WELFARE OF EMUS DURING THEIR HANDLING AND TRANSPORT

by

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Abstract

Emu *(Dromaius novahollandiae)* are native to Australia, but they are commercially farmed in many countries, including Canada, the US, and India. Currently producers transport their emus over long distances for slaughter due to the lack of processing facilities. During such shipments, emus are exposed to stressful and adverse conditions, causing welfare concerns. This study was undertaken to suggest methods to improve the welfare of emus during their handling and transport.

Reference intervals were established for hematological, serum biochemical variables, enzymes and electrolytes in adult male and female emus. Changes in the indices of stress and metabolic homeostasis were used to evaluate the physiological responses of emus to transport for six hours under warm and cool weather conditions. The activities of enzymes were significantly ($P < 0.001$) increased at slaughter, indicating muscle cell wall damage. Transport under warm weather resulted in significantly ($P < 0.05$) higher weight loss, hyperthermia, hyperglycaemia, plasma corticosterone and packed cell volume, and meat pH than cool weather. Meat drip loss after 24 hours storage was greater in emus which had greater weight loss after transport. Oral administration of nutrient supplements (electrolytes, dextrose, and amino acids) pre- and post-transport was effective in protecting against muscle damage and recovery of body weight losses during lairage, but had little effect on meat quality. The clinical findings were suggestive of the incidence of exertional rhabdomyolysis in emus. Meat quality studies revealed the incidence of stress myopathy and dark firm and dry conditions.

The important behavior categories and unusual behavior in emus were identified and described. Handling time greater than 8 minutes significantly ($P < 0.05$) increased the concentrations of corticosterone, glucose and enzyme activities in emus. The significant increase ($P < 0.05$) in the time spent on stereotypies such as pacing, fence pecking and reduction in grooming after transport seemed to be their response to stress.

This study provided insight into the physiological and behavioral responses of emus to transport stress and the efficacy of oral supplements in alleviating the same. Identification of non-invasive indicators of stress from this study would aid to improve the management and welfare of emus.

Preface

Approval for the experiments conducted in connection with this research project was given by the University of British Columbia Animal Care Committee, under permits # A10-0104, # A10-0183, and # A10-0184. Approval for the survey project (permit # H10-0052) was provided by the Behavioral Ethics Research Board, University of British Columbia.

This thesis study was designed in consultation with my supervisors Drs. Kimberly M. Cheng, Darin C. Bennett and Allen L. Schaefer. Planning of experiments and sample collection, were mostly done by me with the help of Dr. Bennett. Drs. Tony Kozak and Val Lemay from the Department of Forestry provided guidance on SAS programming. Data analysis was carried out by me while Dr. Cheng provided necessary directions. I analyzed, interpreted the data and wrote the basic manuscripts. Drs. Bennett and Cheng provided valuable suggestions and edited all the manuscripts. All the chapters benefited from inputs provided by the co-authors and Dr. Rajamahendran.

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8. Menon, D. G. 2012. Why extreme caution is needed while handling emus. Emu Today and Tomorrow. 22:5-15.

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Abbreviations

ACTH	Adrenocorticotropic hormone
AHA	Animal Health Australia
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CARC	Canadian Agri-Food Research Council
CFIA	Canadian Food Inspection Agency
CK	Creatine kinase
cm	centimeter
CORT	Corticosterone
CRF	Corticotropin releasing factor
CS	Corticosteroids
DFD	Dark, firm and dry
DL_{24}	Drip loss measured at 24 hours post-slaughter
DL_5	Drip loss measured at 5 days post-slaughter
DPIW	Department of Primary Industries and Water
g	Gram
GC	Glucocorticoid
GGT	Gamma-glutamyltransferase
H/L ratio	Heterophil / lymphocyte ratio
HPA	Hypothalamic-pituitary-adrenal
IU	International units
Kg	Kilogram
L	Liter
LS	Changes during lairage
m	Meter
NA	North America
OHTG	Ontario Humane Transport group
OS	Overall changes
PCV	Packed cell volume
pH_{45}	pH recorded at 45 minutes post-slaughter
pH_{24}	pH recorded at 24 hours post-slaughter
PIMC	Primary Industries Industrial Council
PSE	Pale soft exudative
RBC	Red blood cell
RI	Reference Interval
SA	Sympathoadrenal
S.E	Standard Error
TS	Changes during transport
UA	Uric acid
WBC	White blood cell

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Dedication

Dedicated to my grandfather Sri. Rama Varma Thirumulpad, who taught me to dream big and instilled in me, the courage to pursue those dreams.

1. INTRODUCTION AND REVIEW OF LITERATURE

1.1 Introduction

Emus are ratites native to Australia. They are farmed globally for their fat, which is rendered into the oil that has therapeutic (Howarth et al., 2008; Lindsay et al., 2010; Abimosleh et al., 2012) and cosmetic properties (Zemstov et al., 1996). Emus are short day (winter) breeders (Martin, 1999; Blache et al., 2000) and their breeding season begins in the months of December or January, and extends up to April or May in Canada (Contini et al., 2010). During the breeding season, emus reduce their feed intake considerably and lose a significant portion of their body weight (Davies, 2002). Emu eggs have a lengthy incubation period of around 52-56 days (Mahato et al., 2006), and it is the male alone that incubates the eggs and broods the young (Davies, 2002). Producers slaughter their emus in fall so that they harvest the maximum amount of fat from them.

Many emu farms are located in areas lacking slaughtering facilities suited to process these birds (Michael, 2000; Stevens, 2012). This necessitates their shipment, often over long distances, resulting in exposure to stressful and adverse conditions (Mitchell and Kettlewell, 2009). Such transport for slaughter has always been a topic of public concern, as exposure to multiple stressors could compromise the welfare of animals (Canadian Agri-Food Research Council (CARC), 2001). The effect of stressors of transport has been examined in depth in traditional poultry species, but not in emus. Though the emu industry caters to a niche market in Canada, ethical treatment of emus is an important issue, which provided the impetus to analyze their welfare status, and suggest methods to improve the same.

Typically in North America, yearlings are used to supply the market for fresh meat, while older birds that have completed at least one breeding season are slaughtered for their fat to supply the oil market. So far, no research has been undertaken to examine the behavioral and physiological responses of emus to handling and transport. Emus are highly susceptible to stress, and hence there is the need to undertake research on this topic, so as to suggest measures to alleviate transport stress. Strategies specific to the species have to be examined to minimize welfare problems. Therefore this project was designed to assess the welfare of emus during handling, transport, and slaughtering.

Several studies have found that nutrient (electrolyte, dextrose, and amino acids) interventions help to mitigate the effects of transport stress (dehydration, electrolyte imbalance, energy deficits) in livestock and poultry (Schaefer et al., 1995; Smith and Wilson, 1999; Olanrewaju et al., 2007; Arp et al., 2011). Their effectiveness in emus has never been documented. Furthermore, the effect of transport on carcass yields and meat quality characteristics of adult birds has not been examined. The behavioral peculiarities and indicators of stress in emus also needed to be identified. Therefore, the objective of this study was to examine the effect of handling and transport before slaughter on adult emus, so as to identify important welfare issues and suggest methods to improve their wellbeing.

In this chapter, I shall be reviewing important aspects of the emu industry, namely, emu farming and associated products; assessment of welfare during transport; stress physiology and its evaluation.

1.2 Review of relevant literature on emus

1.2.1 An introduction to emus

Emu (*Dromaius novaehollandiae*) is the second largest extant bird species in the world, and is native to Australia. Emu belongs to a flightless group of birds called ratites (Sibley and Ahluist, 1990), which also includes ostrich (South Africa), rhea (South America), kiwi (New Zealand), cassowary (New Guinea) and tinamous (Central and South America) (Davies, 2002). The term "ratite" is derived from the Latin word "ratis" for "raft", referring to the emu sternum, which lacks a keel (Hutchins et al., 2002). It is believed that the term 'Emu' originated from the Portuguese word "ema", which means a large bird (Eastman, 1969).

All extant species of ratites are restricted to the southern hemisphere. It was previously believed that ratites emerged as a flightless group in Gondwanaland during the Cretaceous, and then as the continents drifted apart, they were widely distributed to different locations (Cracraft, 1974). Older classification schemes considered ratites as a monophyletic group within a single order *Struthioniformes* (Sibley and Ahluist, 1990). Recent studies have confirmed that ratites are polyphyletic, suggesting that the tinamous, who retained their flight capabilities, also cluster within the ratite lineage (Harshman et al., 2008).

