REDUCING STRESS IN SHEEP BY FEEDING THE SEAWEED *ASCOPHYLLUM NODOSUM*

A Dissertation

by

GREGORY SCOTT ARCHER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2005

Major Subject: Animal Science

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ABSTRACT

Reducing Stress in Sheep by Feeding the Seaweed *Ascophyllum nodosum*.

(August 2005)

Gregory Scott Archer, B.S., Virginia Polytechnic and State University;

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Feeding the extract of the seaweed *Ascophyllum nodosum* (ANOD) has been shown to mediate the response of livestock to certain environmental stressors. To determine if feeding ANOD is useful in alleviating handling and transport stress, two trials were conducted. The dose response trial was conducted to determine at which rate ANOD should be fed to obtain beneficial results. Forty-four lambs received ANOD at either 0 (control), 0.5, 1, or 2% of dry matter intake per day (approximately 0, 0.25, 0.5, and 1 g/kg of body wt per day). Sheep were administered ANOD twice daily for 14d. After 14d of supplementation, IgG and IgM antibody response to ovalbumin was reduced by ANOD. The sheep fed at the 2% rate had a narrower range of body temperature during transport than controls. The 2% rate also had lower body temperatures than the controls during times when the thermal heat index was above 80. The sheep fed the 2% rate had lower cortisol and aldosterone concentrations during walking and transport compared to the controls. Post transport, sheep supplemented at the 1 or 2% rates were less dehydrated as indicated by plasma chemistry profiles and

electrolyte concentrations. In a subsequent trial, the major components of the ANOD (fucoidan, salt, and betaine) were fed to determine which, if any, were responsible for the treatment effects in the dose response trial. After 14d supplementation, the salt and ANOD sheep had a depressed IgG and IgM antibody response to ovalbumin and an increase in white blood cell counts and lymphocyte numbers compared to controls. The ANOD sheep were generally lower in body temperature than the other treatments during transport. The ANOD and salt sheep had lower cortisol concentrations compared to controls. At the end of transport, sheep supplemented with ANOD or salt had lower electrolyte concentrations than control sheep. Supplementation with ANOD was associated with lowered body temperature; however, it also suppressed antibody titer which could leave animals susceptible to bacterial infection. The lowered antibody production is of concern and needs further study before ANOD can be recommended as a useful stress management tool.

DEDICATION

This dissertation is dedicated to all of the sheep that participated in this study. I will have many fond memories of them. Also, this is dedicated to my beloved wife Larisa for being supportive of me throughout my education and for always being there for me.

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I first have to thank my family and God. They were there for me when I needed to talk and always willing to give advice and support. My parents have always helped instill in me a desire to pursue my dreams They have taught me that hard work does pay off and that there are awards, both in the pursuit and in the attainment of one's goals. I would also like to thank Larisa for being there for me and supporting me during my education. Without my loved ones, none of this would have been possible. I hope I have made them all proud.

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INTRODUCTION

The processes of handling and transporting livestock are known to be stressful events for livestock (Kent, 1997; Broom, 2003; Cockram, 2004; Dwyer and Bornett, 2004). Numerous research projects have sought to reduce or alleviate this stress. Researchers have administered injections of tyrosine or naloxone (Tayeb and Marzouki, 1992; Ali et al., 2001), investigated the effect that stocking density (Tarrant, 1990; Cockram et al., 1996), the orientation of animals (Clark et al., 1993; Toscano and Friend, 2001), the length of transportation, the road texture (Hall et al., 1998c; Ruiz del la Torre et al., 2001), and rest periods (Ibanez et al., 2002) as means to reduce stress. However, most of these factors are difficult to control within the animal industry. If animals could be fed a supplement prior to transport that could alleviate some of the causes of stress during transport while also improving the health of the animals, such a supplement would be extremely valuable to producers.

An extract of the seaweed *Ascophyllum nodosum* (ANOD) has been shown to have positive effects on an animal's physiology during hot weather and in relation to fescue toxicity (Allen et al., 2001b). Some of these positive effects could decrease the stress and/or increase the welfare of animals being transported during hot weather.

Two trials were conducted to ascertain whether ANOD would be a useful to reduce stress during transport and handling. The first trial was a dose response trial that determined if ANOD influenced the physiology of sheep under exercise and transport stress, thereby reducing stress and mediating body temperature and hydration. An

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This dissertation is in the style and format of the Journal of Animal Science.

additional objective was to determine if supplementation would impact growth and humoral immune response in sheep. The objective of the second trial was to determine which ANOD component(s) (salt, fucoidan, or betaine) caused any effects seen during the dose response trial.

LITERATURE REVIEW

The objectives of this study were to determine if stress could be reduced and health improved during handling and transport by supplementing the diet with ANOD prior handling and transport. The following review will discuss the common physiological variables used to assess stress and health.

General stress response

Stress occurs whenever an individual is presented with a challenge to homeostasis, in the form of a physiological or psychological stimulus (Chrousos and Gold, 1992). The stress response is necessary for coping with stressors; without the stress response animals can suffer dire consequences, possibly even death, should the stress response continue indefinitely. This illustrates the delicate balance of the stress response. When conditions are favorable animals can spend energy towards functions such as eating. If unfavorable conditions arise the stress response allows animals to survive a stressful situation until they can escape it. When stress becomes excessive an animal's adaptive responses are relatively stereotypic and non-specific, which Selye termed "the general adaptation syndrome" (Tsigos and Chrousos, 2002). Selye hypothesized there were three stages in the stress response; alarm, resistance, and exhaustion. In the first stage, the body prepares to deal with the stressor. In the second stage, the body seeks to return to homeostasis. If stressors are not removed, then the body will not return to homeostasis, but will enter the third stage, exhaustion, in which the body function begins to breakdown. During stress cardiac output and respiration are

accelerated, catabolism increases, and blood flow is redirected to the brain, heart, and muscles (Tsigos and Chrousos, 2002). Without an outlet to appropriately respond to the stressor, problems arise for the animal as it enters the exhaustion stage of the stress response. Normally the stress response is time limited, therefore, making the anti reproductive, anti growth, catabolic, and immunosuppressive effects of the stress response temporarily beneficial. Unfortunately, many common practices related to livestock production put animals in situations they can not alleviate and the normally beneficial results of the stress response become damaging. This extreme response may eventually lead to death if the stress response doesn't end. In order to ease the problems associated with the stress response, one must first understand what physiological reactions occur during stress.

When an individual is undergoing stress, multiple pathways are activated. The two main effector pathways are the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic neurohypothalamus. These two pathways also interact with higher areas of the brain which influence anticipatory phenomena (mesocortical/mesolymbic systems), the initiation, propagation, and termination of the stress response (amygdala/hippocampus complex), and the sensing of pain (arcuate nucleus) (Tsigos and Chrousos, 2002). If the stress is short term, alarm or emergency, the neurohypothalamus is activated, causing a release of the neurotransmitter acytcholine which has receptors on the adrenal medulla. When these receptors are activated, it causes the chromaffin cells to release epinephrine. Epinephrine, a catecholamine, together with norepinephrine, is secreted during times of fear or anger and results in increased heart rate and the

hydrolysis of glycogen to glucose. This reaction, often called the "fight or flight" response, prepares the body for strenuous activity.

If the stress is long-term, then the limbic system is activated, causing the hypothalamus to secrete the corticotrophic releasing factor (CRH). This prompts the pituitary to release adrenocorticotropic hormone (ACTH) and beta-endorphin. The beta-endorphin acts on the brain as a possible form of analgesia. ACTH causes the zona fascilulata and zona reticularis of the adrenal gland to release glucocorticoids (Swenson, 1993). Glucocorticoids, such as cortisol, participate in the control of the body's homeostasis and the animal's response to stress (Tsigos and Chrousos, 2002). Glucocorticoids negatively feed back on the secretion of ACTH in order to limit the duration of the exposure of body tissues to glucocorticoids. This negative feedback minimizes the catabolic, anti reproductive, immunosuppressive affects of these hormones. Other negative feed back compounds include γ-amino-butyric acid (GABA), corticotrophin, and several opioid peptides (Tsigos and Chrousos, 2002). Acetylcholine and serotonin excite CRH neurons and sympathetic neurons (Tsigos and Chrousos, 2002). While the normal stress response allows animals to deal with stress until they can escape it, this does not always occur during livestock production.

Effect on immunity. The stress and the hormones released during stress impact both the cellular and humoral immune response (Galliard, 2001; Murray et al., 2001; Webster, Tonelli, and Sternberg, 2002; Beishuizen and Thijs, 2004). Stress affects the immune system by directly interacting with the parts of the nervous system that

innervate peripheral immune organs. For example, noradrenergic fibers, fired during stress, have been documented to be located in the thymic cortex (Khansari et al., 1990) and developing thymocytes have been shown to possess adrenoreceptors that respond to the hormones released during stress by altering T-cell receptors. It is also suspected that there is an interaction between the noradrenaline releasing nerve fibers that run adjacent to in the spleen and macrophages, T-cells, and B-cells (Khansari et al., 1990). Stress has been demonstrated to increase the number of CD4+ lymphocytes while decreasing CD8+ lymphocytes (Degabriele and Fell, 2001). Changes in gene expression due to gluccocorticoids and catecholamines disregulate the immune system (Padgett and Glaser, 2003).

One of the primary categories of hormones investigated during stress research is glucocorticoids, i.e. cortisol. Cortisol exerts both permissive and regulatory effects, with the permissive effects allowing other hormones maintaining homeostasis to function at a normal level. The regulatory effects, in turn, prevent over reaction of the central immune system and other systems to challenges of homeostasis. Cortisol does this by suppressing cell-mediated immune events (Westley and Kelley, 1984, Bauer et al., 2001). Large infrequent pulses of cortisol cause modification to the cellular immune response (Rhind et al., 1999). Stressful events, such as mixing animals or transporting them, has been demonstrated to lower cell mediated immune response (Sevi et al., 2001). The stress of sudden weaning may lead to higher coccidial infestations than in gradually weaned sheep (Orgeur et al., 1998) an indication of lowered humoral immunity. Cortisol is required in low concentrations for normal development of T cells in the

thymus (Pruett, 2001) and modulates the transcription of many cytokines. Cortisol suppresses the pro-inflammatory cytokines IL-1, IL-2, IL-6, IL-8, IL-11, IL-12, TNF-α, IFN-γ, and GM-CFS. Gluccocorticoids also up regulate the anti-inflammatory cytokines IL-4, IL-10, and TGF-β (Graham, 1999; Elenkov and Chrousos, 2002; Webster et al., 2002). Raised cortisol concentrations during stress cause a decrease in NK cells and lymphocytes, diminish mitogen-induced lymphocyte stimulation, and redistribute T cells from circulation to the bone marrow (Speirs, 1992).

Cortisol also inhibits acetylcholine release. While norepinepherine is released to prime the body for the fight or flight response, acetlycholine is released to help calm an individual. While acetlycholine is inhibited by cortisol, during stress, norepinephrine is free to compromise the immunity of the individual. Beta endorphin, another compound released during stress, is thought to enhance cytotoxic T and NK cell activity and proliferation (Speirs, 1992). Another hormone released during stress is prolactin. Prolactin is thought to have immunodulatory role in two ways: first, by stimulating the immune system directly, and second, by reducing the degree to which gluccocorticoids are secreted in response to stress (Davis, 1998).

Effects of handling (exercise) stress. One instance during livestock production where animals are often put under stress is during handling (exercise) (Pearson et al., 1977). Processes of handling sheep such as shearing and loading have been demonstrated to be stressful (Hargreaves and Hutson, 1990; Parrott et al., 1998a; Mears et al., 1999). If handling of livestock becomes intense and lasting it is similar to

exercise. During exercise or vigorous activity, the body is under stress because of increased energy demand. Exercise causes an increase in the body temperature of sheep (Buckler, 1971; Laburn et al., 2002). During a period of exercise, the body first increases the out put of cortisol (Rudolph and McAuley, 1998) to mobilize sugar stores. It eventually depletes the cortisol stores in the body if the exercise continues for a long period (de Diego Acosta et al., 2001).

Effects of transport stress. Transport is a very stressful part of livestock production which can also lead to an increase in body temperature (Ingram et al., 2002; Kumar et al., 2002). Many factors contribute to stress during transport and each species has a different stress response to transport (Manteca and de la Torre, 1996). It has been demonstrated that excessively high stocking density during transport leads to increased injury and stress (Tarrant, 1990; Cockram et al., 1996). The initial period of transport is the most stressful time for animals (Knowles et al., 1995; Broom et al., 1996). The stress of transport has been shown to cause lower carcass yields and dark cutters in cattle (Tarrant, 1990; Warriss, 1990). Orientation during transport had no effect on stress levels, but transport is stressful and orientation affects balance during transport (Clark et al., 1993; Toscano and Friend, 2001). Driver behavior and road quality are also factors affecting sheep during transport (Cockram et al., 2004).

Effects of heat stress. Numerous environmental factors (i.e., temperature, crowding, etc.) contribute to the stresses of handling and transport. Heat stress is a main

issue in the welfare of livestock. An increase in core body temperature in response to environmental stress has been documented in numerous species (Ingram et al., 2002). This stress-induced hyperthermia has been used to assess the stressfulness of management practices in sheep (Parrott et al., 1999) and goats, with respiration rate also being used in goats (Kasa et al., 1995). Though ruminants have a mechanism for thermoregulation, they do not maintain a strict body temperature (McLean et al., 1983). Cattle, for example, show a diurnal range of 3˚C in body temperature (Finch, 1984). Research has shown that hyperthermia has negative effects on the production of livestock (Finch, 1984). Animals go through four stages in an attempt to regulate hyperthermia. The first is the innocuous stage, where the thermo regulation begins in response to external temperatures. The second is the aversive stage, in which the animal is coping with the external temperatures, but with difficulty. The third stage is the noxious stage, in which body temperature increases and the fitness of the animal is decreased. The final stage is the extreme stage, in which the body temperature continues to rise and if not assuaged, causes the animal to die (Silanikove, 2000). A method for assessing the level of heat stress involves use of the thermal heat index (THI). This index is a combination of the temperature and humidity using the following formula: THI = (Dry-Bulb Temp. °C) + (0.36 dew point Temp., °C) + 41.2). When the THI is above 72 indicates mild stress, above 80 indicates medium stress, and above 90 indicates severe heat stress in cattle (Pennington and Van Devender, 2004).

In sheep, panting is the major evaporatory heat loss mechanism used to counteract heat stress; respiratory frequency correlates with heat loss by evaporation

(Hales and Brown, 1974). When environmental temperatures exceed 25 ºC, sheep increase evaporative heat loss via increased respiration and sweating (Degen and Shkolnik, 1978). In the goat, progressive dehydration leads to suppressed sweating and increased panting (Baker, 1989). A panting animal does not lose salt, thereby causing electrolyte concentrations to increase as water is lost from the body.

An increase in electrolytes after long periods of transport indicates dehydration (Friend et al., 1998; Shaheen, 2001; Jaber et al., 2004). Water loss caused by urination and thermo regulation contribute to dehydration during heat stress. Cortisol increases during stress cause sheep to suffer from a loss of body water in excess of that associated with a loss of electrolytes (Lowe et al., 2002; Parker et al., 2003) as cortisol plays a role in water balance within the body. Finally, increased environmental temperatures have been shown to cause a decrease in RBC, WBC, hemoglobin, and hematocrit in sheep (da Silva, et al., 1992).

Ascophyllum nodosum

Ascophyllum nodosum (ANOD) is a brown seaweed originating from the coastline of the North Atlantic Ocean, from Nova Scotia to Norway. ANOD has many uses in human and animal nutrition, plant growth, and as an industrial product ingredient. The seaweed ANOD is often used as an ingredient in supplements as a vitamin source to improve health and immunity. Alginic acid, which is extracted from ANOD, is used in the form of salts as a viscosity control gelatin and emulsifying agent (Sharp, 2005). The alginic acid contained within ANOD is composed of 65% mannuronic acid, 35% guluronic acid the ratio of these components can range between

1.40 and 1.95 (Cottrell and Kovacs, 1980). Common food uses for alginates include prepared pie fillings, instant mixes and sauces (Sharp, 2005).

The ANOD has also been used as a fertilizer for plants. In fact use of ANOD as a soil conditioner began with the first settlements of Maritime Canada (Sharp, 2005). Extracts of the ANOD have been shown to be chelating agents which improve the utilization of minerals by plants (Lynn, 1972). Extracts of ANOD have also demonstrated increased germination and early growth in seeds (Britton and Nayes, 1964). Fescue treated with ANOD has shown increased superoxide dismutae, gluthaione reductase, and ascorbate peroxidase (Ayad, 1998). Increases in α-tocopherol, β-carotene, superoxide dismutase and asorbic acid have also been seen in turfgrasses treated with seaweed (Schmidt and Zhang, 1997; Zhang, 1997, Zhang and Schmidt, 1999). An increase in superoxide dismutase has been seen to last throughout the grazing season in fescue treated with ANOD (Allen et al., 1997; Fike et al., 2001).

The sodium alginates from ANOD are also used in industrial products and processes. They are also used as a component of a sizing solution for liner-board, for enhancing the penetration of adhesives, as carrier for fire reactive dyes, and as an emulsifier in polishes (Sharp, 2005). The sodium alginates from ANOD are also used for their gelling properties in air fresheners and explosives (Sharp, 2005).

The seaweed ANOD can also be used as an animal feed additive; it can compose up to 5% of the diet of poultry, sheep, cattle, pigs, and horses (Sharp, 2005). Trace elements and vitamins found within ANOD have been shown to affect growth of cattle, milk production, color in eggs, and wool color in sheep (Neeb and Jensen, 1965). An

extract of the ANOD has been reported to lower core body temperature of cattle in hot weather while also stimulating a higher core body temperature in cold weather (Allen et al., 2001a,b). The seaweed ANOD has also demonstrated an increased cell mediated immune function in cattle grazing endophyte infected fescue. Cattle grazing infected fescue and treated with an ANOD extract had increased phagocytic activity and major histocompatibility complex class II expression compared to cattle not grazing treated fescue (Allen et al., 2001b; Saker et al., 2001). This improved immune function lasted while animals were on pasture, during cross-country transport, and throughout 160 d finishing period (Allen et al., 2001a). Saker et al. (2001) did, however, see depression in cellular immunity in cattle not feeding on infected fescue when they were fed ANOD during the grazing season compared to cattle grazing uninfected fescue and not fed ANOD. However, in a separate study, lambs feed hay with ANOD showed enhanced monocyte oxidative burst during both short and long periods of heat stress (Saker et al, 2004). Steers fed ANOD at 1.5 or 3% of dry matter intake in the feedlot exhibited greater immune response to intra dermal injections with phytohemagglutinin than steers not fed ANOD (Allen et al., 2001b).

Supplementation with ANOD has shown promise in lowering stress as well. Lactating mares fed ANOD at 1% of dry matter intake had little change in their neutrophil: lymphocyte ratio during the 56 d post weaning period; however mares not fed ANOD had an increased ratio indicating an increase in stress (Allen et al., 2001b). Supplementation with ANOD has also been shown to increase weight gain in swine (Turner et al., 2002) and salmon (Gabrielsen and Austreng, 1998).

Anti-bacterial characteristics have also been attributed to ANOD. Vacca and Walsh (1954) reported that ANOD had antibacterial properties for 10 bacteria consisting of both gram negative and gram positive organisms. Supplementation with ANOD at the 2% rate in cattle reduced the prevalence of *E. coli* 0157 in both hide swabs and fecal samples (Braden et al., 2004). However, calves given ANOD in water showed no lowering in incidence of bronchopneumonia (Allen et al., 2001b).

Supplementation with ANOD has also demonstrated effects on carcass quality. An increase in meat shelf life, as a result of longer color preservation, was seen in cattle which grazed on ANOD treated fescue prior to feedlot finishing (Montgomery et al., 2001). Steers fed ANOD treated fescue exhibited increased marbling scores (Allen et al., 2001a). Timing of feeding seems to play a role in the marbling score as feeding ANOD during the first 10 d in feedlot increased marbling but feeding during the last 14 d of finishing did not (Allen et al., 2001b). Other forms of seaweed have also been observed to increase feed intake, increase carcass weight, and decrease digestive tract fill (Al-Shorepy et al., 2001).

Seaweeds, such as ANOD, also contain antioxidants (i.e. selenium, vitamin E, phenols, vitamin B, zinc, copper, iron, and manganese) which can reduce the effects of free radicals that can occur during transport (Miller and Brzezinski-Slebodzinska, 1993). Lambs grazing ANOD treated pastures have demonstrated increased serum vitamin A and whole blood Se (Fike et al., 2001). Steers fed ANOD demonstrated concentrations of vitamin E in their livers (Montgomery et al., 2001). Superoxidase dsimutase activity was increased in lambs fed ANOD (Saker et al., 2004). Finally, the glucans contained

within seaweed have been shown to stimulate TNF-α in vitro and in vivo (Jelinkova et al., 2002).

