helix structure of the α-keratin molecule, which is made up of long chains of amino acids, and results in the intertwining of many keratin molecules (Pearson 2008).

![Figure 2.8](image)

**Figure 2.8**  Huacaya (A) and Suri (B) alpacas and their fleece type. Alpaca images sourced from Kobler Alpacas (www.kobler.com.au) and fleece images sourced from Maryland Alpacas and Baarrooka Alpacas (Huacaya - www.marylandalpacas.org; Suri - www.baarrooka.com.au).

In the fibres of the Huacaya, the α-keratin molecule has a bilateral formation of ortho- and paracortical cell bundles, that gives the fleece its sheep-wool like crimp (Figure 2.9, Fraser *et al.* 1988). The Suri fibres do not have bilateral formation of cell bundles, and as a result have a less crimped and straighter fibre than the Huacaya (Antonini 2010). The external cuticle cells on the fibres of both Huacaya and Suri are scaled and protrude approximately 0.4 micrometres (microns; µm), in comparison to 0.8 µm in sheep-wool, from the shaft of the fibre. As such, the fibres have a serrated edge, but it is still softer to touch than sheep-wool due to the fibre curvature (Holt 2005; McGregor 2014).
As Huacaya alpacas constitute 90% of the world alpaca population, and are more commonly used in the Australian alpaca fibre industry, this thesis will focus on the Huacaya alpaca.

![Internal structure of a Huacaya fibre (Holt 2005).](image)

2.5.1. Development and structure of the follicles, fibre and fleece

Animal fibres are produced from follicles in the skin dermis, a process that begins during foetal development. Alpacas have an 11 month (~345 day) gestation period and, like lambs, the young (cria) are born with a fleece covering all of the body (San Martin et al. 1968). Follicle development, particularly the secondary follicles, continues for at least another four months after birth (Antonini et al. 2004). At 210 days of gestation, the foetus has developed hair on the lips, eyebrows and tail. By 240 days, foetal hair is present over the whole body, and at 270 days of gestation the fibre is 2 cm long over the body. At birth, the fibre length at the shoulder is around 4 cm (Walter Bravo and Varela 1993). The follicles are formed in the skin by pre-papilla cells that mass together at regularly spaced initiation sites in the skin. The initiation sites are predetermined based on a biochemical reaction within the epithelial cells (referred to as the reaction-diffusion system) that produce spatial patterns of
evenly distributed chemical components, forming the initiation sites for the primary and secondary follicles (Nagorcka 1995a, 1995b). The closer the initiation sites are clustered together, the higher the density of follicles (and fibres) that are produced. The fewer pre-papilla cells there are per follicle, the finer the fibre that is produced from that follicle as an adult in sheep (Figure 2.10, Moore et al. 1998). Although the number of pre-papilla cells per follicle is known to have a genetic basis and is strongly linked to high fibre density, there is currently no way to guarantee that an offspring of two densely fibred alpacas will also have fine and dense fibre.

![Diagram of follicle development](image)

**Figure 2.10** Development of the follicle by the pre-papilla cells in the papillary layer of the dermis. The dotted line encircles the pre-papilla cells massing together to form the basis of the follicle. Note that in the second illustration there are fewer pre-papilla cells in the primary follicle which results in a thinner fibre. Diagram as found in Moore et al (1998) with slight modification.

The follicles develop in several stages within the papillary layer of the skin, a layer that is rich in blood vessels and nerves. There are two types of follicle formed by pre-papilla cells in the developing foetus, namely, primary and secondary follicles. In Merino sheep, which have a gestation of 5 months, the
Follicles are produced in overlapping waves of development with primary follicles forming at around 60 days and the secondary follicles at around 85 days (Hardy and Lyne 1956; Moore et al. 1996). Follicle development in the alpaca follows the same pattern as the Merino, but due to the longer gestation in the alpaca the time frame of the overlapping follicle development waves is likely to be different (Walter Bravo and Varela 1993; Antonini et al. 2004).

Figure 2.11  Microscope photograph of a skin section taken from a Huacaya alpaca displaying two follicle bundles differentiating the primary and secondary follicles, the sebaceous gland and the sweat duct (Image by K. Moore).

Follicles are arranged in a follicle bundle or group which consists of a single primary follicle, surrounded by multiple secondary follicles (Figure 2.11). The fibres produced from the secondary fibres are finer than the primaries, as there are fewer pre-papilla cells per secondary follicle, given that they arise during the second developmental wave when most of the pre-papilla cells have already massed to the primary follicle initiation sites (Moore et al. 1998; Antonini et al. 2004). Whilst alpacas generally only have one primary follicle per follicle group,
other species can have multiple primaries per group (Ferguson et al. 2012). The ratio of secondary to primary follicles (S:P ratio) per follicle group is a good indicator of the total fibre density (and of the quality of the fleece) as the more secondary follicles there are per follicle group, generally, the higher the total follicle density, as found in Merino sheep (Adams and Cronjé 2003). Because the development of the secondary follicles continues after birth, follicle density may be variable in juvenile animals (Antonini et al. 2004), and any measurement of follicle density in alpacas is unreliable until the animal is fully grown in height and stature (~18 months of age for total follicle density) or more than 10 months of age (for S:P ratio).

The primary follicles have an associated sweat gland and arrector pili muscle that visually differentiates them from the secondary follicles (Figures 2.11 & 2.12, Ryder and Stephenson 1968b). The secondary follicles can also have an associated sebaceous gland, although in alpacas these glands are smaller and less numerous than the sebaceous glands associated with the secondary follicles in Merino sheep (Ferguson et al. 2012). Consequently alpacas do not produce as much sebum (lanolin and other waxes) from the sebaceous glands as sheep (Wuliji et al. 2000), with an average grease content of about 2% in alpaca fibre compared to 10-20% in Merino wool (Wang et al. 2003).
Figure 2.12  Illustration of the differences between the primary and secondary follicles and fibres with the lack of arrector pili muscle and sweat gland in the secondary follicle highlighted. It is also to be noted that the fibres produced from the derived secondary follicles are finer than either the original secondary follicle or the primary follicle. Original illustrations modified from Hardy and Lyne (1956).

Fibre is distributed over almost the entire body of the alpaca, with the face (cheeks and chin) and underbelly region (axillar, lower flanks and thighs) being less densely fleeced, and comprised more of lone thicker primary fibres than the finer secondary fibres (Wuliji et al. 2000; Gerken 2010). The underbelly region has thick fibres that are sparsely placed and lay flat to the skin (Atlee et al. 1997) and are not considered as part of the fleece. There is large variation in the fibre density and distribution on the alpaca’s head (cheeks and muzzle) and how much skin is directly exposed to solar radiation at all times (Figure 2.13). In sheep, face coverage is a heritable trait and can be selected for (or against) if desired (Terrill and Hazel 1946; Terrill 1949; Dun et al. 1964; Cockrem 1966; Cockrem and Rae 1966). In the alpaca it is presumed that face coverage is a heritable trait, but this has never been analysed.
Fibre diameter and density are important determinants of a fleece’s commercial value. In sheep-wool, fibre fineness is a measure of quality whilst density is a measure of quantity (Dunlop and McMahon 1974; Adelson et al. 2002). These two traits are known to be genetically correlated in many breeds of sheep and it has been observed that a denser fleece, with finer fibres, is a result of fewer pre-papilla cells per follicle bulb (Young and Chapman 1958; Hynd 1994; Moore et al. 1998; Adelson et al. 2002). It is the selective breeding for these two traits that produced the Super-fine Merino flock in Australia.

In alpacas, there is some indication that, as in Merino sheep, the fibre diameter and fibre density are correlated (Ferguson et al. 2012), given that these two
traits are selectively bred for, but there is still large variation within the national flock. The large variability in fibre diameter can be due to factors other than genetics, including altitude, nutrition, season, age, gender, sampling method and degree of medullation (Wuliji et al. 2000; Aylan-Parker and McGregor 2002; McGregor and Butler 2004; Lupton et al. 2006; McGregor 2006; Braga et al. 2007).

Medullation, the hollowing of the centre cortex of a fibre, is an undesirable trait for fibre production animals. Commercially, it causes the fibre to lose its crimp and become thicker (decreasing the quality of the fibre), and also can cause breakage during spinning (Pilkington and Purser 1958; Allain and Renieri 2010). Medullation is nearly always found in sheep-wool that is thicker than 30 µm in diameter, although the degree of medullation can vary greatly (Scobie et al. 1998). Medullation can be caused by genetic factors and the number of pre-papilla initiation sites, as well as nutrition as both a foetus and as an adult, and also time of season (Goot 1945; Ryder and Stephenson 1968a). In comparison to wool, alpaca fibres have a higher incidence of medullation, 50.2% in Huacaya alpaca compared to 0.2% in Merino sheep (Ferguson et al. 2012). Medullation is observed in both primary and secondary fibres (Wang et al. 2005; Allain and Renieri 2010). Moreover, the presence of the medulla and the exposed air-pockets within the fibre make commercially dyed wool appear paler than those without medullation due to internal light reflection in the hollow fibre (Chaudhuri and Bandyopadhyay 2009). Conversely, the medullation may contribute to the insulation and help to make alpaca fibre a better insulator than wool as air can be trapped inside the air pockets within the fibre (Calle-Escobar 1984; Wang et al. 2005).
2.5.3. Fleece length

In fibre production animals, fleece length is a controlled variable as the animals are shorn annually, usually in spring, for their fibre or wool as the commercial product. Alpaca fibre has a growth rate between 80-150 mm between annual shearings, with an Australian average length of 127 mm (Arnold et al. 1964; Lupton et al. 2006) and varies with nutrition (Russel and Redden 1997), gender and season (Wuliji et al. 2000). The fleece grows the most during summer, ~0.5 mg/cm²/day, with males producing 0.2 mg/cm²/day more than females (Wuliji et al. 2000). Although some studies have correlated fleece length/growth to other fibre characteristics (Wuliji et al. 2000; Frank et al. 2006), and proposed some suggestion of heritability (Ponzoni et al. 1999), there has been no specific study looking at the genetic influence on the growth of fibre in alpacas.

2.5.4. Colour

The colour in animal fibre and skin is a result of melanin pigmentation that forms in granules inside the cortical cells of the fibre or epidermal cells in the skin (Figure 2.14). There are two types of melanin produced by the melanocytes in the skin and hair fibre; eu-melanin, which is generally black or some derivative of black such as blue grey or very rarely chocolate brown, and pheo-melanin, which is tan and any brown that has a red shade to it (Hoffman 2006). Darker coloured alpacas have both a higher density of pigment granules in the cortical cells under the fibre cuticle and larger pigment granules than the lighter coloured alpacas (Wang et al. 2005). In addition, the cortical cells of the coloured fibres are more loosely bundled than they are in pure white fibres (Wang et al. 2005).
Figure 2.14  Longitudinal view from a brown Huacaya alpaca fibre displaying melanin pigment granules loosely bound within the cortical cells. (Wang et al. 2005)

Varying concentrations of melanin pigmentation in the fibre have resulted in 22 ‘official’ alpaca colours ranging from white to shades of brown to black (Hoffman 2006; Fan et al. 2010). Pure white alpacas have no melanin pigmentation in the fibre. Light-coloured alpacas are commercially farmed more often due to the versatility afforded by being able to colour dye the fleece, but there is also a market for the darker-coloured fleeces producing garments of natural fleece shades.

2.6. Fleece Insulation & Radiant Heat Load

Solar (visible, UV and infrared) radiation can impose a large radiant heat on an animal's body. Although mammals and birds evolved fibrous insulation to facilitate endothermy and reduce heat loss from the body to facilitate homeothermy, the layer of external insulation (fleece for alpacas) reduces the
exchange of heat (including pure absorption of solar radiation) between the animal's body and the ambient environment. Heat is transferred through the coat as thermal energy along a warm to cool gradient by convection, conduction and radiative transfer (Øritsland and Ronald 1978; Campbell et al. 1980). Alpaca fleece is anecdotally known to be a good insulator against heat loss in the cold, but there is no reference as to how good it is at insulating against radiant heat loads from the environment (given that they are from a cold climate), or how specific fleece characteristics will affect the insulating ability.

2.6.1. Colour

Coat colour is potentially important for determining the radiant heat load as to how much solar radiation is reflected from the animal and how much is absorbed by the fibres in the coat. Light-coloured coats are more reflective than dark-coloured coats as found when comparing white and black-fleeced sheep (Cena and Monteith 1975). As the lighter-coloured coats reflect more radiation, the darker-coloured coats absorb more radiation as the melanin pigmentation absorbs the radiation at the fibre tips before it can enter the coat (Cena and Monteith 1975; Walsberg et al. 1978; Burtt 1981; Walsberg 1983; Acharya et al. 1995).

Given that darker pigments absorb more radiant heat, an adaptation of dark skin with white fibres is often adopted by polar animals (Øritsland 1970; Cena and Monteith 1975). The radiation that strikes a coat element but is not reflected away by the white fibre tips is forward scattered towards the skin and the photon is absorbed closer to the skin, along with the radiant heat (Hutchinson et al. 1975; Walsberg et al. 1978). Black-skin with white-fleece is one of the three white-fleece colour genetic varieties identified in alpacas, called the Agouti
genotype (Munyard 2013). As alpacas are from a cooler climate we do not know if this is also a special adaptation (doubtful given that there are so many natural colour varieties), and if so, radiation may penetrate deeper into the fleece. In Australia, radiation penetrating deeper into the fleece may be detrimental thermal stress for an alpaca in an already hot environment.

2.6.2. Fleece structure

Fibre density is important in determining the insulation and radiative properties of the fleece or coat. Denser coats have been found to be proportionally more insulative than sparser coats (Figure 2.15, Scholander et al. 1950b). Additionally, cattle with denser coats are more heat tolerant than cattle with sparser fibres (Dowling 1955; Walker 1957) and a sheep fleece with denser fibres records less radiation penetration into the fleece than an otherwise similar fleece with sparser fibres (King and Millington 2010).
Figure 2.15  Insulation in relation to winter fur thickness in a series of arctic and tropical mammals. The insulation in tropical mammals is indicated by the shaded area. In the aquatic mammals (seal, beaver, polar bear) the measurements in 0°C air are connected by vertical broken lines with the same measurements taken in ice water (Scholander et al. 1950b). This figure has been adapted from the original version for clarity (Schmidt-Nielsen 2002)

The effect of fibre thickness on insulation and heat load has never been investigated in a single-coated species like the alpaca because most studies have been carried out on mammals with double-coats, that is coats that consist of guard hairs and fine under hair resulting in a range of fibre thickness. Therefore it has been difficult to distinguish fibre thickness as an individual effect. For example, rock squirrels, which have a mixture of fine undercoat and thicker guard hair have 76% of their total coat insulation attributed to the fine and densely-fibred layer (Walsberg 1988b). However, as previously discussed,
alpacas are considered to have a single-coated fleece, which means that the fibre thickness between the primary and secondary fibres are not significantly different (Gerken 2010).

It is rare that thicker-fibred coats have the same fibre density as fine-fibred coats (Young and Chapman 1958; Moore et al. 1998). Denser coats are more insulative than sparse coats when affected by high wind speed (Tregear 1965; Ames and Insley 1975), possibly because high fibre density, whether they be fine or thick fibres, make the coat more robust and prevent forced convection penetrating deep into the coat reducing the amount of insulative air between the fibres. Thicker fibres should also be less flexible than thin fibres and prevent flattening of the coat and subsequent reduction in insulation depth as wind speed increases (Moore et al. 2011). This needs to be investigated further and confirmed for alpaca fleeces.

2.6.3. Fleece length

Animals with longer coats can tolerate an environment which has a high solar heat load better than those with shorter coats (Parer 1963; Bennett and Hutchinson 1964; Christopherson and Young 1981; Gebremedhin 1987; Acharya et al. 1995; Heath et al. 2001). The further that solar radiation has to penetrate into the coat, the more insulated the air near the body is from radiant heat. Additionally, a longer fleece reduces the proportion of fleece insulation that is removed from the coat by wind than in shorter fleece (Bennett and Hutchinson 1964; McArthur and Monteith 1980), and the metabolic rates to maintain homeostasis are higher in short fleeced sheep at high wind speed indicating a greater heat loss with shorter fleeces (Joyce and Blaxter 1964). However, shearing (the physical removal of fleece length) does help to aid the
dissipation of heat by convection from the bodies of animals exposed to radiant heat in hot conditions, resulting in lower body temperatures of sheep and camelids than those with longer fleece (Macfarlane et al. 1958; Eyal 1963; Heath et al. 2001; Bulgarella and de Lamo 2005; Gerken 2010). Therefore there must be a balance between having a fleece long enough to prevent radiation from being absorbed at the skin level and short enough to be able to dissipate heat.

How short is too short though? If the fleece is cut too short for summer, then the ability to protect the body from radiant heat would be compromised (Eyal 1963; Parer 1963), and the animal may have to actively thermoregulate to maintain homeothermy (Hammel 1955; Cain III et al. 2006). In Merino sheep, wool that is 40 mm long has been found to be an adequate length to protect the body from the heating effects of radiation in summer, whilst still allowing for the dissipation of heat by convection (Parer 1963). Due to the differences in fleece structure between alpacas and sheep, we are not able to extrapolate an ideal insulating fleece length in summer from sheep data, and therefore it is not known what the idea fleece length would be for alpacas to insulate the body from a high solar heat load when the ambient temperature is high and prevent heat stress.

2.7. Evaporative Heat Loss in Camelids

When radiant heat raises the body temperature, animals must use mechanisms to cool themselves. Heat can be lost from the body by evaporation from either the respiratory tract by panting or from the skin surface via sweating from sweat glands in the skin (Christopherson and Young 1981). As sweat glands are generally associated with primary follicles, they are found all over the body. In
llamas, sweat glands are numerous on the underbelly, muzzle, inside of the ears and on the footpads; although the footpads have specialised glands not associated with hair fibres (Atlee et al. 1997). The hair fibres aid in the dissipation of heat as sweat as the moisture transits from the sweat duct through the fibre shaft and up to the skin surface (Allen and Bligh 1969). Many mammal species also produce a waxy sebum from the sebaceous glands that is combined with the sweat when it reaches the skin surface, however alpacas produce very little sebum and it would not have any effect on the efficiency to sweat (Jenkinson 1973; Fowler 1998). Once the sweat is on the skin surface, heat energy from the skin transforms it from a liquid to a gas and in that transformation heat is removed from the skin (Gavin 2003). The fur or fleece coat of many mammals limits the effectiveness of sweating to dissipate heat, as the coat prevents the evaporated sweat from being removed by convention. Many mammals therefore developed panting to dissipate heat (Hofmeyr 1985; de Lamo et al. 2001; Jessen 2001c), but camelids are not known to pant when heat stressed (Rosenmann and Morrison 1963; de Lamo et al. 2001).

Camels, llamas and guanacos actively sweat in response to increased radiant heat load. They have a lower sweating rate than some other mammals, such as horses (Dowling and Nay 1962; Allen and Bligh 1969; Atlee et al. 1997; de Lamo et al. 2001). Histological studies have not found any indication (from a lack of specific chemicals during cell staining) that sweat glands in alpacas are actively used for thermoregulation (Montalvo and Cevallos 1973), but this is not definitive evidence that alpacas do not sweat for heat dissipation, and as other camelids, sheep and goats sweat to dissipate heat, it is highly likely that alpaca’s sweat glands also have an active role in thermoregulation (Robertshaw 1968; Fowler 1994; Fleis and Scott 2010; Gerken 2010). Because fleece can
hinder sweat evaporation, camelids, like some other mammalian species, have a thermal window region that is less well fleeced and has well developed sweat glands (Fowler 1994; Atlee et al. 1997).