Four species of *Dromaius* have been described: *D. novaehollandiae*, *D. ater, D. baudinianus and D. ocypus* (Eastman, 1969; Boles, 2001; Davies, 2002). Currently, there is only a single living species of emu, *D. novaehollandiae* with the extant subspecies in Australia (Roots, 2006) namely: 1) *D. novaehollandiae novaehollandiae,* 2) *D. novaehollandiae woodwardi,* 3) *D. novaehollandiae rothschildi* (Thompson, 1997) and one extinct subspecies namely, *D. novaehollandiae diemenensis* (Roots, 2006).

1.2.2 Emu farming

Despite a long history of use by the aborigines, it was only in 1970's that the Australian Government permitted the capture of 300 wild emus to establish a primary breeding stock for domestication (Krauss et al., 2002). Although emus were first imported to the US between 1930 and 1950, commercial emu farming began only in the late 1980's (Thompson, 1997). This industry also spread to Europe, and Canada in the eighties (View West Marketing Inc. and Zbeetnoff Agro-Environmental Consulting, 2002) and then to Asia and Africa.

1.2.3 Emu products

Today, emus are farmed around the world and the main products, oil and meat contribute to approximately 80% of the total returns (Stubbs, 1998). The fat is rendered into oil and is reputed to have various therapeutic properties (see Section 1.2.6). Emu meat is low in fat and cholesterol (Beckerbauer et al., 2001), but high in iron (Qiu, 1998; O'Malley and Snowden, 1999). In appearance and taste, it is similar to beef. The leather is supple, yet durable. Carved emu eggs and feathers are the other products from this bird. A description of various emu meat cuts is given by Frapple et al. (1997).

1.2.4 Emu fat

There are two major fat depots: internal (abdominal) and external (breast and back) fat (Yoganathan et al., 2003; Sales, 2007). An adult emu weighing 40-45 kg produces up to 10-12 kg of body fat (Sales et al., 1999), from which oil could be obtained by rendering at temperatures up to 150°C (Whitehouse et al., 1998). The yield of fat and oil varies with genetics of the bird, diet, stress, weather conditions and time of the year (O'Malley and Snowden, 1999). Bulk emu fat sells for $20-$30 per kg, while refined oil sells for $80-$300 per litre (Dwayne Harder, Emu Producer, Try Harder Farms, Saskatchewan, personal communication).

1.2.5 Emu oil

Emu oil is composed of triglyceride esters of saturated and unsaturated fatty acids (Wang et al., 2000). Emu oil has a high proportion of oleic acid, linolenic acid and saturated fatty acids like palmitic and stearic acid. Emu oil contains 56 % monounsaturated fatty acids (MUFA), 31 % saturated fatty acids and 13 % polyunsaturated fatty acid (Wang et al., 2000).

1.2.6 Therapeutic properties

The medicinal value of emu oil has been documented as early as in the 1860s (Power and Cameron, 2004). The anti-inflammatory, anti–arthritic, and emollient properties of emu oil were demonstrated in rats (Whitehouse et al., 1998), and mice (Lopez et al., 1999; Yoganathan et al., 2003). Wound healing properties in tissues were illustrated by Qiu et al. (2005) and Li et al. (2009) in rats; and Lunam (*in vitro* studies; 2008). Emu oil was found to lessen the effects of inflammation in the small intestine of rats, suggesting a new therapeutic strategy for mucositis (Lindsay et al., 2010; Abimosleh et al., 2013). Emu oil was shown to have antioxidant properties and to provide protection against oxidative damage in cells (*in vitro* studies; Bennett et al., 2008). Topical application of emu oil was effective in reducing auricular inflammation in mice (Yoganathan et al., 2003). Recently emu oil was found to be beneficial in reducing tissue damage associated with colitis in rats (Abimosleh et al., 2012). Emu oil was also effective in preventing chemotherapy induced loss of bone tissue (Nandhanan et al., 2012). Thus emu oil has several therapeutic properties, making it a very valuable product. Still, there is a long way to go before these claims get wide acceptance from the scientific community. More successful clinical trials confirming the claims would mean better acceptance of the products and better marketability. This would definitely help improve the prospects of emu industry.

1.2.7 Hide and feathers

The leather is soft and supple, colorable and with an attractive pattern resulting from the removal of feathers. An adult emu can yield up to 0.5 to 0.75 m^2 of leather, which is used to make a variety of soft leather items and even garments (MacNamara et al., 2003). Emu leg skin has a distinctive scale pattern and is in demand for making leather accessories. About 90 % of the emu hides collected are damaged (Michael, 2000), indicating the need to harvest skin with utmost care (Frapple et al., 1997). The emu feathers are also used to make pillows; in automotive and electronic industries as dusters; and by fishermen for tying flies (Adams and Revell, n.d). They are used in the fashion, art and craft industries, but high processing costs are a major issue.

1.3 Assessing the welfare of animals during transport and slaughter

The concept of animal welfare addresses the ethical concerns about the quality of life of animals (Fraser and Weary, 2004). According to the World Health Organization, an animal is in good state of welfare if it is healthy, comfortable, well-nourished, safe, able to express innate behavior, and not suffering from unpleasant states such as pain, fear, and distress (Bousfield and Brown, 2010). The five freedom concept defines animal welfare to include: freedom from hunger and thirst, freedom from discomfort, freedom from pain, injury or disease, freedom to express normal behavior and freedom from fear and distress (Farm Animal Welfare Council, 2005). The three major approaches used by researchers to assess animal welfare are the subjective experiences of animals, biological functioning and freedom for expression of natural behavior (Fraser et al., 1997; Blandford et al., 2002), and they are not mutually exclusive. The use of scientific methods based on these approaches in assessing animal welfare is widely accepted (European Commission, 2002; Duncan, 2005; Terlouw et al., 2008).

1.3.1 Current guidelines on animal transport in Canada

The Office Internationale des Epizooties (OIE) provides recommendations for animal health worldwide, while the minimum welfare standards in many countries are established by legislature (Adams et al., 2008). The Federal obligations under the Health of Animals Regulations, Part XII in Canada are enforced by the Canadian Food Inspection Agency (CFIA) with the assistance of provincial and territorial authorities (CFIA, 2013a). Federally, there are three sections of legislation to protect farm animals namely, the Criminal Code (Section 446 – Cruelty to Animals), Health of Animals Act, and Meat Inspection Act (Wepruk, 2011). Most provinces have their own animal protection laws. In British Columbia, the province's "Prevention of Cruelty to Animals Act" is enforced by the Society for Prevention of Cruelty to Animals (Wepruk, 2011).

It is the responsibility of animal transporters and owners to prevent injuries or undue suffering to transported animals (CFIA, 2013b). Any journey in which animals are transported for more than four hours is considered long distance in Canada (Canadian Agri-Food Research Council (CARC), 2001). There is a mandatory training program for livestock transporters in Canada (Schwartzkopf-Genswein et al., 2008). The recommended code of practice for the care and handling of farm animals during transport in Canada are voluntary (CARC, 2001). These guidelines underline the pre-transport fitness of animals, use of ramps and chutes, need for proper handling, and unloading at stops, resting with access to feed and drinking water during long journeys, and recording injuries at the destination (CARC, 2001). Around 90% of the large slaughter plants in Canada are welfare audited (Grandin, 2005), still this sector is in need of stricter legislature. An unofficial investigation in Canada by the World Society for the Protection of Animals (2010) and the inspections conducted by the CFIA suggested that welfare could be compromised, especially in shipments wherein low-value (spent) animals are involved (Doonan and Appelt, 2008).

Though there are very little guidelines specific to ratites, signs of distress, treatment of unfit animals, and emergency euthanasia procedures are discussed by the Ontario Humane Transport Working Group (OHTG, 2007). Emus are different from other livestock and hence guidelines specific to their well-being needs to be provided to their producers. If legislative changes are needed in this direction, then the suggestions should be based on sound research. Such research should focus on the conditions under which emus are transported, as well as monitor their physiological responses, and behavior. Thorough research is needed to suggest measures to improve the welfare of emus that are transported before slaughter.