Seaweeds contain many biologically active compounds. For example, ANOD is composed of 10% fucoidan (Baardseth, 1970), a complex sulfated polysaccharide that has been shown to increase cellular immunity (Maruyama et al., 2003) while also decreasing the classical compliment system (Tissot et al., 2003). This compound has also been shown to have anti viral activity, anti bacterial activity, and amplified phagocytic activity in shrimp (Chotigeat et al., 2004). Fucoidan was also shown to have anti viral functions in humans (Lee et al., 2004). In mice, fucoidan has been shown to help prevent tumors (Maruyama et al., 2003) and to inhibit the human immunodeficiency virus (HIV) (Sugawara et al., 1989). Fucoidan has been demonstrated to stimulate Il-4 and Il-13 production in human breast milk (Eiwegger et al., 2004). These interleukins are important in lymphocyte maturation and influence the differentiation of T cells into TH2 cells. Fucoidan from ANOD shows excellent metal binding abilities and binds lead most efficiently of the divalent metal cations (Paskins-Hurlburt. 1976). In rats, fucoidan has renal protecting properties (Zhang et al, 2003).

The seaweed ANOD also contains 0.03% betaine (Blunden et al., 1992), which has been shown to decrease the effects of heat stress in poultry (Sheikh-Hamad et al., 1994; Zulkifli et al., 2004). Betaine acts as both an osmolyte and a methyl donor. As an osmolyte, betaine reduces the energy needed to maintain water and ion balance by helping cells retain water (Partridge, 2003). As a methyl donor, betaine suppresses the increase of homocyteine (Yagisawa et al., 2004). Betaine has also been reported to

increase growth (Wray-Cahen et al., 2004) and improve carcass quality (Siljander-Rasi et al., 2003) in swine, though it did not impact body temperature (Matthews et al., 2001). However, in a separate study, betaine lowered body temperature in poultry during heat stress (Belay et al., 1992). Increased feed intake and growth has been seen in prawns fed diets containing beatine (Felix and Sudharsan, 2004). Ducks fed diets containing betaine also exhibited increased growth and improved carcass quality (Wang et al., 2004). In cattle, no effect on growth was found when betaine was added to the diet (Loest et al., 2002). In humans, betaine has been shown to protect internal organs, thereby preventing chronic disease (Craig, 2004).

Since ANOD is a seaweed, it also consists of 3-4% sodium, 3-4% chloride and 2-3% potassium (Baardseth, 1970); therefore, it is of interest to see if salt also could account for some of ANOD's possible effects. Sodium chloride has been shown to have positive effects on sheep physiology in response to transport by maintaining electrolyte balance (Cole, 1998). Electrolytes are thought to alleviate the effect of stress due to the leakage of plasma glutamic oxaloacetic transaminase from damaged tissue (Apple et al., 1993). Electrolyte solutions have also been shown to alleviate the effects of transport stress. Normally during transport, reduced blood pH, glucose concentration, and interstitial water, and increases in serum chloride, hemoglobin, urine sodium, and urine osmolality are seen; however, electrolyte solutions attenuate these changes (Schaefer et al., 1997). Shaefer et al. (1997) found that electrolyte therapy for transported animals resulted in improved live and carcass weights and better meat quality. However in sheep, when sodium chloride intake is high, there is an increase in urinary concentrations

of sodium (Meintjes and Engelbrecht, 1993). Meintjes and Olivier (1992) also found that salt loading directly into the rumen in sheep increased water intake, fractional turnover of body water, plasma potassium concentration, urine sodium concentration, and decreased urine potassium concentration from control values.

Physiological measures of health and stress

The common way to monitor the effect of stress on the health of animals is to collect physiological measurements. These measurements include hormone concentrations, blood constituents, growth, and immune function. Hormones are produced by the body to maintain homeostasis. These hormones in turn, can affect blood constituents, growth, and immune function. When animals are under stress, these values may be altered from basal levels; they may also be used to measure overall health of an animal. If values are altered from the normal range it can indicate disease states.

Cortisol. Blood cortisol concentration has long been used as a measure of stress. An animal's well-being, or homeostasis, is challenged by a series of events in the animal's brain which leads to the secretion of gluccocorticoids from the adrenal cortex (Tsigos and Chrousos, 2002). Cortisol is secreted in a diurnal pattern, with maximum secretion in the morning and minimum secretion at night (Ford et al., 2001). Overall, cortisol affects metabolism, muscle and cardiovascular function, behavior, and the immune system (Ford et al., 2001). Plasma cortisol concentrations have been documented in sheep in numerous studies (Table 1). As the studies in Table 1

illustrate, under different conditions and in different flocks, cortisol levels can vary greatly in sheep. Cortisol ranged from 2 – 350 ng/ml in these studies and the average basal concentration was 10 - 40 ng/ml.

Table 1. Studies of sheep where blood was collected to determine cortisol concentrations

Experimental test	Cortisol concentration ng/ml	Study
Artificial Insemination	50-350	Khalid et al., 1998
Basal	10-15	Parker et al., 2003
Handling	22-78	Pearson et al., 1977
Isolation	20-100	Apple et al, 1993
Loading	2-13	Parrott et al., 1998a
Shearing	58-79	Hargreaves and Hutson, 1990
Shearing	20-80	Mears et al., 1999
Transport	15-54	Parrott et al., 1998b
Transport	21-27	Hall et al., 1998a
Transport	2-18	Hall et al., 1998b
Various	10-100	Mears and Brown, 1997
Weaning	9-15	Orgeur et al., 1998
Weaning	7-29	Sowinska et al., 2001

Aldosterone. Another hormone produced by the adrenal gland, aldosterone, can be used to assess electrolyte homeostasis. Aldosterone is produced by the adrenal gland and helps maintain the sodium/potassium balance within the body (Rabinowitz, 1996). As aldosterone concentration increases, more sodium is retained and more potassium is excreted, thereby increasing water re-absorption. During dehydration due to water loss,

aldosterone concentrations decrease to maintain plasma osmoality. Higher than normal aldosterone concentrations may indicate: primary hyperaldosteronism, cardiac or kidney disease, Cushing's syndrome, or a very low sodium diet. Lower than normal concentrations of aldosterone may indicate: Addison's disease, very high sodium diet, congenital adrenal hyperplasia, or hyporeninemic hypoaldosteronism. During dehydration aldosterone concentrations in sheep range from 60 to 100 pg/ml and normally range from 200 to 250 pg/ml when sheep are properly hydrated (Li et al., 2000; McKinley et al., 2000).

Plasma chemistry profile. Plasma chemistry profiles are an accepted way of documenting the overall health of an animal (Kumar et al., 2003). Chemistry profiles are useful as stress can change the normal concentrations of some blood constituents such as glucose, total protein, and blood urea nitrogen (BUN). This occurs because during stress, corticosteroid concentrations are increased causing an increase in gluconeogenesis and a decrease in protein deposition (Barnett et al., 1983). The following is a list of the constituents measured in a plasma chemistry profile and what values outside the normal range indicate about the health status of an animal. These are useful to monitor when feeding ANOD to determine its impact on the health and general physiology of animals.

Albumin/Globulin (A/G) ratio. The A/G ratio may be elevated in the following situations: hypothyroidism, high protein or high carbohydrate diet with poor nitrogen retention, hypogammaglobulinemia (low globulin), and gluccocorticoid excess (low

globulin). The A/G ratio may also decrease due to liver dysfunction (Swenson, 1993; Fenner, 2000).

Albumin. The liver, using dietary protein, synthesizes albumin. Its presence in the plasma creates an osmotic force that maintains fluid volume within the vascular space. Low albumin is an indication of poor health. The normal range for domestic sheep is 2.7 – 3.7 g/dl (Aiello, 1998). Albumin concentrations may be elevated by: dehydration, congestive heart failure, poor protein utilization, gluccocorticoid excess, and congenital factors. Albumin concentrations may be decreased by: dehydration, hypothyroidism, chronic debilitating diseases, malnutrition - protein deficiency, dilution by excess H_2O (polydipsia or IV fluids), renal losses (Nephrotic Syndrome), protein losing-enteropathy, skin losses (burns, exfoliative dermatitis), and liver dysfunction (Swenson, 1993; Fenner, 2000).

Aspartate amino-transferase. Aspartate amino-transferase (AST(SGOT)) is released into the circulation following the injury or death of cells, particularly by the liver, muscle, and highly metabolic cells. The normal range for domestic sheep is 49 - 123.3 U/l (Aiello, 1998). Causes of increased concentrations include: liver inflammation, heart muscle damage, other tissue damage, red blood cell damage, malignancy, and drugs. Causes of decreased concentrations include a vitamin B_6 deficiency or chronic kidney dialysis (Swenson, 1993; Fenner, 2000).

Blood urea nitrogen (BUN). Blood urea nitrogen is a by-product of nitrogen (protein) utilization. The normal range for domestic sheep is 10.3 - 26 mg/dl (Aiello, 1998). Causes of increased concentrations include: renal dysfunction, diabetes mellitus,

starvation, dehydration, diarrhea, congestive heart failure, gastrointestinal hemorrhage and obstruction, shock, tissue necrosis, third degree burns, renal artery stenosis, renal vein thrombosis, urinary tract obstruction, gout, increased protein catabolism, and high protein diet. Causes of decreased concentrations include: syndrome of inappropriate ADH secretion (SIADH), liver or biliary dysfunction, malnutrition, celiac sprue, advanced stages of acidosis, zinc deficiency, posterior pituitary hypofunction, anabolic hormones, and normal pregnancy (Swenson, 1993; Fenner, 2000).

Calcium. Plasma calcium reflects the metabolic and hormonal state of the individual. Ionic or free calcium is not only the biologically active form of calcium but reflects the amount of albumin and the blood pH. When the blood is acidic, calcium becomes ionized and is liberated from plasma proteins. When the blood is basic, more calcium is bound to proteins as well as precipitating out of solution. The normal range for domestic sheep is 9.3 – 11.7 mg/dl (Aiello, 1998).

Increased concentrations may be due to: primary hyperparathyroidism, secondary hyperparathyroidism, tertiary hyperparathyroidism, aluminum induced bone disease, familial, drugs, and pheochromocytoma, malignancy, hypophosphatasia, toxic effects of non-medicinal metals, granulomatous disease, milk-alkali syndrome, medications, epilepsy, Paget's disease, immobilization from any cause, excess ingestion of Vitamin D or A, and pregnancy and lactation. Causes of decreased concentrations may be: hypoparathyroidism and magnesium deficiency, ovarian hypofunction, vitamin D insufficiency, anticonvulsants, rapid bone deposition, protein malnutrition, digestive

dysfunction, renal dysfunction, pancreas dysfunction, pregnancy, and metabolic acidosis (Swenson, 1993; Fenner, 2000).

Creatinine. Creatinine (creat) is formed in muscles from creatine, which is formed in the liver. It is a substance that in healthy animals is easily excreted by the kidney. The normal range for domestic sheep is 0.9 - 2 mg/dl (Aiello, 1998). Causes of increased concentrations may be: renal dysfunction, congestive heart failure, starvation-dehydration, uncontrolled diabetes, and muscle degeneration. Causes of decreased concentrations include: muscle atrophy, liver disease, and pregnancy (Swenson, 1993; Fenner, 2000).

Gamma glutamyltransferase. The source of gamma glutamyltransferase (GGT) is the liver, making it an excellent and specific indicator of liver damage or problems with the drainage of bile in the liver. The normal range for domestic sheep is 0.9 - 2 U/l (Aiello, 1998). Causes of an increase in the concentration of gamma glutamyltransferase include: hepatic inflammation, myocardial infarction, diabetes mellitus, neurologic disease, sepsis, and obstruction of bile ducts. Decreases in gamma glutamyltransferase include: hypothyroid, low magnesium and hypothalamic malfunction (Swenson, 1993; Fenner, 2000).

Globulins. Globulins are proteins that include gamma globulins (antibodies) and a variety of enzymes and carrier/transport proteins. The gamma fraction usually makes up the largest portion of the globulins; therefore, antibody deficiency should be suspected when the globulin concentration is low. The normal range for domestic sheep is 3.2 - 5 g/dl (Aiello, 1998). Globulin concentrations may be elevated in: chronic

infections, liver disease, carcinoid syndrome, rheumatoid arthritis, ulcerative colitis, multiple myelomas, leukemias, Waldenstrom's macroglobulinemia, autoimmunity, and renal dysfunction. The plasma globulin concentration may be decreased in: nephrosis, alpha-1 antitrypsin deficiency, acute hemolytic anemia, liver dysfunction, and hypogammaglobulinemia/ agammaglobulinemia (Swenson, 1993; Fenner, 2000).

Glucose. The normal range for domestic sheep is 44 – 81.2 mg/dl (Aiello, 1998). Causes of increased concentrations include: diabetes mellitus and insulin resistance syndromes, thiamine (B_1) insufficiency, hemochromatosis, ataxia telangiectasia, endocrine hyperfunction, acute and chronic pancreatitis, drugs, and stress. Causes of decreased concentrations are: excess insulin, impaired glucose tolerance, late/large malignancies, endocrine hypofunction, high dose salicylates, protein malnutrition, pregnancy, various free radical pathologies, hypochlorhydria, liver dysfunction, and hereditary (Swenson, 1993; Fenner, 2000).

Magnesium. Plasma magnesium is not reflective of total magnesium stores. Unfortunately there is not a good test for magnesium. The normal range for domestic sheep is 2.0 – 2.7 mg/dl (Aiello, 1998). Increases in magnesium include: hypothyroidism, adrenal cortical hypofunction, hyperparathyroidism, and renal dysfunction. Causes of decreased magnesium include: excessive urinary losses, renal dysfunction, hypercalcemia or hypophosphatemia, hyperthyroidism, hyperaldosteronism, diuresis, decreased Intake of magnesium, increased intestinal losses, and pancreatitis (Swenson, 1993; Fenner, 2000).

Phosphorus. This mineral is essential to the formation of muscle, red blood cells, ATP, the maintenance of acid-base balance and lowering blood viscosity, nervous system function, and the metabolism of carbohydrates, protein and fat. The normal range for domestic sheep is 4 – 7.3 mg/dl (Aiello, 1998). Causes of increased concentrations are: renal dysfunction, magnesium deficiency, bone sources, endocrine, sarcoidosis, liver dysfunction, diabetes, drug induced, and high calcium concentrations. Causes of decreased concentrations are: intracellular phosphorus shift, alkalosis or recovery from acidosis, carbohydrate intake, beta-adrenergic agents, diarrhea, malabsorption including hypochlorhydria, nasogastric suctioning, hypomagnesemia, hypokalemia, renal disease, diuretics, corticosteroids, xanthine derivatives, protein malnutrition, insufficient vitamin D, liver dysfunction, low serum calcium concentrations, hyperparathyroidism, and trauma (Swenson, 1993; Fenner, 2000).

Plasma protein. Proteins are the most abundant compounds in serum. Because total protein represents the sum of albumin and globulins, it is more important to know which protein fraction is high or low than what is the total protein. The normal range for domestic sheep is 5.9 – 7.8 g/dl (Aiello, 1998). Total protein may be elevated due to: chronic infection, adrenal cortical hypofunction, liver dysfunction, collagen vascular disease, hypersensitivity states, sarcoidosis, dehydration, respiratory distress, hemolysis, cryoglobulinemia, and leukemia. Total protein may be decreased due to: malnutrition and malabsorption, liver disease, diarrhea, severe burns, loss through the urine in severe kidney disease, low albumin, low globulins, and pregnancy (Swenson, 1993; Fenner, 2000).

Electrolytes. Electrolytes are a useful measure of the hydration of an animal. During transport, animals can easily become dehydrated, making hydration monitoring an important factor. It is of interest to examine how ANOD supplementation affects hydration level. The following is a list of commonly measured electrolytes and what factors contribute to their abnormal levels.

Sodium. Sodium maintains acid-base equilibrium for proper osmotic balance. Normally it is the most abundant cation in the fluid outside of the cell. Sodium is the most important factor in osmotic regulation of extra-cellular fluid balance and acid balance, as well as renal, cardiac and adrenal functions. It is needed to maintain the sodium-potassium pump, which transports sodium out of the cell and potassium into the cell. The normal range for domestic sheep is 141.6 – 159.6 meq/l (Aiello, 1998). Sodium concentrations may be elevated in: water deficit, diarrhea, diabetes mellitus, renal disease, excessive intake of sodium, adrenal cortex hyper-function, pyloric obstruction, congestive heart failure, insufficient anti-diuretic hormone production, and insufficient parathyroid hormone. Sodium may be reduced in: pyloric spasm, hyperglycemia and diabetes mellitus, excess perspiration, adrenal cortex hypo-function, excess progesterone, diarrhea and metabolic alkalosis, and renal dysfunction (Swenson, 1993; Fenner, 2000).

Chloride. Chloride contributes to the body's acid/base balance. Along with sodium, potassium and carbon dioxide, it is important in evaluating acid/base relationships, state of hydration, adrenal and renal functions. The normal range for

domestic sheep is 100.8 – 113 meq/l (Aiello, 1998). Causes of increased chloride are: metabolic acidosis, renal dysfunction, excess sodium chloride intake, adrenal cortical hyperfunction, severe dehydration, diabetes insipidus, hyperparathyroidism, and anterior pituitary hypofunction. Causes of decreased chloride are: renal chloride loss, GI chloride loss, metabolic alkalosis, chronic compensated respiratory acidosis, congestive heart failure, over hydration, burns, diabetes, Addison's disease, hypoparathyroidism, and perspiration (Swenson, 1993; Fenner, 2000).

Potassium. Potassium is an extremely valuable electrolyte essential to heart and kidney function as well as to the maintenance of blood and urine pH. It is the chief electrolyte in the fluid of cells. The normal range for domestic sheep is 4.6 – 6.3 meq/l (Aiello, 1998). Plasma potassium is increased in: renal dysfunction, adrenal cortex under function, metabolic acidosis, respiratory dysfunction, bradycardia, and diabetes. Plasma potassium is decreased in: diarrhea and/or vomiting, adrenal cortex over function, anemia, metabolic alkalosis, and hypertension (Swenson, 1993; Fenner, 2000).

Na/K ratio. Na/K ratio is an approximate measurement of ions. The ratio is increased when there are excessive anions/acids in the blood. Common causes of an elevated Na/K ratio include: keto acid overproduction, lactic acid, genetic defects of enzymes of carbohydrate metabolism, nutritional deficiencies, inability to excrete acids, and dehydration. The Na/K ratio is decreased by free radical pathology due to overproduction of alkaloids. Other symptoms that have been associated with a reduced ratio are: hyperchloremic acidosis, multiple myeloma, Hypoalbuminemia, and kidney disease (Swenson, 1993; Fenner, 2000).

Immunity. As mentioned earlier, stress impacts immune function. Therefore, measuring immune function is an important tool in assessing the effects of stress on an animal as well as its overall health. There are two main components of the immune response, cellular and humoral. These two systems can be altered independently of one another and are responsible for removal of different types of antigens, while using different components of the immune system to complete their functions.

Cellular. Cellular immunity is the effector function of the T lymphocytes. The purpose of cellular immunity is to fight infection by pathogens living within cells, such as viruses. Cellular immunity works by infected cells presenting antigen to the T lymphocytes. The cellular immune response consists of two types of reactions. The first consists of the T lymphocytes producing cytokines after recognizing presented antigens. These cytokines activate phagocytes and stimulate inflammation. The second stage of the cellular immune response entailes the cytolitic T lymphocytes causing an infected cell to lyse (Abbas et al., 2000).

Complete Blood Cell Count. A complete blood cell count is used to assess the overall health of an individual. The differential count of the white blood cells allows an assessment of cellular immune function. The differential examines the specific types of white blood cells found within an individual, such as eosoniphils, basophils, etc. The following is a list of the components of the complete blood count and what values outside the normal range indicate about an animal's health status.

White blood cell count. The white blood cell count is the measurement of the number of leukocytes within the blood. The normal range for domestic sheep is 4000 –

12000/µl (Aiello, 1998). Low numbers of WBCs may indicate: bone marrow failure, presence of cytotoxic substance, collagen-vascular diseases, and disease of the liver or spleen. High numbers of WBCs may indicate: infectious diseases, inflammatory disease, leukemia, severe emotional or physical stress, and tissue.

Red blood cell count. The primary reason to assess the RBC is to check for anemia and to evaluate the production of red blood cells. The mature red blood cell carries oxygen attached to the iron in hemoglobin. The normal range for domestic sheep is 9,000,000 – 15,000,000/µl (Aiello, 1998). The number of red blood cells is increased in: chronic respiratory insufficiency, living at a high altitude, cystic fibrosis, adrenal cortical hyper function, and anabolic metabolism. The number of red blood cells is decreased in: iron deficiency, vitamin B6, B12, and/or folic acid deficiency, chronic disease, free radical pathology, toxic metals, and catabolic metabolism.