The thermal window is a relatively fibreless area on the abdomen and the inside of the thighs of SACs where the skin is thin, well vascularised and the sweat glands are well developed in size and fully functional (Figure 2.16, Fowler 1994; Atlee et al. 1997). In guanacos, the thermal window region represents about 20% of the total body surface, but that area has the highest heat loss of the whole body (Figure 2.17, Morrison 1966; Bulgarella and de Lamo 2005). The skin of the thermal window is thin so the blood vessels are close to the skin surface and vasomotor action (vasoconstriction or vasodilation) allows the animal to control the amount of heat delivered to the skin (McArthur 1981). In extreme heat, SACs are known to wallow in water (exposing the thermal window directly to water), which is an effective cooling technique as it allows for direct convection and convection to the water (Figure 2.18, Fowler 1998; de Lamo et al. 2001; Jessen 2001a).

Figure 2.16 Underbelly of a female alpaca displaying the sparsely haired inside of the thighs and lower belly (Photo K.Moore).
Figure 2.17  Mean conductance (heat loss) for the different topographic areas on the guanaco. Lower flank and axillar (thermal windows) are significantly different from the other areas (P<0.05). ● = mean; □ = ±SE; l = ±SD. (Bulgarella and de Lamo 2005)

Figure 2.18  Example of alpacas wallowing in water (Photo sourced from http://www.kysheepdreams.com/).
2.8. Vitamin D

Another aspect of physiology that is impacted by the fleece is the vitamin D levels of alpacas, as the primary source of this crucial hormone is due to a photochemical reaction with UV radiation in the skin. Currently the average alpaca has insufficient levels of vitamin D during winter in Australia (Judson et al. 2008). Vitamin D is a steroid hormone that is formed in the skin (as the D$_3$ form) through a chain of reactions starting with a photochemical reaction between UVB radiation (290-320 nm of the UV spectrum) and a sterol precursor (7-dehydrocholesterol). The sterol precursor is produced in the sebaceous glands in the skin (Gaylor and Sault 1964; Gropper et al. 2008), and under UVB induction produces a pre-vitamin D$_3$ (Figure 2.19). From here the pre-vitamin D$_3$ undergoes a heat-induced isomerisation to vitamin D$_3$, which is then absorbed into the bloodstream, or photo converted to lumisterol and tachysterol (Holick 1987). The latter pathway is an inbuilt mechanism so that the body doesn’t make too much vitamin D$_3$ and prevents vitamin D toxicity as additional sterols are produced that remain in the skin and are sloughed off when the skin cells die (Webb and Holick 1988; Dittmer and Thompson 2011). There is also a secondary form of vitamin D (D$_2$) that is synthesised in plants and is actively available to animals when it is ingested after the plant has been dried and sun-cured, but it contributes only a very small portion of the total vitamin D requirements of alpacas (Judson et al. 2008; Dittmer and Thompson 2011).
Figure 2.19  Production pathway of vitamin D$_3$ in the dermis. Pre-vitamin D$_3$ (produced from a UVB radiation reaction with 7-dehydrocholesterol) undergoes heat-induced isomerisation to vitamin D$_3$ but can also be photoconverted back to 7-dehydrocholesterol or to lumisterol or tachysterol. In turn lumisterol and tachysterol can be converted back to pre-vitamin D$_3$ in the absence of photons (aka darkness). (Norman 1998)

Vitamin D is important to mammals as it contributes to the balance of calcium and phosphorus in the body that is crucial for bone mineralisation and development as well as neuromuscular function (Webb and Holick 1988; Garner-Paulin 2005). A deficiency in calcium and phosphorus can cause health problems such as rickets (weakening and underdevelopment of bones) in young animals and osteomalacia (weakening of the bones) in mature animals (Figure 2.20, Hill et al. 1994; Garner-Paulin 2005; Dittmer and Thompson 2011). In Australian and New Zealand a total (D$_2$ and D$_3$) vitamin D deficiency in alpacas occurs when blood plasma concentrations are below than 50 nmol/L in adults or 20 nmol/L in the young (Hill et al. 1994; Judson and Feakes 1999; Judson et al.)
Adult alpacas with plasma levels below 15 nmol/L are critically at risk of developing osteomalacia, levels 50-80 nmol/L are counted as low to insufficient, and levels 100-250 nmol/L are considered normal (Van Saun et al. 1996). Most alpacas in Australia receive an annual vitamin D supplement during winter (Judson and Feakes 1999).

Figure 2.20 Young alpaca exhibiting signs of rickets with badly bowed legs, a permanent deformity (Photo S. Donahoe, 2013; http://www.openherd.com/articles/725/long-acres-alpaca-farm-vitamin-d-and-alpacas)

Alpacas become vitamin D deficient in Australia probably because they evolved in, and adapted to, a very UV intense environment. Australia is at a much lower altitude and further from the equator than the region of the altiplano where alpacas are found and the UV intensity is much lower, see section 2.2 for values (Dittmer and Thompson 2011). The UV intensity in Australia is also
seasonal (due to the latitude), with the lowest levels in winter. Vitamin D deficiency correspondently occurs during winter (Judson et al. 2008). Winter is also the time of year when the fleece is longest and long fleece probably prevents UVB radiation from reaching the majority of the alpaca’s skin for the synthesis of vitamin D₃.

Another fleece characteristic which affects the synthesis of vitamin D₃ is the colour of the fleece and skin. As the UV intensity is high in the Andes, alpacas developed a wide range of fleece colours without a risk of vitamin D deficiency. The melanin pigmentation in fleece and skin absorbs the UV radiation, outcompeting the 7-dehydrocholesterol in the skin cells for the UVB radiation (Webb and Holick 1988). In areas of high UV intensity exposure, the level of pigmentation does not limit vitamin D₃ synthesis in humans (Holick et al. 1981), but in Australia alpacas with darker-coloured coats are at a higher risk of vitamin D deficiency during winter (Judson et al. 2008). Although the fleece length and colour affect the level of vitamin D in alpacas, it is not known if the fleece structure affects the penetration of UVB radiation to the skin. As the fleece structure characteristics (fibre diameter and fibre density) are the main focus of breeding selection of alpacas in Australia, it would be interesting to see if that selection helped or hindered vitamin D deficiency of alpacas during winter.

2.9. Conclusion

Alpacas have been introduced in Australia 27 years ago and into a climate that is very different to their original habitat. Breeding selection for a high quality and quantity fleece may affect how well an alpaca is able to successfully cope with
the environment, and in particular insulated from solar radiant heat. If the insulative ability is lowered, then alpacas may become heat stressed during summer but may be able to naturally produce higher levels of vitamin D in winter. To what extent selection criteria for breeding objectives (breeding selection) affects the insulating layer is unknown. In this doctoral research, I aimed to test how the fleece characteristics of fibre density, diameter, length and colour impact on the insulating ability of the fleece and the radiant heat load it acquired from solar radiation, the ability to dissipate heat via the evaporation of sweat, and its consequential effect on vitamin D synthesis. The results of this work should help to identify if specific breeding selections are detrimental or beneficial to the physiological health of alpacas in southern Australia.
PART A: FLEECE CHARACTERISTICS AND HEAT STRESS
3.1. Introduction

Alpacas (Vicugna pacos) originated on the altiplano plateaus of the Andes mountain range in South America. The area is a dry semi-arid environment characterised by seasonally low average temperatures of 0 to 20°C in summer and -11 to 13°C in winter (San Martin and Bryant 1989). Evolving in this environment it might be expected that the alpaca has not been exposed to selection pressure for an ability to deal with heat stress (Hoffman 2006). Although very adaptable animals, the exposure of alpacas to a warmer climate may stretch their heat loss abilities because heat loss can be limited in fleeced animals (Hofmeyr 1985; de Lamo et al. 2001). Anecdotally, the lack of adaptations to hot conditions does not seem to be an issue for alpacas in Australia, as there are few records of alpacas presenting heat stress symptoms. The Australian alpaca fibre industry was established as an alternative to sheep-wool, due to the light, soft and warm wool-like fibre produced by alpacas (McGregor 2006). In Australia, alpacas are most commonly farmed in southern regions and are exposed to a climate with higher ambient temperatures in summer than those of the altiplano. For example around Perth, South Western
Australia (31.95° S, 115.86° E), the average summer temperature (mean minimum and mean maximum) ranges from 18 to 31°C, and in Bendigo, central Victoria (36.75° S, 144.27° E), 13 to 29°C (Australian Bureau of Meteorology). The average maximum temperatures in Australia are thus higher than those experienced on the altiplano. Because temperatures in southern Australia can induce heat stress in sheep and goats (McGregor 1985; Stockman 2006) it is therefore very likely that alpacas will need to sweat to maintain heat balance, given that they rarely pant for heat dissipation.

In fleeced animals, the fleece originates from two types of fibre follicle, the primary and the secondary follicles. The follicles are differentiated by the order of their formation in the skin during foetal development and by the glands associated with the follicles (Moore et al. 1998). Follicles are arranged in the skin as follicle bundles, with many secondaries clustered around a single primary. The fibre produced by a primary follicle is typically larger in diameter, and thereby lower in commercial fibre quality, than the fibre produced by the secondaries (Hardy and Lyne 1956; Moore et al. 1996). Associated with the primary follicle, and located in the epidermis alongside the primary follicle, are sweat glands. These glands produce a fluid, derived from the extra cellular fluid that is excreted onto the skin surface. When the fluid evaporates, cooling of the skin occurs (Atlee et al. 1997). Camelids, like many other mammalian species, have thermal windows on the underbelly and inside of the thighs, where the fleece is sparse, and composed of more lone thicker primary follicles, and evaporation is enhanced by greater air movement near the skin (Fowler 1994; Atlee et al. 1997; de Lamo et al. 2001). Although there are fewer follicles and fibres in this region, the follicles that are present are nearly all primary follicles and the sweat glands associated with those follicles are larger and more
developed than those elsewhere on the body (Fowler 1994; Atlee et al. 1997). Although the sweat glands are larger and more developed in the thermal window region, the sweat glands on the rest of the body are also involved in thermoregulation (Atlee et al. 1997).

Studies on the skin morphology and thermoregulation of other camelids, including camels, llamas and guanacos, show that the sweat glands over the entire body are active secretory cells. Furthermore these species all sweat in response to high temperatures although the sweating capacity is lower than it is in some other species, such as horses (Dowling and Nay 1962; Allen and Bligh 1969; Atlee et al. 1997; Fowler 1998; de Lamo et al. 2001). Sweating has never been measured or reported in alpacas. It is highly likely that alpacas sweat in response to heat stress. Sheep and goats, which are known to pant in preference to sweating for evaporative cooling, have functional sweat glands and do sweat when induced (Robertshaw 1968; Fowler 1994; Gerken 2010). Some histological studies have suggested that alpacas might not use their sweat glands as secretory cells for thermoregulation (Montalvo and Cevallos 1973; Fleis and Scott 2010), but the conclusion was based on limited data and since alpacas are rarely observed to pant, it is logical to assume that alpacas use sweating for heat exchange like other camelids.

The mean fibre diameter of the national flock of alpacas in Australia is very variable, ranging from 17.7 to 46.4 µm (McGregor and Butler 2004), with a minimum fibre diameter and range similar to that reported in Peruvian Huacaya alpacas (Montes et al. 2008). In comparison, Merino sheep have been bred to have fibre diameter ranging between 17.5 to 23.0 µm (Adams and Cronjé 2003). Since the introduction of the alpaca industry in 1987, breeding to
improve the quality (fibre diameter) and quantity (fibre density) of fleece produced in Australia has been directed towards decreasing the diameter of the primary and secondary fibres produced from the follicles and increasing the number of fibre follicles produced in the skin. Such selection may possibly reduce the number of primary follicles, and the associated sweat glands per unit area. The aim of this study was to test the hypothesis that alpacas with finer fibre would have fewer primary follicles and thereby sweat glands, than alpacas with thicker fibres. A decrease in sweat gland density would mean that breeding for fibre fineness may potentially reduce the alpaca’s ability to dissipate heat evaporatively in the hotter environments where they are found in Australia.

3.2. Materials and Methods

3.2.1. Experimental design

Skin biopsy samples were taken from mature Huacaya alpacas and sections were made transverse to the plane of the fibre follicles. The primary and secondary follicle density, the sweat gland duct density and the secondary to primary follicle ratio were determined. The fibre diameter of each animal was analysed from a fibre sample taken from a mid-side patch.

3.2.2. Biopsy samples

All animals sampled for this study were adult Huacaya alpacas, mean age 4.1±0.6 years old, from Banksia Park Alpaca Stud, Serpentine, Western Australia. Four dark-coloured and 29 light-coloured animals (2 male, 31 female) were used and skin biopsy samples were collected over a five year period. All surgical work was carried out by an experienced veterinarian and the samples
were used as per permission granted from the University of Western Australia’s Animal Ethics Notification of Use of Animal Tissues approval RA/3/500/003.

3.2.3. Sampling procedure

Alpacas were sedated by intra-muscular injection of a combination of 1 mL of ketamine (100 mg/mL) and 0.6 mL of xylazine (100 mg/mL), and laid out in lateral recumbency with a neck-pillow supporting the head positioned to prevent choking on rumen reflux, as standard practice by the veterinarian who obtained the skin biopsies (a practice similarly used in equine anaesthesiology hydro-pools) (Auckburally and Flaherty 2009). A 10 x 10 cm mid-side patch of fleece was clipped, level with the last rib and about 20 cm ventrally from the spine. The fibre sample was bagged for analysis and the area was washed with a diluted (20 mL per 1 L water) chlorhexidine skin scrub (Chlorhex-C, 50 mg/mL chlorhexidine gluconate, Jurox, NSW, Australia). Either an 8 mm or a 10 mm biopsy skin punch was used to remove a mid-side skin sample, which was placed in a labelled tube filled three quarters with formaldehyde fixative (10% neutral buffered formalin). The biopsy site was sutured closed with a half curved cutting needle using 4.0 chromic catgut. Alpacas were then monitored until they reached full consciousness and were able to stand unaided.

3.2.4. Microscopy protocol and analysis

The biopsy samples were fixed in a formaldehyde solution for a period ranging from eight months to five years. The samples were rinsed in normal saline (0.9% NaCl) to remove the formaldehyde and the remaining wool stubble was trimmed with a scalpel blade before the sample was placed in 70% ethanol overnight to be processed through a Leica ASP200 Tissue Processor the next day, which involved ten stations; five stations of three concentrations of ethanol
(in the order of 70%, 90%, 100%, 100% and 100%), two stations of toluene and
three stations of molten paraffin wax which lasted from 30 minutes to 2 hours
depending on the station and solution. On removal, the samples from the
Tissue Processor were placed in a tray of 60°C molten paraffin wax awaiting
transverse embedding into block moulds. Wax blocks containing the skin
samples were then trimmed, and 7 μm transverse sections were taken at the
mid-sebaceous gland level using a Leica RM2255 microtome. The sections
were floated in lukewarm water onto slides and dried at 40°C overnight in a
drying oven. The sections were then stained using a Haematoxylin, Eosin and
Picric Acid method, modified slightly from Maddocks and Jackson (1988) and a
coverslip was secured using DPX mountant (Biolab Ltd, New Zealand; supplied
by Thermo-Fisher, Australia). The method was modified by using toluene,
instead of xylol, for the dewaxing and clearing before mounting stages, and an
ethanol wash was included between rinsing in the alkaline blueing solution and
the eosin staining stages. Skin shrinkage was taken into account prior to
quantification.

The follicle density and the sweat duct density were determined on
microphotographs taken with a digital camera (Pentax OptioWP) mounted onto
an Olympus CHS microscope (Olympus Optical Co., LTD, Japan) with a 10x
eyepiece, 4x magnification and 3x optical camera zoom (total magnification
equals 120x). Four to six sections were photographed per skin sample. The skin
section area that was photographed was measured using a stage micrometer to
determine a known area for the calculation of follicles per mm$^2$. The number of
follicles and sweat ducts was then counted over the photo area. The primary
follicles were differentiated from secondary follicles by the presence of the
sweat duct and bi-lobed sebaceous glands (Antonini et al. 2004; Ferguson et al.)
Total, primary and secondary follicles and the number of sweat ducts were each quantified. The secondary to primary follicle ratio was determined by counting at least eight follicle bundles, consisting of at least 100 follicles per slide, following methodology used by Ferguson et al. (2012).

### 3.2.5. Fibre analysis

The fibre samples taken at the time of skin sampling were analysed using an OFDA 2000 optical-based fibre diameter analyser, (BSC Electronics, Perth, Western Australia) by the MicronMan (Bibra Lake, Western Australia), which provided data on the mean fibre diameter at the time of skin sampling. The cross-sectional area (CSA) of the fleece, dividing the fleece into proportions of fibre and air space between the fibres for a given skin area, was calculated using the mean fibre diameter in μm (MFD) and total follicle density in follicles/mm² (TFD; Equation 1). As the samples were taken at random times, the time since shearing was not consistent and so the total volume of the fleece using the fleece length could not be calculated. Therefore the CSA provides a reasonable indication of how much of the fleece volume is fibre (Henderson and Hayman 1960).

**Equation 1:**

\[
CSA_{fibre}(\%) = \left( \pi \times \left( \frac{MFD}{2000} \right)^2 \times TFD \right) \times 100
\]

### 3.2.6. Statistical analysis

Linear regression analysis (GenStat v.14) was used to analyse the relationships between total follicle density, primary follicle density, secondary follicle density, sweat duct density, secondary to primary follicle ratio (S:P ratio), mean fibre diameter and the cross-sectional area.
3.3. Results

The follicle bundles from the alpacas in this study consisted of one primary (P) follicle surrounded by secondary (S) follicles, as previously described (Figure 3.1, Antonini et al. 2004; Ferguson et al. 2012). The number of S follicles associated with a P follicle ranged from 4 to 20. In four of the very low follicle density animals, there were some sections that had a lone P follicle that lacked associated S follicles. But these were rare and were not included in the S:P follicle ratio counts. Most (95%) of the P follicles had a sweat gland duct visible and there were less than four sebaceous glands visible per follicle bundle, usually only associated with the P follicle (Figure 3.2). The denser fleeced animals (Figure 3.2A) had smaller follicles than those more sparsely fleeced animals (Figure 3.2B).

Figure 3.1 Photomicrograph of a skin section taken from a Huacaya alpaca showing two follicle bundles (outlined in red) with the primary and secondary follicles, the sebaceous gland, and the sweat gland duct.
Figure 3.2 Photomicrographs of the skin from two alpacas shown at the same magnification. One alpaca with a fine and dense fleece (A) and the other with a thick and sparse fleece (B). Total follicle density (TFD), mean fibre diameter (MFD) and secondary to primary follicle ratio (S:P) of both animals are indicated underneath.