1.3.2 Documentation for transport

Preparing and carrying all necessary documents related to the flock is necessary before embarking on the journey. Such a list is suggested for cattle transport in Canada (Livestock Identification Services Ltd, 2009), but not for ratites. The necessary documents enlisted for transporting ostrich in UK (Wotton and Hewitt, 1999) included a health and "fitness for transport" certificate from the local veterinarian, address and contact details of the owner of the stock, consignment details, including the numbers of emus, the date and time of dispatch, anticipated time of arrival and contact details for the relevant person(s) at destination, contact details of veterinarian, and an intact emergency slaughter protocol, invoices, route-plan, weight ticket, and welfare certificates.

1.3.3 Concerns during transport

The transport process starts with herding, and continues through loading, confinement within the truck, unloading and penning in an unfamiliar environment (Tarrant and Grandin, 2000). Factors such as weather, duration of transport, vehicle design, road and driving conditions, stocking density, and mixing with unfamiliar animals during transport would have a detrimental effect on them (Harding and Rivers, 2008; Ahmed, 2009; Guardia et al., 2009; Mitchell and Kettlewell, 2009; Petracci et al., 2010). Loading, competence of operators, humidity and ambient temperature, handling and restraint, stocking density and headroom, ramps, lighting, ventilation, bedding, feeding and watering, time of transport, vehicles, lairage, slaughter techniques have all been identified as key issues affecting the wellbeing of transported animals (Chambers and Grandin, 2001; Grandin, 2001a; 2006; Animal Liberation Inc. 2008; Doonan and Appelt, 2008). It is also important to ensure the welfare of animals during each and every step in food production (Blandford et al., 2002), as a part of the total quality concept (Mench, 2003).

1.3.4 Fitness for transport

Pre-transport fitness assessment performed on all animals by trained people is an essential part of welfare evaluation (Grandin, 2001b). Emus need to be inspected for ocular discharge, enteritis, respiratory distress, and injuries involving leg and neck (Glatz, 2000). Non-ambulatory, severely debilitated, moribund, weak, diseased and injured emus should not be transported (Doonan, and Appelt, 2008; Harding and Rivers, 2008; Van Reenen et al., 2008). The necessary guidelines for the same in Canada are provided in the Compromised Animals Policy of the Transportation of Animals Program- (CFIA, 2013a). The guideline helps in decision making and suggests situation specific appropriate measures including emergency euthanasia (Adams and Thornber, 2008; Ontario Farm Animal Council, 2009).

1.3.5 Role of handlers

Trained and compassionate handlers can improve the welfare of animals during handling (Humane Farm Animal Care, 2009). Regular contact and training of animals would help to make them more comfortable in the presence of people (Grandin, 1999; Speer et al., 2001). The animal’s relationships to handlers are identifiable from their approach and avoidance strategies (Lensink et al., 2000; Winckler, 2008), which also indicate welfare (Waiblinger et al., 2004). Handlers should be able to identify behavioral changes early enough, and for this, trained or experienced handlers are needed (Wotton and Hewitt 1999). Awareness of normal behavior repertoires is important to interpret activities (Sanotra and Weeks, 2004; Mollenhorst et al., 2005). Changes in the time spent on each behavior (time budgets) could indicate welfare issues (Aland, 2007). It is important that animals are prepared well ahead of the day of transport.

1.3.6 Preparing for transport

A very common pre-transport practice is feed and water withdrawal, which includes the time for which the birds are deprived feed or water while on the farm, during transport and while resting in lairage (Petracci et al., 2010). Withholding water for six hours prior to transport has been long accepted as a measure to reduce slipperiness on floor (Tarrant and Grandin, 2000). The Australian guidelines (Glatz, 2000; SCARM, 2003; Harding and Rivers, 2008) suggest a maximum feed withdrawal period of 12 and 24 hours respectively, for chicks and adult emus. Feed withdrawal causes weight losses (Warriss et al., 2004; Nijdam et al., 2006) due to dehydration, evacuation of gut contents, and catabolism, which could be as high as 10 % in ratites after a 24 hour transport (Schaefer et al., 1995). Some animals refuse to eat and drink at lairage, which would further aggravate the condition (Knowles and Warris, 2000). Appropriate fasting time is important as it influences carcass yield, and contamination, ultimate muscle pH and keeping quality (Petracci et al., 2010). However, providing feed and water until the start of transport has a settling effect on the animal (Chambers and Grandin, 2001) and is currently practiced in many species including ratites (A. L. Schaefer, Agriculture and Agri-Food Canada, Lacombe, Edmonton, personal communication).

1.3.7 Ramp

Ramps and chutes are used to facilitate easy movement of animals. Vehicle ramps designed for use with emus should have high, closed side gates, steps less than 15 cm in height and an inclination not exceeding 25^{o} (Minka and Ayo, 2008). There should be no gap between the ramp and the vehicle (Wotton and Hewitt, 1999). A curved single-file chute, 1.8 m tall, 50-60 cm in width can be used to prevent emus from turning back during loading and unloading (Glatz, 2000). Behavioral principles outlining the point of balance, flight zone and visual field, flags and lighting arrangements should be utilized to facilitate easy movement (Chambers and Grandin, 2001).

1.3.8 Design of vehicle and stocking density

One major factor deciding animal safety and comfort during transport is the type of vehicle used. These vehicles must be fully enclosed, but with adequate ventilation to ensure the comfort of emus (Glatz, 2000). Sufficient gap between the roof and sides of the vehicle provides good ventilation (Wotton and Hewitt, 1999) on hot days (Kettlewell et al., 2003). The suggested crate design for the transport of emus is a steel frame with plywood lining on sides and tall enough (1.8 m) to let the emus stand erect (Glatz, 2000). Dim light, appropriate stocking density and provision of sufficient space to sit and stand improves the welfare of emus during transport (Glatz and Miao, 2008). Adult emus may be transported in groups of 12 and chicks in groups of 20 (Glatz, 2000). Stocking density during transport should be based on weather conditions, duration of transport, body weight, and age of the birds (Elrom, 2000; DEFRA, 2007). Both high and low stocking densities can result in carcass bruising (Eicher, 2001; Emanuela et al., 2007). The appropriate stocking density is 8 birds/m^2 for emus less than 7 kg live weight, 3 birds/m^2 for emus weighing 25-30 kg and 2 birds/m^2 for mature emus of 35-45 kg live weight (Department of Primary Industries and Water, 2008).

Strong animals appear to fight with others for best positions inside the transport truck (Kannan et al., 2002). Providing partitions inside the compartment would help reduce such interactions. Individual compartments were found to reduce injuries in ostrich chicks (Minka and Ayo, 2011a), but to what extend it is applicable to emus is not clear. Temperature regulated and air sprung trucks are increasingly being used in animal transport to reduce the impact of road conditions (Animal Health Australia (AHA), 2012). However good the truck used may be, driving skills play a vital role in the welfare of birds transported.

1.3.9 Driving conditions

In Canada, livestock are generally transported by the owners themselves or on company controlled trucks (Woods and Grandin, 2008). Truck drivers need to be trained in transport and livestock-handling techniques (Schwartzkopf-Genswein et al., 2008). Falling down during transport is a major risk, which increases with stocking density and reckless driving (Tarrant and Grandin, 2000). As far as possible, constant speed should be maintained, and roads which are bumpy and with heavy traffic may be avoided (Terlouw et al., 2008). Sudden stops and acceleration, sharp turns or tight corners should be avoided to prevent bruises, loss of body weight, injuries and mortality (Black and Glatz, 2011).

1.3.10 Well-being during transport

Since the duration of transport significantly affects welfare (Gebresenbet et al., 2005), journeys should be as short as possible and carefully undertaken (Wotton and Hewitt, 1999; Crowther et al., 2003; Glatz and Miao, 2008). Stressors during transport cause hypo/ hyperthermia, postural instability, vasodilatation, internal bleeding, pooling of blood in organs and heart failure (Wotton and Hewitt, 1999; Crowther et al., 2003; Earley et al., 2007). Animals tighten the abdominal muscle to counter the displacement of organs by vibrations, resulting in poor expansion of air sacs, anoxia and related tissue injuries (European Union Directive, 2005).