Packed cell volume. The hematocrit or packed cell volume (PCV) represents the volume of red blood cells in 100ml of blood and is therefore reported as a percentage. The normal range for domestic sheep is 27 – 45 % (Aiello, 1998). A low hematocrit and hemoglobin usually indicate decreased production, excessive loss, or destruction of red blood cells. The conditions associated with an increased or decreased hematocrit are the same as for hemoglobin. Transport of sheep has been shown to decrease packed cell volume (Hall et al., 1998a).

Hemoglobin. Hemoglobin is what gives the blood its characteristic red color. It contains iron, which carries the oxygen to the cells. The hemoglobin concentration indicates the amount of intracellular iron; hence, its value in determining anemia. The

normal range for domestic sheep is 9 – 15 g/dl (Aiello, 1998). Hemoglobin is increased in: dehydration, emphysema, severe asthma, macrocytosis, adrenal cortex over activity, polycythemia vera, high altitude adaptation, splenic hypofunction, and testosterone supplementation. Hemoglobin is decreased in: Digestive inflammation, free radical pathology, adrenal cortical hypofunction, hereditary anemia, hemodilution, blood loss, vitamin or mineral deficiency, chronic disease, and, bone marrow insufficiency.

Mean corpuscular volume. The MCV relates to the average size of the red blood cell. The normal range for domestic sheep is 28 – 40 fl (Aiello, 1998). The MCV is increased in: hereditary anemia, reticulocytosis, liver disease, and hypothyroidism. The MCV is decreased in: copper deficiency, low stomach acid, vitamin C insufficiency, vitamin B6 deficiency, rheumatoid arthritis, and iron deficiency.

Mean corpuscular hemoglobin concentration. The average hemoglobin concentration per unit volume (100 ml) of packed red cells is indicated by MCHC. The normal range for a domestic sheep is 31 – 34 g/dl (Aiello, 1998). MCHC is increased and decreased under the same conditions as the MCV.

Eosinophil. Eosinophils are bone marrow derived granulocytes which are abundant during hypersensitivity late phase reactions. They play an important role in the defense against extra cellular parasites. The normal range for domestic sheep is 0 – 1000/µl (Aiello, 1998).

Basophil. Basophils are granulocytes which are recruited into tissue sites where antigen is present and may contribute to immediate hypersensitivity reactions. The normal range for domestic sheep is 0 – 300/µl (Aiello, 1998).

Monocyte. Monocytes are the precursors to the macrophages. They are recruited to the inflammatory sites where they differentiate into macrophages. The normal range for domestic sheep is 0 – 75/µl (Aiello, 1998).

Neutrophil. Neutrophils are the most abundant white blood cell. They are recruited to inflammatory sites and are capable of phagocytising and digesting microbes. The normal range for domestic sheep is 700 – 6000/µl (Aiello, 1998).

Lymphocyte. Lymphocytes are the B and T cells of the immune system. They are responsible for both the humoral and cellular immune responses, respectively. The normal range for domestic sheep is 2000 – 9000/µl (Aiello, 1998).

Fibrinogen. Fibrinogen is a protein produced by the liver which aids in forming clots in order to stop bleeding. The normal range for domestic sheep is 0.1 – 0.5 g/dl (Aiello, 1998).

Humoral. The humoral immune response utilizes antibodies to neutralize and eliminate microbes that are in the extra cellular spaces, such as bacteria and toxins. Antibodies are a structurally related family of glycoprotiens produced by B lymphocytes. Membrane bound antibodies mediate the activation of the B cells. Once activated, B cells secrete antibodies in order to eliminate foreign microbes. The binding region of antibodies are highly variable, allowing individuals to produce 10^9 different antibodies, each of which is antigen specific. There are also different types of antibodies, known as isotypes. Different isotypes predominate during initial versus

secondary exposure to an antigen. The IgM isotype is the predominating isotype in the initial exposure, while IgG predominates during the subsequent exposures.

The first function of the antibodies produced during a humoral immune response is to neutralize the microbe or toxin by binding to them in order to prevent their binding to cellular receptors. Many microbes bind to a specific cellular surface molecule and use these to enter the host cells. Antibodies interfere with this process by binding to the microbes, making it physically impossible for the microbe to bind to the host cell; this is called "steric hindrance." In rare cases, the binding of an antibody to a microbe causes a conformational change to the microbe's surface receptors which prevents the microbe from interacting with the host cell. Antibodies block toxins from affecting cells through steric hindrance. Microbes can be neutralized by any isotype as it only involves the antigen binding region of the antibody. The most abundant type of neutralizing antibody is IgG. Many vaccines work by this process of stimulating the production of neutralizing antibodies (Abbas et al., 2000).

The second function of antibodies is for the opsinization of microbes and to promote phagocytosis. Antibodies, specifically IgG, coat antibodies and promote phagocytosis by binding the Fc receptor on phagocytes. Mononuclear phagocytes and neutrophils can digest microbes without the aid of antibodies, as they possess surface receptors that will bind to the microbes. However, the phagocytosis is much more effective at aiding antibodies as the phagocytes bind to the Fc receptor of the antibody. When the Fc is bonded by a phagocyte, it sends signals to the phagocyte to ingest the microbe (Abbas et al., 2000).

The third function of antibodies in the humoral immune response is to aid natural killer cells in their ability to lyse cells. The Fc receptor of the natural killer cell is called CD16. When the CD16 is bound by an antibody coated microbe it causes the natural killer cell to release cytokines and discharge its granules into the microbe. These granules cause the microbe to lyse thereby killing it (Abbas et al., 2000).

The final function of antibodies is to activate the compliment system. The compliment system consists of serum and membrane bound proteins which act together to create biologically active products. There are two major pathways of the compliment system: classical and alternative. The classical pathway is activated by antigen-antibody complexes, while the alternative pathway is activated by microbial surfaces without antibodies. Both pathways produce enzymes that divide the C3 protein. The products of this enzymatic reaction become covalently bound to microbial surfaces or antibodies. The complement system functions to opsonize microbes and immune complexes, leading to binding by phagocytes. The activation of the compliment system enhances the humoral immune response (Abbas et al., 2000).

The humoral immune response is important in sheep as it is necessary for proper immunization and disease resistance. The common bacterial infections against which sheep are vaccinated include: *Clostridium perfringens* type C and/or D, *Campylobacter (Vibrio) fetus intestinalis, Chlamydia psittaci, Leptospira pomona, Leptospira canicola, Leptospira hardjo, Leptospira grippotyphosa, Leptospira icterohaemorrhagiae, Clostridium tetani, Clostridium chauvoei, Clostridium septicum, and Clostridium novy* (Grotelueschen, 2005). These vaccines are usually considered “killed” vaccines, in

which the animal is challenged by an innocuous form of the bacteria. These types of vaccines require two doses of the vaccine administered between three and six weeks apart. The initial dose stimulates the immune system to respond to the bacteria but a second dose is needed to raise the immunity to a protective level (Rook, 2005).

The bacteria *Clostridium perfringens* type C and/or D causes the disease enterotoxemia, commonly called overeating disease, in sheep. Toxins produced by this bacteria cause sudden death in sheep (Grotelueschen, 2005). *Campylobacter (Vibrio) fetus intestinalis* causes abortions, dead, and weak lambs (Grotelueschen, 2005). This bacterium is the most common cause of abortions in sheep. *Chlamydia psittaci* is classified as a rickettsia, smaller than bacteria and larger than a virus. This organism generally causes abortions, conjunctivitis, arthritis in lambs, epididymitis, pneumonia, and diarrhea. It is another major cause of abortions in ewes (Grotelueschen, 2005). The Leptospirosis bacteria cause abortions, anemia, and systemic disease in sheep (Grotelueschen, 2005). *Clostridium tetani* is a bacterium which inhabits contaminated soil and enters the body through open wounds such as castration, tail docking, or lacerations. The death rate in lambs is usually over 75% in affected animals. Tetanus usually manifests itself as muscle spasms, stiffness, and other nervous system signs (Grotelueschen, 2005). Other clostridial diseases such as Blackleg, Malignant Edema, and Braxy are caused by *Clostridium chauvoei, Clostridium septicum, and Clostridium novyi.* Animals affected by these diseases usually exhibit lameness and subcutaneous swelling followed by rapid death (Grotelueschen, 2005).

While these diseases can occur in non stressed animals, stress can intensify the susceptibility of animals to other bacterial infections. Stressful events, such as transport, have been shown to lower antibody production in response to ovalbumin intramuscular injection (Krawczel et al., 2005). Heat stress has been shown to lower primary and secondary humoral immunity in chicken (Barlett and Smith, 2003). Emotional stress has also been seen to lower humoral immune response to ovalbumin in rats (Shao et al., 2003). Social defeat in hamsters leads to a suppression in antibody production (Jasnow et al, 2001).

This lowered humoral immunity due to stress can leave animals open to bacterial infections. For instance, Bronchopneumonia caused by *Pasteurella* spp. which affects sheep and goats of all ages worldwide, occurs most often in those animals that have undergone recent stress such as transportation, weaning, or commingling with animals from unrelated farms (Aiello, 1998). Stress appears to be an important factor in the breakdown of respiratory tract defense mechanisms, allowing bacteria to invade lung tissue causing pneumonia (Aiello, 1998). Besides *Pasteurella* spp., other bacteria such as *Mycoplasma* spp., *Chlamydia* spp., *Haemophilus* spp., and *Salmonella* spp. are associated with causing either primary or secondary bronchopneumonia in sheep and goats (Aiello, 1998). Infection with these pathogens is also increased in stressful situations. Another form of pneumonia, shipping fever, which is commonly found in cattle can also be found in sheep being transported. The stress of transport is what causes an animals' susceptibility to shipping fever. In the year 2000, respiratory problems were the fifth leading cause of death in sheep following predators, old age,

lambing problems and unknown causes (USDA, Part 1: Reference of Sheep Management in the United States, 2001). It is, therefore, necessary to reduce stress and improve humoral immune status of animals prior to transport and handling.

Average daily gain in sheep. Since sheep are raised predominantly for the purpose of meat and fiber production, the rate at which they grow is very important. The faster sheep grow, the earlier a producer may sell them. This has led to many different growth supplements and intense breeding for faster growing sheep. The average daily gain of domestic sheep is 0.2 – 0.3 kg/day (Nutrient Requirements of Sheep, 6th ed, 1985). If an animal's growth is depressed from the normal rate, disease or nutrition is usually the contributing factor. It is worthwhile to ascertain the effects of supplementation with ANOD on the growth of an animal, as normal growth points to good overall health.

Objective

Stress during handling and transport is a major problem in the livestock industry, especially when it occurs during hot weather. The health of the livestock is often at risk during these procedures. It would be beneficial to find methods of alleviating the stress of these processes, thereby improving the health and well being of the livestock. Dietary supplementation with ANOD has been reported to have many positive effects on animals. The primary objective of this study was to use physiological measurements to ascertain if and at what rate ANOD should be fed to animals prior to handling and

transport in order to obtain beneficial effects such as mediating body temperature and lowering stress. The secondary objective of this study was to which of the biologically active components of ANOD were responsible for the effects observed in the first trial.

DOSE RESPONSE TRIAL

As ANOD has been shown to have beneficial effects on animals during heat stress by lowering body temperature and increasing cellular immunity (Allen et al., 2001a,b), it is of interest to determine if ANOD would be useful in mediating transport or handling stress during hot conditions. Transport often occurs during the hottest periods of the year, which raises the stress levels of animals. The initial goal of this project was to establish how much ANOD is needed to obtain benefits during transport or handling.

Methods and materials

General. Forty-four crossbred sheep (23 ewes and 21 whethers), averaging 26.4 kg were used in this study. These sheep were used in a prior transport study (Krawczel et al., 2005) six wk earlier, but six wk allowed for sufficient recovery. The sheep were randomly assigned to receive ANOD (Tasco 14, Acardian Agritech, Dartmouth, Nova Scotia, Canada) at the rate of 0 (control), 0.5, 1, or 2% of daily dry matter intake (DMI) estimated by NRC (Nutrient Requirements of Sheep, 6th ed, 1985), approximately 0, 0.25, 0.5, and 1 g/kg of body wt per day.. The 2% rate was used in previous studies with cattle (Allen et al., 2001b) and the goal was to determine if lower concentrations would have the same effects. Feeding more than 2% was reported to decrease intake in cattle when the ANOD was mixed with feed (Allen, personal communication).

The sheep in this study were kept on pasture and fed a 16% pelleted supplement on an ab litum basis. Twice daily starting on day 0 and continuing until day 14, the

sheep were brought down from the pasture to the working pens. Sheep were then administered three gelatin capsules (size 14, Torpac, NJ, USA) containing either the appropriate amount of ANOD or their standard grain supplement using a balling gun. After receiving boluses, the sheep were released back into the pasture.

During the 2 wk ANOD supplementation, the sheep were weighed on day 0, 7, and 14 to determine growth rate. On day 11, all sheep were injected intramuscularly (I.M.) in each side of their hindquarters with 0.5 mg ovalbumin (egg protein) suspended in 0.25 ml of saline to test humoral immune response (antibody production). This was a booster vaccination as the sheep were given an initial ovalbumin injection in an earlier study six wk prior to this study. Blood samples (10 ml) were taken via jugular vena puncture on day 11, 15, and 18. An indirect ELISA modified from Ameiss et al. (2004) was performed on all plasma samples using 96-well plates coated with 5 µg of ovalbumin per well. Following overnight incubation of the coated plates at room temperature, plates were blocked with a BSA solution (1X PBS, 0.05% Tween 20, 0.5% BSA) for 30 min and then rinsed with PBS-T (pH 7.4, 0.5% Tween 20). Plates were again rinsed and 100 µl of each sample was added to the first and sixth column of the plates and serial dilutions were performed for columns 2-5 and 7-10. The plates were then incubated for one h at room temperature after which they were rinsed again and 150 µl of donkey anti-sheep IgG or chicken anti-sheep IgM (Bethyl Laboratories, Montgomery, TX) was added to each well and plates were agitated for one h at room temperature. Plates were rinsed and a substrate solution was added (Immuno Pure TMB Substrate kit, Pierce, Rockford, IL). After 15 min, the reaction was stopped by adding

100µl of 2 M sulfuric acid. Absorbance was read by a SunriseÂ® multi-well plate reader (Tecan, Austria) at a wavelength of 450 nm. Positive and negative control samples were also run on each plate. The positive controls were used to standardize the results of each plate and were obtained by vaccinating three ewes every three weeks (over a four month period) with ovalbumin. The titer for each ewe was checked and the ewe with the highest titer was used for the positive control.

Walking trial. All sheep had temperature data loggers (Thermochrons, Maxim/Dallas Semiconductor Corp., Sunnyvale, CA) placed in one of their ear canals to measure body temperature on day 14, one hour prior to the walking trial. Each data logger was first placed into the toe of an infant size cotton sock. The sock was then filled with cotton and placed in the ear with the toe as far as possible into the ear canal. Next, the ear was taped shut to seal it and to ensure the sock did not fall out during the trials. Loggers were set to record a temperature every 5 min. At the end of the trial, all sheep had their rectal temperature taken using a digital thermometer.

All sheep were relocated to an enclosed pen (9 X 9 m) formed from a section of their home pasture on day 14 at 1300 h. Sheep were walked in a circular motion around a 3 X 3 m rectangular structure within the pen for 30 min in order to increase the body temperature of the sheep and to simulate vigorous extended handling. At the start of the walking trials, the environmental temperature was 29.5 ºC. The sheep were walked at a brisk pace until their respiration rate increased and they started panting. The pace was then slowed and maintained for the remainder of the 30 min period. Increased

respiration rates occur during hyperthermia (Hales and Brown, 1974; Entin et al., 1998, 1999; Friend et al., 1998). The sheep were then allowed to rest for 30 min to allow respiration rates to return to normal, after which the 30 min walk was repeated.

Ten ml of blood was taken from all sheep via jugular vena puncture immediately prior to the first walking period and immediately after the second walking period. Samples were analyzed for cortisol concentration using commercially available RIA kits (DPC, Los Angeles, CA). Duplicates of each plasma sample were assayed and the mean concentration was calculated by a computer from a logit-log representation of the calibration curve. First, the program calculated the average of raw counts per minute of the two duplicate samples and subtracted from this number the average non-specific binding count. Next, the corrected count was divided by the maximum binding of the tracer to get the percent bound. This percent bound was then compared to the calibration curve to get the concentration of cortisol in each sample. Any duplicates that differed by more than 12% were re-assayed. The intra-assay and inter-assay CV for the cortisol assays were less than 9%.

Transport trial. Sheep were transported on day 15 over local roads near College Station, Tx for 12 h (730-1930) during the month of September in a goose neck trailer. Treatments were equally divided into three groups of 15 sheep between three 2.1 X 2.3 m compartments within the trailer. An extra sheep, not used in the study, but part of the same flock, was used to equalize the stocking density of 15 sheep per compartment. The

external temperature during transport ranged between 19.0-30.7 ºC and the temperature inside the trailer ranged between 18.7-32.0 ºC.

Temperature loggers were left in the ears of the sheep following the walking trial so they could be used to monitor body temperature the next day during transport. Ten ml of blood was taken from all sheep via jugular vena puncture at 0, +4, +8, and +12h of transport and on day 18. Plasma was frozen until analyzed. All samples were analyzed for cortisol and the 0 and +12h samples were analyzed for aldosterone using commercially available RIA kits (DPC, Los Angeles, CA). Any duplicates that differed by more than 12% were re-assayed. The intra-assay and inter-assay CV for the cortisol and aldosterone assays were less than 9%. A portion of the blood sample taken at 0 and +12h of transport was used for determination of electrolyte concentrations and plasma chemistry profiles by the Texas Veterinary and Medical Diagnostic Laboratory. Lambs were weighed before and prior to being offered feed and water after transport.

Following transport, the sheep were simultaneously released into a 9 X 9 m pen sectioned off from their home pasture; this section contained feed and water troughs. The feed was offered in the same round feeder that the sheep fed from daily and two additional 4 m troughs were filled with feed (16% pelleted ratio) to allow room for all sheep to eat. The water trough was 1 m long with a float valve for automatic refilling. The latency to eat, drink, and lay down was recorded for all sheep by observers.

Statistical analysis. Humoral immune response and cortisol during transport were analyzed following a repeated measures design. Proc GLM (SAS version 8.2, SAS

Institute Inc., USA) was used with treatment, time, and the treatment by time interaction, and individual sheep nested within treatment as the factors. When significant treatment by time interactions were found, mean separation was performed using the pdiff function. Average daily gain, cortisol during the walking trial, body temperature, plasma chemistry, electrolyte, aldosterone, weight loss, and behavior data were analyzed using ANOVA followed by the LSD procedure for post hoc analysis of pair wise comparisons. Significant differences were $P < 0.05$, unless otherwise stated. A regression analysis was performed on all data to determine dose response ($P < 0.05$). Curve estimation was performed to visualize the best fit for significant dose response effects.

Results

General. During the 14 d supplementation period, the sheep fed the 1% rate grew at a faster rate (0.30 kg/day) than control sheep (0.15 kg/day, $P < 0.05$). This was due to the 1% rate having higher ($P = 0.06$) gain than the control sheep during the second week of supplementation.

All sheep had similar titers of IgG specific to ovalbumin prior to the booster (d 11, Table 2). On day 15, only the control sheep exhibited a normal immune response. All other treatments had a significantly lower IgG immune response and this effect was dose dependent even at day 18 (Table 2). The dose response effect for IgG on d 15 was linear ($P < 0.05$) and best fit a cubic line ($R^2 = 0.37$). A similar effect was also seen in

the IgM response, again IgM decreased linearly as dose of ANOD increased ($P < 0.05$) on d 11 and d 15 and best fit a cubic line ($R^2 = 0.32, 0.32$).

Table 2. Mean IgG and IgM titers (absorbance at 450 nm ± SE) of sheep during dose response trial

	IgG			IgM		
Treatment	Day 11	Day 15	Day 18	Day 11	Day 15	Day 18
0%	0.43 ± 0.10	2.39^{abc} ± 0.44	1.62^{ab} ± 0.25	1.16± 0.05	1.24^{abc} ± 0.09	1.33^{ab} ± 0.06
0.5%	0.56 ± 0.11	1.24^{ad} ± 0.45	1.37^{c} ± 0.30	1.26^{a} ± 0.07	0.83^{a} ± 0.12	1.34^{cd} ± 0.07
1%	0.54 ± 0.11	0.87^{b} ± 0.19	1.09^{b} ± 0.21	1.04 ± 0.04	0.83^{b} ± 0.06	1.01^{ac} ± 0.14
2%	0.38 ± 0.06	0.17^{cd} ± 0.03	0.62^{ac} ± 0.13	0.91^{a} ± 0.07	0.69^{c} ± 0.09	1.00^{bd} ± 0.17

Means with same superscript within columns are different ($P < 0.05$).