There was a strong association between the MFD and the TFD, with the animals that had a lower MFD being more densely fleeced than animals with higher MFD (Figure 3.3A; r=-0.8; P<0.001). The alpacas with finer fibre also had a higher density of both S and P follicles, and sweat gland ducts, than animals with thicker fibres (Figure 3.3B, C and D; P<0.001 for all). The slope of the relationship between MFD and TFD was steeper for the S follicles than for the P follicles, meaning that the S:P ratio was also higher in animals with finer fibre (Figure 3.3E; P<0.001).
Figure 3.3  Linear regressions showing the correlations between total follicle density (A), secondary follicle density (B), primary follicle density (C), sweat duct density (D) and the S:P ratio (E) when compared to the mean fibre diameter of the alpacas (n=33).
The S follicle density was nearly perfectly correlated with the TFD; alpacas with a higher follicle density had more S follicles (Figure 3.4A; r=0.998; P<0.001). The P follicle density, sweat gland duct density and the S:P ratio were also higher in animals with a higher follicle density than in animals with sparser follicles (Figure 3.4B, C and D; P<0.001 for all). The CSA of the fleece that was fibre was not higher for alpacas with a higher TFD (Figure 3.4E; r=0.12; P=0.52). There was a significant positive relationship between the sweat gland duct density and the P follicle density, as expected, indicating that there was one sweat gland duct per P follicle (Figure 3.4F; r=0.87; P<0.001).
Figure 3.4  Linear regressions showing the correlations between secondary follicle density (A), primary follicle density (B), sweat duct density (C), the S:P ratio (D) and the cross-sectional area of the fleece as fibres (E) when compared to the total follicle density (n=33). Linear regression showing the correlation between sweat gland duct density and the primary follicle density (n=33).
3.4. Discussion

The hypothesis that alpacas possessing a fleece with finer fibre would have fewer sweat glands, over a given area, than animals with thicker fibres was rejected. The number of sweat gland ducts was positively correlated with the number of primary follicles, and while the secondary to primary follicle (S:P) ratio was higher in alpacas with finer fleeces, the increase in total follicle density in the finer fibred animals more than outweighed the increased S:P ratio (including those samples with lone primary follicles). The result was that alpacas with finer fleece had more sweat gland ducts per unit area of skin than alpacas with thicker fibres. The total follicle density of the animals in this study was higher than has been reported in the literature (Antonini et al. 2004; Ferguson et al. 2012). These findings indicate that the potential sweating ability of alpacas should not be hindered by increased fibre density.

In the animals with high follicle density, not only was there a greater abundance of secondary follicles per primary follicle, but there were more primary follicles per given area, than in the animals with low follicle density. Because the increase in density was due predominantly to an increase in the abundance of secondary follicles, the fibre of those animals was finer than in those alpacas with sparser fleeces. Similar results have been reported in sheep (Moore et al. 1996; Purvis and Swan 1997; Adams and Cronjé 2003), where the relationship between follicle density and fibre diameter is explained by the pre-papilla cell theory.

Pre-papilla cells form the basis of all hair or wool fibre follicles in animal skin. It is suggested that each animal has a programmed number of pre-papilla cells that mass at the predetermined initiation sites and are available for follicle
formation approximately equidistant from each other during foetal development due to biochemical reactions with the epithelial cells (Nagorcka 1995a, 1995b; Moore et al. 1998). The pre-papilla cell theory states that as follicle density increases the number of pre-papilla cells available to form each follicle decreases, whether it be a primary or secondary follicle, and the fibre that is produced from that follicle becomes finer (Moore et al. 1998). The pre-papilla cell theory provides a basis for producers aiming to improve the quality (finer fibre diameter) and quantity (fibre density) of fleece produced by their alpacas. Therefore the main focus of further developing the quality and quantity of fibre produced within the industry can be towards increasing the follicle density, in that it promotes finer fibre, without reducing the number of primary follicles. In fact, the interpretation data suggests that such selection increases primary follicle density as well.

Alpacas that had high follicle density also had both more primary follicles and more sweat gland ducts per unit area. The ratio of primary follicles to sweat gland ducts found in this study was close to one, which is consistent with current literature for the skin histology of South American camelids (Atlee et al. 1997; de Lamo et al. 2001; Ferguson et al. 2012). What has not been determined in camelids however, is whether a higher follicle density affects the size of the sweat glands present. In Merino sheep the wool follicles are packed tightly together due to the high density of follicles, resulting in the sebaceous glands that are slender and oblong (Nay 1966). If the same applies to sweat glands, then it is likely that the sweat glands in the skin of animals with a high follicle density will be smaller (Moore et al. 1998). But this does not necessarily mean that reducing the size of the sweat glands will reduce the sweating capacity of the animal. Egyptian cattle have six times the number of sweat
glands (although smaller in size) over a given area than Egyptian buffalo, although the cattle have finer, denser fibres than the buffalo (Hafez et al. 1955). Therefore increasing follicle density and theoretically reducing the size of sweat glands should not reduce the sweat output per mm$^2$ of skin and be detrimental to the ability of the alpacas to dissipate heat as sweat, presuming that the glands produce sweat to their full capacity.

In the present study the total follicle density of the denser fleeced alpacas was higher than values that have previously been reported for alpacas (Antonini et al. 2004; Ferguson et al. 2012). That improvement may be due to genetic selection, but the follicle density in Merino lambs can be increased by increasing the energy intake of the ewe during late pregnancy, or of the lamb up to 18 weeks after birth (Short 1955; Kelly et al. 2006). The mechanism seems to involve the production and survival of all potential secondary follicles (Schinckel 1955; Kelly et al. 2006). Primary follicles, on the other hand, are not altered by nutrition and appear to be genetically programmed, setting a limit to the number of follicle groups that an animal has (Corbett 1979). Since 90% of the animals used in the present study were stud animals (animals specifically selected for breeding purposes due to their genetic potential), it is highly likely that their mothers were intensively managed during pregnancy and that additional nutrition was supplied during late pregnancy and lactation. Thus it is possible that the fleece characteristics we observed were the result of good management and not necessarily a reflection of improvement in the gene pool. However, if the primary follicle density is genetically determined in alpacas, as it is in sheep, then the higher primary follicle density in those alpacas with finer and denser fleeces suggests that genetic improvement has indeed occurred. That my values are higher than previously reported could also reflect the
genetics and/or nutrition of the animals previously tested (or by human error in the processing of the samples). The alpacas in previous studies were probably not intensively selected or managed for fleece properties (Antonini et al. 2004; Ferguson et al. 2012). If alpaca producers could identify when, during late pregnancy, nutritional manipulation is important, and which feed supplement to use, then secondary follicle density could be increased as observed in sheep. Supplementary feeding during pregnancy across a whole breeding flock would also help identify the offspring with the highest genetic potential. Then, stud breeders across the industry could fast track their selective breeding programs to improve the quality and quantity of fleece produced.

It is not known how much alpaca fleece acts as a barrier to evaporative heat loss. It has been reported that in sheep and goats sweat is not easily dispersed from the skin through the fleece (Robertshaw 1968); but in llamas it is reported that the fibre produced by the thicker primary follicles may act as a wick at the skin level, drawing the moisture produced by sweat glands up along the fibre, dispersing sweat through the thick coat for evaporative heat exchange (Atlee et al. 1997). It is unknown if a reduction in the size of the primary fibre, as would be the case in my alpacas given the increase in follicle density, might limit the effectiveness of moisture loss due to the ‘wick effect’. The length of the fleece would also be a major influence on how well moisture is dispersed from the skin, and the distance at which the wick effect is effective.

Sweating through the fleece would be a secondary resort of evaporative heat loss when the upper critical temperature is exceeded, given that alpacas have a thermal window region on the underbelly and flanks for both evaporative and passive heat exchange (Fowler 1998; Gerken 2010). The sweating ability of the
glands would also be determined by the size, shape and functionality of the individual glands (Atlee et al. 1997). Alpacas might only be required to sweat through the fleece if exposed to high environmental temperatures beyond the upper critical temperature, as suggested by the interpretation of the data. The cross-sectional area data indicate that although the fibre yield was higher for the fine and dense fleeces than the thick and sparse, the volume of fleece per unit area of skin was not different between the fleece types, as the thicker fibres take up the same amount of space as several finer fibres. Therefore the air to fibre volume ratio of the fleece was the same and the 'barrier' effect of the fleece on sweat evaporation and water vapour dispersal might be similar between the fleece types, dependent of course on the length of the fleece. In turn, the length of the fleece affects the amount of sweat produced by sheep exposed to sun, with lower sweat rate in sheep with longer fleece (Hofmeyr et al. 1969), likely because the increase in the insulative ability of longer fleece reduces the radiant heat gain (Parer 1963). As camelids are not known to pant for evaporative heat loss purposes (Rosenmann and Morrison 1963; de Lamo et al. 2001), a more comprehensive study is needed on the effect of the fleece as a barrier for evaporation. Because a large proportion of an animals' heat load can be due to direct solar radiation, it also would be useful to know how the fleece characteristics (fibre density, fibre diameter and fleece length) affect the ability of the fleece to insulate against radiant heat, thereby potentially reducing the need for sweating.
3.5. Conclusion

The secondary to primary follicle ratio has been found to indeed be higher in individuals that produce finer fibre, but that the more favourable ratio did not come at the expense of primary follicle density. Instead it was found that as the secondary to primary follicle density increased, so did the total follicle density, and as a result the primary follicle density increased. The result was that individuals that produce fine fibre had a higher sweat gland density than those individuals that produce thicker fibres. Increasing follicle density should not hinder potential sweating ability, assuming that the functionality of the glands is consistent, over the whole body surface.
4.1. Introduction

In the previous chapter, it was reported that alpacas with higher fibre density (and finer fibres) do not have fewer sweat glands over a given area than alpacas with lower fibre density (and thicker) fibres, indicating that sweating potential, assuming that functionality of all the sweat glands remains consistent, for heat exchange (if heat stressed) would not be limited for alpacas with all types of fleece structure. However, if the fleece of the alpaca is able to competently insulate against radiant heat then the need to sweat for heat dissipation would only be relied upon in circumstances when the environmental temperature exceeds that of the alpacas' upper critical temperature.

Heat exchange between an animal and the environment is complex. When heat gains equal heat loss, an animal is in thermal balance. There are four main forms of heat transfer between animals and their environment; conduction, convection, evaporation and radiative transfer (Øritsland and Ronald 1978; Campbell et al. 1980). Conductive heat is exchanged between the animal and the environment by direct physical contact with another surface or medium; this can be the ground, another animal, a plant, water or the air that is stationary around the animal. Heat exchanged with moving air or water is forced convective heat exchange. Evaporative heat loss is the latent heat lost when
water changes phase from a liquid to a gas on the surface of an animal, either as insensible evaporation, sweat from the general body surface, or via panting from the respiratory tract (Figure 4.1).

**Figure 4.1** Forms of heat exchange for a fleeced mammal. Solid lines represent heat gain and dotted lines represent heat loss. Alpaca image taken from Australian Alpaca Fleece Limited (www.aafl.com.au)

In terms of heat gain, a radiant heat load is imposed on animals from two sources. First (and most importantly) direct incident radiation from the sun can either be absorbed by the animal or reflected. Second, as thermal radiation, from objects surrounding the animal, such as the ground or trees (Figure 4.1).
The animal itself will also emit radiant heat, as an animals' surface body temperature is always going to be higher than absolute zero. Although external insulation (fur coat or fleece) on many mammals evolved initially to reduce heat loss from the body and maintain homeothermy, it also secondary acts to retard radiant heat gain. The characteristics of the coat determine to the amount of reflected or absorbed solar radiation from the animal, and the amount of heat lost by convection (Macfarlane 1968; Christopherson and Young 1981). Of the radiation that is not reflected back to the environment, some will be absorbed at the coat surface and some will penetrate and be absorbed further into the coat (Hutchinson and Brown 1969). When radiation is absorbed by the fibres of the coat, it becomes heat energy. The deeper into the coat that the radiation is absorbed, the closer the radiation is to the skin and the greater the heat load is on the animals body (Walsberg et al. 1978; Walsberg 1988a).

The total insulation a fleece or fur coat is composed of two layers, the fleece or fur layer ($I_{\text{fleece}}$) itself and the air boundary layer (ABL; $I_{\text{ABL}}$), which is a thin layer of still air at the interface of the fleece surface and the ambient environment (Figure 4.2). Together these two layers "trap" air, the fleece between the fibres of the coat and the ABL above the coat. The trapped layer of unstirred air between the fibres serves as the insulation of the fleece, as the air is the key ingredient in that it is less conductive that the coat fibres (Tregear 1965). Through the air within the coat there is a gradient of temperatures, ranging from near body temperature at the skin surface to near ambient temperature at the fleece surface. The animal does not need to use any additional energy to warm or cool itself as long as the body temperature does not fall or rise above the animals lower and upper critical temperatures (Christopherson and Young 1986). Increased convection, or wind speed, decreases the insulation of the
fleece by removing the air boundary layer of still air, and disturbing the air trapped between the fibres (Jessen 2001b). In summer this can benefit the animal, as it can promote the removal of heat from within the fleece if the body temperature increases, but it also decreases the insulation layer that prevents radiant heat from penetrating to the skin.

The depth that radiation penetrates into the fleece is termed the level $Z$ or $I_z$ (Figure 4.2). The deeper that $I_z$ extends into the fleece, the closer radiant heat is to the skin level and reduces the layer of insulation between the absorbed radiation and the skin, potentially causing heat stress on the body. The integrity of the insulation layers of the fleece determines how deep radiation can penetrate into the fleece ($I_{fleece} - I_z$), and the radiant heat load that may be imposed on the animal. The integrity of the insulation layers is determined by the fleece characteristics (length, colour, fibre density and fibre diameter). For the purpose of this thesis, the insulation will be only discussed as the total insulation ($I_{total}$), and not the individual fleece and ABL layers. While the value of the individual layers is understood, it is the total insulation that is more important for the concepts discussed here. These layers (fleece and ABL) have been discussed above only to provide foundations for the concepts of radiation penetration ($I_z$) and radiant heat load.
Figure 4.2  Schematic of insulation components of the fleece. $I_{\text{total}} =$ total insulation, $I_{\text{fleece}} =$ fleece insulation, $I_{\text{ABL}} =$ air boundary layer insulation and $I_Z =$ insulation of the depth of radiation penetration to level $Z$.

The length, or depth, of the coat is a critical aspect of insulation and has an impact on radiant heat exchange in many species of animals. The longer the coat, the greater the insulation of the coat (Scholander et al. 1950a), and the further away the point of radiation penetration and absorption ($I_Z$) is from the skin (Hutchinson and Brown 1969). Given these considerations, a longer coat should reduce the radiant heat load on an animal, as illustrated by the lower respiration rate (i.e. lower heat gain) of unshorn sheep compared to shorn sheep standing in full sun (Parer 1963). Similarly, unshorn alpacas did not show any signs of heat stress, indicating that the fleece was providing insulation against radiant heat gain, when compared to shorn alpacas exposed to hot humid conditions that did show signs of heat stress, including open-mouth breathing, nasal flaring, drooling, anorexia, depression and lethargy, tachycardia and tachypnoea (Heath et al. 2001; Navarre et al. 2001; Duncanson 2012). Additionally, longer fleeces should be affected less by convection, as there is a deeper layer of insulation that can act as a barrier to heat flow even if the top layer of insulation is reduced.
Coat colour can also affect the radiant heat load. Incident solar radiation is either reflected away or absorbed by the coat, and it is the colour of the coat that determines the ratio of reflected to absorbed solar radiation (Cena and Monteith 1975). Dark-coloured animals have more melanin pigment in the skin and fibres, and therefore more radiation is absorbed. With less melanin, light-coloured animals reflect more radiation. For example, the differences in melanin concentration result in a difference in reflectivity results and radiant heat load in dark and light-coloured pigeons (Walsberg et al. 1978). Although dark-coloured coats absorb radiation closer to the fleece surface, and more radiant heat as a whole, the penetration of radiation tends to be deeper in light-coloured coats, because while incident radiation can be reflected backwards out of the coat, some can be reflected further into the coat (Walsberg 1983; Acharya et al. 1995). Because the light-coloured coats reflect more solar radiation, but also have deeper penetration of radiant heat, the heat load is not a simple function of colour.

The fibre structure (thickness and density of fibres) of the coat affects the stability of the air being trapped between and above the fibres for the insulation. High fibre density (denser coat) increases the insulating capacity of a coat, particularly when exposed to high wind speed (Scholander et al. 1950b; Tregear 1965; Ames and Insley 1975; McArthur and Monteith 1980), and decrease the radiation penetrability of sheep fleece (King and Millington 2010). The role of fibre thickness to insulating capacity is less known. Newborn lambs with thicker-fibre coats had lower metabolic rates than lambs with finer fibre when exposed to wind (Alexander 1962), indicating that the thicker fibres reduces heat loss.
The alpaca (*Vicugna pacos*) is a fleeced camelid originating from the high altitudes of the Andes and are now raised in Australia as part of a niche fibre industry. The Australian environment is on average 10°C hotter in summer than the native range of the alpaca in South America, raising the possibility that alpacas may be subjected to a higher heat load than they are adapted to and may become heat stressed during an Australian summer (San Martin and Bryant 1989; Australian Bureau of Meteorology 2013b). Within the developing alpaca fibre industry, there is strong selection for fine and dense fibre to increase the quality and quantity of fibre output. Fibre thickness and density are generally genetically correlated in Merino sheep and alpacas (Young and Chapman 1958; Moore *et al.* 1998; Ferguson *et al.* 2012). As there is currently a wide variety of fleece types (diameter and density combinations) among the national flock (McGregor and Butler 2004), it is imperative to know how alpacas with all combinations of fleece types insulate against radiant heat.

This study aimed to identify which fleece characteristics (length, colour, fibre density, and fibre diameter) had the greatest influence on the insulation and radiant heat load of an alpaca to potentially reduce the chance of heat stress during summer, under realistic environmental conditions of solar radiation exposure and wind. To test this, a series of hypotheses are posed. Firstly, I predicted that alpacas with darker-coloured fleeces would have a higher radiant heat load than alpacas with light-coloured fleeces; secondly, alpacas with longer fleece will have more total insulation and a lower radiant heat load; thirdly, fibre density will be more important than fibre thickness for radiation penetration and alpacas with denser fleeces will have a lower radiant heat load; and lastly, alpacas with denser fleeces will have higher levels of insulation when exposed to high wind speed than alpacas with sparse fleeces.
4.2. Materials and Methods

4.2.1. Experimental design

Pelt samples from alpacas (n=18), that varied in colour from light to dark, were used. The insulation provided by each pelt was measured at 1 and 6 m/s wind speed, and also the heat load resulting from short wave incident radiation at the top of the pelt at the same wind speeds. The measurements were made at three distinctly cut fleece lengths of 10, 20 and 40 mm.

4.2.2. Pelt samples

Pelt samples from light-coloured (white or light fawn; n=9) and dark-coloured (brown or black; n=9) Huacaya alpacas were obtained from Western Australian producers from animals that were either culled or died of natural causes, as per approval from the University of Western Australia's Animal Ethics Notification of Use of Animal Tissues approval RA/3/500/003. A pelt sample (skin and fleece) of approximately 40x40 cm was taken from the mid-dorsal region of the back, salted on collection, cleaned to remove subcutaneous fat and tanned using the protocol provided with a commercial tanning kit (Pizzari Home Tanning Kit, Victoria, Australia). Once tanned, a circular 21 cm diameter sub-sample was cut out of the larger pelt sample for study in the wind tunnel.

4.2.3. Fibre characteristics

Fleece colour was assessed according to a colour classing chart provided by the Australian Alpaca Association, and for the purpose of this study categorised as light or dark. The categorisation was supported by the quantitative reflectance data (see below).
Fibre samples from each pelt were taken from the remnants of the original pelt sample by shaving off at the skin level (10x10 cm) using a hand held cordless Lazor Laube clipper (Kim Laube &Co. Inc., USA). The mean fibre diameter (MFD; µm) was measured using an OFDA 2000 (optical-based fibre diameter analyser, BSC Electronics, Perth, Western Australia) by the MicronMan (Bibra Lake, Western Australia). The total fibre density (TFD; fibres/mm²) was calculated from fibre weight and fibre diameter, assuming a constant specific gravity of the fibre, using the following equations, based on a method commonly used in the wool industry to estimate fibre density (courtesy of Anthony Schlink, Department of Agriculture Western Australia).

Equation 1: \( V(mm^3) = \pi \times \left(\frac{MFD}{2000}\right)^2 \times FL(mm) \)

Calculations of the volume of alpaca fibre (mm³) by using the formula for a cylindrical shape, as the samples were taken from a biopsy punch which was 5 mm in diameter. The radius was determined by dividing the MFD in µm by 2000 to be in mm and this squared was multiplied by pi and the length of the fibres in mm, assuming the fibres are mostly straight and not curled.