Ambient temperature, humidity, air velocity and levels of noxious gases such as ammonia, and carbon dioxide are important factors affecting the comfort (Kettlewell et al., 2003) and health (Gebresenbet et al., 2005) of birds during transport. The livestock weather safety index chart (Grandin, 2010a) cautions about the hazards of exposure to extremes of temperature, humidity and wind chill. Hot weather seems to be a greater stressor than cold, as mortality rates were higher in chicken transported under warm weather (17ºC), while no mortality occurred under cold (-10ºC) weather (Warriss et al., 2005). Monitoring body temperature would provide vital information on the well-being of the birds, since a rise or fall in core body temperature by 1.5°C will have a significant impact on health and that by 4-5ºC will almost always be fatal (Kettlewell et al., 2003). As ambient temperature affects the well-being of animals, information on the microclimate (temperature and humidity) inside the truck compartment collected using data loggers is essential to ensure welfare (Atkins, n. d; Gupta et al., 2005).

1.3.11 Bedding

Provision for bedding in the transport compartments is a common practice. Emus excrete large amount of droppings (Skadhauge et al., 1991) during transport, which causes slippery conditions (Wotton and Hewitt, 1999). Being bipedal, they have great difficulty in maintaining body balance while in transport (Stewart, 1984). Materials such as straw, hay, and wood shavings absorb moisture, while rubber mats provide a non-slip footing (Glatz, 2000), thus prevent slipping, falling and bruising. When the humidity is too high, providing absorbent bedding is useful. Lighter bedding is used under very warm weather.

1.3.12 Transport losses

It is necessary to inspect animals during and after transport to assess their health. Welfare of animals during transport can be assessed based on mortality, number and extent of injuries, and changes in carcass quality (Broom, 2005). Most injuries observed in ratites after transport are related to handling and associated activities (Glatz, 2000). Injuries compromise the welfare of animals, downgrade carcasses, and even lead to mortality (Nijdam et al., 2004), thus resulting in economic losses due to trimming and general carcass value depreciation (NanniCosta et al., 2006). The color of the bruising should also be recorded as it would indicate the probable cause and age of the injury (Glatz, 2000). All such incidents should be critically analyzed to find probable cause of the injury and steps should be taken to avoid them in future.

1.3.13 Lairage conditions and handling at the abattoir (processing plant)

Lairage is the period after being unloaded at the abattoir, until the animals are slaughtered. Upon reaching the destination, emus should be immediately unloaded and inspected so that injured birds can be attended to urgently and humanely slaughtered, if necessary (Environment and Heritage Service, 2004; OHTG, 2007). Good unloading skills are extremely important as emus are very panicky in nature (Glatz and Miao, 2008). Overnight lairage in spacious pens help them rest, rehydrate and settle down (Villaroel et al., 2001; Wotton and Sparrey, 2002; Glatz and Miao, 2008). However, the ultimate benefits would depend on the conditions in the lairage (reviewed by Villaroel et al., 2001; Smith et al., 2004; Minka and Ayo, 2009).

Cattle transported for less than six hours are generally rested for at least 2 hours before slaughter (Colditz et al., 2006), while longer rest period may be needed with longer journeys (Smith et al., 2004). Regular auditing of the handling and lairage conditions are necessary to identify problems in animal handling (Grandin, 2001b). It is important to take necessary measures to overcome these problems and ensure that animals are properly rested at lairage (Villaroel et al., 2001).

1.3.14 Stunning and slaughter techniques

Australia and New Zealand have set standards for the hygienic production of emu meat for human consumption. The most common stressors during pre-slaughter handling, such as stalling of the lines, and slipping (NanniCosta et al., 2003) would lead to aggression, and carcass bruising, thus affecting meat quality (Grandin, 2006; NanniCosta et al., 2006). Maintaining calmness while handling, using appropriate stunning methods and proper bleeding would considerably improve their welfare during slaughter (Wotton and Sparrey, 2002; Gupta et al., 2007). The methods of stunning ratites include use of captive bolt pistol, concussion stunning, electronarcosis, and exposure to carbon dioxide (Lambooij et al., 1999; Glatz and Miao, 2008). Equipments should be clean and sterilized, and well maintained to ensure effective stunning (Glatz, 2000; Terlouw et al., 2008). The variables to be evaluated during slaughter, based on the hazard analysis critical control point system are effective stunning, bleed rail insensibility, falls, vocalization, use of electric prod, and wilful abuse of animals (Grandin, 2006). Abattoir personnel should be trained and experienced to ensure effective stunning. The rhythmic breathing movements indicate recovery and its absence is an important sign of effective stunning (Wotton and Sparrey, 2002). Insensitivity should be ascertained before hoisting. Birds should be bled completely by severing both the carotid artery and jugular vein within 20-30 seconds after stunning (Wotton and Sparrey, 2002). Emergency procedures should be in place to avoid potential problems (OHTG, 2007).

Evaluating the sufficiency of welfare measures is extremely difficult; still essential as the positive outcomes that follow, benefit the animals, producers and consumers. Genetics, individual differences and previous experience greatly influence animal responses. Properly designed facilities and well trained personnel are essential to ensure better welfare. Effective evaluation of the welfare measures, stressors and their effects on animals would dictate the need for further research to identify potential losses and quantification of shortfalls with respect to transportation economics (Speer et al., 2001).

1.4 Transport stress

1.4.1 The physiology of stress

There have been several approaches to the concept of stress and hence more than one definition is in use. Stress is any environmental impact on an animal, which taxes its body systems, reducing fitness (Broom and Kirkden, 2004) or health and performance (Rosales, 1994). Stress also refers to the consequences of the failure of an animal body to “respond appropriately to emotional or physical threats, whether actual or imagined” (Heidenreich and Pruter, 2009).

Stressors are perceived by the higher centres of the brain, resulting in the activation of two major response mechanisms. The sympathoadrenal (SA) system responses explained by Walter Cannon, who first used the term stress to report his work on the postganglionic neurons and adrenal medullary tissue (Sapolsky et al., 2000). The rapid SA responses result in the release of catecholamines, adrenaline and nor-adrenaline (Sapolsky et al., 2000), to elicit the fight or flight response (Romero and Butler, 2007). This response causes “elevated blood pressure, muscle tone, nerve sensitivity, respiration rate and blood sugar concentrations and antibody production” (Elrom, 2000). The mechanism of stress responses is depicted in Figure 1.1.

Hans Selye, the pioneer researcher on stress recognized the role of stressors and explained the working of Hypothalamo-pituitary-adrenalcortex (HPA) axis (Siegel, 1980; Rushen, 1986). The slower, generalized and long term effects of stress are due to the activation of the HPA axis, leading to the production of corticosteroids, GCs, and mineralocorticoids, which are called as stress hormones (Sapolsky et al., 2000). Hypothalamus releases corticotropin releasing factors (CRF) which enter the portal circulation, reach the anterior pituitary, bind to receptors and stimulate the secretion of adrenocorticotropic hormone (ACTH; Rich and Romero, 2005). After reaching the adrenal cortex, ACTH binds to receptors (Romero and Butler, 2007) and stimulates the production of steroid hormones. Corticosterone secretion is stimulated by both CRF and arginine vasotensin from the hypothalamus (Carsia and Harvey, 2000). ACTH stimulates the synthesis and release of steroids from the adrenal cortex by promoting the uptake of cholesterol and its enzymatic conversion to corticosterone and cortisol (Schmidt and Soma, 2008).

Vasopressin potentiates the effects of CRF on pituitary to release ACTH, while the beta endorphins stimulate the release of GCs. Stress hormones act on a variety of target tissues and organs to maintain homeostasis (Matteri et al., 2000). Corticosteroids also exert a negative feedback effect on the hypothalamus to inhibit the release of CRF and hence the stress response would subside (Downing and Bryden, 2002). So also when the system is exhausted, it would stop producing CRF.