Walking Trial. Ear canal temperature followed rectal temperature, but was between 2 and 3 ºC lower (Table 3). There was no difference between treatments in the maximum, minimum, or range in ear canal temperature during the walking trial. There was no treatment effects on rectal temperature response post walking.

Table 3. Comparison of ear canal and rectal temperature (ºC ± SE) at the end of walking trial. Ear canal temperature (ºC ± SE) range during 12h of transport.

	Temperature		
Treatment	Rectal end walk	Ear canal end walk	Ear canal range transport
0%	41.18 ± 0.12	38.81 ± 0.13	1.28^{a} ± 0.10
0.5%	41.18 ± 0.10	38.62 ± 0.11	1.21 ± 0.11
1%	41.24 ± 0.10	38.59 ± 0.11	1.27 ± 0.18
2%	41.16 ± 0.09	38.69 ± 0.13	0.92^{a} ± 0.07

Means with same superscript within columns are different ($P < 0.05$).

Prior to walking, the control sheep had significantly higher plasma cortisol concentrations than those fed the 2% rate of ANOD (Table 4), with the 0.5 and 1% rates of ANOD being intermediate, though non-significant, concentrations of cortisol (P = 0.14, 0.10). The same trend was true post walk, except for the 0.5% rate of ANOD which increased in cortisol concentration.

Table 4. Mean cortisol concentration (µg/dl ± SE) pre and post walking

Treatment	Pre walk	Post walk
0%	$1.90^{a} \pm 0.41$	1.3 ± 0.28
0.5%	1.32 ± 0.26	$1.54^{ab} \pm 0.24$
1%	1.25 ± 0.21	$0.87^{a} \pm 0.15$
2%	$0.97^{a} \pm 0.10$	$0.83^{b} \pm 0.15$

Means with same superscript within columns are different ($P < 0.05$).

Transport Trial. During the 12 h transport period, all sheep reached similar minimum body temperatures (Fig. 1). The sheep fed at the 2% rate of ANOD had a lower maximum body temperature (38.3 ºC, $P < 0.05$) than the control sheep (38.6 ºC) and a lower average body temperature (37.9 ºC, $P < 0.05$) during hot periods of transport (Thermal heat index above 80) than the control sheep (38.2 ºC). Additionally, the sheep fed the 2% rate of ANOD had a significantly smaller range (0.92 ºC) in their body

temperature throughout transport compared to the control sheep (1.28 ºC, Table 3). All sheep had similar variation in body temperature throughout transport (avg. 0.12).

Throughout transport, the control sheep tended to have higher cortisol concentrations than the other sheep (Fig. 2). However, this was significant only at 4 and 8 h of transport when the control sheep had significantly higher concentrations than the sheep fed the 2% rate of ANOD (P = 0.05, 0.10).

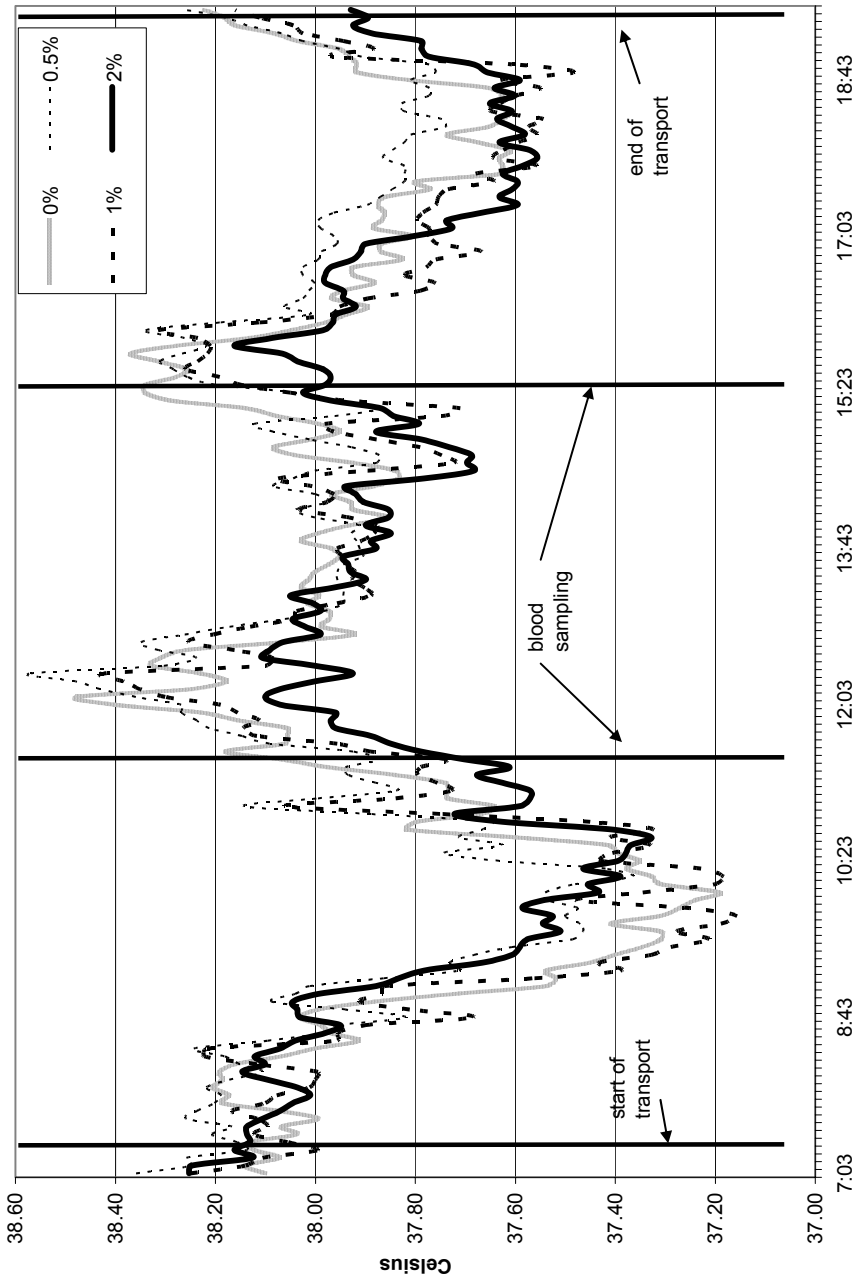

Figure 1. Ear canal temperature of sheep (°C) during transport during dose response trial.

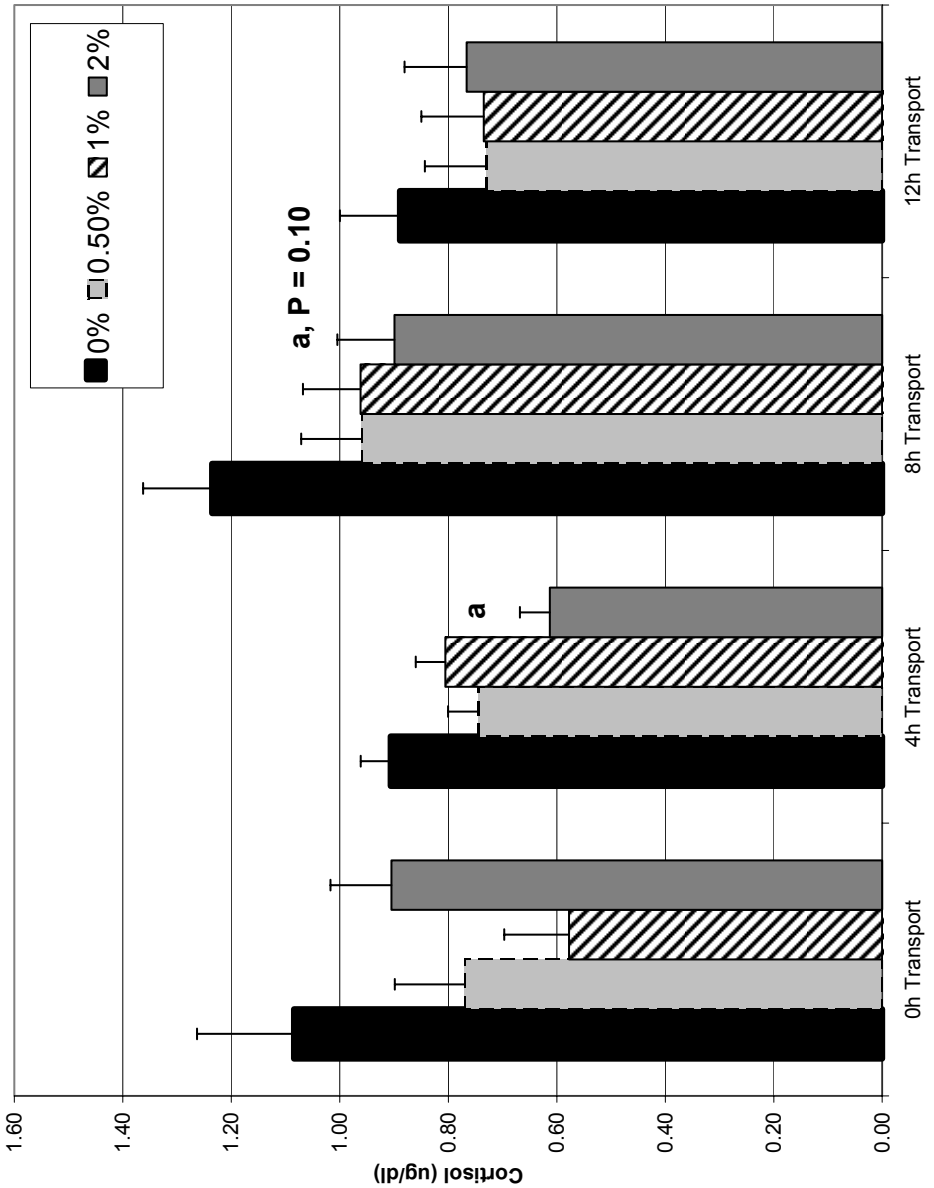

Figure 2. Cortisol concentration (µg/dl ± SE) of sheep being transported during dose response trial.
[a] Significantly different from control $P < 0.05$, unless otherwise stated.

Higher supplementation lowered pre-transport concentrations of aldosterone (Table 5). After transport all sheep had similar concentrations of aldosterone, except for the sheep fed the 1% rate of ANOD which were significantly higher than the other treatments for no known reason.

Table 5. Aldosterone concentrations (ng/dl ± SE) pre and post 12h transport

Treatment	Pre transport	Post transport
0%	35.34^{ab}± 6.10	16.08^{a}± 1.46
0.5%	32.14± 4.95	15.70^{b}± 2.15
1%	22.50^{a}± 3.36	24.75^{abc}± 2.04
2%	21.99^{b}± 3.41	18.27^{c}± 2.44

Means with same superscript within columns are different ($P < 0.05$).

There were no differences between treatments in pre-transport plasma chemistry profiles. However, there were many differences in the post transport plasma chemistry profile (Table 6). Generally, plasma protein, albumin, phosphorus, glucose, BUN, magnesium, calcium, AST(SGOT), and creatainine were significantly higher in controls and/or the sheep fed the 0.5% rate of ANOD than in the sheep fed the 2% rate of ANOD and occasionally the 1% rate.

The control sheep significantly increased in concentrations of calcium, phosphorus, and magnesium post transport (Table 6). All sheep had significant increases in glucose concentrations post transport except the sheep fed the 2% rate of ANOD. The sheep fed the 1% and 2% rates of ANOD also had a significant decrease in

their BUN concentrations. Globulins, CK, the A/G ratio, and gamma glutamyltransferase (GGT) were not influenced by treatments and therefore are not shown in Table 6.

Table 6. Plasma chemistry panels pre and post 12h of transport

Treatments	P. protein g/dl	Album. g/dl	C mg/dl	P mg/dl	Glucose mg/dl	BUN mg/dl	Creat. mg/dl	T. bili. mg/dl	AST (SGOT) U/l	Mg meq/l
Pre transport										
0%	5.98	3.15	10.25	6.56	69.36	26.56	0.55	0.25	133.36	2.1
0.5%	5.89	3.15	10.42	6.35	65.09	24.96	0.56	0.28	103.55	1.99
1%	5.88	3.14	10.47	6.5	67.82	26.24	0.57	0.26	116.82	2.01
2%	6.32	3.24	10.75	7.04	69.55	24.05	0.56	0.25	118.73	2.15
Post transport										
0%	6.04^{a}	3.24^{ab}	11.22^{az}	7.68^{abz}	85.27^{abz}	24.36^{ab}	0.59	0.22	137.27^{a}	2.30^{abz}
0.5%	6.23^{bc}	3.31	11.33^{bc}	7.21	86.09^{z}	25.15^{cd}	0.61^{a}	0.23	104.45	2.34^{cdz}
1%	5.56^{c}	2.97^{a}	10.30^{c}	6.46^{a}	80.91^{az}	20.17^{acz}	0.58	0.20^{z}	102.09^{a}	2.05^{ac}
2%	5.45^{ab}	2.83^{b}	9.84^{ab}	6.63^{b}	74.82^{b}	20.44^{bdz}	0.52^{a}	0.36	103.73	1.95^{bd}

Means with same superscript within columns and within pre or post transport sampling are different ($P < 0.05$).
[z] Significant change from pre-transport concentrations.

Treatments caused no differences in the pre-transport concentrations of Na, K, or Cl. However, the pre-transport Na/K ratio of the sheep fed the 1 and 2% rates of ANOD were significantly lower than the control sheep ($P = 0.03, 0.06$). There were several differences in the post transport electrolyte concentrations where higher supplemented sheep had lower electrolyte concentrations than the lower supplemented sheep (Table 7).

There were several significant changes between pre to post transport electrolyte concentrations. The control sheep significantly increased in sodium, potassium, and chloride concentrations post transport (Table 7). The sheep at the 0.5% rate significantly

increased in potassium concentration by the end of transport; otherwise, all other treatments showed no significant change in electrolytes as a result of transport.

Table 7. Electrolyte concentrations (meq/l) pre and post transport

Treatments	Na	K	Na/K ratio	Cl
	Pre transport			
0%	134.73	4.06	33.20[a]	101.4
0.5%	131.27	4.11	32.05	99.73
1%	132.27	4.2	31.60[a]	100.2
2%	136.91	4.32	31.79	102.9
	Post transport			
0%	141.45[abz]	4.79[abz]	29.67[a]	107.82[abz]
0.5%	141.45[cd]	4.55[z]	31.16[a]	107.91[cd]
1%	129.27[ac]	4.36[a]	29.8	97.91[ac]
2	126.27[bd]	4.17[b]	30.32	95.45[bd]

Means with same superscript within columns and within pre or post transport sampling are different ($P < 0.05$).
[z] Significant change from pre-transport concentrations.

The percentage of body weight lost during transport was significantly more in the sheep fed the 1% and 2% rates of ANOD than in the control sheep.

No differences were seen in latency to eat as all sheep initially went to feed upon release into the pen after transport. There was a trend for a dose dependent effect on time to drink, with the control sheep taking the longest and the sheep fed the 2% rate of ANOD taking the shortest time to drink after transport ($P = 0.20$). Finally, no difference was seen in latency to lay after transport as all sheep lay at approximately 1.5h post transport.

Discussion

Supplementation did not affect overall growth during the dose response trial, contrary to what was reported by Turner et al. (2002) as a result of their 14 d supplementation. Supplementation with ANOD at the 1% rate appeared to increase daily gain. However, the higher rates had no effect on average daily gain. Therefore, it is likely that the increased growth at the 1% rate was an aberration, as there was not a significant quadratic dose response even during the second week ($P > 0.05$).

The ANOD dramatically decreased the antibody titer. There was a very strong dose dependent response which raises concern. It is important to note that this decreased antibody production was not only seen in the post boost response in both IgG and IgM, but IgM was already lower in high supplemented animals before the booster. This is indicative of a decreased antibody titer even before the booster, demonstrating a suppression of existing antibody titer. This decreased humoral immune response is noteworthy as it has been reported that cellular immune response was increased after ANOD supplementation (Allen et al., 2001b; Saker et al., 2001). It is possible that ANOD supplementation is causing one immune system to predominate.

Although it has been reported that ANOD decreased basal body temperature during the summer (Allen et al., 2001b), that effect was not seen in this study in response to walking. Although ANOD helps maintain lower body temperature during hot weather, it does not have the same effects during vigorous handling of livestock. This is not unexpected, as the physiology which causes and controls body temperature during exercise is different from what occurs in resting animals during ambient heat stress.

Exercise causes an increase in body temperature due to energy expenditure from muscle movement. As long as an animal continues to exercise, heat is continually being produced internally.

The ability of ANOD to moderate body temperature was observed during transport as the sheep fed the 2% rate had a narrower range in temperature. The 2% rate also caused the sheep at that rate to have lower average and maximum body temperature during periods of medium heat stress (THI above 80). This is consistent with previous research in cattle (Allen et al., 2001a,b) that investigated the effects of ANOD on body temperature.

The ANOD did decrease cortisol plasma concentrations in the higher supplemented sheep compared to the control sheep in both the walking and transport trials. It was not clear whether this is due to a decreased perception of stress or a decrease in the HPA axis's function. Supplementation did not affect the release of cortisol in response to walking as the differences before walking were maintained post walking. This indicates that the initial differences between sheep were due to the supplementation itself and not the walking. The control sheep also had significantly higher cortisol concentrations than the sheep fed the 2% rate of ANOD during the hottest part of transport and cortisol was generally higher in the controls at all times. This indicates that the sheep fed the 2% rate of ANOD were possibly less stressed during these periods.

Supplementation also appeared to affect the regulation of water balance. Prior to transport, the higher supplemented sheep had lower concentrations of aldosterone

indicating that they were excreting sodium and retaining potassium (Dickson, 1993) before transport began. Low aldosterone concentration before transport may have allowed the supplemented sheep to remain hydrated throughout transport. Supplemented sheep were already excreting sodium, counteracting the increase usually seen in electrolyte concentrations due to the water loss from excretion and thermo regulation. This hypothesis was supported by the changes in plasma chemistry, as highly supplemented sheep also maintained plasma chemistry concentrations during transport. Aldosterone concentrations after transport were similar among all treatments, possibly indicating that the control sheep were compensating for their altered electrolyte concentrations.

COMPONENT TRIAL

Once the appropriate supplementation rate was determined through the dose response trial, several major components of the ANOD were evaluated to determine their contribution to the treatment effects observed in the dose response trial. The results of the component trial may possibly eliminate the need to feed the ANOD as a whole to get beneficial effects. It might also be possible to determine if a specific component was causing the detrimental effects seen in the previous trial. If any negative components could be identified, that component could possibly be removed from the seaweed supplement.

Methods and materials

General. Fifty-three crossbred sheep averaging 25 kg were used in this study. There were nineteen ewes and thirty-four whethers, all of which were immunized in each side of their rump with 0.5 mg ovalbumin (egg protein) suspended in 0.25 ml of saline and 0.25 ml of Fruend's incomplete adjuvant three weeks prior (-21 d) to day 0. The whethers and ewes where housed in adjacent pastures. Each sheep was randomly assigned to receive ANOD (Tasco 14, Acardian Agritech, Dartmouth, Nova Scotia, Canada) at the rate of 0 (control) or 2% of daily dry matter intake as estimated by the NRC (1985), or 3 g of fucoidan (PRI, City of Industry, CA), or 1 g of sodium (Na/Cl) and 1 g of potassium (potassium glutamate), or 0.25 g of betaine (Betafin, Dansico, Copenhagen, Denmark). The fucoidan, salt, and betaine were fed at a rate to mimic the amount consumed within the 2% ANOD treatment. Twice daily, starting on day 1 and

continuing until day 14, the sheep were brought down from the pasture to the working pens. Sheep were each given three boluses (gelatin capsules, size 14, Torpac, NJ, USA) containing the appropriate amount of substance based on their treatment. After receiving the boluses, they were released back into the pasture.

During the two week ANOD supplementation, the sheep were weighed on day 0 and day 15 to determine growth rate. On day 11, all sheep were injected I.M. in each side of their rump with 0.5 mg ovalbumin (egg protein) suspended in 0.25 ml of saline to test humoral immune response (antibody production) using the same ELISA procedure as in the dose response trial. Blood samples (10 ml) were taken via jugular vena puncture on day 11, 15, and 18 to determine antibody production. The samples on day 15 also had a CBC with differential count performed by the Texas Veterinary and Medical Diagnostic Laboratory, College Station, Tx.