Equation 2: \( Wt(kg) = V(mm^3) \times SG(kg/mm^3) \)

\[ Wt(g) = Wt(kg) \times 1000 \]

The calculated weight of the fibre samples was calculated by multiplying the volume (mm³) by the specific gravity of alpaca fibre, and then converted from kg to g. Specific gravity is the ratio of the density of a substance to the density (mass of the same unit volume) of a reference substance. Alpaca fibre has a specific gravity of 1.31 but when converted to a mass per volume value of kg/mm³ is equal to \( SG = 1310 \times 1000^{-3} kg/mm^3 \).
Equation 3: \( TFD (\text{fibres/mm}^2) = \frac{\text{Act Wt(g)}}{\text{Wt(g)}} / \text{Area (mm}^2) \)

The TFD can then be calculated in fibres per \( \text{mm}^2 \) by first dividing the actual weight of the fibres (g) by the calculated weight of the fibres (g), which is then divided by the area of the base of the sample which was 5 mm in diameter.

The cross-sectional area of fibre (CSA) as a percentage of the total fleece cross-sectional area is a measurement of the proportion of a fleece that is made up of fibre. The remainder is air, or space between the fibres. This was calculated to quantify if some fleeces had more insulating air than others.

Equation 4: \( \text{CSA(\%)} = \left( \pi \times \left( \frac{MFD}{2000} \right)^2 \right) \times TFD \times 100 \)

4.2.4. Fleece length

Fleece length was controlled (± one to two millimetres by human error) by clipping using a hand held cordless Lazor Laube clipper fitted with a size 10, 1.5 mm Andis ultraedge blade (Andis Company, USA). Andis clipper comb guides of 10 mm and 19 mm, and a 38 mm Wahl comb guide were used to create controlled fleece lengths (averaging 10, 20 and 40 mm) for all pelts.

4.2.5. Heat load and insulation

Thermal insulation was measured using the technique described for the measurement of thermal conductance for emu pelts by Maloney and Dawson (1995). Insulation \( (\text{m}^2\cdot\text{C} \cdot \text{W}^{-1}) \) was calculated as the inverse of thermal conductance. Pelt samples were secured (using masking tape) in a wind tunnel (Figure 4.3) onto a metal hot plate (100 mm diameter) embedded with a heat flux transducer (HFT; 20 x 30 mm, model HA13-18-10P, Thermonetics)
Corporation, USA). The temperature of the hot plate was maintained using a circulating, thermostatically controlled, water bath (IsoTemp S150, ThermoFisher, Pittsburgh, USA) set at 38°C to mimic the average core body temperature of an adult alpaca (Fowler 1994). The heat flux transducer was calibrated using two insulating polystyrene blocks of known thermal conductance (Boral Industries, Sydney, Australia).

![Schematic diagram of the wind tunnel and solar heat lamp setup.](image)

Figure 4.3  Schematic diagram of the wind tunnel and solar heat lamp setup. The pelt was attached to the water filled hotplate (attached to the circulating water bath) inside of the tunnel that has polypipe ends (to allow wind to travel through) and clear glass sides. A fan was situated at one end to provide convection and an anemometer is located side on to the pelt to record the wind speed. An ARRI Daylight solar lamp was suspended above the tunnel with an aluminium foil covered glass sheet below to block radiation access except through a circular hole above the centre of the pelt. Thermocouples are placed above the fleece tips, on the upper skin and on the hotplate to measure heat flow through the fleece.

Plate temperature ($T_p$), air temperature ($T_a$), fleece tip temperature ($T_{tip}$) and skin surface temperature ($T_{sk}$) were measured using copper/constantan (Type-T) thermocouples of 0.7 mm diameter. The skin surface temperature was measured by feeding two thermocouples through the skin from below using an
18G x 1½” needle and resting the bent ends of the thermocouple wires on the upper skin surface amongst the fibres. The wires were taped to the underside of the pelt. Fleece surface temperature was measured by two thermocouples attached to flexible wires positioned so that the thermocouple tips rested on the fleece surface. Air temperature was measured near the inlet of the wind tunnel. The output from all thermocouples and heat flux transducers was logged on a personal computer via an analogue/digital converter (Datataker, Data Electronics Australia P/L, DT500). Prior to the experiment, the thermocouples were calibrated against a certified (National Association of Testing Authorities, Australia) mercury in glass thermometer.

Thermal insulation was measured at a ‘low’ wind speed of 1 m/s and a ‘high’ wind speed of 6 m/s at the three fleece lengths (10, 20 and 40 mm). Wind speed was monitored by a thermo-anemometer (Schiltknecht 39400, Technical and Scientific Equipment Co., Melbourne, Australia) behind and above the pelt and controlled by regulating the power supplied to a fan driving air through the wind tunnel (Figure 4.3). Each pelt was measured twice at each fleece length and wind speed, once without and once with an ARRI solar heat lamp (ARRI Daylight 575W metal halide lamp; ILC Technology, DM1575) providing 590 W/m² of radiation to the pelt sample, at the level of the hot plate, suspended above the wind tunnel. At each fleece length the radiation intercepted at the fleece surface was; 581.7 (10 mm), 627.2 (20 mm) and 754.9 (40 mm) W/m² of radiation. A piece of glass, covered in aluminium foil, except for a hole above the pelt sample allowing light to penetrate over the entire sample, was placed between the lamp and the wind tunnel to reduce heating of the wind tunnel around the pelt sample (Figure 4.3). Radiation was measured level with the top of each fleece (10, 20 and 40 mm), with a radiometer (Eppley Laboratory, INC.;
Model: 8-48; Newport, Rhode Island, USA) after the radiation from the ARRI had passed though the two sheets of glass, the same as in the wind tunnel set-up (Figure 4.3).

Temperatures and heat flow were measured for at least 100 minutes or until readings were stable for more than 20 minutes. The mean values of the period, when values were stable, were used to calculate insulation. Total pelt insulation ($I_{\text{total}}$; fleece plus air boundary layer), fleece insulation ($I_{\text{fleece}}$) and the air boundary layer insulation ($I_{\text{ABL}}$) (Figure 4.2) were all calculated (equations can be found in Appendix Two). The heat load from radiation, expressed as a percentage of incident radiation (RHL%) was calculated as:

$$\text{Equation 5: } RHL\% = \left( \frac{\text{heat flow without radiation} - \text{heat flow with radiation}}{\text{incident radiation at that fleece length}} \right) \times 100$$

4.2.6. Reflectivity

The total reflectance of the pelts at 10, 20 and 40 mm fleece lengths was measured under the ARRI solar lamp, using an Eppley radiometer (model 6-1.8, Eppley Inc., Newport, Rhode Island), and the strength of reflected radiation of each pelt relative to black and white standards was recorded. The samples were placed flat on a horizontal surface and the ARRI solar lamp spotlight was directed onto the sample at an angle of 25° from vertical. The reflected radiation was collected by the Eppley radiometer placed opposite the spotlight and also directed down at an angle of 25° from vertical. A 20 cm long tube, painted black on the inside was placed over the top of the radiometer so only radiation directly from the sample was detected. The same procedure that was carried out for each sample was also done on two 5 mm thick pieces of board, one flat black and the other flat white. To establish the black and white standards as
calibrated comparisons for the calculation of the pelt reflectivity, the total visual (and UV) spectral reflectance (200-700 nm) was measured against a magnesium oxide standard (setting the percent reflectivity to 0 so that it read 100% reflectivity at 400 nm) on a diffuse reflectance spectrophotometer (Varian Techtron UV-Visible Spectrophotometer, Varian Techtron Pty. Ltd., Mulgrave, Australia). Published values for the reflectance of the magnesium oxide standard (Janecek 2012) were used to calculate the reflectance of the black and white boards at each wavelength. The spectral power of the ARRI spotlight was then used to calculate the total reflectance of each standard board under the ARRI spectrum. The total reflectance of each pelt sample was calculated relative to the black and white standards. The calculated reflectivity of the white standards was 85.5% and the black standard was 9.1%.

4.2.7. Solar Penetrance

The distance that solar radiation penetrated into the fleece was calculated using the fleece length, fleece insulation and $I_z$ (Figure 4.2) from the following equations.

Equation 6: Penetranse (mm) = \left( \frac{I_z}{I_{fleece}} \right) \times FL(mm)

where:

$I_z = \left( \frac{HLR}{(absorbance\ of\ radiation)} \times I_{pelt} \right) - I_{abi}$

and where:

$HLR$ (heat load from radiation) = $\frac{heat\ flow\ without\ radiation}{heat\ flow\ with\ radiation}$
\[
\text{Absorbance of radiation} = \left(\frac{(100 - \text{reflectance})}{100}\right) \times \text{ARRI power}
\]

\[
\text{ARRI power} = \frac{W}{m^2} \text{ of radiation at that fleece length}
\]

4.2.8. Statistical analysis

Regression analysis was used to determine whether the total fibre density or mean fibre diameter accounted for variation between pelts in total insulation, radiant heat load or radiation penetrance at each wind speed and at each fleece length. The percent decrease in total insulation was calculated between 1 and 6 m/s wind speed, with the proportions arcsine transformed prior to the regression analysis. Regression analysis was also used to determine if the total fibre density or mean fibre diameter accounted for any of the variation in the reflectance of the pelts at each fleece length.

Three-way ANOVA’s with a Student-Newman-Keuls comparison test were used to determine main effects and interactions between the fleece length, wind speed and fleece colour for penetrance, heat load % and total insulation, using the pelt number as a block (residual degrees of freedom equals 80). A two-way ANOVA was used to examine the effect of fleece length and colour on reflectance (residual degrees of freedom equals 32). When there was an indication of a significant effect of fleece colour or wind speed (see Results) then two-sample paired t-tests were further used at each wind speed and fleece length to determine if there were differences in penetrance, radiant heat load, reflectance, or total insulation. Genstat v.14.11 (VSN International Ltd., United Kingdom) was used for all analyses.
4.3. Results

4.3.1. Fleece characteristics

The mean fibre diameter and the total fibre density of the pelts (Appendix Three) were correlated, with the pelts with finer fibres having higher fibre densities than those with thicker fibres (P<0.001). The cross-sectional area (CSA) was influenced by the total fibre density of the fleece (P=0.02), but not by the mean fibre diameter (P=0.78). Because the denser fleeces were made up of finer fibres, and the sparse fleeces had thicker fibres, there was no difference between the fleece types in CSA. The CSA of the fleeces was not related to the colour of the fleeces (P=0.48).

4.3.2. Reflectance

The light-coloured fleeces were significantly more reflective than the dark-coloured fleeces at all fleece lengths (10 mm P<0.001; 20 mm P<0.001; 40 mm P=0.002; Figure 4.4A). Within each colour, the reflectance of the 10 and 20 mm long fleeces did not differ (dark: 10-20 mm P=0.6; light: 10-20 mm P=0.3), but the 40 mm long fleeces were slightly more reflective for both the light (20-40 mm P=0.4) and dark fleeces (20-40 mm P=0.1) visually, but when both colour groups were combined and averaged at each fleece length, the mean of the 40 mm fleeces were more reflective than the 10 and 20 mm fleeces (Figure 4.4A). The light-coloured pelts were quite dirty and dusty towards the skin base. There was no interaction found between fleece length and fleece colour. The mean fibre diameter and total fibre density did not affect the reflectivity of the pelts (Figure 4.5). In summary, light-coloured fleeces reflected more radiation than dark-coloured fleeces, which in turn absorb more radiation. Fibre density and diameter did not affect the reflectivity of the fleeces.
4.3.3. Solar penetration

The penetration of radiation into the fleece was not affected by the fleece length (P=0.88), but did differ between the fleece colours (P=0.002; Figure 4.4B). Light-coloured pelts absorbed radiation deeper into the fleece than the dark-coloured pelts at 1 m/s for both 20 mm (P=0.007) and 40 mm (P=0.02) fleece lengths, as well as at 6 m/s (P=0.012) for the 10 mm fleece length. Regardless of fleece colour, wind speed had an effect (P<0.001) with deeper penetration at low wind speed than at high wind speed (Figure 4.4B). There was an interaction between wind speed and fleece colour (P=0.024) and between fleece length and wind speed (P=0.006), but no three way interaction, most probably due to the light-coloured pelts having a deeper penetration at 1 m/s wind speed for the 20 (P=0.007) and 40 mm (P=0.02) fleece lengths, which was largely contributed by one pelt which had noticeably deeper penetrance at both of these fleece lengths (Figure 4.4B).

The total fibre density affected the distance that solar radiation penetrated into the fleece. Radiation penetrated less into the denser fleeces at 1 m/s wind speed for the 10 mm (P=0.004) and 20 mm (P=0.04) fleece lengths than it did in the sparser fleeces. The total fibre density had no effect on the penetration of radiation into the pelts at 40 mm fleece length (Figure 4.5). The mean fibre diameter (Appendix Three) did not affect the distance that radiation penetrated into the fleece at any fleece length or wind speed (1 m/s: 10 mm P=0.1, 20 mm P=0.4, 40 mm P=0.6; 6 m/s: 10 mm P=0.4, 20 mm P=0.7, 40 mm P=0.5).

In summary, at low wind speed, radiation penetrated deeper into the light-coloured pelts. At high wind speed there was less of a difference in radiation
penetration between the fleece colours but still followed the same trend as at low wind speed. Fibre diameter did not affect the penetration of radiation.

Figure 4.4 Reflectance (panel A) and depth of radiation penetrance (panel B) of dark (dark grey) and light (light grey) coloured fleeces at 10, 20 and 40 mm fleece lengths, and at low (1 m/s) and high (6 m/s) wind speeds. Different italicised letters indicate difference between fleece length (P<0.05) and * indicates differences between winds speeds within one fleece length (P<0.05)
4.3.4. Insulation

The total insulation did not differ between the dark and light-coloured fleeces (P=0.69) at any wind speed or fleece length. The total insulation decreased significantly as wind speed increased (P<0.001), and total insulation increased with fleece length (P<0.001), but only between the 10 mm and the 20 and 40 mm long fleeces, with the latter two not significantly different from each other (Figure 4.6). There was an interaction between wind speed and fleece length (P<0.001) for total insulation, due to a greater effect of wind speed (1 m/s) on the longer fleeces (Figure 4.6).
Figure 4.6  Total insulation (m\(^2\cdot\text{C} \cdot \text{W}^{-1}\), mean±SE) of all pelts at 10, 20 and 40 mm fleece lengths, at both low (1 m/s) and high (6 m/s) wind speeds. Different italicised letters indicate differences between fleece length (P<0.05) and P-values indicate differences between wind speeds within one fleece length.

There was no effect of the mean fibre diameter on the total insulation at any wind speed or any fleece length. The total fibre density influenced the total insulation at 1 m/s wind speed for the 10 mm (P=0.008) and 20 mm (P=0.022) long fleeces, with the denser fleeces being more insulative than the low density fleeces (Figure 4.7). At 6 m/s the denser fleeces had a significantly higher total insulation than the less dense fleeces at both the 20 mm (P=0.015) and 40 mm (P=0.008) fleece length (Figure 4.7).

When wind speed increased from 1 to 6 m/s, the percent decrease in the total insulation was significantly less for the denser fleeces at 20 mm in length (P=0.04) and, there was a trend (P=0.06) in the 40 mm fleeces of a smaller percent decrease in total (Figure 4.8). The percent decrease in total insulation was neutral across all fibre densities when the fleece was only 10 mm long (P=0.2).
In summary, fleece colour did not affect the insulation of the fleeces but at low wind speed (1 m/s), longer fleeces were significantly more insulative than short fleeces. Denser fibred fleeces were also more insulative than sparse fleeces at all wind speeds and as wind speed increased the dense, longer fleeces lost the least amount of insulation. Fibre diameter did not affect insulation.

**Figure 4.7** Relationship between the total fibre density (fibres/mm$^2$) and the total insulation, at 10, 20 and 40 mm fleece lengths, and at both low (x; 1 m/s) and high (○; 6 m/s) wind speeds. A thick trendline indicates a relationship with a P<0.05

**Figure 4.8** Relationship between the total fibre density (fibres/mm$^2$) and the percent decrease in total insulation from 1 to 6 m/s at 10 (x; solid line), 20 (○; thick broken line) and 40 mm (■; fine dotted line) fleece lengths. A thick trendline indicates a relationship with a P<0.05
4.3.5. Radiant heat load

The radiant heat load, as a percentage of incident radiation (proportional radiant heat load) was not affected by the fleece colour at any fleece length or wind speed ($P=0.35$; Figure 4.9). Nor did the radiant heat load relate with the reflectance values at any fleece length (10 mm – 1 m/s $P=0.6$, 6 m/s $P=0.6$; 20 mm – 1 m/s $P=0.8$, 6 m/s $P=0.9$; 40 mm – 1 m/s $P=0.6$, 6 m/s $P=0.6$). There was a significant decrease in radiant heat load when wind speed increased in for all fleeces ($P<0.001$), with there being no difference in the decrease between fleece colour. An increase in fleece length was associated with a significant reduction in the radiant heat load ($P<0.001$; Figure 4.9). An interaction between wind speed and fleece length was recorded ($P=0.047$), most probably due to the substantial effect of wind at 10 and 20 mm fleece lengths, with a smaller effect at 40 mm fleece length. The fibre diameter did not affect the radiant heat load at any length or wind speed, but the total fibre density did affect the radiant heat load in the 10 mm fleece at 6m/s ($P=0.047$) with a decrease in the radiant heat load as density increased (Figure 4.10).

In summary, fleece colour did not affect radiant heat load but short fleeces had a far greater radiant heat load than longer fleeces. Fibre density did not affect the radiant heat load of the longer fleeces, but did have a small effect on the shortest fleece at low wind speed. Fibre diameter did not affect the radiant heat load.
Figure 4.9  Radiant heat load, expressed as a percentage of incident radiation of dark (dark grey) and light (light grey) coloured fleeces at 10, 20 and 40 mm fleece lengths, and at low (1 m/s) and high (6 m/s) wind speeds. Different italicised letters indicate difference between fleece length (P<0.05)

Figure 4.10  Relationship between the total fibre density (fibres/mm²) and the radiant heat load, as a percentage of incident radiation at both low (x; 1 m/s) and high (©; 6 m/s) wind speeds, at 10, 20 and 40 mm fleece lengths. A thick trendline indicates a relationship with a P<0.05
4.4. Discussion

This study was designed to identify which fleece characteristics (length, colour, fibre density or fibre diameter) had the greatest influence on the insulation and radiant heat load of an alpaca’s fleece to potentially reduce the chance of heat stress during summer, under realistic environmental conditions of solar radiation exposure and wind.

The fleece length was found to be the most important fleece characteristic to influence the penetration of radiant heat into the insulation, as longer fleece lengths reduced the radiant heat load but also increased the insulation of the fleece, leading us to accept the second hypothesis. It was found that neither fleece colour nor structure (fibre diameter or density) affected the radiant heat load, despite fibre density having a small influence on the depth of penetration of radiation into the fleece. Thereby the first hypothesis that dark-coloured fleeces would have a higher radiant heat load than light-coloured fleeces is rejected. There was some support for our third hypothesis in that radiation penetrated less into denser fleeces, but only at the shortest fleece length at low wind speed. At longer lengths and at high wind speed, there was no effect of fibre density on radiation penetration.