1.4.2 Stress responses

The HPA response is also called as ‘general adaptation syndrome’ (GAS; Rosales, 1994; Scott and Schaefer, 1999) and it occurs in three stages namely, the stage of alarm reaction, the stage of resistance and the stage of exhaustion (Schaefer et al., 2001). Animals try to maintain the

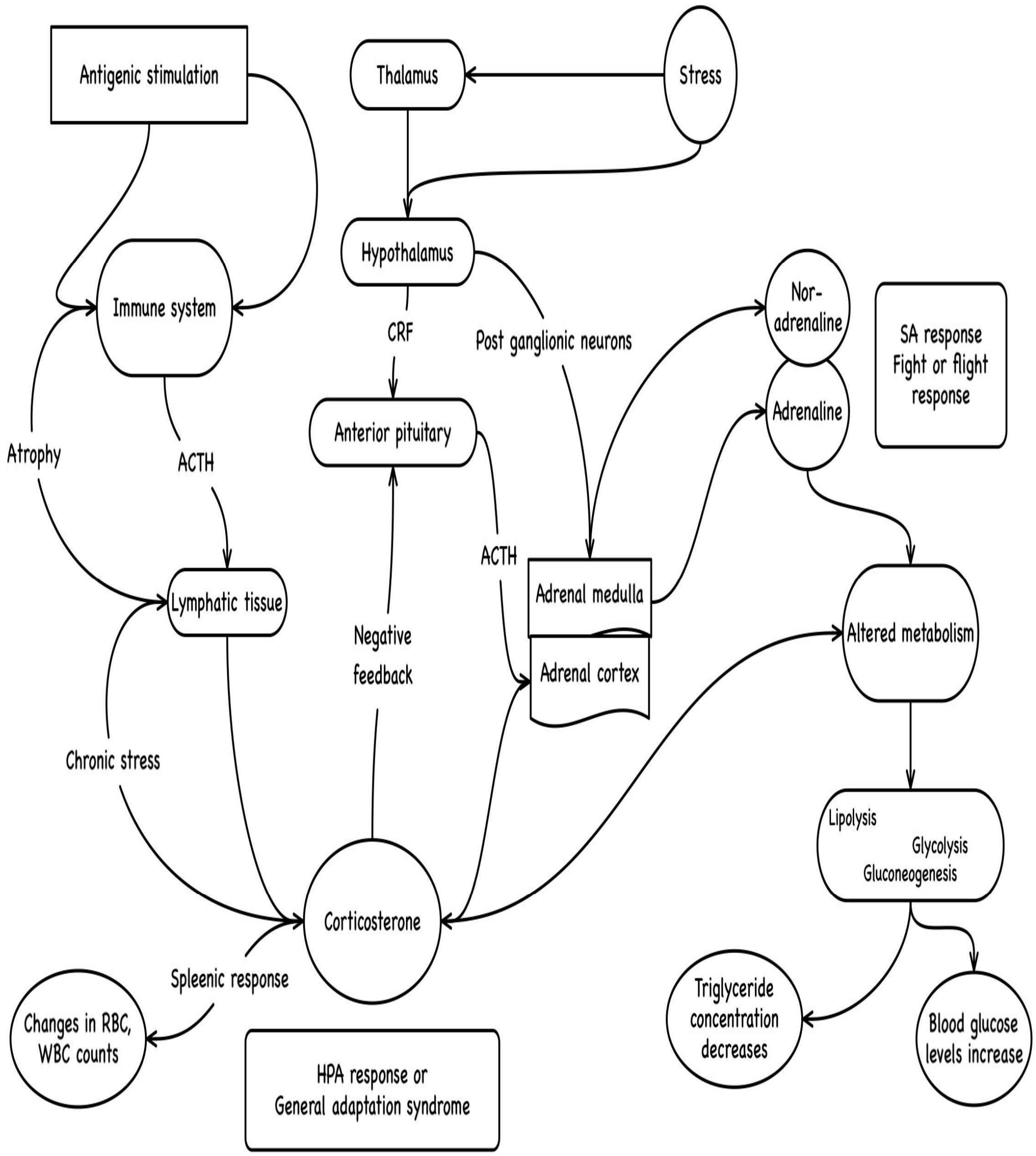

Figure 1.1. Mechanism of stress responses: Sympathoadrenal (SA) and Hypothalamic-pituitary-adrenal (HPA) responses.
CRF = Corticotrophin releasing factors; RBC = Red blood cell, WBC = white blood cell; ACTH = Adrenocorticotropic hormone.

internal environment within the normal range (homeostasis; McEwen, 2000) through behavioral, physiological or metabolic mechanisms, and activation of the immune system (Blecha, 2000; Bilgili et al., 2006; Gupta et al., 2007; Suchy et al., 2007; Hoffman et al., 2012). These coping strategies are distinct responses, which are consistent over time and context, and characteristic of a group of individuals (Koolhaas et al., 1999).

The perception, responses and effect of stress on animals can be summarised as given in Table 1.1. Animals adjust to most stresses by modifying their physiological functions and all favourable adjustments made are referred to as acclimatization (King, 2010). Unnatural or prolonged stress disrupts homeostasis, becomes distress, which when severe can culminate in illnesses and death (Smith et al., 2004). One of the main challenges in animal operations involving confinement is managing stress, so that it does not progress to distress (King, 2010).

Table 1.1. The perception, responses and effect of stress on animals.

Strength of stimuli	Slight	Moderate	Intense
Animals' perception	Imperceptible	Stress	Distress
Animals' response	None	Physiological	Pathological
Effect on animal	None	Adaptation	Abnormality

Adapted from King (2010).

GCs mobilize glucose from the body's reserves including glycogen, lipids, and proteins to combat the stress (Virden and Kidd, 2009). If the stressor continues, body reserves and the hormones from the adrenal gland are exhausted. This phase leads to fatigue of the homeostatic mechanisms and ultimately, death (Rosales, 1994). Animal responses to stress include those to combat, to adapt, or submit to stress. They can be differentiated from the animal's behavior, which could be to flee, fight, or fright i.e., freeze responses (Bracha et al., 2004) or tonic immobility (Broom, 1991; Adams and Thomber, 2008). Physiological responses would affect the functioning of several body systems, resulting in reduced feed intake, altered blood chemistry, muscle metabolism, and immunity and even mortality (Bradshaw et al., 1996; Loerch and

Fluharty, 1999). Thus, it is beyond doubt that stress compromises the welfare of animals and causes considerable economic losses. The physiology of stress differs in mammals and birds.

1.4.3 Differences between mammals and birds

The releasing factors from the median eminence of the hypothalamus pass through a "blood capillary portal system to the anterior pituitary gland" (Deeming, 1999). There is no division of the avian adrenal gland into distinct regions as in mammals (Downing and Bryden, 2002). Corticosterone secretion is controlled by the hypothalamus and pituitary in mammals. But the pituitary is less subject to hypothalamic control in birds (Siegel, 1980). Avian leukocytes are also capable of secreting ACTH, when stimulated by CRF (Elrom, 2000). In most mammals and fishes, the primary corticosteroid is cortisol, while in birds, reptiles, amphibians and some rodents it is corticosterone (Matteri et al., 2000; Hull et al., 2007; Breuner, 2008).

1.4.4 Stress hormones

Within seconds of stimulation, the catecholamines, adrenaline and nor-adrenaline are released (Raj, 2010). They cause lipolysis, glycogenolysis, and gluconeogenesis (Elrom, 2000) to combat the stress (Romero and Butler, 2007). Catecholamines are not the best indicators of stress, as their concentration shows wide variation and their effects last only for a few minutes (Romero and Butler, 2007). On the other hand, GCs exert their effect only minutes after stimulation. Their effect lasts for around 60 minutes, if the stressor does not persist (Romero and Butler, 2007). Similar to catecholamines, GCs also produce changes in glucose and mineral metabolism, gastro-intestinal ulcers, cardiovascular irregularities, hypercholesterolemia, alterations in immune function (Sapolsky et al., 2000) and even death of the animal, if not properly managed (Guardia et al., 2009). It is therefore not easy to differentiate between the effects of catecholamines and GC on systems. However, collecting samples within three minutes has been suggested to eliminate the effect of corticosterone on several physiological variables measured (Romero and Reed, 2005). The corticosterone-blinding globulins have an important role to play in regulating the GC concentrations and hence are considered by some researchers as a better indicator of stress than GCs responses (Malisch, et al., 2010).