Transport trial. All sheep had temperature loggers (Thermochrons, Maxim/Dallas Semiconductor Corp., Sunnyvale, CA) placed in one of their ear canals on day 15 immediately prior to transport to measure body temperature during transport. The procedure for placing the data loggers within the ear was the same as in the dose response trial. Loggers were set to record a temperature every 5 min.

Sheep were transported on day 15 over local roads near College Station, Tx for eight h (1000-1800h) during the month of September in a goose neck trailer. Treatments were equally divided among seven compartments within the trailer. Each compartment had a similar stocking density. The external temperature during transport ranged between 26.0-33.6 ºC and the temperature inside the trailer ranged between 26.0-34.9 ºC.

Ten ml of blood was taken from all sheep via jugular vena puncture at 0, +4, and +8 of transport. Samples were then frozen until analyzed. All samples were analyzed for cortisol concentration, and the 0 and +8h were analyzed for aldosterone concentrations using commercially available RIA kits (DPC, Los Angeles, CA). Any duplicates that differed by more than 12% were re-assayed. The intra-assay and inter-assay CV for the cortisol and aldosterone assays were less than 8%. The ten ml of blood that was taken at 0 and +8h of transport was also analyzed for electrolyte concentration by the Texas Veterinary and Medical Diagnostic Laboratory, College Station, Tx.

Weights were taken before and after transport to measure the amount of body weight lost during the transport period. All animals were led onto the scale one by one before transport, and the process was repeated after the transport; after transport, sheep were not offered feed and water until after they were weighed.

Following transport, the latency to eat and drink was recorded for all sheep by observers. The sheep were simultaneously released into their home pastures containing a feed and a water trough. The feed trough was the same as the round feeders that the sheep fed from daily and the whethers' pasture was supplemented with two 4 m troughs filled with feed (16% pelleted ratio) to allow all sheep room to eat. A water trough in both pastures was equipped with a float valve for automatic refilling.

Statistical analysis. Humoral immune response and cortisol during transport were analyzed following a repeated measures design. Proc GLM (SAS version 8.2, SAS Institute Inc., USA) was used with treatment, time, sex, and the treatment by time

interaction, and individual sheep nested within treatment as the factors. When significant treatment by time interactions were found, mean separation was performed using the pdiff function. Average daily gain, body temperature, CBC, electrolyte, aldosterone, weight loss, and behavior data were analyzed using ANOVA followed by the LSD procedure for post hoc analysis of pair wise comparisons. Significant differences were $P < 0.05$, unless otherwise stated.

Results

General. There was no difference between treatments for growth rates of any of the treatments during the 14 d supplementation period.

All sheep had similar amounts of IgG specific to ovalbumin on day 11 (Table 8). On day 15, the ANOD sheep had a significantly lower IgG titer than the control sheep. The salt sheep showed a slight trend for a reduced IgG immune response ($P = 0.20$) relative to the controls. By day 18, all sheep had similar IgG titers once again (Table 8). A similar effect was also seen in the IgM response; the exceptions were seen in the betaine sheep starting with lower IgM titer and in the salt sheep ending with higher IgM titer than the control sheep on day 18.

Table 8. Mean IgG and IgM titers (absorbance at 450 nm ± SE) of sheep during component trial

Treatment	IgG			IgM		
	Day 11	Day 15	Day 18	Day 11	Day 15	Day 18
ANOD	0.15 ± 0.05	0.63^{a} ± 0.07	0.69 ± 0.13	1.07^{a} ± 0.03	0.85^{abc} ± 0.07	1.48 ± 0.19
Fucoidan	0.22 ± 0.02	0.70 ± 0.05	0.71 ± 0.10	1.05± 0.02	1.27^{ad} ± 0.04	1.36± 0.08
Salt	0.19 ± 0.03	0.69 ± 0.04	0.68 ± 0.13	1.19^{b} ± 0.07	0.79^{def} ± 0.08	1.57^{ab} ± 0.11
Betaine	0.16 ± 0.02	0.70 ± 0.04	0.72 ± 0.07	0.91^{abc} ± 0.08	1.22^{be} ± 0.08	1.27^{a} ± 0.06
Control	0.20 ± 0.03	0.80^{a} ± 0.08	0.67 ± 0.15	1.19^{c} ± 0.20	1.42^{cf} ± 0.21	1.31^{b} ± 0.12

Means with same superscript within columns are different ($P < 0.05$).

The ANOD sheep tended to have higher ($P = 0.10$) white blood cell counts, eosoniphils ($P = 0.10$), and lymphocyte counts ($P = 0.06$) than the control sheep following 14 day supplementation (Table 9). The salt sheep had higher lymphocyte counts ($P = 0.06$) than the controls following supplementation (Table 9). The betaine sheep had higher plasma cell volume and hemoglobin ($P = 0.06$) than the control sheep following supplementation. There was no difference in neutrophil/lymphocyte ratio between any treatments. All other measurements in the complete blood count data (Appendix Tables 37-40) were not significantly different from the controls.

Table 9. Mean white blood cell count (WBC), packed cell volume (PCV), hemoglobin, eosoniphil, and lymphocyte count (± SE) on day 15.

Treatment	WBC	PCV	Hemoglobin	Eosoniphil	Lymphocte
ANOD	6440^{a} ± 527	27.98 ± 0.87	9.58^{a} ± 0.31	507.7^{abcd} ± 176	3555^{ab} ± 232
Fucoidan	4982 ± 416	28.61 ± 0.66	9.95 ± 0.23	123.3^{a} ± 170	2581^{ac} ± 214
Salt	6009 ± 519	28.14 ± 0.75	9.72 ± 0.31	150.9^{b} ± 175	3543^{cd} ± 247
Betaine	5320 ± 571	29.93^{a} ± 0.79	10.37^{ab} ± 0.30	162.3^{c} ± 89	2891 ± 324
Control	4964^{a} ± 635	27.47^{a} ± 1.26	9.46^{b} ± 0.46	276.0^{d} ± 58	2486^{bd} ± 328

Similar superscripts significantly different ($P < 0.10$).

Transport. During the eight h of transport, all sheep reached similar maximum and minimum body temperatures (Table 10). The temperature range for the ANOD sheep tended to be smaller than the control sheep, though they were not significantly different. The THI was above 80 for all of the transport. The ANOD supplemented sheep often maintained a lower body temperature during hot periods of transport than the control sheep (Fig. 3). There was no difference between the variance of body temperature during transport (avg 0.34). The mean number of sheep used to collect temperature data was 7.5 ± 1.0 per treatment during transport. Some sheep lost their temperature loggers during transport. The number of sheep with working loggers did not steadily diminish as most loggers were found and replaced in the ear canal midway through transport.

Table 10. Ear canal maximum, minimum, and temperature range (ºC ± SE) during 8h of transport

	Temperature		
Treatment	Maximum	Minimum	Range
ANOD	38.58 ± 0.12	37.25 ± 0.37	1.19 ± 0.22
Fucoidan	38.69 ± 0.25	37.06 ± 0.18	1.44 ± 0.20
Salt	38.77 ± 0.16	36.98 ± 0.24	1.86 ± 0.21
Betaine	38.76 ± 0.10	37.11 ± 0.30	1.65 ± 0.21
Control	38.77 ± 0.14	36.91 ± 0.36	1.61 ± 0.23

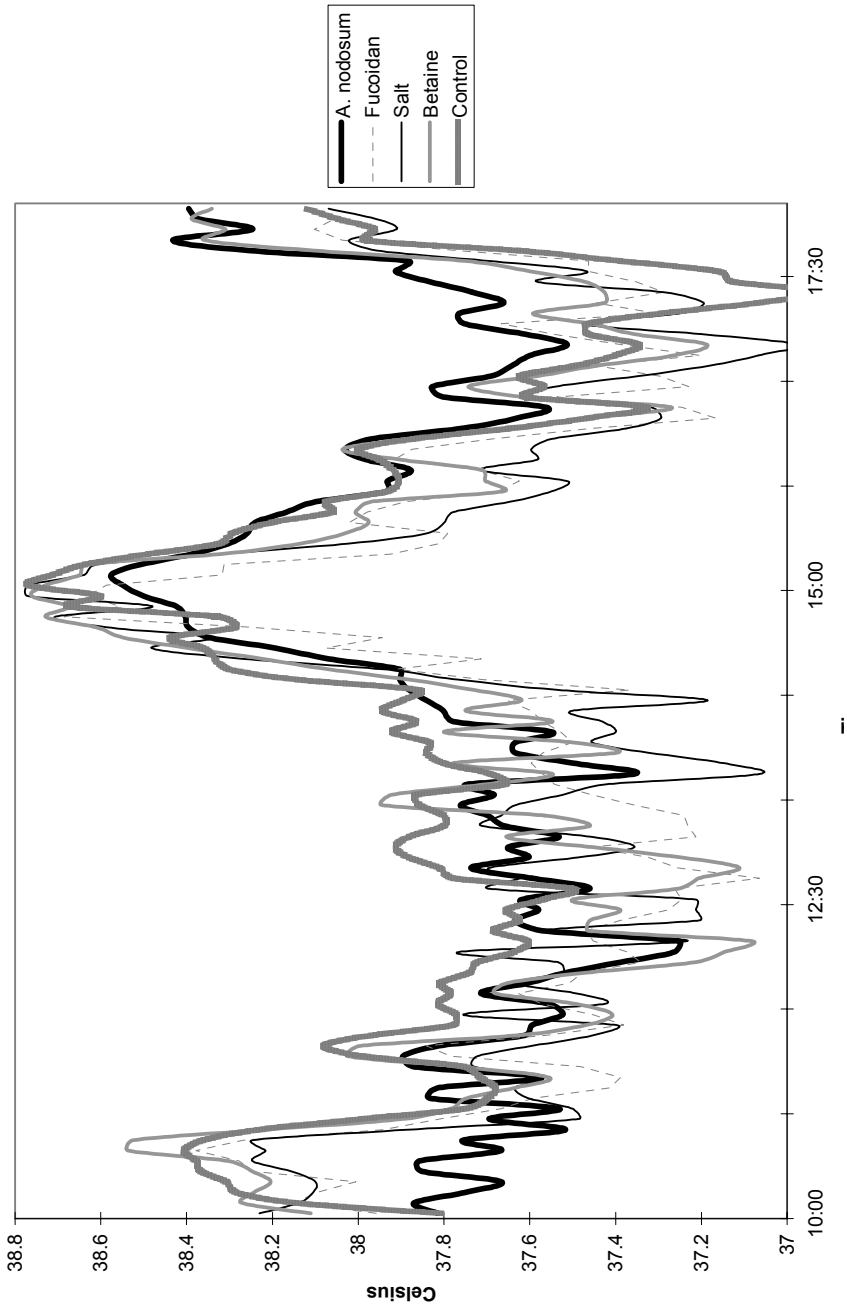

Figure 3. Ear canal temperature of sheep (˚C) during transport during component trial.

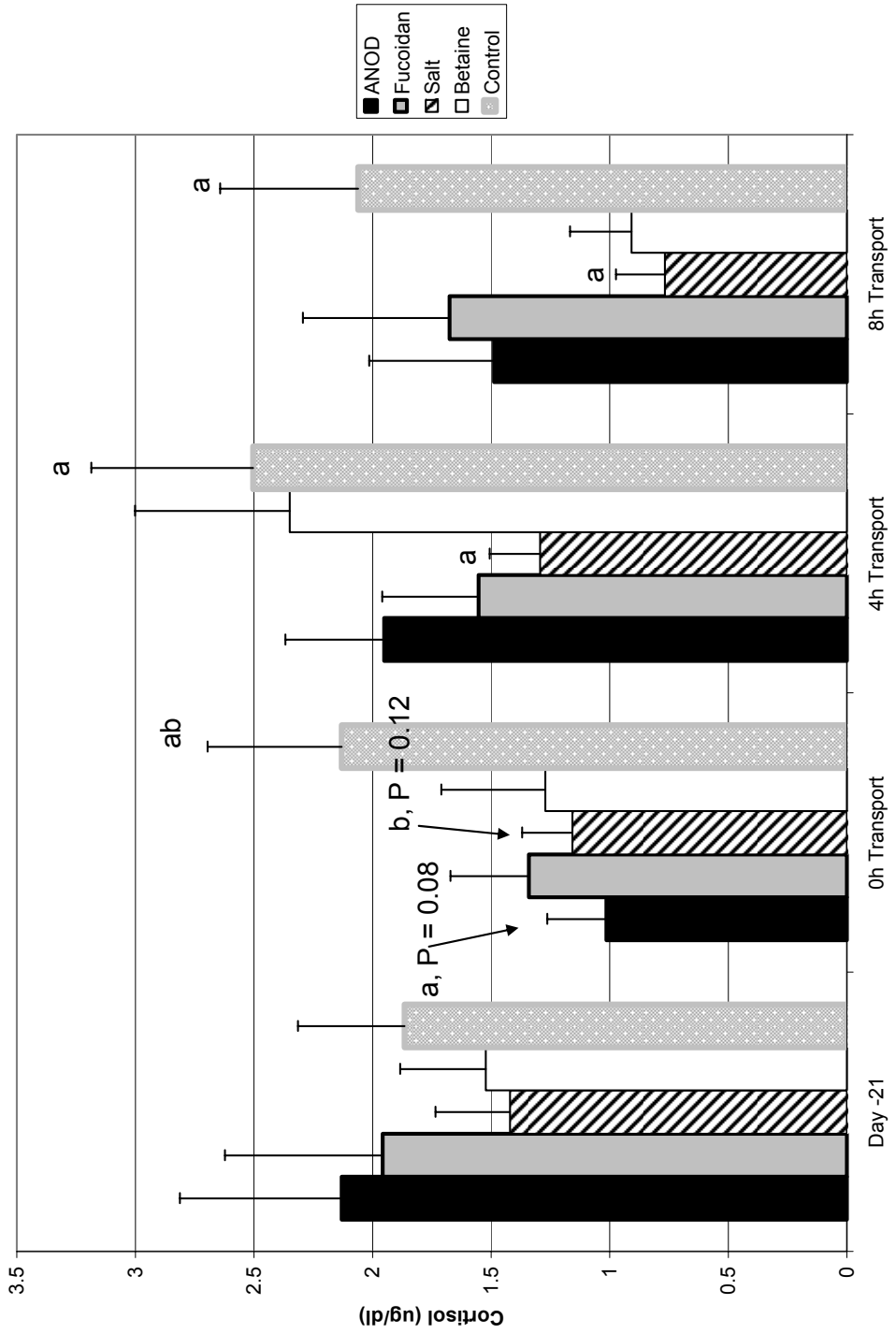

Figure 4. Cortisol concentrations (µg/dl ± SE) of sheep before supplementation (day -21) and while being transported during the component trial.
Similar superscripts within time period indicate significantly different, $P < 0.05$, unless otherwise stated.

Prior to the start of supplementation (d -21) all sheep had similar cortisol concentrations (Figure 4). At the start of transport the ANOD and salt sheep tended to have lower cortisol concentrations than the control sheep (P = 0.08, 0.12). The salt sheep continued to have significantly lower cortisol concentrations than the control sheep throughout transport, while the ANOD sheep generally had lower, though non-significant concentrations (Figure 4).

On day -21 all sheep had similar aldosterone concentrations (Table 11). Pre-transport, the ANOD, fucoidan, salt, and betaine sheep all showed a slight trend for lower aldosterone concentrations than the control sheep (P = 0.10, 0.07, 0.14, 0.09). After transport all sheep had similar concentrations of aldosterone when compared with the control sheep (P > 0.05).

Table 11. Aldosterone concentrations (ng/dl) pre and post 12h transport

Treatment	Day -21	Pre transport	Post transport
ANOD	13.39 ± 3.84	5.51 ± 1.19[a]	5.08 ± 1.26[a]
Fucoidan	11.15 ± 1.76	5.19 ± 1.65[b]	7.85 ± 1.90
Salt	8.80 ± 1.60	5.97± 1.76	7.31 ± 1.57
Betaine	9.66 ± 2.06	5.37 ± 1.06[c]	10.07 ± 2.27[a]
Control	12.32 ± 2.33	9.30± 1.98[abc]	6.83± 1.70

Means with same superscript within columns are different (P < 0.10).

There were no differences between the control and the other treatments in pre-transport concentrations of Na, K, Na/K ratio, or Cl (Table 12). The one exception was

with the salt sheep, which had lower pre-transport Na/K ratio and chloride concentrations than the control sheep (P = 0.07, 0.01). There were several differences in the post transport electrolyte concentrations, in which control sheep had higher sodium and chloride concentrations than other treatments (Table 12).

All sheep significantly increased in sodium concentrations post transport (Table 12). Na/K ratio was impacted by transport in the control and salt sheep but not in the other sheep. All sheep, except for the fucoidan and salt sheep increased in chloride concentrations post transport as well.

Table 12. Electrolyte concentrations (meq/l) pre and post transport

Treatments	Na	K	Na/K ratio	Cl
	Pre transport			
ANOD	144.40	4.62[a]	31.32[a]	109.60
Fucoidan	144.27	4.75	30.42	110.36
Salt	144.36	4.82	30.04[b]	108.64[a]
Betaine	144.10	4.67[a]	31.05	109.80
Control	145.27	4.65	31.29[ab]	110.64[a]
	Post transport			
ANOD	148.50[abz]	4.72	31.50	111.00[aez]
Fucoidan	148.09[acz]	4.64	32.22	111.55[be]
Salt	149.73[cz]	4.63	32.75[z]	109.18[cdef]
Betaine	149.50[dz]	4.61	32.55	111.70[fz]
Control	151.18[bdz]	4.54	33.63[z]	113.55[abcz]

Means with same superscript within columns and pre and post transport period are different (P < 0.10).
[z] Significant change from pre-transport concentrations.

There was no difference between the control and any other treatments in the percentage of weight lost as a result of transport as all sheep lost between eight and ten percent of body weight.

No differences were seen in latency to eat as all sheep initially proceeded to feed upon release into the pen after transport. Control sheep tended to drink sooner than ANOD and fucoidan sheep post transport (P = 0.17, 0.09).

Discussion

The suppression of humoral immunity (antibody titer) which was seen during the dose response trial was also seen in the component trial in the ANOD and salt treatments. While all treatments did not have as high IgG or IgM titers as the controls, only the salt sheep and ANOD sheep had a significantly reduced IgM titer on day 15. Salt appears to be the component which is contributing to the decreased humoral immune response as a result of feeding ANOD. Excess chloride, twice required levels, when sodium is not increased can decrease humoral immunity (Latshaw, 1991). Because the salt in the pelleted diet and mineral supplement was in the form of sodium chloride, and the drinking water had four times as much sodium as chloride, it is unlikely that chloride levels reached the amount needed to suppress humoral immunity. Further research is needed to determine the mechanism of suppression caused by the salt supplementation in this study.

It was reported that ANOD increased cellular immunity in cattle (Allen et al., 2001b; Saker et al., 2001). This trial appears to support that assessment as the salt and

ANOD sheep in the component trial had higher white blood cell counts and lymphocyte counts. This, coupled with the humoral data, suggests that a component of ANOD is causing a switch to a predominately cellular immune response (Abbas et al., 2000).

Similar body temperature responses were seen in this trial as in the dose response trial with the ANOD sheep, though much of the results were non-significant due to lower numbers of successfully recorded ear canal temperatures. The mean number of sheep from which viable data could be collected from during this trial was 7.5 ± 1.0 due to sheep losing their temperature loggers. It was also not clear as to which of the studied components, if any, could be causing the reduced body temperature as all components showed no consistent trend body temperatures in relation to the controls during transport.

To determine if supplementation was impacting plasma cortisol, we sampled the sheep prior to the start of supplementation. Supplementation with ANOD caused a decrease in plasma cortisol concentration. This was also true for the salt sheep which had lower cortisol throughout transport. This suggests that ANOD, and specifically the salt contained within it, are causing a decrease in adrenal function.

If the decreased cortisol concentrations are further combined with the lowered aldosterone concentrations seen in this trial in the sheep fed the 2% rate of ANOD prior to transport, it does appear that the adrenal function might have been affected by the ANOD. If this decreased adrenal function results in insufficiency of adrenal hormones, it can lead to chronic fatigue (Siegel and Melby, 1994). The affect of ANOD on the adrenal gland, however, requires further study.

While the component trial's lower aldosterone levels were not as dramatic as in the dose response trial, the experimental treatments did have lower sodium and chloride concentrations post transport than the control sheep. This again indicates they were better physically hydrated, since their electrolyte balance was maintained. The betaine sheep had higher packed cell volume and hemoglobin, indicating dehydration due to water loss. It is plausible that betaine may cause increased water loss if fed for two weeks, though more research is needed to confirm this conclusion. There is very little published data available on the effect of feeding betaine to ruminants. Betaine also did not impact growth as seen in previous studies (Felix and Sudharsan, 2004; Wray et al., 2004; Wang et al., 2004) and is likely not the component responsible for increased growth seen by Turner et al. (2001).