There was some support for the fourth hypothesis, that denser fleeces would provide better insulation at high wind speed, but only in the 20 and 40 mm long fleeces. At 10 mm fleece length there was no effect of fibre density on total insulation. On the other hand, at low wind speed a denser fleece provided better insulation at 10 and 20 mm fleece lengths, but had no effect at 40 mm. Additionally, denser fleeces also had a smaller change in insulation as the wind speed increased, but only for the longer fleeces.
As fleece colour did not have any effect on the radiant heat load and the fleece structure only had a minor influence on the insulation, alpacas with any combination of fleece structure and colour should not be at risk of accumulating a heat load that may invoke heat stress during summer as long as the fleece is at an adequate length.

Fleece length was the most influential characteristic for the insulation and radiant heat load. The total insulation of the alpaca fleeces increased with fleece length and the percent of incident radiation contributing to the radiant heat load was lower with longer fleece, as previously shown in sheep. Sheep with 25 mm long wool had twice the fleece insulation of sheep with 13 mm long wool (Bennett and Hutchinson 1964) and sheep with longer fleece had lower respiration rates and were less heat stressed than shorn sheep, standing in full sun (Macfarlane et al. 1958). Because there is more depth for air to be trapped between the fibres of the fleece (insulating the animal), and more distance between the skin and the ambient environment (reducing the impact of the penetration and effect of radiant heat), longer fleece should thereby also reduce the chance of heat stress in alpacas. From the results of this study, it could be suggested that a fleece length longer than 20 mm would be adequate to protect an alpaca from heat stress under radiant heat.

The insulation of alpaca fleece in still air (when extrapolated from the relationship between wind speeds, at 1 and 6 m/s, and total insulation) is comparable to other fleeced mammals, at similar coat lengths (Scholander et al. 1950b). However, when affected by wind speed, the differing coat structure causes a different response for alpacas. Shorter fleeces reputedly should have less insulation than longer fleeces when exposed to high wind speed.
Increasing wind speed reduces the insulation from sheep fleece, with shorter fleece being more affected than longer fleeces (Parer 1963; Bennett and Hutchinson 1964; Ames and Insley 1975; McArthur and Monteith 1980). However, for the alpaca fleeces the effect of the increasing wind speed on insulation was independent of fleece length as all fleeces displayed a reduction in insulation to a similar level, despite the fact that high wind speed nearly halved the radiant heat load of the alpaca fleeces at each fleece length. Perhaps a reduction in insulation due to wind speed, independent of fleece length, differed from the sheep studies cited above because the coat structure (fibre diameter and density) plays a role in maintaining insulation once wind speed increases in the sheep. The fleece types (combinations of fibre diameter and density) of the alpacas were much more variable than what would be found among a group of research sheep with similar fleece types, due to intensive selective breeding (Parer 1963; Bennett and Hutchinson 1964). In this study, it was the fibre density that was found to be the greatest influence for the percent decrease in insulation of the whole coat as wind speed increased.

The total insulation of the alpaca coats was higher for denser, in comparison to less dense coats, at both low and high wind speeds for the short and longer fleeces respectively. In species ranging from cattle to rabbits to polar bears, it has been clearly demonstrated that denser coats provide a better insulation than sparsely fibred coats when exposed to high wind speed as the air within the coat is better held between the fibres (Scholander et al. 1950b; Tregear 1965; Gebremedhin et al. 1997). Unlike previous literature, the fibre density did not affect the radiant heat load on alpaca fleeces. Although at low wind speed the denser coats were not as deeply penetrated by radiation, the radiant heat load was not greater for the sparsely-fibred fleeces as had been found in cattle.
(Dowling 1955). The 10 mm fleeces, which were more sparsely-fibred, did have a slightly higher radiant heat load than the denser coats at high wind speed. I would suggest that this significant result was due to the short fleece length and the high wind speed, rather than the fleece structure given that fleece structure had no further effect on the radiant heat load. Overall, this study shows the insulation is decreased by wind no matter the structural properties of the coat, and the fleece structure has little effect on the radiant heat load. So, in terms of the animal's thermoregulatory capacity, the fleece length is more important than fleece structure or fleece colour on the radiant heat load.

Fleece colour also did not affect the radiant heat load of the fleeces in this study. Although previous literature suggested that dark-coloured coats would accumulate a greater radiant heat load than light-coloured coats (Hamilton and Heppner 1967; Heppner 1970; Lustick 1971; Walsberg et al. 1978; Lustick et al. 1980), this was not found in the present study. It would seem that the melanin pigmentation in the darker coats absorbs the radiation at the fleece surface rather than deeper into the coat and while the light-coloured alpaca fleeces had a higher reflectance of radiation, they also had a deeper penetration of radiation than dark-coloured fleeces. For the light-coloured fleeces, the penetration of the radiation into the fleece was at most 4 mm in the 40 mm long fleece, and is proportionally a small amount, given the depth of insulation (from I\textsubscript{s} to the skin level). The length of the fleece prevents the penetrated radiation, or that absorbed at the fleece surface, from influencing the radiant heat load and therefore, the colour of the fleece was found to be irrelevant to the insulation and radiant heat load, as also found in cattle, polar bears and koalas (Hutchinson and Brown 1969; Blackshaw and Blackshaw 1994; Dawson et al. 2014).
4.5. Conclusion

In the present study fleece length is the most important fleece characteristic to reduce the radiant heat load and increase the insulation of an alpaca’s fleece. As wind speed increased, insulation decreased less for denser fibred coats than for sparsely fibre coats. Coat colour did not affect the radiant heat gain on the animal. Overall, to prevent heat stress in alpacas over summer, producers should make sure that their alpacas have an adequate fleece length (at least 20 mm) to insulate and protect the alpacas from radiant heat as well as ensuring that adequate shade structures or trees are provided to shelter under.
PART B:
FLEECE CHARACTERISTICS AND VITAMIN D
5.1. Introduction

In the previous chapter, it was reported that alpaca fleece is very effective at reducing the radiation that reaches the skin surface. Longer fleece provided the best reduction in radiant heat gain, but fibre density also had a small impact, particularly when the fleece was exposed to wind. Vitamin D synthesis is reliant upon radiation reaching the skin and triggering a photochemical reaction for its production. Given that alpaca fleece is so effective at stopping radiation penetration to the skin, this experiment was designed to investigate the impact of the fleece length and structure on the production of vitamin D in alpacas.

Vitamin D is a steroid-hormone that helps to regulate the absorption of calcium and phosphorus in the intestine. These elements are important in bone mineralisation, development, and general growth (Webb and Holick 1988; Garner-Paulin 2005). A vitamin D deficiency can cause debilitating bone diseases such as rickets (in the young) and osteomalacia (in adults) and reduce the overall growth, health and production efficiency of an animal (Judson and Feakes 1999; Smith and Van Saun 2001; Garner-Paulin 2005). Vitamin D can be either ingested in the diet (mainly as D$_2$) or synthesised in the skin via a photochemical reaction between ultraviolet B (UVB; 280-320nm) radiation and a steroid pre-cursor (7-dehydrocholesterol) that produces vitamin D$_3$. Vitamin D$_2$
is produced in sun-cured feeds in the summer and autumn months when there are increased seasonal levels of solar and UV radiation and is absorbed when these feeds are digested (Parker et al. 2002). During the winter and spring months in Australia, green pasture is a poor source of vitamin D₂ (Van Saun et al. 1996). Even in the summer, vitamin D₂ provides only a small portion of the required levels of vitamin D in herbivores and therefore the synthesis of D₃ in the dermal tissue is the predominant source (Smith and Van Saun 2001).

The alpaca (*Vicugna pacos*) is a South American camelid that originates from the high-altitude altiplano plateaus in the Andes mountain range. In their native habitat, characterised by high altitude (4,100-4,700 m above sea level) and low latitude (15-20°S), the UV intensity is high (UV index of 22), alpacas are generally sufficient in vitamin D (Charrier and Muñoz 1997; Liley and McKenzie 2006). However, the incident UV levels are quite different in some countries to which alpacas have been exported. In southern Australia and New Zealand, the UV radiation levels are lower than in the altiplano and are more seasonal due to low altitude (on average 10-300m above sea level in Australia and about 600m above sea level in New Zealand) and high latitude (30-45°S).

While Australia has high UV intensities during the summer relative to altitude and latitude (UV index of 11-12), the levels are low in winter (UV index of 1-3) (Australian Bureau of Meteorology 2013b). Cloud cover during winter also can decrease or increase UVB depending on the level of cloud cover. If there is more than 90% cloud cover then UVB levels are reduced by up to 99% on that incident on the top of the cloud, if there is less than 90% cloud cover then UVB can be increased by 24-27% due to radiation scattering within the cloud (Estupiñán et al. 1996). Either of these cloud cover scenarios contribute to the
low UV index levels (1-3) recorded at ground level. During winter, the alpacas also have a thick fleece cover because they are generally shorn once a year, in the spring. The combination of low UV input and thick fleece cover in late winter is associated with vitamin D insufficiency in alpacas in Australia. In winter, vitamin D supplementation of alpacas is a common practice, particularly for young or dark coloured animals that are the most susceptible to insufficiency (Judson and Feakes 1999; Judson et al. 2008; Judson et al. 2011). A total vitamin D (both D\textsubscript{2} and D\textsubscript{3}) level in blood plasma between 100 and 250 nmol/L is considered normal, while anything below 80 nmol/L is low, and below 50 nmol/L is considered inadequate (Hill et al. 1994; Van Saun et al. 1996; Judson and Feakes 1999; Judson et al. 2008; Oonincx et al. 2010; Judson et al. 2011). Clinical rickets occurs at a total plasma vitamin D level below 15 nmol/L (Van Saun et al. 1996; Judson and Feakes 1999). During a two-year survey of alpacas in South Australia and Victoria, it was found that the average vitamin D level of alpacas in winter was 35 nmol/L. The minimum occurred in August, about a month after the lowest UV intensity levels in July, suggesting there is a time lag between environmental UV and vitamin D levels in alpacas as well as the effect of green pasture for grazing (low vitamin D\textsubscript{2}), increased fleece length and a higher incidence of cloud cover (Estupiñán et al. 1996; Smith and Van Saun 2001; Judson et al. 2008; Australian Bureau of Meteorology 2013b).

The alpaca industry in Australia is selectively breeding for finer fibre because the finer (thinner) fibre is more valuable for commercial use (McGregor 2006). By selecting for finer fibre, alpacas are concurrently being selected for increased fibre density because there is a correlation between fibre thickness and the density of fibres (Chapters 3 and 4, Young and Chapman 1958; Hynd 1994; Moore et al. 1998; Adelson et al. 2002; Ferguson et al. 2012). When a
decrease in fibre density of Merino sheep fleece was simulated, without altering the fibre thickness, there was a 50% increase in light reaching the skin level (King and Millington 2010), indicating that denser fleeces might compound UV deficiency. However, thinner and denser fibres may intercept more UV radiation than thicker and sparser fibres, potentially permitting more UV radiation to reach the skin by forward scattering. The present study was designed to first test the hypothesis that alpacas with finer fibre, and denser fleeces, would have higher levels of vitamin D$_3$ in comparison to alpacas with thicker fibres, and sparser fleeces.

Despite any combination of fleece structure, a longer fleece is an effective barrier against the penetration of incident radiation in alpacas (Chapter 4). Long fleeced sheep have vitamin D levels one half to one third of those of shorn sheep (Parer 1963; Zintzen and Boyazoglu 1973), indicating long fleece prevents UVB reaching the skin for the synthesis of vitamin D$_3$. It is likely that alpacas, which typically have longer fleece in the winter months, would have lower vitamin D levels than alpacas with shorter fleece in winter. Shearing alpacas when their vitamin D levels are low may therefore be an effective way to increase the levels of vitamin D naturally without supplementation. However, because there is a time-lag between natural vitamin D production in alpacas and changes in UV intensity (Judson et al. 2008), it is unknown how quickly vitamin D$_3$ levels increase after shearing. It is hypothesised that shearing will lead to an increase in vitamin D$_3$ levels of alpacas with both fine and thick fibred fleeces.
5.2. Materials and Methods

5.2.1. Experimental design

To test how fibre structure and fleece length might affect the level of vitamin D\(_3\) in alpacas, two experiments were conducted. From a herd of mature female Huacaya alpacas, the eight with the finest fibre, and the eight with the thickest fibres were selected and their plasma vitamin D\(_3\) concentration was measured over a week in each of winter and summer (Experiment 1). The plasma vitamin D\(_3\) concentration in the same 16 alpacas were also measured during one week prior to, and also during one week after, shearing during spring (n=16; Experiment 2). All procedures were approved by The University of Western Australia's Animal Ethics Committee (RA/3/100/988).

5.2.2. Animals

The 16 animals were supplied by Banksia Park Alpaca Stud (Serpentine, Western Australia). All but one of the alpacas were used in both experiments. One animal from the fine-fibre group that was used in the winter experiment was removed before the spring experimentation due to ill health and replaced with an animal of similar mean fibre diameter and body mass for the remainder of both experiments. The alpacas were 2-4 years of age, all white to light fawn in colouring and had an initial mean live-weight (± SE) of 54.5 ± 2.7 kg and condition score of 2.8 ± 0.1 units. Condition score was determined by palpating the muscle and fat coverage along the backbone, level with the last rib and was given a scale between 1 = emaciated and 5 = obese (Fysh 2008). Live-weight and condition score were recorded weekly during both the habituation and experimental periods to ensure that maintenance requirements were met. For Experiment 1, the alpacas were habituated for two weeks to the surrounds,
handlers, and daily feeding routine in both the winter and summer blood sampling periods. During Experiment 2, the alpacas were maintained on-farm and did not require any habituation period.

5.2.3. Experimental groupings

The alpacas were selected based on the mean fibre diameter determined from a 10 x 10 cm fibre sample taken from the mid-side (caudal of the last rib and 40 cm ventral from the backbone) one month before the start of Experiment 1. The fibre patches were analysed using an OFDA 2000 (optical-based fibre diameter analyser, BSC Electronics, Perth, Western Australia) by the MicronMan (Bibra Lake, Western Australia). After selection the two groups differed significantly in mean fibre diameter (± SE), with the fine-fibred group having a mean of 18.8 ± 0.66 μm and the thick-fibred group having a mean of 26.7 ± 0.77 μm (P<0.001). The total follicle density (follicles per mm²) was determined from skin biopsies taken from the mid-side, provided by Banksia Park Alpaca Stud, and analysed as described in Chapter 3. The fine-fibred group had a mean total follicle density of 63.9 ± 6.14 follicles/mm² making them significantly different from the thick-fibred group which had mean total follicle density of 37.9 ± 4.21 follicles/mm² (P=0.008). The mean fibre diameter and the total follicle density were correlated (r=-0.85; P<0.001) and the cross-sectional area of the fleece was calculated, as an indication of the intercept likelihood of radiation with the fibres (Equation 1).

Equation 1: \( CSA_{fibre}(\%) = \left( \pi \times \left( \frac{MFD}{2000} \right)^2 \times TFD \right) \times 100 \)
Where $\text{CSA}_{\text{fibre}}$ (%) = percentage of the cross-sectional area of the fleece as fibre, $\text{MFD}$ = mean fibre diameter in µm, and $\text{TFD}$ = total follicle density in follicles per mm$^2$.

5.2.4. Experiment 1: fleece structure and vitamin $D_3$ synthesis

Once in winter (July 2011) and once in summer (January 2012), the two groups of alpacas were transported from Banksia Park Alpaca Stud to the University of Western Australia's Shenton Park Research Facility in Perth, Western Australia (latitude 31°57'S ~ 14 m above sea level) where they were maintained as a single herd in a grassed paddock. The animals had access to ad libitum water and received a daily maintenance ration of dry feed diet consisting of 22 g/kg metabolic body weight (MBW – body weight to the power of 0.75) barley straw chaff, 8 g/kg MBW 101 maintenance pellets (Macco Feeds, Williams, Western Australia), 4.6 g/kg MBW canola meal, 20 g dried sugarcane molasses (Palabind, Probiotec, Laverton North, Victoria, Australia) and 20 g of sheep minerals (formulated by Independent Lab Services, Serpentine, Western Australia). Sheep minerals were used, as opposed to alpaca minerals, because the alpaca minerals contain a vitamin D supplement. During both the winter (July) and the summer (January) periods a blood sample was taken from each animal on three separate days over one week (Day 1, 3 and 5; three samples per animal) between noon and 2pm.

5.2.5. Experiment 2: fleece length (shearing) and vitamin $D_3$ synthesis

During the months of October and November, the same two groups of alpacas were maintained as a single herd in a grassed paddock with ad libitum water at Banksia Park Alpaca Stud (Serpentine, Western Australia; latitude 32°23'S ~15 m above sea level). The alpacas were shorn on the 1st of November 2011 (Day
0; spring). Over the course of 19 days, a blood sample was taken from each animal on three separate days over one week before and one week after shearing (Day -9, -7 and -5 pre-shearing; Day +5, +7 and +9 post-shearing; total of six samples per animal), with nine days between the two sampling periods. The length of fibre was measured before and after shearing using a 15 cm metal ruler placed into the fleece to the skin at three random locations on the mid-side, and the depth of the fleece was averaged.

5.2.6. Blood sampling and analysis

Blood samples (8 mL) were collected by jugular venipuncture using lithium heparin Vacuette tubes (Greiner Bio-One International AG, Kremsmuenster, Austria). The plasma was separated by centrifugation at 1800 g for 10 minutes and then frozen at -20°C for later analysis. Plasma vitamin D₃ (25-hydroxy vitamin D₃) was measured by LC-MS/MS mass spectroscopy, which analyses the vitamin D separately as D₂ and D₃, a more accurate measure than the old RIA method that produced an accumulated value of the two (PathWest, Royal Perth Hospital, Western Australia). Eight separate replicate analyses of quality control material at the specified concentrations were used to calculate the coefficient of the assays. The intra-assay %CV for vitamin D₃ was 3.7%, 3.2% and 2.1% for the concentrations of 25.0, 66.7 and 206.3 nmol/L and the inter-assay %CV was 6.8%, 6.8% and 4.9% for the concentrations of 26.3, 64.8 and 190.1 nmol/L. The intra-assay %CV for vitamin D₂ was 9.3%, 7.2% and 9.2% for the concentrations of 20.9, 60.4 and 127.7 nmol/L, and the inter-assay %CV was 11.4%, 9.2% and 8.9% for the concentrations of 29.6, 118.6 and 184.1 nmol/L. The mean of the triplicate samples for each individual from each sampling period was calculated and used for statistical analysis.
5.2.7. Meteorological data

For the experiment at UWA Shenton Park Research Facility (Experiment 1), the daily maximum and minimum ambient temperatures were obtained from the Department of Agriculture Floreat weather station located within 1 km of the UWA Shenton Park Research Facility. For the experiment at Banksia Park (Experiment 2), the weather data were obtained from the Bureau of Meteorology climate data online service, using the Karnet weather station, 12.7 km away from Banksia Park Alpacas stud. The UV radiation data for both Experiment 1 and 2 were obtained from the Radiation Health Services Branch of the Australian Radiation Protection and Nuclear Safety Agency.

5.2.8. Statistical analysis

Data were analysed using GenStat v.14.11 (VSN International Ltd, United Kingdom). The effect of fibre structure and fleece length on vitamin D₃ and total vitamin D was analysed using two-way ANOVAs with repeated measures. For Experiment 1, the factors were fleece type and season, and for Experiment 2 the factors were fleece type and time (pre versus post-shearing). Standard linear least-squares regression analysis was used to test the correlation between fibre diameter and follicle density, and between fleece length and vitamin D₃ levels in winter and summer.

5.3. Results

5.3.1. Fleece characteristics

The mean fibre diameter and the total follicle density were strongly negatively correlated with the fine-fibred alpacas being more densely fleeced than the
thick-fibred alpacas (r=-0.85; P<0.001; Figure 5.1). The average total follicle density differed significantly between the groups that had been chosen based on fibre diameter (P=0.01). Consequently, the two groups are referred to as “fine and dense” and “thick and sparse”. There was no difference between the groups in the percentage of the cross-sectional area of the fleece that was fibre (fine and dense=1.7% ± 0.11; thick and sparse=2.0% ± 0.21; P=0.14).