1.4.4.1 Corticosterone and stress

Acute stress has the potential to alter corticosterone receptor mediated behaviors and physiological processes up to 24 hours after the termination of the stressor (Malisch et al., 2010). The increase in plasma corticosterone concentrations is detectable in a few minutes after initial exposure to a stressor (Romero and Reed, 2005). The concentration of corticosterone in blood is used as an indicator of stress, but responses vary with the type of stressor, individual susceptibility, time of the day, circadian rhythm (Shaw and Tume, 1992) and concentration of corticosterone binding globulin (Breuner and Orchinik, 2002) in many species. The typical plasma corticosterone response during handling is an initial rise that starts several minutes after capture, reaching a peak 15 to 30 minutes later, and remaining constant or dropping thereafter (Cockrem and Silverin, 2002). Therefore, the time for sample collection could also affect the results (Koolhaas et al., 1999; Leche et al., 2013). As stressors produce both short-term and long term effects on the animal, multiple variables need to be examined (Broom, 2000) to evaluate transport stress. Plasma corticosterone concentrations alone may not reveal the exact effect of the stress on the animal, as they are less sensitive to some stressors and influenced by animal responses (Rushen, 1986). Therefore, considering the multidimensional nature of welfare, the assessment of an animal's wellbeing should not be based on a single variable (Aland, 2007; Botreau et al., 2007).

The greatest challenge in evaluating stress is to choose the best list of indicators for a particular species. Usually a combination of hormonal, physiological, enzymatic and behavioral parameters are used to assess stress (Knowles and Warriss, 2000; Smith et al., 2004; Earley et al., 2007; Huff et al., 2008; Sporer et al., 2008). Variables such as breathing rate, heart rate, heart rate variability, changes in body temperature, plasma activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatine kinase (CK), osmolality of the blood, white blood cell (WBC) and red blood cell (RBC) counts, differential leukocyte counts, electrolytes, heterophil/lymphocyte (H/L) ratio, calcium, magnesium, free fatty acids, glucose, lactate and thyroid hormones, presence of injuries, conditions of body and integumentary system, mortality rates, behavioral reactions to the presence of people, stereotypic behavior such as pacing, excessive aggression, cannibalism, pecking, have all been used in different studies to evaluate stress, but with varying results (Broom, 2000; Yuill and Broom, 2003; Ghareeb and Bohm, 2009; Prieto and Campo, 2010; Manhiani et al., 2011).

1.4.5 Classification of stress

Types of stress involved in animal transport are several: climatic, environmental, nutritional, physiological, physical, social, psychological, and immunological (Rosales, 1994; Grandin, 1997; Duncan, 2005). In the current study, I am mostly interested in the stress arising during handling and transport.

1.4.6 Evaluation of stress

Managing the welfare of animals during transport is quite challenging, as it involves many variables, which can greatly influence their health, well being, meat and fat quality and economics of production. During transport, animals are exposed to a variety of stressors (Villarroel et al., 2001) such as rounding up, dehydration, feed withdrawal, mixing with unfamiliar animals, and extremes of weather (Broom and Kirkden, 2004). Stress responses differ markedly between individuals (Cockrem, 2005), depending on factors such as sex, and environmental conditions. Wide variations in the avian stress responses have been reported among individuals (Sih et al., 2004; Blas et al., 2005). It is necessary to consider the basal concentrations and their fluctuations over time (European Commission, 2002) while using physiological parameters to evaluate stress and interpret welfare. The commonly used physiological indicators of stress during transport are discussed by Broom et al. (2004).

1.4.7 Challenges in evaluating stress

Studies to evaluate stress in farm animals during handling and transport generally provide variable results and are hard to generalize and interpret (Blackshaw, 1986; Grandin, 1997). There is marked between individual differences in stress responses (Littin and Cockrem, 2001; Ellis et al., 2006; Cockrem, 2007). Handling, stunning and slaughter could release corticosterone and catecholamines, thus confounding the concentrations of many of the hematological and biochemical variables (Shaw and Tume, 1992). The objective therefore should be to collect samples with minimum handling. The physiological changes caused by transport require comprehensive studies to identify all the associated responses (Obernier and Baldwin, 2006). The variation in individual response to stress (Siegel and Gross, 2000) depends on genetics, adaptive responses developed early in life (Blas et al., 2005), and environmental interactions (Ellis et al., 2006). Environmental changes and weather conditions were found to account for 35 to 88 % of the individual variations (Romero et al., 2000). Schematic representation of body responses to stress and the framework to evaluate stress are given in Figure 1.2.

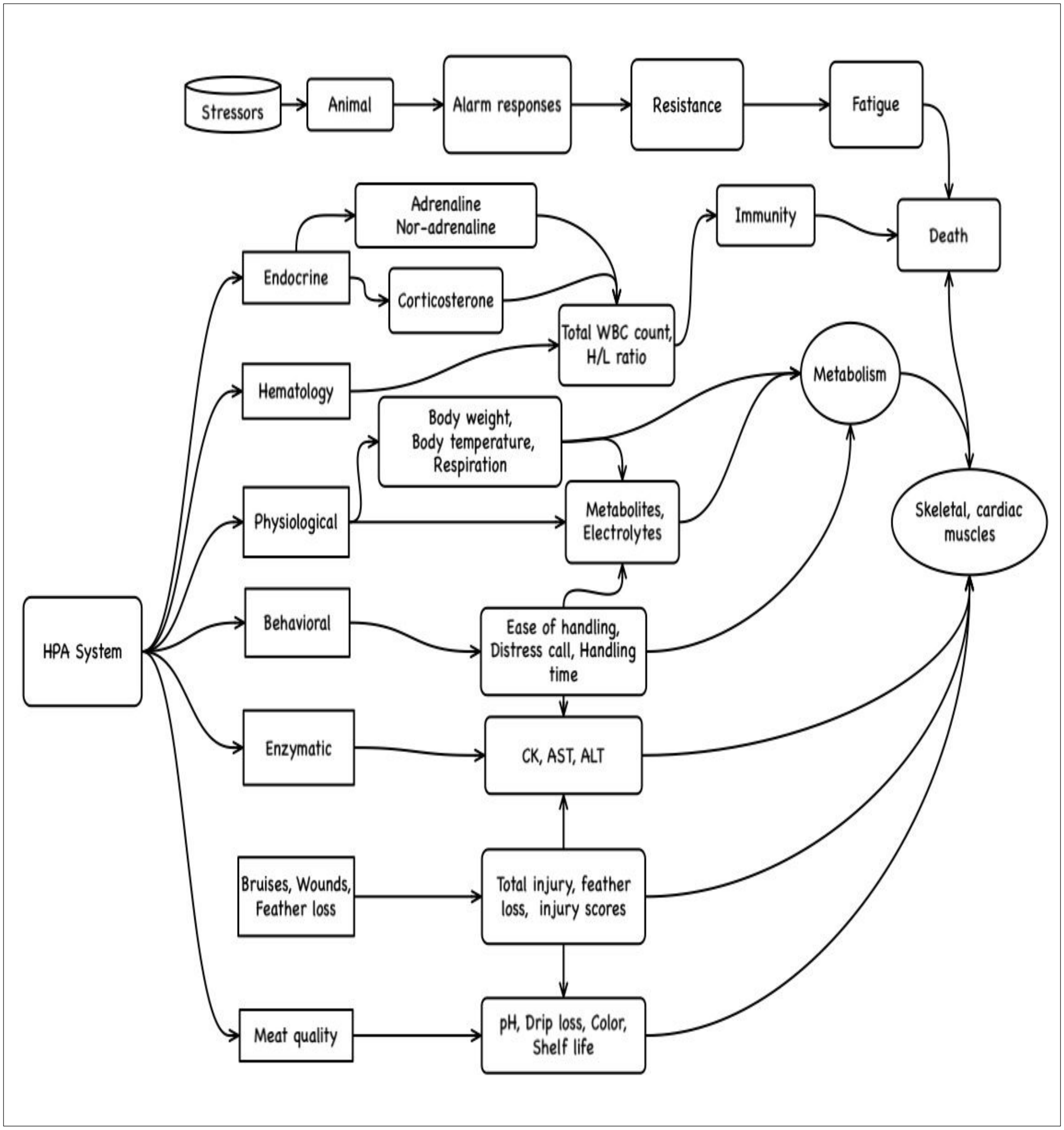

Figure 1.2. Framework used for choosing the variables to evaluate stress.

HPA = Hypothalamic-pituitary-adrenal; H/L ratio = Heterophil/lymphocyte ratio; CK = Creatine kinase, AST = Aspartate aminotransferase, ALT = Alanine aminotransferase.