GENERAL DISCUSSION

All previous studies either sprayed an ANOD extract on the grass the animals were grazing, mixed it in the feed (Allen et al., 2001a,b), or fed it as a mineral supplement (Allen, personal communication). In those studies, it was assumed that the animals ate ANOD at the 2 % rate; however, there is no way of knowing if these animals uniformly consumed the ANOD at the expected rate. In the current study, sheep were given ANOD boluses to guarantee that each one received the specified concentration of ANOD. This enabled a more accurate investigation of the effects of different supplementation concentrations than in any previous study.

The dose response trial found that ANOD had no effect on the regulation of body temperature during exercise. The supplement also had no impact on average daily gain during that trial, contrary to what was seen in Turner et al. (2002). However, ANOD was only fed for two weeks and a longer feeding period may be necessary to influence daily gain. The dose response trial did reveal that ANOD can reduce body temperature during transport in conditions where the THI is at least 80. This trial also confirmed that the 2% rate of supplementation suggested in other studies (Allen et al., 2001a,b) is the appropriate rate for sheep to get the desired lowering of body temperature. However, as the amount of ANOD was increased, suppression of antibody titer increased in a dose dependent manner. There also appeared to be a greater effect on adrenal hormones as the supplementation rate was increased. Since the 2% rate decreased pre-transport aldosterone concentrations, this may have allowed sheep at the 2% rate to maintain

electrolyte concentrations during transport as they increased salt excretion through urination.

This study also sought to determine the active components of ANOD which caused the treatment effects seen in the dose response trial. By giving boluses to the sheep with equal proportions of the components as those found within ANOD when fed at 2% of DMI, we were able to determine if those components were responsible for the effects on body temperature, immune function, adrenal hormones, and hydration that were found in the dose response trial.

The ANOD treatment in the component trial again indicated lowered body temperature and a suppressed humoral immune response. The ANOD also appeared to increase cellular immunity as suggested by Allen et al. (2001b) and Saker et al. (2001). There was an increase in total WBC number and specifically lymphocytes. Though it needs confirmation, one can infer that the increase in lymphocytes indicated an increase in T cells. If B cells increased instead of T cells, humoral immune response would not have been suppressed. This trial also confirmed that the supplementation of ANOD caused a reduction in adrenal function, as prior to supplementation all sheep had similar cortisol and aldosterone concentrations.

Of the components studied, only the salt seemed to be causing the effects on immunity and adrenal function demonstrated by ANOD supplementation. The salt affected humoral and cellular immunity, and adrenal function in a similar manner as the ANOD. After supplementation, betaine sheep showed higher PCV and lower aldosterone concentrations than the control sheep. This indicates that betaine sheep were

less hydrated than controls, which differs from previous research which found that betaine helped to retain water (Partridge, 2003). Since such a small amount of betaine was fed, it is possible that it was not enough to see the positive effects seen in other studies. Belay et al. (1992), fed betaine at a rate of 0.10% of total feed intake. In this study, however, betaine was fed at a substantially lower rate. Fucoidan caused similar affects compared to those seen in the dose response trial on post supplementation aldosterone concentrations and electrolyte concentration maintenance. Although previous research (Maruyama et al., 2003; Tissot et al., 2003; Chotigeat et al., 2004; Eiwegger et al., 2004; Lee et al., 2004) found that fucoidan affected immune function, it had no effect in this study.

The reduced antibody titers observed in both trials is troubling as it leaves supplemented animals open to bacterial infections. Without antibodies to respond to a bacterial infection, bacteria will likely prosper in an ANOD supplemented animal, though more research is needed to confirm it. Antibodies produced during a humoral immune response are needed to neutralize bacteria by binding to them, to opsinize bacteria to promote phagocytosis, to aid natural killer cells in their ability to lyse bacteria, and to activate the compliment system (Abbas et al., 2000). It is also important to note that it would be counterproductive to vaccinate animals who are being supplemented with ANOD as they would produce little to no antibodies. Vaccinating prior to supplementation with ANOD may be advisable, though suppression of all antibodies is likely no matter when vaccination occurs. Recent research has shown that ANOD supplementation can reduce the amount of *E. coli* found in feces and on the

bodies of cattle (Braden et al., 2004). The reduction in *E. coli*, however, is likely not occurring as a result of the humoral immune system as other studies have not shown the impact of feeding ANOD on bacterial infections (Allen et al., 2001b). It is likely some change in intestinal pH or other mechanism is the reason for the decreased *E. coli* numbers and anti bacterial effects of ANOD (Vacca and Walsh, 1954) though research is needed to confirm this.

The trend for lower body temperature in the supplemented sheep in both trials could have resulted in a reduced loss of water through thermo regulation as the need to cool their bodies was decreased. Water loss through excretion was likely higher during the dose response trial than in the component trial because the average percentage of weight loss during transport were 12.4% and 8.9%, respectively. Weight loss as a result of transport is due mostly to water loss. In the dose response trial, the higher supplemented sheep lost more weight than the control sheep, while in the component trial all sheep lost similar amounts. The difference between the trials is likely due to the greater difference in aldosterone concentrations in the dose response trial compared to the component trial. During the dose response trial the higher supplemented sheep were excreting more salt via urination than the control sheep based on their aldosterone concentrations. The aldosterone concentrations of the treatments in the component trial were more similar to each other, indicating they were likely urinating similar amounts (Borresen et al., 1982; Dick et al., 1994). Urine volume was not measured in this study, but should be quantified in future studies. The pattern for which treatment drank first after transport was not consistent. Highly supplemented sheep in the dose response trial were possibly

urinating more which accounted for their increased body weight loss and increased thirst. The ANOD supplemented sheep had lower plasma electrolytes post transport than the control sheep in both trials, although it was more noticeable in the dose response trial. Because the ANOD supplemented sheep had less concentrated electrolytes in their blood, water with electrolytes will be most useful in rehydrating those sheep. Water without electrolytes could be detrimental as it would further decrease the concentration of electrolytes within their plasma. Sex of the animals did not have an effect in any treatment in either trial.

CONCLUSION

Livestock are often transported long distances in hot weather. This common practice has led to the need to find methods of decreasing the effects of transport and heat stress on these animals. One possible option is to find dietary supplements that could alleviate some of the stress. The seaweed ANOD has shown numerous beneficial results that could make it useful in the alleviation of stress associated with handling and transport of livestock. An extract of the ANOD has been reported to lower core body temperature of cattle in hot weather while also stimulating a higher core body temperature in cold weather (Allen et al., 2001a,b), increase cell mediated immune function (Allen et al., 2001b; Saker et al., 2001), increase weight gain (Turner et al., 2002), increase marbling (Allen et al., 2001a), increase shelf life of meat (Montgomery et al., 2001), and has anti-bacterial characteristics (Allen et al., 2001b). All of these would likely improve the well being and production of livestock.

In this study, ANOD showed some positive effects in the alleviation of heat stress during transport, but it also had a significant negative impact. The 14 d supplementation of ANOD at the 2% rate lowered body temperature during transportation and maintained electrolyte and plasma chemistry throughout transport. However, the humoral immune system was negatively affected by supplementation with ANOD. It appears that salt is the component of ANOD which caused the reduced antibody titers. It is still unclear which component of ANOD is causing the decrease in body temperature as none of the tested components caused similar effects as the ANOD. Supplementation also lowered cortisol and aldosterone concentrations, indicating a

possible decrease in adrenal function. This suppressive effect that ANOD appears to have on the adrenal gland is troubling as proper functioning of the adrenal is needed to maintain the bodies homeostasis and for the stress response (Swenson, 1993); however, future trials are needed to ascertain whether the decreased cortisol and aldosterone concentrations are truly detrimental. Although ANOD showed promise in dealing with heat stress during transport, its negative effects on antibody production may outweigh the positive effects. If animals have no defense against bacterial infections, such as those affecting the respiratory system, they will likely become ill. This makes the supplementation of livestock with ANOD unadvisable until further research can confirm that the health of supplemented animals is not at risk and until the mechanism by which ANOD is affecting antibody production is determined.

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APPENDIX

Table 13. Group number and corresponding treatment

Trial	Treatment #	Ttreatment
1	1	0% (control)
1	2	0.5%
1	3	1%
1	4	2%
2	1	2%
2	2	fucoidan
2	3	salt
2	4	betaine
2	5	0% (control)

Table 14. Weights of sheep collected during the dose response trial (kg)

Treatment #	Sex	Sheep #	Wt 1	Wt 2	Wt 3	Wt 4	Wt 5 pre-transport	Wt 6 post transport	Wt 7
1	F	1	22	24	26	27	29	25	29
1	F	10	27	31	33	34	34	29	34
1	F	11	27	30	32	34	35	31	35
1	F	12	27	28	30	29	29	25	30
1	F	13	23	24	27	29	26	24	29
1	M	14	37	39	41	44	43	39	43
1	M	15	23	26	28	29	30	26	31
1	M	16	24	25	31	32	31	28	34
1	M	17	30	32	36	37	37	33	38
1	M	18	33	35	37	38	39	35	40
1	M	19	27	29	32	33	34	30	34
2	F	2	23	24	27	26	28	23	27
2	F	20	27	27	31	31	33	29	34
2	M	21	33	35	39	39	40	36	39
2	M	22	27	29	33	35	34	30	35
2	F	23	26	26	26	29	30	27	30
2	F	24	27	25	26	27	29	24	29
2	F	25	22	23	24	25	26	23	26
2	F	26	28	29	32	34	32	29	35
2	M	27	17	18	21	21	22	19	24
2	M	28	28	28	30	33	34	29	34
2	M	29	27	29	29	33	35	31	34
3	F	3	24	24	28	30	29	25	30
3	F	30	22	23	26	27	27	24	28
3	F	31	21	22	24	26	26	23	27
3	M	32	34	35	36	39	38	34	39
3	M	33	34	35	36	39	39	34	40
3	M	34	33	36	36	38	39	34	39
3	F	35	27	30	32	34	34	30	34
3	M	36	23	25	29	31	32	27	32
3	M	37	26	27	30	33	33	29	32
3	F	38	26	28	30	33	34	29	34
3	F	39	26	28	33	34	35	30	35
4	M	4	20	23	22	26	27	24	28
4	F	40	24	24	29	29	28	24	29
4	F	41	26	28	30	32	32	28	31
4	M	42	34	33	34	34	38	34	39
4	M	43	27	29	32	34	36	30	35
4	F	44	27	27	29	32	32	27	32
4	M	45	18	19	21	24	24	20	24
4	F	46	26	29	31	32	31	27	33
4	F	47	26	28	30	31	30	27	32
4	M	48	31	32	32	36	38	33	37
4	F	49	25	24	28	30	29	26	30

Table 15. Cortisol concentrations (ug/dl) collected during the dose response trial

Sheep #	Basal	Pre-walk	Post-walk	Pre-transport	4h transport	8h transport	12h trans	2d post-trans
1	1.41	2.65	0.83	2.39	0.79	0.52	0.62	0.94
10	2.38	4.32	1.06	1.41	1.11	0.87	0.43	0.66
11	1.11	0.79	1.33	0.50	0.92	1.72	1.05	0.87
12	1.31	1.75	3.21	1.70	0.81	1.53	0.65	0.56
13	1.41	3.63	0.55	0.42	0.85	1.18	0.85	0.49
14	1.25	0.35	0.79	0.47	0.84	0.93	0.66	0.82
15	2.50	1.74	2.99	0.91	0.81	1.84	1.22	1.45
16	1.31	1.68	0.64	0.92	0.61	1.72	1.77	0.61
17	1.78	0.47	1.10	0.90	1.30	1.01	0.77	0.88
18	2.64	0.42	0.44	0.98	0.93	1.29	1.00	0.49
19	2.23	3.11	1.31	1.33	1.00	0.98	0.76	0.83
2	0.60	1.21	2.49	0.80	0.76	1.01	0.39	0.12
20	1.70	0.80	1.54	0.84	0.78	0.85	0.83	0.55
21	0.67	1.27	2.61	0.63	0.62	0.73	1.16	0.60
22	1.35	1.08	1.27	0.98	0.68	0.95	0.45	0.50
23	0.92	0.78	0.66	0.59	0.80	0.16	0.75	0.39
24	2.23	1.03	2.85	0.55	0.70	0.88	0.50	0.45
25	0.96	1.23	0.88	0.88	0.88	1.03	0.93	0.97
26	1.18	3.78	0.86	1.16	0.95	0.79	0.56	0.95
27	2.00	0.82	1.89	0.75	0.82	0.80	0.43	0.54
28	1.09	1.76	0.57	0.68	0.48	2.28	0.47	0.57
29	0.74	0.74	1.37	0.61	0.71	1.08	1.56	0.67
3	1.22	1.56	1.39	0.70	1.03	2.62	0.86	0.74
30	1.95	0.99	0.70	0.72	0.55	0.93	1.75	0.63
31	1.32	1.26	1.86	0.81	0.60	0.93	0.54	0.40
32	0.80	0.90	1.09	0.32	0.77	0.99	0.40	0.24
33	1.30	0.59	0.46	0.68	0.88	1.20	0.38	0.25
34	0.92	0.98	0.32	0.55	0.89	0.78	0.74	0.35
35	0.82	1.02	0.34	0.52	0.82	0.25	1.03	0.25
36	0.85	0.90	0.51	0.29	0.67	0.79	0.66	0.28
37	0.51	0.93	0.70	0.16	0.61	0.35	0.50	0.32
38	0.63	3.19	0.89	0.75	1.42	0.77	0.67	0.44
39	0.49	1.48	1.33	0.85	0.62	0.97	0.55	0.24
4	0.59	0.41	0.99	0.26	0.61	1.06	0.48	0.84
40	0.65	1.48	1.20	0.69	0.68	0.91	0.56	0.58
41	1.96	0.88	1.74	0.70	0.74	0.47	1.14	0.89
42	2.37	1.13	1.54	0.59	0.12	0.89	0.61	0.49
43	0.42	0.85	0.68	0.52	0.67	0.88	0.62	0.49
44	2.91	1.12	0.82	0.27	0.74	1.05	0.98	0.41
45	1.15	0.53	0.54	0.81	0.32	0.76	1.13	0.54
46	2.24	0.89	0.54	3.20	1.46	1.57	0.86	0.37
47	0.54	0.99	0.21	1.26	0.12	0.73	1.00	0.47
48	1.03	0.82	0.21	0.94	0.64	0.73	0.34	0.53
49	1.22	1.55	0.62	0.71	0.64	0.84	0.71	0.48

Table 16. Aldosterone concentrations (ng/dl) collected during the dose response trial

Treatment #	Sheep #	Pre-transport	Post-transport
1	1	42.36	23.80
1	10	17.71	9.76
1	11	4.55	21.71
1	12	65.84	17.86
1	13	61.01	17.77
1	14	19.01	13.56
1	15	25.84	7.54
1	16	21.21	16.87
1	17	48.67	19.35
1	18	27.15	14.01
1	19	55.41	14.69
2	2	39.65	29.29
2	20	15.47	8.70
2	21	11.63	12.29
2	22	22.63	8.34
2	23	24.47	12.99
2	24	50.88	22.28
2	25	38.29	26.94
2	26	24.18	13.61
2	27	55.54	13.87
2	28	15.71	12.89
2	29	55.05	11.51
3	3	49.64	16.78
3	30	17.93	33.61
3	31	29.98	22.08
3	32	22.38	26.10
3	33	29.87	21.16
3	34	15.27	36.92
3	35	10.07	29.71
3	36	12.22	21.65
3	37	14.62	28.85
3	38	23.05	17.94
3	39	22.50	17.50
4	4	19.52	16.01
4	40	18.94	25.21
4	41	18.84	8.73
4	42	12.22	16.22
4	43	22.45	8.39
4	44	29.85	11.57
4	45	24.58	13.41
4	46	10.67	27.55
4	47	8.87	15.86
4	48	26.08	32.05
4	49	49.87	25.97

Table 17. IgG and IgM titers (abs 450nm) collected during the dose response trial

Sheep #	IgG d 11	IgG d 15	IgG d 18	IgM d 11	IgM d 15	IgM d 18
1	0.15	0.44	0.88	1.06	1.38	1.57
10	0.68	3.32	1.97	1.06	1.01	1.27
11	1.17	2.85	1.30	1.14	0.98	1.44
12	0.74	2.80	0.86	1.14	1.49	1.48
13	0.39	3.50	2.10	1.11	0.80	1.19
14	0.26	0.82	1.46	1.06	1.33	1.72
15	0.15	4.16	2.15	1.09	1.25	1.11
16	0.12	0.45	0.17	1.14	1.94	1.32
17	0.24	0.99	1.43	1.68	1.20	1.12
18	0.40	4.33	3.06	1.14	1.16	1.15
19	0.43	2.63	2.42	1.17	1.05	1.27
2	0.30	1.29	1.75	1.09	1.36	1.31
20	1.27	5.13	3.60	1.44	1.41	1.69
21	0.86	2.76	2.57	1.46	0.85	1.22
22	0.52	0.43	0.17	1.12	1.29	1.05
23	0.27	0.58	0.63	1.32	1.07	1.25
24	0.92	1.37	1.53	1.01	0.44	1.22
25	0.77	0.08	0.82	1.09	0.56	1.48
26	0.62	0.33	0.82	1.13	0.62	1.71
27	0.23	1.02	1.53	1.05	0.64	1.45
28	0.26	0.48	0.95	1.73	0.47	1.36
29	0.17	0.21	0.71	1.48	0.37	1.02
3	0.08	1.82	1.78	1.21	0.44	1.25
30	0.51	0.16	0.31	1.27	0.59	2.11
31	0.52	0.75	1.55	1.10	0.92	0.69
32	0.86	1.52	2.05	1.12	0.72	1.12
33	1.08	1.33	0.68	1.13	0.77	0.68
34	0.98	0.10	0.94	1.06	0.90	0.78
35	0.94	1.16	1.37	1.02	0.89	0.67
36	0.50	0.20	0.19	0.95	0.91	0.72
37	0.10	1.10	0.33	0.84	0.95	0.82
38	0.14	1.35	1.99	0.85	1.25	0.67
39	0.21	0.06	0.80	0.89	0.81	1.60
4	0.50	0.32	1.04	0.97	1.33	2.02
40	0.83	0.06	0.78	0.87	1.02	1.55
41	0.49	0.24	1.15	0.64	0.83	1.12
42	0.53	0.28	1.25	0.81	0.80	1.13
43	0.45	0.11	0.33	0.71	0.55	1.26
44	0.32	0.27	0.93	0.73	0.60	1.17
45	0.30	0.15	0.26	0.72	0.65	1.35
46	0.10	0.10	0.05	1.42	0.43	0.23
47	0.19	0.07	0.22	1.18	0.49	0.41
48	0.26	0.07	0.41	0.96	0.40	0.32
49	0.19	0.16	0.43	1.03	0.52	0.43

Table 18. Behavior data (latency min) collected during the dose response trial

Sheep #	Lying	Drinking	Eating
1	5700	0.25	6.53
10	5700	0.25	10.93
11	5700	0.25	14.47
12	5700	0.25	19.15
13	5700	0.25	10.23
14	5379	0.25	13.23
15	5700	0.25	15.80
16	5700	0.25	12.80
17	5700	0.25	25.88
18	5700	0.25	4.77
19	4174	0.25	17.05
2	5220	0.25	5.35
20	5700	0.25	16.42
21	5700	0.25	23.68
22	5700	0.25	9.78
23	5700	0.25	10.30
24	5700	0.25	18.93
25	5210	0.25	10.10
26	5700	0.25	1.50
27	5700	0.25	23.97
28	5700	0.25	0.50
29	5700	0.25	16.42
3	4871	0.25	18.53
30	5700	1.25	0.23
31	5478	0.25	14.15
32	5700	0.25	0.65
33	5700	0.25	10.33
34	5700	0.25	9.97
35	5700	0.25	2.25
36	5700	0.25	15.27
37	5700	0.25	15.80
38	4491	0.25	0.47
39	5700	0.25	21.37
4	5528	0.25	17.05
40	5700	0.25	2.00
41	5700	0.25	2.00
42	5700	0.25	7.00
43	5483	0.25	8.57
44	5700	0.25	15.80
45	4140	0.25	21.83
46	5700	0.25	7.33
47	5700	0.25	10.40
48	5700	0.25	1.83
49	5700	0.25	14.20

Table 19. Temperature collected during walking (w) and transport (t) during the dose response trial (˚C)