Figure 5.1 Correlation between mean fibre diameter and total follicle density. The circles indicate the fine-fibred alpacas and the squares the thick fibred alpacas. Due to the relationship between the mean fibre diameter and the total follicle density the groups are reclassified as “fine and dense” and “thick and sparse”.

5.3.2. Experiment 1: Effect of fleece structure

The level of UV radiation was lower in winter than in summer (P<0.001; Figure 5.2A). The mean maximum (MAX) and minimum (MIN) air temperatures were higher in summer than in winter (MAX P=0.03; MIN P=0.02; Figure 5.2A).

There was an effect of season (P<0.001) and fleece type (P=0.001) on plasma vitamin D₃ levels and no interaction between season and fleece type (P=0.06). Plasma vitamin D₃ levels were lower in winter than summer (P<0.001; Figure
and lower in the fine and dense alpacas than the thick and sparse alpacas (winter $P=0.01$; summer $P=0.01$; Figure 5.2B). The average total vitamin D ($D_2$ plus $D_3$) in winter was $29.2 \pm 8.2$ nmol/L for the fine and dense group and $70.5 \pm 10.2$ nmol/L for the thick and sparse group. The average total vitamin D in summer was $230.0 \pm 24.9$ nmol/L for the fine and dense group and $360.9 \pm 29.3$ nmol/L for the thick and sparse group (Figure 5.3). Because the animals were shorn in the spring, the fleece was significantly shorter in the summer (23.1 mm) than in the winter (71.1 mm; $P<0.001$) but individual fleece length and vitamin $D_3$ levels of the alpacas were not correlated in either winter ($r=0.4$) or summer ($r=0.44$).
Figure 5.2  

A: Daily meteorological data (Days 1, 3 and 5) during the winter and summer sampling periods. Mean MIN (light grey squares) and mean MAX (black circles) air temperatures (°C) are on the left y-axis and the mean UV (light grey bars) radiation (W/m²) is on the right y-axis. 

B: Mean vitamin D₃ levels (nmol/L; ± SE) of the fine and dense (light grey bars) and thick and sparse (dark grey bars) groups of alpacas for days 1, 3 and 5 in the winter and summer sampling periods.
5.3.3. Experiment 2: Effect of fleece length

The ambient temperatures and UV radiation levels were not different during the periods pre and post-shearing (MAX: P=0.08; MIN: P=0.93; UV: P=0.39; Figure 5.4A). The maximum and minimum temperatures and UV radiation levels were higher during spring, Experiment 2, than in winter, Experiment 1 (MAX: P=0.05; MIN: P=0.02; UV: P<0.001).

Prior to shearing in the spring, the fleece length of the alpacas was the same in the two groups (111.9 ± 4.0 mm; P=0.36). The alpacas were all shorn to 5 ± 2 mm in length, and so there was a significant effect of time (pre vs post-shearing) on fleece length (P<0.001).

Shearing the animals in spring had an effect on the plasma concentration of vitamin D₃, with the level increasing in both groups from pre to post-shearing (All: P<0.001, fine and dense: P<0.001, thick and sparse: P<0.001; Figure 5.4B). Pre-shearing, there was no difference in vitamin D₃ between the fine and
dense and the thick and sparse groups (P=0.12), with the alpacas in the thick and sparse group having higher average plasma vitamin D₃ levels but more variation between animals. Post-shearing, the fine and dense group exhibited significantly lower levels of vitamin D₃ (P=0.01) than the thick and sparse group (Figure 5.4B). There was an effect of time (Day -9 to +9) on the vitamin D₃ levels (P<0.001) and an interaction between time and fleece type group (P=0.02), but no interaction when all pre-shearing and all post-shearing values were separately averaged by the fleece type (P=0.28).

The average total vitamin D (D₂ plus D₃) pre-shearing was 46.1 ± 6.3 nmol/L for the fine and dense group and 77.2 ± 17.4 nmol/L for the thick and sparse group. The average total vitamin D in post-shearing was 90.9 ± 12.2 nmol/L for the fine and dense group and 154.9 ± 18.3 nmol/L for the thick and sparse group (Figure 5.3).
Figure 5.4  A: Daily meteorological data during the pre (Days -9, -7 and -5) and post-shearing (Days +5, +7 and +9) sampling periods. Mean MIN (light grey squares) and mean MAX (black circles) air temperatures (°C) are on the left y-axis and the mean UV (light grey bars) radiation (W/m²) is on the right y-axis. B: Mean vitamin D₃ levels (nmol/L; ±SE) of the fine and dense (light grey bars) and thick and sparse (dark grey bars) groups of alpacas during the pre and post-shearing sampling periods in spring for days. Lowercase letters indicate significant differences between sampling time points for the fine and dense alpacas and uppercase letters indicate significant differences between sampling time points for the thick and sparse alpacas. Shearing occurred on day 0.
5.4. Discussion

The hypothesis that alpacas with finer fibre would have higher levels of vitamin D$_3$ in comparison to alpacas with thicker fibres was rejected. In fact, precisely the opposite was found, and the plasma concentrations of vitamin D$_3$ in the fine and dense group was lower than in the thick and sparse group in both summer and winter. Even accounting for the higher density of fibres in the fine-fibred alpacas, this was an unexpected finding, since the cross-sectional area of the fleece in the two groups was the same, which should mean that the penetration of UV radiation into the fleece would be similar in the two groups (Chapter 4). The higher plasma vitamin D$_3$ level of the thick and sparse alpacas was also found post-shearing in the second study.

The hypothesis that shearing would lead to an increase in vitamin D$_3$ levels of alpacas with both fine and thick fibred fleeces was accepted. The vitamin D$_3$ levels of all the alpacas increased significantly after they were shorn with no change in ambient UV levels. The timing of shearing could be an important management tool in increasing vitamin D$_3$ levels. Because of the differences found in vitamin D$_3$ levels of the alpacas in these experiments that could not be explained by fleece characteristics, I looked for other characteristics that differed between the groups. A characteristic that emerged was wool coverage on the face as the fine and dense alpacas seemed to have woollier faces. Having a woollier face would expose less of their face directly to UV radiation, and correspondingly reduce the synthesis of vitamin D$_3$ in the skin than in the alpacas with thick and sparse fibre which had a barer appearance on their faces.
The UV radiation levels were five times higher in summer than they were in winter, which corresponded with the higher vitamin D levels in the alpacas tested in summer. In regions of the northern and southern hemispheres the vitamin D levels of alpacas, llamas and sheep are reported to fluctuate with the seasonal UV levels, unlike in the Andes where UV radiation does not change with the season and is consistently high due to the altitude and latitude (closeness to the equator) (Quarterman et al. 1964; Smith and Van Saun 2001; Parker et al. 2002; Judson et al. 2008). The alpacas tested in this study were very seasonal in their vitamin D levels, as they were at their lowest when tested in July (Australian winter) and were on the rise in October (spring) and then at their highest in the January (summer) sampling period. Of these sampling periods, the alpacas in the fine and dense group were classed as having insufficient levels of total vitamin D (below 50 nmol/L) in both winter and spring (pre-shearing). In the winter, 10 of the 16 alpacas have levels of vitamin D$_3$ that were below the total vitamin D insufficiency level of 50 nmol/L (Judson and Feakes 1999). If these alpacas were relying on natural forage in the winter, they would have been at risk of developing osteomalacia and generally have lower body mass maintenance and fibre production. However, the small amount of barley straw chaff provided to the alpacas must have contained some vitamin D$_2$, because total vitamin D was higher than vitamin D$_3$ alone, and it was enough to raise total vitamin D above the insufficiency level in two of those alpacas, highlighting the importance supplementary feed in winter (Garner-Paulin 2005). Nonetheless, 8 of the 16 alpacas were still deficient in total vitamin D.

The alpacas in this study had shorter fleece in summer, due to shearing the previous spring, than they did in the winter. We know that shorter fleece
provides less insulation against solar radiation than longer lengths of fleece (Chapter 4) and shorn sheep are reported to have higher vitamin D levels than unshorn sheep (Zintzen and Boyazoglu 1973). Therefore the influence of fleece length on vitamin D$_3$ levels in alpacas was further explored. As expected the vitamin D$_3$ levels did increase when alpacas were shorn, but what was unexpected was how quickly the plasma vitamin D$_3$ levels rose after shearing. I found that after shearing the plasma vitamin D$_3$ levels doubled by nine days after shearing. These results stand in contrast to the reports of a three month time-lag in vitamin D levels when UVB levels increase naturally with season (McMillan et al. 1995; Judson et al. 2008) and a reduction in cloud cover (Estupiñán et al. 1996). Given that vitamin D$_3$ levels in these alpacas were at their lowest in the winter sampling period, and it is known that even short lengths of fleece can provide some insulation for the alpaca (Chapter 3), producers may consider shearing their alpacas towards the end of winter (August in Australia). Shearing may be a very useful tool for producers to increase in vitamin D$_3$ levels in their alpacas without affecting the production efficiency of fleece length, as there could still be a fleece growth for a full year.

The relationship between the structure of the fibres in the fleece and vitamin D$_3$ had not previously been examined. The correlation of fibre thickness with follicle density, and the corresponding similarities in the cross-sectional area of the fleeces, suggests that UVB would penetrate similarly into the fleece of both fleece types. The structure of the fibres making up the fleece, the density and diameter of the fibres, does not seem to impact the amount of vitamin D$_3$ synthesised in the skin. Measurements of solar radiation penetrance into alpaca fleece indicate that the fleece provides a ‘blanket-effect’ from solar radiation reaching the skin and generating a heat load. Nevertheless, radiation is not all
absorbed at the outside of the fleece and it is fleece colour, not the fibre structure, which has the greatest influence on how deep radiation penetrates into the fleece (Chapter 4). Solar radiation penetrates deeper into light-coloured fleeces (Chapter 4). Since dark-coloured alpacas are found to have lower levels of vitamin D than lighter coloured alpacas (Smith and Van Saun 2001; Judson et al. 2008), it is likely that the penetration of UV radiation into dark-coloured fleeces is less than in light coloured fleeces. The ‘blanket-effect’ was illustrated in this study by the large increase in vitamin D$_3$ levels of the animals in spring after shearing, removing the layer of insulation between solar radiation and the alpacas skin. Given that the alpacas used in this study were all the same light-colour and had similar cross-sectional area of fibre, they all had the same potential for exposure to UVB through the fleece. However, the alpacas with thick and sparse fleeces had higher levels of vitamin D$_3$ at all sampling periods. What was different between the two fleece type groups that could account for this difference in vitamin D$_3$? The area of face-wool coverage was noted to be different among the alpacas, with the fine and dense group being more ‘woollier’ faced. Potentially this would reduce the area of ‘non-woolly’ skin directly exposed to solar radiation and perhaps these animals would be able to produce higher levels of vitamin D$_3$.

Sheep also have individual variability in face-wool coverage. Although face cover has never been directly linked to vitamin D or vitamin D deficiency (Terrill 1949; Cockrem and Rae 1966), there is circumstantial evidence that vitamin D levels might be influenced by the coverage of wool around the face in sheep. Australian Merino and New Zealand Romney ewes that have bare faces produce better growing lambs than ewes of the same breeds with woolly faces (Fail and Dun 1962; Cockrem 1968). Since vitamin D$_3$ is known to be important
for the growth of young animals it is possible that the better growth is a result of higher vitamin D levels subsequent to reduced face-wool coverage (Van Saun et al. 1996; Judson and Feakes 1999; Dittmer and Thompson 2011). Because alpacas tend to be vitamin D deficient, a more comprehensive study of the influence of face coverage on vitamin $D_3$ levels in alpacas is warranted.

5.5. Conclusion

The fine and densely fibred alpacas had lower levels of vitamin $D_3$ but we suspect that it was not structure of the fibres in the fleece that affected how much vitamin $D_3$ was synthesised by the alpacas, but more how the fleece was distributed. The fleece creates a "blanket-effect", which reduces the penetration of UVB radiation through the fleece to the skin. Longer fleece lengths provide a stronger shield-effect. The relationship between face-coverage and the plasma level of vitamin $D_3$ has not been described previously but needs to be investigated further to assess if selection for low percentage of face coverage could become an effective breeding strategy to reduce the need for vitamin D supplementation. The link between fleece colour, face-wool cover and vitamin $D_3$ synthesis should also be investigated due to the significant influence that fleece colour is known to have on vitamin D levels in alpacas.
6.1. Introduction

In the previous chapters it has been reported that alpaca fleece is an effective insulator against radiation and blankets out most radiation penetrating into the fleece (Chapter 4) and therefore fleece length, not fleece structure, was the only characteristic to have any effect on the vitamin D levels of alpacas (Chapter 5). I did find that the fleece distribution around the face was a strong indication for vitamin D levels. Given the importance of vitamin D for healthy livestock, this chapter reports on the experiment conducted to investigate the effect of face-wool cover on vitamin D levels. Additionally, due to the lack of effect of fleece colour on radiant heat load and the absorption of radiation at the surface of dark-coloured fleeces (Chapter 4), the effect of fleece colour on vitamin D levels was also investigated.

Alpacas (Vicugna pacos) are particularly susceptible to vitamin D deficiency in southern Australia. They are native to the altiplano plateaus of the Andes in South America, which range from 4,100-4,700 m above sea level and are at a latitude less than 30°S (Charrier and Muñoz 1997). At this altitude, ultraviolet radiation (UV, 200-400 nm) intensity on clear days can be up to 20% higher than at sea level (Cabrera et al. 1995). Although the radiation at this altitude is
high, the maximum ambient temperature is about 20°C in summer and daily
temperatures can fluctuate as much as 30°C, resulting in quite a cool climate
(Thomas and Winterhalder 1976; Bryant and Farfan 1984). In the Andean
environment there has probably been strong natural selection pressure for
alpacas to adapt to the cold and the high incident UV levels. The fleece
structure fits with that selection scenario, being thick and covering the entire
body except for small areas on the face and underbelly (Fowler 1994).

In the last 20-30 years, the high quality fleece of alpacas has been recognised
outside South America and alpacas have been exported to many countries to
establish alpaca fibre industries, including Australia and New Zealand (Wuliji et
al. 2000). In Australia, most alpaca production occurs in the southern part of the
continent at higher latitudes, but much lower altitudes than the altiplano.
Consequently, alpacas in southern Australia are exposed to lower UV intensity
than is normal on the altiplano. Ultraviolet B radiation (UVB, 280-320 nm) is
required by most mammals for the synthesis of vitamin D₃ in the skin. Vitamin
D₃ is crucial for body functions, particularly for bone mineralisation (Dittmer and
Thompson 2011). Small amounts of vitamin D₂ are available to herbivores from
sun-cured feeds but is a poor source for the maintenance of total vitamin D
levels required by alpacas (Garner-Paulin 2005). Alpacas have insufficient
levels of vitamin D during the winter and spring, when UV radiation is
particularly low (Judson and Feakes 1999; Judson et al. 2008). As a result,
there is a high incidence of metabolic bone disorders particularly in cria and
darker coloured alpacas (Hill et al. 1994; Van Saun et al. 1996; Garner-Paulin
2005). To prevent these bone disorders most producers supplement their
alpacas annually with a vitamin D injection (Judson and Feakes 1999).
The concentration of vitamin D in the plasma of alpacas is generally higher in summer than in winter due to more solar radiation of higher UV intensity, \textit{in situ} with a shorter length of fibre after being shorn in spring (Chapter 5, National Research Council 2007; Judson \textit{et al.} 2011). Dark-coloured alpacas tend to have lower concentrations of vitamin D because the melanin pigmentation in their fleece and skin absorbs UVB strongly and so competes with the sterol precursor and limits the synthesis of vitamin D$_3$ in the skin (Judson \textit{et al.} 2008; Dittmer and Thompson 2011). In winter, the concentrations of total vitamin D (D$_2$ and D$_3$) of most alpacas are insufficient and often considered as deficient, with less than 50 nmol/L in mature females (Van Saun \textit{et al.} 1996; Judson and Feakes 1999). For mature alpacas vitamin D concentrations of 100-250 nmol/L throughout the year are considered ‘normal’ and concentrations below 80 nmol/L are considered low (Oonincx \textit{et al.} 2010).

The previous study showed that the fleece structure (fibre diameter and density) did not influence vitamin D$_3$ levels in the plasma. However, in that study it was noted that the vitamin D$_3$ levels in plasma were related to the area of the face exposed to solar radiation (Chapter 5). In sheep, heavy wool cover on the face decreases the synthesis of vitamin D$_3$ (Fail and Dun 1962; Cockrem 1968; Horst \textit{et al.} 1981). In the present study the hypothesis that in winter, when UV radiation is low, mature alpacas with barer-faces, with either light or dark fleeces, will have higher levels of vitamin D$_3$ than those with more face-cover was tested. It was also hypothesised that the plasma concentration of vitamin D$_3$ will be lower in dark-coloured alpacas than in light coloured alpacas.
6.2. Materials and Methods

6.2.1. Experimental design

Light and dark-coloured Huacaya alpacas were grouped according to their percentage of face coverage. Plasma concentrations of vitamin D$_3$ and total vitamin D were measured in three plasma samples taken every second day during winter.

6.2.2. Animals, procedure and analysis

White, light fawn, dark fawn, brown and black female Huacaya alpacas from a breeding herd, plus two black males, were provided by Banksia Park Alpaca Stud. The alpacas all had a similar length of body fibre. The animals were kept as a herd and maintained on grassed paddocks with supplementary forage (barley straw), at Banksia Park Alpaca Stud (Serpentine, Western Australia; latitude 32°20’S; ~15 m above sea level) during July and August (winter). During the study the UV radiation level (mean ± SE) was 0.07 ± 0.004 W/m$^2$ and the minimum and maximum temperatures (mean ± SE) were 3.0 ± 1.5°C and 15.9 ± 0.96°C. All procedures were approved by the Animal Ethics Committee of The University of Western Australia (RA/3/100/1109). Body condition score was determined by palpating the muscle and fat coverage along the backbone, level with the last rib and given a scale from 1 = emaciated to 5 = obese (Fysh 2008).

The face cover of each alpaca was measured by photographing the face of the alpaca side on 40 cm away and calculating the percentage of the face and head that was covered with face-wool and that which was immediately exposed to solar and UV radiation as described in Chapter 5. Briefly, outlines of the face areas were traced on a digital photograph. The proportion of each of three areas was determined according to the level of wool coverage; exposed, face
fleece and head fleece. After printing these outlines onto 5 mm² graph-paper, the area of each type of face cover was measured and expressed as the percentage of the face and head covered in wool. Animals were labelled "woolly-faced" when more than 75% of the head was covered, "bare-faced" when less than 60% of the head was covered and "average coverage" when 65-70% of the head was covered (see Figure 6.1 for example)

![Woolly, Average, Bare](image)

**Figure 6.1** Examples of the face-wool cover of light and dark-coloured alpacas used to differentiate the groups used in this study.

A total of 30 light-coloured, white and light fawn, alpacas were allocated into three different face coverage groups (n = 10 per group); woolly-faced, average cover and, bare-faced. Ten alpacas of dark-colouring, dark fawn, brown and black, were allocated into two different groups according to the percentage of face coverage (n = 5 per group); woolly-faced and, bare-faced. Live-weight and body condition score were measured at the beginning of the sampling period. The initial live weight (mean ± SE) was 64.4 ± 1.7 kg and condition score was 2.4 ± 0.1 units and there was no significance between the groups.
Three blood samples were taken over five days (8 mL, Days 1, 3 and 5) during winter by jugular ventipuncture using Vacuette 9 mL lithium heparin tubes (Greiner Bio-One International AG, Kremsmuenster, Austria).