1.4.8 Effects on physiological variables

1.4.8.1 Reduction in body weight

This topic has received much attention in livestock (Coffey et al., 2001; Kannan et al., 2003; Schwartzkopf-Genswein et al., 2012) and broilers (Petracci et al., 2001), but not in emus. Transportation leads to greater loss of body weight than feed withdrawal alone (Kannan et al., 2002), as there could be tissue losses, evaporative and respiratory water losses, voiding of gut contents, and urination (Parker et al., 2007; Schwartzkopf-Genswein et al., 2008). Loss of body weight after transport at varying rates (0.2 - 0.6 % per hour) has been reported by many researchers in poultry (Buhr et al., 1998; Bianchi et al., 2005; Nijdam et al., 2006; Delezie et al., 2007). Similar to other species, loss of body weight could lead to compromised welfare, economic losses and altered meat quality in emus. Therefore, it is necessary to consider methods to minimize it.

1.4.8.2 Body temperature

Increase in body temperature after transport is consequent to catabolic biochemical events (Schaefer et al., 2001; Ingram et al., 2002) and the inability to thermoregulate (Yalcin et al., 2004; Piccione et al., 2005; Minka and Ayo, 2008; Giloh et al., 2012) during a corticosterone response. However, body surface temperatures were found to dip down, after transport, especially with feed withdrawal and depletion of the energy reserves (Schaefer et al., 1997). High correlation has been reported between the temperatures of featherless body parts of chicken and air temperature (Nascimento et al., 2011), indicating that such birds are more susceptible to environmental stress. Therefore monitoring body temperature changes would provide useful information on the wellness of birds.

1.4.8.3 Effects on blood biochemical composition and metabolism

As the body prepares to respond to stress, GCs increase the metabolic processes (Gonzalez et al., 2007; Zhang et al., 2009) in animals (Earley et al., 2007; Adams and Thornber, 2008). Enhanced mobilization of carbohydrates, protein, and fat from liver and muscle tissue, occurs to protect the function of important tissues and organs in the body. This results in increased concentrations of metabolites in circulation (Li et al., 2009). Transport increases body metabolism, and when this continues for long periods, blood glucose concentrations become exhausted. Once there is an energy deficit, body reserves (fat and glycogen) are broken down (European Commission, 2002; Chloupek et al., 2008). The extent of physiological and metabolic changes would affect yields and meat quality characteristics (Schaefer et al., 2001). The altered metabolic rates after transport would take at least a week's time to stabilize, depending on age, severity of stress, and genetics (Obernier and Baldwin, 2006) and could compromise the health and welfare of animals.

1.4.8.3.1 Glucose

High glucose concentrations are needed in ratites as their predominant anti-predatory strategy is running (flight-type stress response; Leche et al., 2009). Elevated blood glucose concentrations are observed after exposure to stress. This is due to glycogenolysis and or gluconeogenesis caused by catecholamines and GCs released during the stress response (Nijdam et al., 2006; Bedanova et al., 2007; Zhang et al., 2009; Vazquez-Galindo et al., 2013). GCs also inhibit glucose absorption from jejunum (Li et al., 2009). Blood glucose concentration was found to be correlated to the meat pH and hence is important in determining meat quality characteristics (Aksit et al., 2006). The increase in concentration after transport would last till the body reserves are exhausted, or the release of corticosterone stops, or until rested at lairage with access to feed (Tadich et al., 2005).

1.4.8.3.2 Triglycerides

Triglycerides are the major energy source in birds, and their level in circulation is fairly stable, without circadian fluctuations (Smith et al., 2004). GCs cause the mobilization of fats and gluconeogenesis. Triglycerides are broken down (Petracci et al., 2010) into non-esterified fatty acids, which are then utilized by liver and other tissues to produce energy (Remage-Healey and Romero, 2001). Under the action of GCs, the synthesis of triglycerides from non-esterified fatty acids will also be inhibited (Remage-Healey and Romero, 2001). Hence a decrease after transport is due to the combined effects of nutritional (Nijdam et al., 2006; Chloupek et al., 2008; Huff et al., 2008; Voslarova et al., 2011), and handling stress (Kannan et al., 2003).

1.4.8.3.3 Total protein

When energy deficits continue, after carbohydrates and fats, the next target would be proteins. Concentration of total proteins, especially albumin, decreases during stress, indicating nutritional deficits (Sporer et al., 2008). But when there is dehydration, their concentrations go up (Earley et al., 2007; Parker et al., 2007). Increased corticosterone secretion or food deprivation leads to the breakdown of proteins and nucleic acids in muscles (Minka and Ayo, 2009), elevating the concentrations of protein metabolites in response to transport (Schaefer et al., 1997; Earley et al., 2007). Creatinine is the by-product of muscle protein turnover (Hochleithner, 1994) and its concentration is used to assess hydration status and renal function (Scope et al., 2002; Giovannini et al., 2006). At times, the concentration of creatinine is too low to make any logical interpretation in birds (Harris, 2000). Handling and transport leads to the damage and lysis of muscle cell walls (Wotton and Hewitt, 1999; Petracci et al., 2010), thus elevating creatinine. Uric acid is the main end product of protein metabolism in birds (Hochleithner, 1994). The uric acid / creatinine ratio (Lumeij, 1987a; Hochleithner, 1994) is a more reliable indicator of renal dysfunction.

1.4.8.4 Haematological changes

Constituents of blood are altered during stress, owing to immune responses and dehydration. Elevated packed cell volume (PCV; hematocrit) and RBC counts seen during transport are due to dehydration (Minka and Ayo, 2008), sympathetic nerve activity, circulating catecholamines (Ndlovu et al., 2008; Minka and Ayo, 2009) or spleenic response (Scope et al., 2002; Lopez-Olvera, 2004). PCV is lowered during lairage, when animals are rehydrated (Tadich et al., 2005; Parker et al., 2007). PCV was found to decrease immediately after loading, during transport (Werner and Gallo, 2008), restraint or isolation (Minka and Ayo, 2009). White blood cell (WBC) counts could either increase or decrease, depending on species, corresponding to lymphopaenia and hetero/ neutrophilia. The heterophil: lymphocyte (H/L) ratio is a reliable indicator of stress (Schaefer et al., 1997) and has been successfully used in assessing poultry welfare (Moneva et al., 2009). This ratio increases significantly, after an initial lag period and could remain so for variable periods, depending on species, and hence should be interpreted along with other stress indicators (Moneva et al., 2009).

1.4.8.5 Enzymes

Though basically liver enzymes, the plasma activities of AST and ALT increase following handling, loading and transport (Schaefer et al., 1997; Scope et al., 2002; Maria et al., 2004; Huff et al., 2008) due to muscles damage. Reduced muscular tissue perfusion, hypoxia, and fatigue from transport causes increased permeability of muscle cell membranes (Williams and Thorne, 1996) and greater leakage of these enzymes into circulation. The CK activity in ratites is generally higher than that in other birds (Quintavalla et al., 2001; Doneley, 2006). Ratites are highly susceptible to exertional rhabdomyolysis (Tully et al., 1996; see Section 4.4.6.3 for a review). The increases in enzymes show seasonal influence i.e. lower concentrations in winter than in summer, but were unaffected by lairage in cattle (Tadich et al., 2005) and hence a more reliable indicator of stress.

1.4.8.6 Electrolytes

Imbalances in plasma electrolyte concentrations are seen consequent to transport in association with increased muscle activity (Ahmed, 2009) and dehydration (Bilgili et al., 2006; Earley et al., 2007; Gonzalez et al., 2007). As a result, there could be increased serum sodium, chloride, bicarbonate, urine sodium, and urine osmolality during stress such as transport (Schaefer et al., 1992, 1997; Olanrewaju et al., 2007; Ahmed, 2009; Vazquez-Galindo et al., 2013). Electrolyte imbalance is measured as anion gap, obtained as the sums of bicarbonates and chloride reduced from the sum of sodium and potassium: Anion Gap (mEq/L) = $[(Na^+ + K^+) - (Cl^- + HCO_3^-)]$, which could be widened during stress (Schaefer et al., 2001). Bicarbonate concentrations could decrease during heat stress (Debut et al., 2005), due to the rapid deep respiration, wherein carbon dioxide is lost. Heavy panting leads to arterial hypoxemia, which affects cell functions and vitality (Jones et al., 1983). Therefore electrolyte imbalances could affect meat quality, which needs to be redressed through oral supplementation (Schaefer et al., 1997).