#	Rectal	Max (t)	Min (t)	Range (t)	Max (w)	Min (w)	Range (w)	Basal
1	40.78	38.39	36.89	1.50	38.78	38.00	0.78	38.33
10	41.83	38.50	37.61	0.89	39.28	38.78	0.50	38.83
11	41.39	38.22	37.11	1.11	38.89	37.94	0.94	38.20
12	40.78							
13	40.61	38.00	36.28	1.72	38.11	38.11	0.00	38.16
14	41.33	38.50	37.39	1.11	39.28	38.61	0.67	39.10
15	41.56	38.89	37.61	1.28	39.28	38.89	0.39	38.80
16	40.78	38.50	37.22	1.28	38.39	38.00	0.39	38.37
17	41.22				38.39	37.89	0.50	38.09
18	41.56	38.78	37.11	1.67	39.11	38.78	0.33	38.92
19	41.17	38.50	37.50	1.00	38.61	38.22	0.39	38.85
2	41.44	38.50	37.00	1.50	38.78	38.39	0.39	38.53
20	40.33	38.89	37.89	1.00	38.78	38.39	0.39	38.73
21	41.11	38.89	37.61	1.28	38.89	38.89	0.00	39.03
22	40.94	38.89	38.00	0.89	39.28	38.89	0.39	39.16
23	41.17	38.00	36.39	1.61	38.22	37.78	0.44	37.90
24	41.28	38.11	37.11	1.00	38.61	38.39	0.22	38.11
25	41.22	38.89	37.50	1.39	38.89	38.50	0.39	38.88
26	41.72	38.50	36.78	1.72	39.11	38.39	0.72	38.81
27	41.22	38.11	37.22	0.89	38.50	38.00	0.50	38.08
28	41.39	38.89	38.39	0.50	39.11	39.00	0.11	38.73
29	41.11	38.61	37.11	1.50	39.11	38.61	0.50	38.61
3	40.78	37.78	37.00	0.78	38.61	38.11	0.50	38.04
30	41.39	38.11	36.11	2.00	38.39	37.78	0.61	38.23
31	41.39	38.00	36.50	1.50	38.00	37.78	0.22	38.15
32	40.89	38.61	37.50	1.11	39.11	38.50	0.61	38.84
33	40.89	38.89	37.00	1.89	39.28	39.28	0.00	38.93
34	41.56	38.39	38.00	0.39	40.00	38.50	1.50	38.58
35	41.17	38.22	36.11	2.11	38.61	38.39	0.22	38.67
36	41.39	38.78	37.78	1.00	39.00	38.89	0.11	38.99
37	41.89	39.11	37.78	1.33	39.39	38.61	0.78	39.05
38	41.22	38.39	37.78	0.61	39.00	38.39	0.61	38.68
39	41.06							
4	41.50	37.78	37.22	0.56	38.39	37.78	0.61	38.20
40	40.83	38.22	37.39	0.83	38.78	38.11	0.67	38.75
41	40.83	38.11	37.22	0.89	38.39	37.89	0.50	38.14
42	41.11	38.22	37.00	1.22	38.50	38.22	0.28	38.43
43	41.11	38.39	37.50	0.89	38.78	38.22	0.56	38.49
44	40.83	38.50	37.61	0.89	38.50	38.22	0.28	38.36
45	41.67	38.39	37.11	1.28	38.22	38.50	-0.28	38.41
46	41.33	38.11	37.39	0.72	38.89	38.50	0.39	38.43
47	41.28		37.89	1.39	39.50	39.11	0.39	39.34
48	40.89	38.00	37.39	0.61	38.78	38.50	0.28	38.40
49	41.39	37.78	36.89	0.89	38.89	38.50	0.39	38.30

Table 20. Pre-transport plasma chemistry data collected during the dose response trial: part one

#	P. protein (g/dl)	Album. (g/dl)	C (mg/dl)	P (mg/dl)	Glucose (mg/dl)
1	5.2	2.8	10.1	7.2	72
10	5.6	2.8	8.9	6.8	63
11	6.3	3.4	10.7	7.3	64
12	6.2	3.5	10.5	6.9	74
13	6.4	3.4	10.2	8.1	61
14	6.7	3	10.9	5.8	97
15	5.9	3.1	9.8	8.2	67
16	6.1	3.3	10.9	5.9	61
17	5.6	3.1	10.9	5.7	68
18	6.4	3.2	10.6	6	67
19	5.4	3	9.3	4.3	69
2	6.5	3.2	10.8	7.3	74
20	5.9	3.2	10.4	5	74
21	5.8	3.1	10.9	6.7	67
22	6.5	3.5	10.8	7.2	71
23	5.8	3.3	10.4	5.2	65
24	6.1	3.2	11.1	6	67
25	3	1.4	4.4	3.3	28
26	6.4	3.1	11.4	8	71
27	6	3.4	10.8	8.6	47
28	6.7	3.7	12	6.8	70
29	6.1	3.5	11.6	5.8	82
3	6.9	3.5	11.1	8.3	62
30	3.1	1.5	5	4	31
31	6	3.1	11	8.7	73
32	5.6	3.1	10.3	5.9	74
33	6.3	3.4	10.8	6	72
34	6.5	3.6	12.6	5.1	81
35	5.6	2.7	9.4	6	54
36	6.3	3.5	11.8	8.4	83
37	6.3	3.4	11.4	6.4	66
38	6.1	3.3	11.1	5.4	76
39	6	3.4	10.7	7.3	74
4	6.7	3.1	10.9	7.2	57
40	3.2	1.7	5.8	3.4	38
41	6	3.2	11.4	6.3	77
42	6.8	3.7	12.1	7.7	86
43	6.5	3.6	11.5	6.4	78
44	6.5	3.2	10.6	7.4	80
45	7.8	3.2	11.2	7.4	64
46	6.4	3.3	10.8	7.2	68
47	7	3.7	12	8.5	70
48	6.9	3.7	11.7	8.8	64
49	5.7	3.2	10.2	7.1	83

Table 21. Pre-transport plasma chemistry data collected during the dose response trial: part two

#	BUN (mg/dl)	Creat. (mg/dl)	T. bilirubin (mg/dl)	CK (U/l)	Ast(sgot) (U/l)
1	23.3	0.4	0.2	168	249
10	22.5	0.5	0.2	66	113
11	30.3	0.8	0.3	79	129
12	27.7	0.7	0.2	61	123
13	30.8	0.6	0.2	207	123
14	22	0.5	0.2	86	113
15	33	0.5	0.3	92	141
16	26.7	0.4	0.3	85	106
17	25.6	0.5	0.3	82	95
18	29	0.5	0.2	92	147
19	21.3	0.7	0.3	64	128
2	17.4	0.6	0.3	99	121
20	21.6	0.5	0.3	67	99
21	27.2	0.7	0.3	63	79
22	26.8	0.7	0.3	97	83
23	28	0.6	0.4	117	74
24	28.8	0.5	0.3	124	114
25	9	0.2	0.1	42	59
26	24.1	0.5	0.2	75	165
27	27.6	0.5	0.4	88	120
28	33.5	0.8	0.2	81	145
29	30.6	0.6	0.3	120	80
3	29.2	0.6	0.3	115	136
30	15.8	0.3	0.2	50	41
31	30.2	0.5	0.2	113	153
32	22.2	0.6	0.3	91	103
33	30	0.7	0.3	290	134
34	32.3	0.8	0.3	93	187
35	23.7	0.5	0.3	65	138
36	26	0.5	0.2	290	120
37	25.9	0.5	0.3	133	105
38	24.4	0.6	0.2	90	74
39	28.9	0.7	0.3	112	94
4	23.5	0.5	0.3	114	89
40	15.4	0.3	0.2	77	63
41	26.1	0.6	0.3	53	84
42	25.8	0.6	0.2	114	125
43	23.4	0.6	0.2	101	87
44	25.1	0.6	0.3	110	127
45	20.7	0.5	0.2	76	89
46	30.2	0.7	0.4	596	118
47	23.9	0.5	0.3	980	231
48	28.6	0.8	0.2	271	111
49	21.9	0.5	0.2	76	182

Table 22. Pre-transport plasma chemistry data collected during the dose response trial: part three

#	Globulins (g/dl)	A/G ratio	GGT (U/l)	Mg (meq/l)
1	2.4	1.17	90	2.2
10	2.8	1	81	1.8
11	2.9	1.17	103	2.5
12	2.7	1.3	89	2.3
13	3	1.13	97	2
14	2.9	1.31	66	2.2
15	2.8	1.11	94	2
16	2.8	1.18	90	1.8
17	2.5	1.24	82	2.2
18	3.2	1	142	2.1
19	2.4	1.25	74	2
2	3.3	0.97	101	2
20	2.7	1.19	86	2
21	2.7	1.15	132	2.2
22	3	1.17	68	2.3
23	2.5	1.32	63	1.7
24	2.9	1.1	85	2.1
25	1.6	0.88	48	0.7
26	3.3	0.94	100	2.1
27	2.6	1.31	4	2
28	3	1.23	93	2.5
29	2.6	1.35	54	2.3
3	3.4	1.03	102	2.3
30	1.6	0.94	42	0.9
31	2.9	1.07	85	2.4
32	2.5	1.24	68	2.1
33	2.9	1.17	119	2.1
34	2.9	1.24	171	2.4
35	2.9	0.93	106	1.7
36	2.8	1.25	68	2
37	2.9	1.17	81	2.1
38	2.8	1.18	47	2
39	2.6	1.31	70	2.1
4	3.6	0.86	71	2.2
40	1.5	1.13	59	1.1
41	2.8	1.14	95	2.1
42	3.1	1.19	77	2.3
43	2.9	1.24	78	2.3
44	3.3	0.97	84	2.5
45	4.6	0.7	161	2.2
46	3.1	1.06	99	2.4
47	3.3	1.12	182	2.2
48	3.2	1.16	99	2.5
49	2.5	1.28	121	1.8

Table 23. Pre-transport electrolytes data collected during the dose response trial

#	Na (meq/l)	K (meq/l)	Na/K ratio	Cl (meq/l)
1	135	4.1	32.9	102
10	127	3.7	34.3	93
11	141	4.4	32	105
12	142	4.3	33	106
13	138	4.2	32.9	104
14	139	4.3	32.3	101
15	133	4	33.3	100
16	129	4.1	31.5	97
17	136	3.9	34.9	105
18	132	4	33	101
19	130	3.7	35.1	101
2	136	4.3	31.6	102
20	137	4.5	30.4	106
21	136	4.1	33.2	103
22	139	4.4	31.6	104
23	135	3.7	36.5	104
24	134	4.3	31.2	104
25	75	2.4	31.2	57
26	140	4	35	107
27	140	4.3	32.6	104
28	133	4.5	29.6	100
29	139	4.7	29.6	106
3	142	4.4	32.3	109
30	84	2.7	31.1	63
31	137	4.9	28	104
32	135	3.9	34.6	103
33	141	4.3	32.8	108
34	141	4.3	32.8	107
35	121	3.9	31	92
36	142	4.8	29.6	106
37	136	4	34	103
38	137	4.6	29.8	103
39	139	4.4	31.6	104
4	138	4.5	30.7	105
40	92	2.8	32.9	70
41	144	4.3	33.5	109
42	143	4.5	31.8	105
43	140	4.4	31.8	107
44	140	4.4	31.8	106
45	140	4.6	30.4	103
46	142	4.3	33	107
47	145	4.9	29.6	108
48	144	4.6	31.3	107
49	138	4.2	32.9	105

Table 24. Post-transport plasma chemistry data collected during the dose response trial: part one

#	P. protein (g/dl)	Album. (g/dl)	C. (mg/dl)	P (mg/dl)	Glucose (mg/dl)
1	5.7	3.1	12.4	10	101
10	6.2	3	10.2	7.8	86
11	5.7	3.1	11.7	7.3	85
12	6.3	3.4	11.3	8.2	92
13	6.3	3.3	11.2	7.7	75
14	6.3	3.7	11.4	8.1	85
15	5.1	2.7	9.6	7.2	79
16	6.2	3.4	11.4	7	79
17	5.7	3.2	11.9	7.6	85
18	6.8	3.3	11.5	6.6	85
19	6.2	3.4	10.8	7	86
2	6	3	11.1	6.2	86
20	5.6	3	11.2	6.3	92
21	6.2	3.2	11.1	7.7	85
22	6.6	3.5	11.2	7.3	82
23	6.2	3.6	11.8	8.1	92
24	6	3.3	11.5	6.5	84
25	6.4	3.1	10.2	7.9	76
26	6.5	3.1	10.9	7.7	95
27	6.2	3.4	11.2	7.4	84
28	6.8	3.7	12.5	7.1	85
29	6	3.5	11.9	7.1	86
3	5.4	2.7	9.1	7.3	74
30	6.1	3.1	10.6	7.2	87
31	4.9	2.5	9.2	6.8	81
32	5.8	3.2	11.3	7.4	95
33	5.7	3.1	10.3	5.8	82
34	3.9	2.2	7.8	4	57
35	5.5	2.7	10	6.9	75
36	6.2	3.5	11.9	8	87
37	6.5	3.5	12.7	6.6	87
38	6.2	3.3	11.2	5.5	90
39	5	2.9	9.2	5.6	75
4	4.2	2	7.5	5.2	55
40	5.5	2.9	10.5	6.5	85
41	4.7	2.5	9.5	6.4	75
42	6.4	3.5	11.6	6.8	90
43	3.9	2.2	7.4	4.4	56
44	5.7	2.8	9.8	8.3	84
45	7.2	2.9	10.9	8.3	72
46	5.3	2.9	10	7	72
47	5.9	3.3	10.8	6.6	78
48	5.9	3.2	10.4	7.2	78
49	5.2	2.9	9.8	6.2	78

Table 25. Post-transport plasma chemistry data collected during the dose response trial: part two

#	BUN (mg/dl)	Creat. (mg/dl)	T. bilirubin (mg/dl)	CK (U/l)	Ast(sgot) (U/l)
1	22.7	0.5	0.2	348	287
10	21.2	0.6	0.2	76	112
11	27.9	0.7	0.2	80	120
12	22.8	0.7	0.2	91	129
13	25.5	0.7	0.2	279	125
14	21.5	0.6	0.2	107	111
15	25.9	0.5	0.2	79	121
16	25	0.4	0.3	96	110
17	24	0.5	0.2	221	100
18	28.3	0.6	0.2	74	148
19	23.2	0.7	0.3	280	147
2	14.9	0.5	0.3	95	106
20	24.8	0.5	0.2	68	94
21	30.1	0.7	0.2	65	82
22	28.1	0.7	0.2	85	83
23	24.5	0.7	0.3	92	72
24	27.2	0.6	0.2	108	114
25	22.1	0.6	0.2	81	125
26	21	0.6	0.3	85	133
27	24.1	0.5	0.2	104	122
28	31.1	0.7	0.2	78	143
29	28.7	0.6	0.2	63	75
3	20.6	0.5	0.2	92	103
30	20.7	0.7	0.2	95	73
31	21.7	0.5	0.2	87	117
32	21.1	0.7	0.2	249	107
33	24.7	0.6	0.2	194	114
34	17.9	0.5	0.2	74	109
35	23.5	0.6	0.2	68	134
36	9.2	0.5	0.2	90	120
37	19.2	0.6	0.2	89	103
38	20.9	0.6	0.2	99	64
39	22.4	0.6	0.2	69	79
4	12.7	0.3	2	207	54
40	23.5	0.6	0.2	132	108
41	23.3	0.6	0.2	53	64
42	23.3	0.6	0.2	102	117
43	17.1	0.3	0.2	59	50
44	20.7	0.6	0.2	97	112
45	18.4	0.4	0.2	85	74
46	21.8	0.6	0.2	72	93
47	23.6	0.5	0.2	104	206
48	19.1	0.7	0.2	124	75
49	21.3	0.5	0.2	277	188

Table 26. Post-transport plasma chemistry data collected during the dose response trial: part three

#	Globulins (g/dl)	A/Gratio	GGT (U/l)	Mg (meq/l)
1	2.6	1.19	104	2.6
10	3.2	0.94	89	2.1
11	2.6	1.19	95	2.4
12	2.9	1.17	94	2.5
13	3	1.1	98	2.5
14	2.6	1.42	63	2.3
15	2.4	1.13	85	2
16	2.8	1.21	87	1.9
17	2.5	1.28	85	2.5
18	3.5	0.94	150	2.3
19	2.8	1.21	84	2.2
2	3	1	97	2
20	2.6	1.15	81	2.2
21	3	1.07	138	2.4
22	3.1	1.13	67	2.5
23	2.6	1.38	70	2.3
24	2.7	1.22	90	2.4
25	3.3	0.94	108	2
26	3.4	0.91	105	2.2
27	2.8	1.21	86	2.6
28	3.1	1.19	92	2.6
29	2.5	1.4	55	2.5
3	2.7	1	79	2
30	3	1.03	8	2.1
31	2.4	1.04	69	2.1
32	2.6	1.23	69	2.3
33	2.6	1.19	110	2.2
34	1.7	1.29	102	1.4
35	2.8	0.96	104	1.9
36	2.7	1.3	70	2.2
37	3	1.17	86	2.1
38	2.9	1.14	47	2.2
39	2.1	1.38	58	2
4	2.2	0.91	44	1.5
40	2.6	1.12	103	2.3
41	2.2	1.14	75	1.8
42	2.9	1.21	70	2.2
43	1.7	1.29	45	1.3
44	2.9	0.97	75	2.3
45	4.3	0.67	149	1.8
46	2.4	1.21	82	2.1
47	2.6	1.27	157	2
48	2.7	1.19	83	2.1
49	2.3	1.26	111	2.1

Table 27. Post-transport electrolyte data collected during the dose response trial

#	Na (meq/l)	K (meq/l)	Na/K ratio	Cl (meq/l)
1	150	6	25	113
10	142	4.8	29.6	107
11	144	4.7	30.6	110
12	147	4.7	31.3	113
13	146	4.8	30.4	113
14	144	5.1	28.2	108
15	127	4.5	28.2	99
16	133	4.4	30.2	100
17	142	4.8	29.6	109
18	141	4.6	30.7	107
19	140	4.3	32.6	107
2	139	4.4	31.6	105
20	142	4.5	31.6	108
21	143	4.5	31.8	108
22	144	4.3	33.5	108
23	145	4.8	30.2	108
24	140	4.7	29.8	111
25	136	4.6	29.6	105
26	142	4.6	30.9	109
27	142	4.4	32.3	109
28	144	4.7	30.6	110
29	139	4.5	30.9	106
3	126	4.1	30.7	97
30	135	4.3	31.4	102
31	119	4.1	29	91
32	145	5.3	27.4	110
33	132	4.5	29.3	101
34	97	3.2	30.3	72
35	126	4.1	30.7	94
36	141	4.7	30	107
37	142	4.9	29	108
38	139	5.2	26.7	104
39	120	3.6	33.3	91
4	99	3.6	27.5	76
40	141	4.4	32	109
41	127	4	31.8	95
42	140	5.2	26.9	103
43	93	3.1	30	71
44	131	4.2	31.2	100
45	133	4.5	29.6	98
46	132	4.1	32.2	100
47	134	4.1	32.7	102
48	131	4.4	29.8	98
49	128	4.3	29.8	98

Table 28. Treatment, sex, and weights of sheep during second summer (a)

Treatment #	Sex	Sheep #	Weight 1	Weight 2	Weight 3
1	M	1	18.14	21.55	19.28
1	F	10	33.11		
1	F	11	34.47	37.19	34.70
1	F	12	42.18	47.40	42.86
1	F	13	39.92	40.37	38.10
1	M	14	27.22	32.66	30.39
1	M	15	18.60	22.45	20.87
1	M	16	19.50	23.59	21.77
1	M	17	27.67	34.47	31.52
1	M	18	19.50	24.95	23.59
1	M	19	12.25	17.46	15.88
2	M	2	21.32	29.03	26.31
2	F	20	32.66	34.70	31.30
2	F	21	32.20	32.89	29.48
2	F	22	36.29	36.29	33.57
2	F	23	33.57	33.57	29.94
2	M	24	21.32	26.76	24.04
2	M	25	15.88	19.73	18.60
2	M	26	19.96	27.22	24.49
2	M	27	15.88	22.23	19.28
2	M	28	17.24	33.34	29.94
2	M	29	17.24	23.36	21.32
3	M	3	19.50	26.31	23.13
3	F	30	32.66	36.06	32.89
3	F	31	39.01	43.77	38.10
3	F	32	37.65	39.01	36.74
3	F	33	31.30	33.34	31.52
3	M	34	22.23	26.31	24.27
3	M	35	15.42	21.77	20.41
3	M	36	23.59	29.03	24.04
3	M	37	22.68	27.44	25.17
3	M	38	25.85	33.11	30.62
3	M	39	24.04	27.90	25.85

Table 29. Treatment, sex, and weights of sheep during second summer (b)