Plasma was separated by centrifugation at 1800 g for 10 minutes and stored at -20°C until analysis. Each plasma sample was analysed for vitamin D$_3$ (25-hydroxy vitamin D$_3$) and vitamin D$_2$ (25-hydroxy vitamin D$_2$ – for the calculation of total vitamin D) by LS/MS/MS mass spectrometry by PathWest (Royal Peth Hospital, Western Australia). In contrast to the most common commercial assays for vitamin D which do not discern between vitamin D$_2$ and D$_3$ in the measurement, LS/MS/MS mass spectrometry separates and measures the two forms vitamin D. Eight separate replicate analyses of quality control material were used to calculate the coefficient of the assays. The intra-assay %CV for vitamin D$_3$ was 3.7%, 3.2% and 2.1% for the concentrations of 25.0, 66.7 and 206.3 nmol/L and the inter-assay %CV was 6.8%, 6.8% and 4.9% over the concentration range of 26.3, 64.8 and 190.1 nmol/L. The intra-assay %CV for vitamin D$_2$ was 9.3%, 7.2% and 9.2% for the concentrations of 20.9, 60.4 and 127.7 nmol/L, and the inter-assay %CV was 11.4%, 9.2% and 8.9% over the concentration range of 29.6, 118.6 and 184.1 nmol/L.

6.2.3. Statistical analysis

The three samples taken over five days for each alpaca were averaged and those values were used to analyse vitamin D$_3$ and total vitamin D by two-way ANOVA with the face cover and fleece colour as the treatment variables. One-way ANOVAs were used to test within fleece colour and student’s t-tests were used to further identify differences within face cover groups of both fleece
colours. Data were analysed using GenStat v.14.11 (VSN International Ltd, United Kingdom).

6.3. Results

There was an effect of face cover (P=0.007), no significant effect of colour (P=0.07), and an interaction between the face cover and colour (P=0.03) for plasma concentrations of vitamin D$_3$.

In the light-coloured alpacas, the plasma concentration of vitamin D$_3$ was higher in the bare-faced group than in the woolly-faced (P=0.002; Figure 6.2A). There was no significant difference in vitamin D$_3$ between the average group and woolly-faced group (P=0.07; Figure 6.2A) or between the average group and the bare-face group (P=0.07; Figure 6.2A), although there is a visual difference between the groups and it is close to being significant.

In the dark-coloured alpacas, the level of face coverage did not affect the plasma concentration of vitamin D$_3$ (P=0.89; Figure 6.2A). The colour of the fleece of the alpacas had an effect on the plasma concentration of vitamin D$_3$ only in the bare-faced alpacas and not in the woolly-faced alpacas. The plasma concentrations of vitamin D$_3$ was higher in the light-coloured alpacas with bare-faces than in the dark-coloured alpacas with bare-faces (P=0.03; Figure 6.2A) but there was no difference between dark and light-coloured alpacas with woolly-faces (P=0.60; Figure 6.2A).

For the total vitamin D (D$_2$ and D$_3$) there was an effect of face cover (P=0.03), no effect of colour (P=0.46) and an interaction between the two (P=0.02). Within the light-coloured alpacas, there was an effect of face cover (P=0.004), with the
bare-face group having significantly higher vitamin D than both the average and the woolly-face groups (Figure 6.2B). In the dark-coloured alpacas there was no effect of face cover (P=0.70). The total vitamin D of all of the alpacas was below the insufficiency level of 50 nmol/L (Figure 6.2B).

**Figure 6.2** Plasma concentrations of vitamin D$_3$ (A) and total vitamin D (B) of light (light grey) and dark-coloured (dark grey) alpacas in the woolly, average and bare face-cover groups (± SE). Significant differences between groups within fleece colours are indicated by the italicised letters (lowercase for the light coloured and uppercase for the dark coloured) and significant difference between fleece colour is indicated by an *. Total vitamin D is separated into D$_2$ (top) and D$_3$ (bottom), standard error is for total vitamin D.
6.4. Discussion

The hypothesis that alpacas with bare-faces would have higher plasma concentrations of vitamin D$_3$ in both the light and dark-coloured fleeces in winter was partially accepted. There was an effect of face cover in the light-coloured alpacas, while the vitamin D$_3$ levels in the dark-coloured alpacas was not affected by face cover. The hypothesis that light-coloured alpacas would have higher concentrations of vitamin D$_3$ than dark-coloured alpacas was also partially accepted. The light alpacas with bare faces did have higher levels of vitamin D$_3$ than the dark-coloured alpacas with bare faces, but there was no effect of colour in the alpacas with woolly faces. My results indicate that the amount of skin on the head that is directly exposed to UV radiation in winter is the most important factor determining vitamin D$_3$ levels in fleeced alpacas that are unshorn, given the outcome from Chapter 5. However, there was no effect of face-cover on vitamins D$_3$ in dark-fleeced alpacas possibly because the melanin pigmentation in the skin absorbs the UV photons before photosynthesis can occur, rendering any effect of face-cover redundant. The lack of difference between the light and dark-coloured woolly-face alpacas is most probably due to being tested during winter when vitamin D levels in alpacas are naturally depressed. Although increased exposure on the face of light-coloured alpacas increases the amount of vitamin D$_3$ produced, my data suggests that alpacas are still at risk of vitamin D deficiency during the winter months and therefore should be supplemented.

Selecting for alpacas with less long fibre on the face, by increasing the skin exposure to UV radiation, improved plasma concentrations of vitamin D$_3$ for alpacas with light-coloured skin. It is well-know that the exposure of even a
relatively small surface of the body to UV radiation can stimulate vitamin D synthesis in both humans and farm animals (Zintzen and Boyazoglu 1973; Matsuoka et al. 1992). Dairy cows have a different coat type and internal fibre structure to alpacas since the hair fibres are flattened against their body and cows are able to synthesise vitamin D over their entire skin surface (Hymøller and Jensen 2010). Dairy cows covered in horse blankets, leaving only the head and neck or 24% of their surface area exposed, were found to have vitamin D levels 17% less than naturally uncovered cows, illustrating that although the head is only a small region of their body, vitamin D is still able to be synthesised (Hymøller and Jensen 2010). In comparison, a woolly-face alpaca has approximately 3% of their body exposed and a bare-face alpaca about 6% (including their ears), which is a very small proportion of exposure when compared with the blanketed dairy cow. Even so, bare-faced alpacas with only 6% of their body exposed were able to produce vitamin D₃ levels which were much closer to being sufficient that alpacas with woollier faces. In addition, alpacas have a fleece made of standing fibre (not flattened), and the length of the fibre can affect the capacity of animals to synthesise vitamin D, as the UV photons do not have to penetrate deep into a cows coat. This is best demonstrated when there is an artificial reduction of fibre length by shearing, which increased vitamin D levels substantially (Chapter 5, Zintzen and Boyazoglu 1973).

The darkness of the melanin pigment in the fleece and the skin impairs the capacity of alpacas to synthetise vitamin D₃ even if they had less face cover, because of the absorption of radiation by the melanin pigmentation of the skin and fibre. For example, in humans, black woollen clothing, which contains a variation of pigment, is the most effective blocker of radiation in the UVB and
UVC spectrums (Matsuoka et al. 1992). The time and intensity of exposure to UV radiation that is adequate for a human to reach sufficient levels of vitamin D$_3$ is positively correlated to the depth of melanin pigment or colour in the skin (Holick 1995). For alpacas in their native habitat of the Andes, the extreme UV intensity all year round does not limit vitamin D$_3$ synthesis in dark-coloured alpacas (Webb and Holick 1988). In alpacas based in south eastern Australia and north western United States, darker-coloured animals had lower plasma levels of total vitamin D than lighter-coloured animals (Smith and Van Saun 2001; Judson et al. 2008). Those studies were both carried out during spring and summer when vitamin D$_3$ levels would be in their annual peak, which is when the greatest difference between dark and light-coloured animals would be expected.

The intensity and length of radiation exposure is a limiting factor to the synthesis of vitamin D$_3$. The low levels of UV radiation during winter in Australia proved to be insufficient for vitamin D$_3$ synthesis resulting in plasma levels of vitamin D that are considered insufficient, even in light-coloured alpacas (Chapter 5, Judson et al. 2008). Despite the higher levels of vitamin D$_3$ in the light-coloured alpacas with barer-faces than those with dark-fleeces and face-wool, all animals tested in this study had deficient or insufficient levels, as per the suggested insufficiency threshold of 50 nmol/L (Judson and Feakes 1999; Judson et al. 2008). To prevent the risk of developing rickets or osteomalacia during winter, all alpacas in southern Australia still require a vitamin D supplementation in winter and should not rely on naturally produced vitamin D$_3$, but barer-faced light-coloured animals may only require a smaller dose.
6.5. Conclusion

Decreased face-wool increases vitamin D$_3$ levels of light-coloured alpacas but does not make any difference for dark-coloured alpacas. Manually reducing the length of fibre on the face of the woolly-faced alpacas might be an effective management tool for producers. All alpacas tested in this study were vitamin D deficient regardless of their face-cover. However, the higher levels seen in light-coloured alpacas with bare-faces may mean that they would require a smaller dose of vitamin D supplement annually.
CHAPTER SEVEN: GENERAL DISCUSSION

The aim of this thesis was to identify if specific selection for breeding objectives could be detrimental or beneficial to the physiological health of alpacas in southern Australia. This was achieved by testing how the fleece characteristics (fibre density, fibre diameter, fleece length and fleece colour) impact on the insulating ability of the fleece from radiant heat gain, the ability to exchange heat as sweat, and its consequential effect on vitamin D synthesis. My results suggest that the physiological health of alpacas raised in southern Australia to thermoregulate and produce sufficient levels of vitamin D is not affected by the fleece type, but instead it is the length and the colour of the fleece that may have consequences on animal health. Therefore, a specific breeding selection of alpacas towards a higher quality (finer) fleece and density of fibres should not be detrimental to alpaca health. The factors of greatest importance to maintain heat balance and vitamin D levels are management practices, regarding the appropriate timing of shearing for both insulation against radiant heat and the natural increase of vitamin D levels, and the administration of vitamin D supplements for animals identified to be at a higher risk.

The fleece length of alpacas was found to be the most influential characteristic, affecting both radiation penetration to the skin and the availability of naturally synthesised vitamin D. Longer fleece increased the insulation of the fleece from radiant heat, that then reduced the heat stress on the body when ambient temperatures approach or exceed body temperature, as the radiant heat forced upon the animal from solar radiation is not adjacent to the skin level and should
prevent any unnecessary induction of evaporative heat loss. Unfortunately, a limitation of my research is that I was not able to test the influence of fleece length on evaporative heat loss \textit{in vivo}. However, Cheviot sheep (with thick fibres) ranging in wool length from 1 to 10 cm in length displayed similar sweat rates despite the length of the wool (Brockway \textit{et al.} 1965). Several differences between sheep and alpaca fleece have been found in these studies and so I am are unable to conclude whether longer fleece limits or benefits evaporative heat loss via sweating in alpacas. However, it can be concluded that having longer fleece reduces the radiant heat load of the fleece and therefore should prevent heat stress.

Longer fleece also reduces the radiation seen at the skin surface, and limits the synthesis of vitamin D. Once shorn, the vitamin D levels of alpacas promptly increases. The alpacas in these studies were shorn in mid to late spring, and their vitamin D levels rose from insufficiency to near insufficiency levels, pre-shearing, to significantly higher and adequate levels post-shearing. Given the impact of shearing on vitamin D levels, I would recommend that alpacas are shorn earlier in spring to promote adequate levels forthwith from winter, when vitamin D tends to be deficient. In addition, early spring shearing would allow more time for the fleece to regrow and provide an adequate length for insulation from radiant heat. Vitamin D levels in summer are normally very high (due to the higher UV radiation intensity levels) and my data suggest that having longer fleece to insulate the body from heat stress wouldn't put alpacas at any risk of becoming vitamin D deficient.

Alpacas with less face-wool (longer and more abundant wool on the face) had consistently higher levels of vitamin D than those with woolly-faces. Given that
the fleece insulates against radiation penetrating through to the skin level (at least when the fleece is longer than 10 mm), the face is the only exposed area of the body that is always available for vitamin D synthesis. The woolly-faced alpacas in my studies had consistently lower levels of vitamin D than their barer-faced counterparts, and during the winter sampling period (Chapter 5), some of the alpacas with the most face-wool had vitamin D levels below critical level. The amount of face-wool is a feature that can be easily identified by producers who can then manage these alpacas better by either providing a higher supplement dose or by manually removing some of the face-wool to expose more skin directly to radiation. Increased face-wool tended to be associated with the alpacas with fine and dense fleeces, indicating that there may be a genetic link between face-wool cover and fibre density and thickness. The suggested management tools of extra supplementation or clipping wool from around the face would not affect breeding programs to produce high quality and quantity alpaca fibre.

The fleece colour did not negatively influence the radiant heat load on the alpacas as I thought it would, but it did have a negative effect on the natural synthesis of vitamin D. Darker coloured fleece absorbs more radiation than lighter fleeces due to the higher concentrations of melanin pigment in the fibre (and skin). Darker pigmentation causes both the radiation to be absorbed by the fibre closer to the fleece tip and not penetrate deeply into the coat, thus lowering the radiant heat load, and prevents UV radiation being absorbed by the skin for the synthesis of vitamin D for alpacas with all types of face-cover. The implications from this is that producers should provide their dark-coloured alpacas with a higher or more frequent dose of vitamin D supplements during winter when they are not able to produce sufficient levels on their own.
Additionally, the dark-coloured females used in the Chapter 6 study were to be mated soon after the experiment finished. As alpacas have an 11 month gestation, and vitamin D is unable to cross the placenta from mother to young, their cria would be born the following winter with very low levels of vitamin D and be at high risk of developing rickets. If the mother is also low in vitamin D, then the milk provided to the young would not be very rich in vitamin D, calcium and phosphorus to promote strong bone development and muscle growth of the cria. Alpaca producers should therefore ensure that all breeding females, of any fleece colour that are either pregnant or lactating, are adequately supplemented with vitamin D to ensure the healthy growth of their young and the maintenance of their own levels. Further research may involve the investigation of the vitamin D status of mothers on the young and their growth (furthering the work of Judson and Feakes (1999), as well as the best delivery method for vitamin D supplementation, whether it would be from a dietary or injectable vitamin D.

The fleece type, a combination of the fibre density and fibre diameter characteristics, did not have any significant effect on the overall physiological health status of alpacas in southern Australia. Fibre diameter (thickness) and fibre/follicle density were related in all studies, with alpaca fleeces consisting of fine fibres being more densely fleeced, and vice versa, which is also seen in sheep (Young and Chapman 1958; Hynd 1994; Moore et al. 1998; Adelson et al. 2002; Ferguson et al. 2012). Denser fleeces were slightly more insulative than sparser fleeces, particularly when exposed to strong air movement but this did not result in a lower radiant heat load than sparse fleeces, indicating that neither fleece type would be more susceptible to heat stress. Therefore breeding selection for fine and dense fleeces would not be detrimental to alpaca's health.
The fleece type should not affect the sweating potential of alpacas and should not provide any advantage if they were to become heat stressed and required to sweat to dissipate heat. Alpacas with fine and dense fibres had more sweat glands, over a given area, than alpacas with thick and sparse fibres and, presuming the functionality of the glands, should be able to sweat out heat over the body surface (if heat induced) and not just through the thermal window region on the underbelly. However, it isn’t known what rate of sweat production would occur from the glands, or the thermoregulatory efficiency of sweating into the fleece would be. Future research should investigate the sweating capacity of alpacas when heat stressed and the dispersal of the sweat from the fleece at different fleece lengths to give an indication of the ability to dissipate heat when stressed. Methods to test the functionality of sweat glands include taking skin biopsies from various body regions and sectioning the samples vertically so that a whole profile of the sweat gland may be seen and the size and shape of the gland may be measured, and also the amount of sweat produced (indicating activity of the glands) could be measured by attaching enclosed cups containing silica beads on small areas of shorn skin of alpacas and measure the difference in weight of the silica beads absorbing the sweat over a period of time.

Vitamin D levels were also not affected by the fleece type, but this was not surprising given how efficient all fleeces were to insulate against solar radiation penetration. A speculative relationship between fleece type and vitamin D could perhaps be the size of the sebaceous glands associated with the fibre follicles. The sebaceous glands produce the precursor (7-dehydrocholesterol) for pre-vitamin D₃ in the skin and if these glands differed among fleece type perhaps less or more of the precursor may be produced. However, the face-wool cover and fleece length (as well as the environmental UV intensity levels) remain as
the primary fleece related determinants of vitamin D levels of alpacas. Further research may investigate the effect of face-wool cover on juvenile alpacas, as less face-wool may promote a superior growth of both bone and muscle than individuals with woolly-faces and these animals may therefore be more productive as adults. The implications for potentially identifying if face cover effects growth in juveniles and productiveness in adults may identify some masking of genetic superiority as well as identify juvenile animals which need some additional supplementation in order to allow them to reach their full genetic potential. In turn, identifying genetic potential of animals as juveniles will assist the national industry to breed towards the highest genetic quality and quantity fleece possible.

In conclusion, breeding selection towards higher quality and quantity of fleece does not appear to have negative effect on physiological health parameters measured in his study for alpacas raised in southern Australia. Husbandry practices are the key to healthy livestock and producers should carefully manage the timing of shearing, to both increase vitamin D levels in spring and ensure that there is adequate regrowth of wool for insulation from the radiant heat in summer. Supplementation of vitamin D should be provided to all alpacas during winter, but additional supplementation should also be provided to those alpacas that have been identified to be at a higher risk of deficiency.
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APPENDIX ONE

High follicle density does not decrease sweat gland density in Huacaya alpacas
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ABSTRACT
When exposed to high ambient temperatures, mammals lose heat evaporatively by either sweating from glands in the skin or by respiratory panting. Like other camelids, alpacas are thought to evaporate more water by sweating than panting, despite a thick fleece, unlike sheep which mostly pant in response to heat stress. Alpacas were brought to Australia to develop an alternative fibre industry to sheep wool. In Australia, alpacas can be exposed to ambient temperatures higher than in their native South America. As a young industry there is a great deal of variation in the quality and quantity of the fleece produced in the raised flock. There is selection pressure towards animals with fine and coarse fibre. Because the fibre from secondary follicles is finer than that from primary follicles, selecting for finer fibres might alter the ratio of primary and secondary follicles. In turn, the selection might alter sweat gland density because the sweat glands are associated with the primary follicle. Skin biopsy and fibre samples were obtained from the mid-section of 33 Huacaya alpacas and the skin sections were processed into histological sections at the sebaceous gland level. Total, primary, and secondary follicles and the number of sweat gland ducts were quantified. Fibre samples from each alpaca were further analysed for mean fibre diameter. The fibre-finned animals had a higher total follicle density ($p < 0.001$) and more sweat glands ($p < 0.001$) than the thicker-finned animals. The fibre diameter and total follicle density were negatively correlated ($r^2 = 0.56, p < 0.001$). Given that the fibre-finned animals had higher follicle density and more sweat glands than animals with thicker fibres, we conclude that alpacas with high follicle density should not be limited for potential sweating ability.

1. Introduction

Alpacas (Vicugna pacos) originated on the altiplano plateaus of the Andes mountain range in South America. The area is a dry semi-arid environment characterised by seasonally low average temperatures of $0-20^\circ C$ in summer and $-11$ to $-13^\circ C$ in winter (San Martin and Bryant, 1980). Evolving in this environment it might be expected that the alpaca has not been exposed to selection pressure for an ability to deal with heat stress (Hoffman, 2003). Although very adaptable animals, the exposure of alpacas to a warmer climate may stretch their heat loss abilities because heat loss can be limited in fleece animals (De Lamo et al., 2001). Although there are a few records of alpacas presenting heat stress symptoms, the Australian alpaca fibre industry was established as an alternative to sheep wool, due to the light, soft, and warm wool-like fibre produced by alpacas (McGregor, 2006). In Australia, alpacas are most commonly farmed in southern regions and are exposed to a climate with higher ambient temperatures in summer than those of the altiplano. For example around Perth, South Western Australia ($31.95^\circ S, 115.86^\circ E$), the average summer temperature (mean minimum and mean maximum) ranges from $18^\circ$ to $31^\circ C$, and in Bendigo, central Victoria ($36.75^\circ S, 144.27^\circ E$), $13^\circ$ to $29^\circ C$ (Australian Bureau of Meteorology). The average maximum temperatures in Australia are thus higher than those experienced on the altiplano. Because temperatures in southern Australia can induce heat stress in sheep and goats (McGregor, 1985; Stoddart, 1996) it is therefore very likely that alpacas will need to sweat to maintain heat balance.

In fleece animals, the fleece originates from two types of fibre follicle, the primary and the secondary follicles. The follicles are differentiated by the order of their formation in the skin during foetal development and by the glands associated with each the follicles (Moore et al., 1998). follicles are arranged in the skin as

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follicle bundles, with many secondaries clustered around a single primary. The fibre produced by a primary follicle is typically larger in diameter, and thereby lower in commercial fibre quality, than the fibre produced by the secondaries (Hardy and Lyne, 1956; Moore et al., 1996). Associated with the primary follicle, and located in the epidermis alongside the primary follicle, are sweat glands. These glands produce a fluid, derived from the extra cellular fluid that is secreted onto the skin surface. When the fluid evaporates, cooling of the skin occurs (Alder et al., 1997). Camels, like many other mammals, possess a terminal window of the sweat gland located on the underbelly and inside the thighs, where the fascia is sparse, and more heat-labile and evaporation is enhanced by greater air movement near the skin (Alder et al., 1997; de Lano et al., 2001; Forester, 1984). Although there are fewer follicles and fibres in this region, the follicles that are present are nearly all primary follicles and the sweat glands associated with those follicles are larger and more developed than those elsewhere on the body (Alder et al., 1997; Fowler, 1964). Although the sweat glands are larger and more developed in the thermal window region, the sweat glands on the rest of the body are also involved in thermoregulation (Alder et al., 1997).

Studies on the skin morphology and thermoregulation of other camels, including camels, llamas and guanacos, show that the sweat glands over the entire body are active secretory cells. Further these species all sweat in response to high temperatures although the sweating capacity is lower than it is in some other species, such as horses (Allen and Bigg, 1969; Alder et al., 1997; de Lano et al., 2001; Dowling and Noy, 1962; Fowler, 1961). Sweating has never been measured or reported in alpacas. It is highly likely that alpacas sweat in response to heat stress. Sheep and goats, which are known to pant in preference to sweating the evaporative cooling, have functional sweat glands and do sweat when induced (Fowler, 1964; Geiker, 2016; Roberts and Swain, 1968). Some physiological studies have suggested that alpacas might not use their sweat glands as secretory cells for thermoregulation (Frisa and Scott, 2010; Maitland and Cleva, 1973), but the conclusion was based on limited data and since alpacas are rarely observed to pant it is logical to assume that alpacas use sweating like other camelids.

The mean fibre diameter of the national flock of alpacas in Australia is very variable, ranging from 17.7 to 48.4 μm (McGregor and Burke, 2004), with a minimum fibre diameter and range similar to that reported in Peruvian Huacaya (Montes et al., 2008). In contrast, Merino sheep have been bred to have fibre diameter tending between 17.5 to 23.0 μm (Nalbandian and Gooch, 2003). Since the introduction of the alpaca industry in 1987, breeding to improve the quality (fibre diameter) and quantity (fibre density) of fibres produced in Australia has been directed towards decreasing the diameter of the primary and secondary fibres produced from the follicle and increasing the number of fibre follicles produced in the skin. Such selection may possibly reduce the number of primary follicles, and the associated sweat glands per unit area. The aim of this study was to test the hypothesis that alpacas with finer fibre would have fewer primary follicles and thereby sweat glands, than alpacas with thicker fibres. A decrease in sweat gland density would mean that sweating for fibre fineness may potentially be detrimental to alpacas in the Australian environment.

2. Materials and methods

2.1 Experimental design

Skin biopsy samples were taken from mature Huacaya alpacas and horizontally sectioned. The primary and secondary follicle density, the sweat gland duct density and the secondary to primary follicle ratio were determined. The fibre diameter of each animal was analysed from a fibre sample taken from a mid-side patch.

2.2. Biopsy samples

All animals sampled for this study were adult Huacaya alpacas, mean age 4.1 ± 0.6 years old, from Banania Park Alpaca Stud, Serpentine Western Australia. Four dark and light-coated males and 29 light-coated females were used and skin biopsy samples were collected over a five year period. All surgical work was carried out by an experienced veterinarian and the samples were used as per permission granted from the University of Western Australia’s Animal Ethics Committee at the University of Western Australia’s Animal Ethics Committee. Approval 9/21/2011/003.

2.3. Sampling procedure

Alpacas were sedated by intramuscular injection of a combination of 1 mL of ketamine (100 mg/mL) and 0.6 mL of xylazine (100 mg/mL), and laid out in lateral recumbency with a neck-collar supporting the head positioned to prevent choking on rumen reflux, as standard practice by the veterinarian who obtained the biopsy samples (a practice similar used in equine anaesthesia hydro-pool) (Hockley and Hatherley, 2008). A 10 x 10 cm2 mid-side patch of skin was clipped, level with the last rib and about 20 cm ventrally from the spine. The fibre sample was tagged for analysis and the area was washed with a clotted (20 mL per 1 L water) chlorhexidine skin scrub (chlorhexide-C. 5 mg/5 ml chlorhexidine gluconate) (Zenra, NSW Australia). Either an 8 mm or a 10 mm biopsy punch was used to remove a mid-side skin sample, which was placed in a labelled tube filled three quarters with formaldehyde fixative. The biopsy site was sutured closed with a half curved cutting needle using 4/0 Chromic catgut. Alpacas were then monitored until they reached full consciousness and were able to stand unaided.

2.4. Microscopy protocols and analysis

The biopsy samples were fixed in a formaldehyde solution for a period ranging from eight months to five years. The samples were rinsed in normal saline (0.9% NaCl) to remove the formaldehyde and the remaining waxed samples was trimmed with a scalpel blade before the sample was placed in 70% ethanol overnight to be processed through a Leica ASP900 Tissue Processor the next day, which involved 10 stations; five stations of three concentrations of ethanol (in the order of 70%, 90%, 100%, 100% and 100%), two stations of toluene and three stations of mordant paraffin wax which lasted from 20 min to 2 h depending on the station and solution. On removal, the samples from the Tissue Processor were placed in a tray of 80°C molten paraffin wax to avoid transverse embedding into block moulds. Wax blocks containing the skin samples were then trimmed, and 7 μm transverse sections were taken at the mid-sebaceous gland level using a Leica RM2255 microtome. The sections were floated in lukewarm water onto slides and dried at 48°C overnight in a drying oven. The sections were then stained using a Haematoxylin, Eosin and Picric Acid method, modified slightly from Hockley and Hatherley (2008) and a coverslip was secured using DPX mountant (Bielak Ltd, New Zealand; supplied by Thermo-Fisher, Australia). The method was modified by using toluene instead of xylol, for the dewaxing and clearing before mounting stages, and an ethanol wash was included between raising in the alkaline bluing solution and the resin hardening stages.

The follicle density and the sweat duct density were determined on microphotographs taken with a digital camera (Pentax OptioWf) mounted onto an Olympus CH5 microscope.
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2.5. Fibre analysis

The fibre samples taken at the time of skin sampling were
analysed using an OFDA 2000 optical-based fibre diameter
analyzer, (ESC Electronics, Perth, Western Australia) by Microfibre
(Fibra Ltd, Western Australia), which provided data on the mean
fibre diameter at the time of skin sampling. The cross-sectional
area of the fibre: dividing the fibre into proportions of fibre and
air space between the fibres for a given skin area, was calculated
using the mean fibre diameter in µm (MFD) and total follicle
density in follicles/mm² (TFD). Equation given below. As the
samples were taken at random times, the time since shearing
was not consistent and so the total volume of the fibre using
the fibre length could not be calculated. Therefore the cross-sectional
area (CSA) provides a reasonable indication of how much of the
fibre ‘volume’ is fibre (Henderson and Rayman, 1966).

\[
\text{CSA}_{\text{fibre}} (\mu) = \left( \frac{\text{MFD}}{2000} \right)^2 \times \text{THI} \times 100
\]

2.6. Statistical analysis

Linear regression analysis (Gerstal v1.4) was used to analyse
the relationships between total follicle density, primary follicle
density, secondary follicle density, sweat duct density, secondary
to primary follicle ratio (S:P ratio), mean fibre diameter and the
cross-sectional area.

3. Results

The follicle bundles from the alpacas in this study consisted of
one primary (P) follicle surrounded by secondary (S) follicles, as
previously described by Antonini et al. (2004) and Ferguson et al.
(2012). (Fig. 1) The number of S follicles associated with a P follicle
ranged from 4 to 20. In four of the very low follicle density ani-
mals, there were some sections that had a lone P follicle that
tacked associated S follicles, but these were rare and were not
included in the S:P follicle ratio counts. Most (75%) of the P follicles
had a sweat gland duct visible and there were less than four
sebaceous glands visible per follicle bundle, usually only asso-
ciated with the P follicle (Fig. 2). The densest follicle animals
(Fig. 2A) had smaller follicles than those more sparsely follicled
animals (Fig. 2B).

There was a strong association between the MFD and the TFD,
with the animals that had a lower MFD being more densely
focussed than animals with higher MFD (Fig. 3A; \( R^2 = 0.64; P<0.001 \)). The alpacas with finer fibre also had a higher density
of both S and P follicles, and sweat gland ducts, than animals with
thicker fibres (Fig. 3B; \( P<0.001 \) for all). The slope of the
relationship between fibre diameter and follicle density was
steeper for the S follicles than for the P follicles, meaning that the
S:P ratio was also higher in animals with finer fibre (Fig. 3E; \( P<0.001 \)).

Looking at the characteristics of the follicles, the S follicle
density was nearly perfectly correlated with the TFD: alpacas with a
higher follicle density had more S follicles (Fig. 4A; \( R^2 = 0.908; P<0.001 \)).
The P follicle density, sweat gland duct density and the S:P ratio were also higher in animals with a higher follicle density
than in animals with sparser follicles (Fig. 4B-D; \( P<0.001 \) for all). The
CSA of the fibre that was fibre was not higher for alpacas with a
higher TFD (Fig. 4E; \( R^2 = 0.0130; P = 0.52 \)). There was a sig-
nificant positive relationship between the sweat gland duct
density and the P follicle density indicating that there was one sweat
gland duct per P follicle (Fig. 4F; \( R^2 = 0.75; P<0.001 \)).

4. Discussion

The hypothesis that alpacas producing a fibre with finer fibre
would have fewer sweat glands, over a given area, than animals
with thicker fibres was rejected. The number of sweat gland ducts
was positively correlated with the number of primary follicles, and
while the secondary to primary follicle (S:P) ratio was higher in
alpacas with finer fibres, the increase in total follicle density in
the finer fibred animals more than outweighed the increased S:P
ratio (including these samples with low primary follicles).

Fig. 1. Photomicrographs of a skin section taken from a Huacaya alpaca showing
two follicle bundles (outlined in red) with the primary and secondary follicles,
the sebaceous glands, and the sweat gland ducts. (Key interpretation of the reference to
colour in this figure legend, the reader is referred to the web version of this article.)

TFD=88.6 MFD=15.6 S:P=16.7:1
TFD=24.7 MFD=28.9 S:P=6.7:1

Fig. 2. Photomicrographs of the skin from two alpacas shown at the same magni-
fication. One alpaca with a fine and dense fibre (A) and the other with a thick
and sparse fibre (B). Total follicle density (TFD), mean fibre diameter (MFD) and
secondary to primary follicle ratio (S:P) of both animals are indicated underneath.
result was that alpacas with finer fleece had more sweat gland ducts per unit area of skin than alpacas with thicker fibres. The total follicle density of the animals in this study was higher than has been reported in the literature (Antonini et al., 2004; Ferguson et al., 2012). Our findings indicate that the potential sweating ability of alpacas should not be hindered by increased fibre density.

In the animals with high follicle density, not only was there a greater abundance of secondary follicles per primary follicle, but there were more primary follicles given area, than in the animals with low follicle density. Because the increase in density was due predominantly to an increase in the abundance of secondary follicles, the fibre of those animals was finer than in those alpacas with sparser fibres. Similar results have been reported in sheep (Adams and Cronje, 2003; Moore et al., 1996; Purvis and Swan, 1997), where the relationship between follicle density and fibre diameter is explained by the pre-papilla cell theory.

Pre-papilla cells form the basis of all hair or wool fibre follicles in animal skin. It is suggested that each animal has a programmed number of pre-papilla cells that mass at 'initiation sites' and are available for follicle formation approximately equidistant from each other during foetal development (Moore et al., 1998). The pre-papilla cell theory states that as follicle density increases the number of pre-papilla cells available to form each follicle decreases, whether it be a primary or secondary follicle, and the fibre that is produced from that follicle becomes finer (Moore et al., 1998). The pre-papilla cell theory provides a basis for producers aiming to improve the quality (fibre diameter) and quantity (fibre density) of fleece produced by their alpacas. Therefore the main focus of further developing the quality and quantity of fibre produced within the industry can be towards increasing the
follicle density, in that it promotes finer fibre, without reducing the number of primary follicles. In fact, our data suggest that such selection increases primary follicle density as well.

Alpacas that had high follicle density also had both more primary follicles and more sweat gland ducts per unit area. The ratio of primary follicles to sweat gland ducts found in this study was close to one, which is consistent with current literature for the skin histology of South American camelids (Adler et al., 1997; de Lamo et al., 2001; Ferguson et al., 2012). What has not been determined in camelids however, is whether a higher follicle density affects the size of the sweat glands present.

In Merino sheep the wool follicles are packed tightly together due to the high density of follicles, resulting in sebaceous glands that are slender and oblong (Nay, 1966). If the same applies to sweat glands, then it is likely that the sweat glands in the skin of animals with a high follicle density will be smaller (Moore et al., 1998). But this does not necessarily mean that reducing the size of the sweat glands will reduce the sweating capacity of the animal. Egyptian cattle have six times the number of sweat glands (although smaller in size) over a given area than Egyptian buffalos, although the cattle have finer, denser fibres than the buffalo (Hafiez et al., 1955). Therefore increasing follicle density and theoretically reducing the size of sweat glands should not be detrimental to the ability of the alpacas to release heat as assumed, presuming that the glands produce sweat to their full capacity.

In the present study the total follicle density of the densely fleece alpacas was higher than values that have previously been reported for alpacas (Antonini et al., 2004; Ferguson et al., 2012). That improvement may be due to genetic selection, but the follicle density in Merino lambs can be increased by increasing the energy intake of the ewe during late pregnancy, or of the lamb up to 18 weeks after birth (Kelly et al., 2006). The mechanism seems to involve the production and survival of all potential secondary follicles (Kelly et al., 2006; Schinzel 1955). Primary follicles, on the other hand, are not altered by nutrition and appear to be genetically programmed, setting a limit to the number of follicle groups that an animal has (Corbett, 1979). Since 90% of the animals used in the present study were stud animals (animals specifically selected for breeding purposes due to their genetic potential), it is highly likely that their mothers were intensively managed during pregnancy and that additional nutrition was supplied during late pregnancy and lactation. Thus it is possible that the fleece characteristics we observed were the result of good management and not necessarily a reflection of improvement in the gene pool. However, if the primary follicle density is genetically determined in alpacas, as it is in sheep, then the higher primary follicle density in these alpacas with finer and denser fibres suggests that genetic improvement has indeed occurred. That our values are higher than previously reported could also reflect the genetics and/or nutrition of the animals previously tested. The alpacas in previous studies were probably not intensively selected or managed for fleece properties (Antonini et al., 2004; Ferguson et al., 2012). If alpaca producers could identify when, during late pregnancy, nutritional manipulation is important, and which feed supplement to use, secondary follicle density could be increased as observed in sheep. Supplementary feeding during pregnancy across a whole breeding flock would also help identify the offspring with the highest genetic potential. Then stud breeders across the industry could fast-track their selective breeding programs to improve the quality and quantity of fibre produced.

It is not known how much alpaca fleece acts as a barrier to evaporative heat loss. It has been reported that as sheep and goats sweat is not easily dispersed from the skin through the fleece (Robertshaw, 1968); but in llamas it is reported that the fibre produced by the thicker primary follicles may act as a wick at the skin level draining the moisture produced by sweat glands up along the fibre, dispersing sweat through the thick coat (Adler et al., 1997). It is unknown if a reduction in the size of the primary fibre, as would be the case in our alpacas given the increase in follicle density, might limit the effectiveness of moisture loss due to the "wick effect". The length of the fibre would also be a major influence on how well moisture is dispersed from the skin, and the distance at which the wick effect is effective along the fibre.

Sweating through the fleece would be a secondary result of evaporative heat loss, given that alpacas have a thermal window region on the underline and flanks for heat exchange (Fowler, 1998; Gerken, 2010). Alpacas might only be required to sweat through the fleece if exposed to extreme heat stress as suggested by our data. The cross-sectional area data indicates that although the fibre yield was higher for the fine and dense fleeces than the thicker and coarser, the volume of fleece per unit area of skin was not different between the fleece types, as the thicker fibres take up the same amount of space as several finer fibres. Therefore the air to fibre volume ratio of the fleece was the same and the 'barrier' effect of the fleece on sweat evaporation and water vapor dispersion should be similar between the fleece types, dependent on course of length of the fleece. In turn, the length of the fleece affects the amount of sweat produced by sheep exposed to the sun, with lower sweat rate in sheep with longer fleece (Hedley et al., 1989), but because the increase insulative ability of longer fleece reduces the radiant heat load (Parer, 1962). As camelids are not known to pant for evaporative heat loss purposes (de Lamo et al., 1991; Rosenmann and Morrison, 1963), a more comprehensive study is needed on the effect of the fleece as a barrier for evaporation. Because a large proportion of an animal's heat load can be due to direct solar radiation, it also would be useful to know how the fleece characteristics (fibre density, fibre diameter and fibre length) affect the ability of the fleece to insulate against radiant heat, thereby reducing the need for sweating.

Acknowledgements

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References


APPENDIX TWO

Equation for total pelt insulation:

\[ I_{\text{total}} = \frac{1}{\text{total conductance}} \]

Total conductance = \( \frac{\text{heat flow}}{(T_{sk} - T_a)} \)

Equation for fleece insulation:

\[ I_{\text{fleece}} = \frac{1}{\text{fleece conductance}} \]

Fleece conductance = \( \frac{\text{heat flow}}{(T_{sk} - T_{tip})} \)

Equation for air boundary layer insulation:

\[ I_{\text{ABL}} = \frac{1}{\text{ABL conductance}} \]

ABL conductance = \( \frac{\text{heat flow}}{(T_{tip} - T_a)} \)
## APPENDIX THREE

Pelt Characteristics:

<table>
<thead>
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<th>Pelt #</th>
<th>Colour Group (Light or Dark)</th>
<th>Mean Fibre Diameter</th>
<th>Fibre Density</th>
<th>Cross Sectional Area (% as fibre)</th>
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