Treatment #	Sex	Sheep #	Weight 1	Weight 2	Weight 3
4	M	4	12.25	17.46	16.10
4	F	40	41.28	45.13	41.96
4	F	41	32.20	36.29	33.57
4	F	42	25.85	30.62	27.67
4	F	43	25.85	30.16	28.12
4	M	44	16.33	17.92	17.24
4	M	45	14.51	20.87	17.46
4	M	46	19.50	27.22	22.23
4	M	47	24.49	30.16	27.67
4	M	48	14.06		
4	M	49	18.14	24.49	21.55
5	M	5	23.59	28.12	26.31
5	F	50	35.38	39.46	35.38
5	F	51	33.57	30.39	29.26
5	F	52	29.94	33.57	30.39
5	F	53	37.65	43.32	39.01
5	M	54	19.96	27.44	24.72
5	M	55	24.95	30.84	28.12
5	M	56	20.87	26.31	24.27
5	M	57	24.04	30.39	28.35
5	M	58	21.32	27.22	24.95
5	M	59	20.41	27.22	24.72

Table 30. Observed latency to drink (sec) post transport during the component trial

Sheep #	Drink	Sheep #	Drink
1		4	563
10		40	1273
11	801	41	
12	1219	42	627
13	489	43	1200
14	450	44	
15	22	45	360
16		46	359
17	327	47	410
18	1024	48	
19	1604	49	549
2	516	5	17
20	511	50	296
21	1720	51	
22	1154	52	478
23	1636	53	84
24	772	54	612
25	279	55	253
26	1017	56	709
27	258	57	564
28	12	58	398
29	846	59	849
3	24		
30	1821		
31	807		
32	877		
33	1564		
34	10		
35	510		
36	386		
37	18		
38	631		
39	555		

Table 31. Cortisol data collected during the component trial (a)

#	-21 d	0h transport	4h transport	8h transport
1	0.55	1.02	0.48	1.76
10				
11	7.12	2.68	2.03	0.69
12	2.59	0.21	2.61	0.10
13	0.20	2.04	4.54	4.81
14	0.58	0.44	0.42	0.24
15	2.65	0.68	0.71	0.45
16	2.82	0.94	1.18	2.24
17	0.47	1.23	2.63	3.86
18	3.67	0.46	3.11	0.45
19	0.67	0.45	1.80	0.28
2	0.47	0.99	1.20	0.94
20	3.17	1.55	0.89	1.70
21	1.11	0.81	1.69	0.26
22	3.92	0.68	0.48	4.26
23	7.65	4.43	0.36	0.46
24	0.99	0.78	0.83	1.58
25	0.73	0.97	0.49	6.81
26	0.33	0.90	1.12	0.41
27	0.56	0.79	4.16	1.23
28	1.10	2.01	1.82	0.38
29	1.52	0.85	4.07	0.42
3	0.69	1.57	0.76	2.18
30	0.97	1.17	2.26	1.32
31	2.38	0.76	0.49	0.38
32	2.77	2.32	0.97	1.12
33	0.92	2.38	0.33	1.63
34	0.68	0.63	2.24	0.33
35	0.67	0.65	1.42	0.31
36	0.51	0.46	1.41	0.28
37	3.57	0.70	1.50	0.21
38	0.67	0.52	2.14	0.39
39	1.79	1.56	0.74	0.32

Table 32. Cortisol data collected during the component trial (b)

#	-21 d	0h transport	4h transport	8h transport
4	0.61	0.58	1.58	0.32
40	0.91	0.83	0.47	0.26
41	2.71	0.53	5.00	0.15
42	3.52	1.80	1.36	0.50
43	2.73	4.95	1.00	0.34
44	2.00	1.62	4.24	1.78
45	0.44	0.20	1.52	1.05
46	0.40	0.48	1.04	2.60
47	0.57	1.13	0.90	1.57
48				
49	1.35	0.60	6.40	0.52
5	1.34	2.29	0.75	0.94
50	2.29	0.91	0.39	2.55
51	4.92	0.83	7.20	5.35
52	3.24	6.67	4.35	4.96
53	3.70	4.24	0.53	0.34
54	0.80	0.62	3.17	0.41
55	1.55	2.80	0.45	4.40
56	0.60	0.93	3.05	1.19
57	0.52	1.52	0.19	0.65
58	0.60	0.66	3.18	0.58
59	0.98	2.01	4.31	1.31

Table 33. Aldosterone data (ng/dl) collected during the component trial (a)

#	-21 d	Pre-transport	Post transport
1	26.93	12.90	3.34
10			
11	4.18	2.27	1.51
12	42.25	4.77	4.63
13	7.75	8.42	1.27
14	10.95	5.59	7.32
15	5.67	3.40	6.06
16	15.18	10.10	2.28
17	5.73	2.33	14.66
18	8.67	3.30	6.68
19	6.59	2.04	3.04
2	4.89	3.88	1.11
20	12.37	7.30	5.58
21	7.60	3.99	2.58
22	17.93	1.03	1.78
23	6.28	1.93	6.67
24	7.06	0.83	12.20
25	22.73	14.85	13.78
26	7.01	6.45	2.87
27	7.32	0.50	7.77
28	12.31	0.72	21.78
29	17.10	15.66	10.26
3	8.14	0.33	0.80
30	5.98	3.67	10.94
31	9.58	1.48	1.61
32	18.77	10.00	4.68
33	17.99	6.96	11.82
34	5.51	0.80	10.91
35	4.49	15.80	10.40
36	5.73	5.52	5.30
37	8.92	2.41	16.98
38	1.92	2.12	5.22
39	9.80	16.62	1.76

Table 34. Aldosterone data (ng/dl) collected during the component trial (b)

#	-21 d	Pre-transport	Post transport
4	3.52	4.88	19.68
40	15.14	4.44	25.31
41	6.50	6.56	7.81
42	3.17	5.44	8.56
43	19.58	13.50	2.93
44	14.65	3.89	8.45
45	13.09	4.84	3.11
46	15.29	6.89	5.27
47	1.54	1.31	11.42
48			
49	4.16	1.92	8.16
5	11.93	14.38	3.00
50	11.53	7.60	9.04
51	21.61	16.24	9.61
52	11.81	22.46	3.77
53	25.67	14.41	8.67
54	4.32	4.54	5.00
55	6.94	2.15	2.30
56	19.55	7.26	20.56
57	4.21	5.87	1.32
58	15.96	2.96	1.85
59	1.98	4.49	10.01

Table 35. IgG and IgM (abs 450nm) data collected during the component trial (a)

#	IgG d11	IgG d15	IgG d18	IgM d11	IgM d15	IgM d18
1	0.08	0.53	0.61	1.08	0.70	1.16
10						
11				1.05	0.83	1.41
12				1.10	0.77	1.31
13				1.27	0.83	2.35
14	0.06	0.43	0.39	0.97	0.66	1.02
15	0.42	0.96	1.02	1.01	0.69	1.45
16	0.15	0.58	1.06	1.22	0.84	2.78
17	0.07	0.51	0.11	1.07	0.70	1.19
18	0.14	0.72	0.89	0.93	1.19	1.05
19	0.12	0.67	0.78	1.00	1.24	1.09
2	0.22	0.88	0.67	1.06	1.39	1.77
20	0.25	0.44	0.23	1.15	1.38	1.57
21	0.35	0.93	1.23	0.95	1.39	1.08
22	0.21	0.56	0.55	1.10	1.39	1.39
23	0.11	0.71	0.65	1.10	1.31	1.27
24	0.20	0.54	0.23	1.02	1.08	1.17
25	0.36	0.87	0.57	1.04	1.33	1.28
26	0.16	0.73	0.86	1.03	1.21	1.12
27	0.15	0.66	1.01	1.05	1.03	1.23
28	0.24	0.69	0.62	1.06	1.38	1.75
29	0.18	0.66	1.14	1.02	1.07	
3	0.15	0.62	0.25	1.02	0.98	2.24
30	0.30	0.83	1.35	1.07	1.13	1.63
31	0.15	0.68	0.49	1.04	1.32	1.87
32	0.23	0.82	0.91	1.33	0.67	2.00
33	0.16	0.60	0.42	1.40	0.77	1.17
34	0.40	0.88	1.36	1.27	0.68	1.59
35	0.11	0.49	0.34	1.78	0.57	1.38
36	0.12	0.62	0.31	0.95	0.84	1.05
37	0.07	0.49	0.23	1.01	0.61	1.56
38	0.23	0.90	0.91	1.06	0.62	1.35
39	0.18	0.72	0.87	1.14	0.45	1.40

Table 36. IgG and IgM (abs 450nm) data collected during the component trial (b)

#	IgG d11	IgG d15	IgG d18	IgM d11	IgM d15	IgM d18
4	0.17	0.72	0.67	0.69	1.37	1.39
40	0.15	0.48	0.56	1.46	1.51	1.42
41	0.17	0.61	1.08	1.08	1.36	1.04
42	0.30	0.61	0.91	0.88	1.53	1.38
43	0.14	0.74	0.42	0.63	1.14	0.89
44	0.19	0.75	0.87	1.16	1.31	1.38
45	0.16	0.63	0.85	0.78	1.23	1.08
46	0.14	0.90	0.71	0.72	0.89	1.44
47	0.10	0.72	0.66	0.78	0.93	1.38
48						
49	0.10	0.82	0.44	0.94	0.94	1.28
5	0.30	0.58	0.24	0.62	1.02	1.00
50	0.11	0.63	0.41	0.87	1.10	1.20
51	0.09	0.66	0.31	0.76	1.01	1.21
52	0.42	1.01	0.94	0.69	1.22	0.84
53	0.32	0.72	0.24	1.19	0.84	1.93
54	0.17	0.63	0.30	0.65	1.31	0.93
55	0.24	1.25	1.18	2.44	3.29	1.07
56	0.24	1.16	1.74	2.52	1.35	1.63
57	0.12	0.65	0.37	1.22	1.94	1.88
58	0.12	0.55	0.97	1.15	1.46	1.62
59	0.07	0.99	0.67	0.98	1.05	1.08

Table 37. Complete blood cell count data collected during the component trial: part one (a)

#	WBC	RBC	PCV	Hemoglobin	MCV	MCH	MCHC
1	9600	8.09	22.3	7.69	27.6	9.5	34.5
10							
11	7600	7.96	24.4	8.01	30.7	10.1	32.8
12	5500	10.5	29.3	10.2	27.9	9.7	34.8
13	7800	11.3	28.8	9.78	25.5	8.7	34
14	6700	10.1	30.9	10.5	30.6	10.4	34
15	4400	10.6	29.9	10.2	28.2	9.6	34.1
16	6900	10.1	28.4	10.1	28.1	10	35.6
17	5800	9.52	27	9.14	28.4	9.6	33.9
18	4000	9.23	30.6	10.3	33.2	11.2	33.7
19	6100	9.53	28.2	9.88	29.6	10.4	35
2	2700	9.33	28.5	9.49	30.5	10.2	33.3
20	2400	10.1	28.1	9.87	27.8	9.8	35.1
21	2400	9.59	29.1	9.9	30.3	10.3	34
22	8200	9.32	25.1	8.65	26.9	9.3	34.5
23	5300	10.4	27.1	9.25	26.1	8.9	34.1
24	8400	11.2	31.6	11.2	28.2	9.6	34.2
25	8200	11.1	32.1	10.9	28.9	9.8	34
26	5100	9.31	28.3	10.1	30.4	10.8	35.7
27	3400	9.13	27.1	10	29.7	11	36.9
28	3700						
29	5000	10.8	29.1	10.1	26.9	9.4	34.7
3	4200	7.74	27.7	9.24	35.8	11.9	33.4
30	6000	11.7	30.1	10.6	25.7	9.1	35.2
31	8400	9.98	26.1	8.79	26.2	8.8	33.7
32	3800	9.33	24.6	8.17	26.4	8.8	33.2
33	9200	9.71	26.8	9.39	27.6	9.7	35
34	5100	9.31	26.8	9.34	28.8	10	34.9
35	4900	11.5	32.5	11.3	28.3	9.8	34.8
36	7700	10.1	29.7	10.3	29.4	10.2	34.7
37	4900	10.4	30.1	10.7	28.9	10.3	35.5
38	3800						
39	8100	9.42	27	9.38	28.7	10	34.7

Table 38. Complete blood cell count data collected during the component trial: part one (b)

#	WBC	RBC	PCV	Hemoglobin	MCV	MCH	MCHC
4	4800	10.1	30.4	10.3	30.1	10.2	33.9
40	5700	11.7	30.9	10.8	26.4	9.2	35
41	6900	11.2	32.1	11.1	28.7	9.9	34.6
42	3200	9.01	29.6	10.3	32.9	11.4	34.8
43	10100	8.22	24.2	8.53	29.4	10.4	35.2
44	5700	11	32.3	11.8	29.4	10.7	36.5
45	3600	8.77	29.4	9.86	33.5	11.2	33.5
46	1500						
47	6300	10.4	30.7	10.3	29.5	9.9	33.6
48							
49	5400	9.72	29.8	10.3	30.7	10.6	34.6
5	4000	9.63	29.6	10.4	30.7	10.8	35.1
50	4000	9.65	24.3	8.34	25.2	8.6	34.3
51	9200	8.62	24.7	8.24	28.7	9.6	33.4
52	3200	6.81	22.4	7.38	32.9	10.8	32.9
53	8400	11.9	28.4	10.1	23.9	8.5	35.6
54	4300						
55	4100	12.3	35.6	12	28.9	9.8	33.7
56	5400	9.83	27.7	9.89	28.2	10.1	35.7
57	6900	8.85	27.5	9.36	31.1	10.6	34
58	3300	9.13	27	9.39	29.6	10.3	34.8
59	1800						

Table 39. Complete blood cell count data collected during the component trial: part two (a)

#	Eosoniphils	Basophil	Monocyte	Neutrophil	P. protein	Lymphocytes	Fibrinogen
1	864	96		4224	6.5	4416	600
10							
11	1292			2888	6.9	3420	400
12	605			1430	7.5	3465	500
13	546		78	3042	8.2	4134	500
14	67	67		2546	6.2	3819	200
15				1672	6.6	2728	300
16				4623	6.4	2277	200
17	58	58	58	1160	6.9	4466	500
18				1080	6.3	2920	200
19	122			2074	6.3	3904	300
2	54			675	6.5	1971	300
20	24			1032	7.1	1344	500
21	72			504	7	1824	200
22	492		82	5330	8.2	2296	800
23	159	106		2756	8.2	2279	600
24	84	84	168	4956	6.2	3108	100
25				2460	6.4	5740	200
26	51	51	255	2601	6	2142	400
27		68	34	1292	5.7	2006	200
28		37	74	962		2627	
29	50	50	50	1800	6.2	3050	100
3	84			1512	6	2604	300
30	300	60	180	3240	7	2220	400
31	168	168	336	2772	7.6	4956	300
32			76	2394	7.4	1330	500
33	92			2484	7.6	6624	400
34			51	2499	6.5	2550	500
35	98			2352	6.4	2450	400
36				1771	6.3	5929	300
37				1372	6.2	3332	100
38	152		38	1406		2204	
39	162			3159	6.1	4779	300

Table 40. CBC Complete blood cell count data collected during the component trial: part two (b)

#	Eosoniphils	Basophil	Monocyte	Neutrophil	P. protein	Lymphocytes	Fibrinogen
4	192			2304	6.6	2304	200
40	114			1824	7.2	3762	500
41	483			1932	7	4485	400
42	32			896	7.1	2272	300
43		101		4646	6.4	5353	400
44	171		57	1995	6.6	3477	400
45	36		36	1728	5.7	1800	200
46			60	480		960	
47		252	126	3150	6.2	2772	200
48							
49	108	54	108	3402	6.2	1728	200
5				1280	5.7	2720	100
50	40		240	1120	6.6	2600	400
51			644	7544	5.7	1012	200
52	32		64	1376	6.5	1728	300
53	840	168	672	2688	7.5	4032	500
54	86		86	602		3483	
55	82	41	82	2624	6.9	1271	500
56	378	108	54	1296	7	3564	100
57	759	69	207	1794	6.2	4071	500
58	231		66	1287	5.8	1716	200
59	36		36	576		1152	

Table 41. Electrolyte concentrations collected before and after transport during the component trial (a)

	Electrolytes before transport				Electrolytes after transport			
Sheep #	Na	K	Na/K ratio	Cl	Na	K	Na/K ratio	Cl
1	144	4.8	30	110	148	4.6	32.2	113
10								
11	144	4.6	31.3	110	145	4.4	33	109
12	146	4.3	34	109	151	4.9	30.8	111
13	146	4.7	31.1	109	152	4.9	31	109
14	145	4.7	30.9	111	149	4.9	30.4	113
15	144	4.8	30	110	149	4.7	31.7	112
16	143	4.5	31.8	109	149	5	29.8	111
17	145	4.4	33	110	147	4.6	32	111
18	143	4.8	29.8	108	146	4.6	31.7	110
19	144	4.6	31.3	110	149	4.6	32.4	111
2	145	4.9	29.6	110	151	4.8	31.5	112
20	148	4.9	30.2	116	147	5.6	26.3	113
21	147	4.4	33.4	111	148	4.5	32.9	112
22	143	4.5	31.8	109	147	4.3	34.2	105
23	144	4.5	32	108	150	4.1	36.6	110
24	143	5	28.6	111	150	4.2	35.7	115
25	145	4.7	30.9	110	147	5.2	28.3	110
26	144	4.8	30	110	146	4.7	31.1	113
27	139	4.7	29.6	108	148	4.8	30.8	113
28	145	5.2	27.9	111	148	4.4	33.6	113
29	144	4.7	30.6	110	147	4.4	33.4	111
3	143	5.3	27	109	151	5.2	29	110
30	147	4.9	30	107	151	4.9	30.8	106
31	145	4.6	31.5	108	149	4.5	33.1	109
32	145	5	29	106	153	4	38.3	110
33	146	4.5	32.4	108	149	4.5	33.1	106
34	143	4.5	31.8	109	150	4	37.5	112
35	146	4.7	31.1	113	153	5.9	25.9	112
36	144	5	28.8	109	147	4.7	31.3	107
37	143	5	28.6	110	148	4.7	31.5	111
38	144	4.8	30	109	149	4.2	35.5	110
39	142	4.7	30.2	107	147	4.3	34.2	108

Table 42. Electrolyte concentrations collected before and after transport during the component trial (b)

	Electrolytes before transport				Electrolytes after transport			
Sheep #	Na	K	Na/K ratio	Cl	Na	K	Na/K ratio	Cl
4	145	4.9	29.6	110	146	4.5	32.4	109
40	147	4.9	30	109	149	4.8	31	108
41	147	4.5	32.7	111	147	4.9	30	108
42	140	4.3	32.6	106	151	4.6	32.8	111
43	143	4.4	32.5	110	153	4.3	35.6	114
44	146	5.3	27.5	111	152	5.1	29.8	114
45	143	4.7	30.4	111	149	4.8	31	114
46	142	4.5	32.6	110	149	4.1	36.3	115
47	145	4.6	31.5	112	150	4.7	31.9	113
48								
49	143	4.6	31.1	108	149	4.3	34.7	111
5	145	4.5	32.2	110	150	4	37.5	113
50	144	4.6	31.3	107	148	4.4	33.6	110
51	147	4.2	35	111	159	3.8	41.8	117
52	149	5	29.8	112	153	4.5	34	114
53	146	4.6	31.7	112	154	4.5	34.2	117
54	144	5	28.8	112	152	4.5	33.8	115
55	145	4.4	33	111	151	4.9	30.8	114
56	145	4.8	30.2	111	148	4.4	33.6	110
57	145	4.8	30.2	110	149	4.9	30.4	113
58	144	4.8	30	111	149	4.6	32.4	113
59	144	4.5	32	110	150	5.4	27.8	113

Table 43. Temperature data (˚C) collected during the component trial

Sheep #	Maximum	Minimum	Sheep #	Maximum	Minimum
1	38.6	37.9	4		
10			40	38.4	36.1
11	38.8	37.5	41	38.5	36.8
12	38.0	36.0	42	38.8	37.9
13		36.0	43	39.0	36.3
14	38.9	38.4	44	38.8	37.0
15			45	39.1	37.6
16	38.6		46	38.8	38.1
17	38.1		47		
18	38.6	37.5	48		
19	39.0	37.5	49		
2	39.6		5	38.5	37.9
20	39.0	37.0	50	38.5	37.0
21	38.2	37.1	51	38.9	36.9
22	37.9	36.3	52	38.4	37.1
23	38.5	37.5	53	37.9	36.6
24			54	39.1	
25			55	38.9	
26			56	39.5	
27			57	38.9	38.0
28		37.1	58	39.3	
29	38.9	37.4	59	38.6	34.9
3	38.8	37.9			
30	38.1				
31	38.1	37.1			
32	39.0	37.1			
33					
34	39.1				
35	38.8				
36					
37					
38	39.4	36.5			
39	38.9	36.3			

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