1.4.8.7 Immune responses

Stress responses involving corticosteroids affect the immune systems in animals as evidenced by lymphopaenia, regression of the lymphoid organs, spleenic degeneration, immuno–incompetency, immunoglobulin and cytokine production, (Elrom, 2000), leukocytosis and leucopenia (Dalin et al., 1993; Gupta et al., 2007). Release of acute-phase cytokines (monokynes and lymphokynes) have also been reported during stress (Rosales, 1994).

1.4.8.8 Injuries

The physical insults on animals, consequent to transport include abrasions, feather loss, fracture, dislocation and mortality (Minka and Ayo, 2011b). Injuries during transport are consequent to trampling, slipping and falling (Wotton and Hewitt, 1999; Glatz, 2000; Meyer et al., 2002). They significantly compromise the welfare of animals and are a major concern. Feather losses are caused by handling, rubbing against each other or surfaces (Wotton and Hewitt, 1999; Orlic et al., 2007) and possibly stress induced moulting (Moller et al., 2006). Poor meat quality occurs as a result of bruising, rough handling, slipping, or aggression during movement (von Holleben et al., 2003; Colditz et al., 2006; Grandin, 2006; Mounier et al., 2006; NanniCosta et al., 2006), leading to suppressed capacity for protein synthesis, and augmented protein catabolism in skeletal muscles (Dong et al., 2007). All precautions should be taken to avoid injuries and losses consequent to transport.

1.4.9 Behavioral changes

Animals express their feelings through specific behaviors which can be considered as indicators of their welfare (Broom, 2000; Wemelsfelder et. al., 2000; Manteuffel et al., 2004). The behavior of animals while responding to herding, regrouping, and environmental conditions (Grandin, 1999) would provide useful information for welfare assessment. Affective states such as fear, aggression, frustration, content, playfulness, interest, relaxation, friendliness, anxiousness (Wemelsfelder et al., 2000; Haynes, 2008), unusual behaviors, and handling time during loading and unloading need to be examined to reveal the welfare of transported animals (Minka and Ayo, 2008). When animals are kept in their social groups and not mixed with unfamiliar animals, stress levels can be kept low (Mounier et al., 2006).

Stereotypies like continuous, purposeless pacing, chewing, licking, and jumping also provides insight into the problems faced by animals (Koene, n. d). Usually, animals stand more during transport, but lie down more during the recovery period (Tarrant and Grandin, 2000; Eicher, 2001). During transport, restlessness may be evident in frequent changes in position, and interactions with other animals (Smith et al., 1999). Fighting or aggression are other observable indicators of stress during transport (Smith et al., 2004), in large groups of ratites (Glatz and Miao, 2008).

Fear is an aversive feeling, occurring in response to a visible danger (Manteca et al., 2005), handling, transport, pre-slaughter procedures, novel locations, causing adrenal cortex activity, leading to freezing, tonic immobility, and escape attempts (Broom, 1991; Scott and Schaefer, 1999). It is important to consider the relationship between behavior and the physiological indices of stress to identify non-invasive indicators of stress. Vocalization made immediately after aversive events were found to be correlated to increased concentrations of plasma cortisol and hence is considered to be reliable indicator of fear or pain in several species (Grandin, 2001b; 2006). In another study, chicken with the shortest tonic immobility (fear) response were found to have the lowest corticosterone levels, while those with increased concentrations had prolonged tonic immobility (Koolhaas et al., 1999), thus confirming the relationship between behavior and hormone concentrations.

Behavioral changes are important in this study as ratites make use of several physiological and thermoregulatory mechanisms including postural changes to maintain body temperatures (Mitchell, 1999). Usually, ratites sit in the darkness of a moving vehicle (Crowther et al., 2003) and this need not necessarily be stress related. Evaluating behavioral responses in emus along with the physiological and hormonal variables would help to identify non-invasive indicators of stress as well as to evaluate stress. Non-invasive indicators of stress are important in a species like emu, which is highly susceptible to handling stress.

1.4.9.1 Non-invasive methods

Sample collection, confinement or handling of animals may elicit stress responses that confound blood biochemical and haematological variables' concentration. To overcome these problems, researchers have utilized telemetric data loggers, special remote catheters, habituated animals to handling, used sedatives to tranquilize, and even resorted to non-invasive samples such as saliva, faecal matter, urine, egg, hair and feathers in several species (Washburn and Millspaugh, 2002; Singh et al., 2009). Droppings are not considered a suitable material for stress evaluation as it will be mixed with urine, which is likely to contain more GCs, and hence the results may not be reliable (Palme, 2005; Johnstone et al., 2012). Therefore more innovative techniques are needed to evaluate stress in emus.

The use of IRT cameras has revolutionised monitoring of stress in animals (Bench et al., 2008). Thermal imaging is used to capture the thermal energy emitted from different body parts of animals. Infrared visualizes invisible heat radiation, which is not usually visible to human eye, regardless of lighting conditions (Stewart et al., 2008) and this is used to quantify stress or pain based on orbital temperature changes and thermodynamics in animals. During stress, the heat emitted from superficial capillaries around the eye changes as blood flow is regulated under autonomic nervous system control and these changes can be quantified using IRT (Stewart et al., 2008). This will also successfully detect different stress responses to various aversive procedures. This technique can be used to evaluate stress in emus during transport.

1.4.9.2 Scoring techniques

The method of scoring events during loading and unloading could be used to evaluate stress non-invasively (Webster, 2001; Van de Water et al., 2003). Alarm responses including vocalization, attempts to escape, avoidance, aggression, and fighting during rounding up (European Commission, 2002; Maria et al., 2004), slipping, falling, balking, excessive struggling, kicking, defecation, and panting (Minka and Ayo, 2007; Richter and Freegard, 2009; Grandin, 2010a), turns, slips, balks and falls (Maria et al., 2004) could be scored and are considered indicators of welfare. These scores were significantly and positively correlated with physiological parameters of stress during handling in ostriches such as neutrophil/ lymphocyte ratio, body temperature and the extent of injuries sustained (Minka and Ayo, 2008). The plasma concentrations of corticosterone, glucose, lactate, and CK were found to be correlated to the scores in one study (Maria et al., 2004), but while in another, there was no correlation between the loading scores and heart rate which are considered as reliable physiological indicator of stress (Ball and Balthazart, 2008).

1.5 Changes in meat quality

Quiet, careful handling of livestock during each and every step of production is necessary to ensure meat quality (Chambers and Grandin, 2001). Stress prior to slaughter will lead to the depletion of glycogen reserves in muscles, causing lower lactic acid production in meat. Such meat will have high pH, dark color, toughness, lower keeping quality and lower water-holding capacity (Chambers and Grandin, 2001; Warriss, 2010). Meat from animals subjected to stress undergoes increased proteolysis and gluconeogenesis (Gao et al., 2008). Another suggested theory is that greater free H+ ions in mitochondria as well as those from hydrolysis of adenosine triphosphate (ATP) would increase the production and accumulation of hydrogen ion (H+) in the cytoplasm, leading to a decrease in intracellular pH rather than lactic acid accumulation (Apple et al., 2005). Temperature (Bianchi et al., 2006), environmental conditions, duration, distance of transport and feed withdrawal affects meat color (Lyon et al., 2004), processing yield and meat quality characteristics (Petracci, et al., 2001; Bianchi et al., 2004). Animals with poor welfare during transport tend to show conditions like pale, soft exudative meat (PSE) with a high drip loss due to sudden drop in muscle pH after slaughter (Hambrecht et al., 2005). Efficient, experienced and quiet handling of livestock, using recommended techniques and facilities, avoiding mixing of unfamiliar animals will reduce stress in the animals and prevent degradation of meat quality (Chambers and Grandin, 2001; European Commission, 2002).

1.6 Methods to alleviate transport stress

In addition to improving the conditions of transport, scientists have experimented with stress hormones, electrolytes, and special diets to overcome the effects of stress on animals. The major effects of transport are those on metabolic and acid-base imbalances and dehydration (Swanson and Morrow-Tesch, 2001; Parker et al., 2007). Providing energy sources and vitamins, electrolytes, energy sources, essential amino acids (Eicher, 2001; Crowther, 2002; Coenen, 2005; Minka and Ayo, 2011b) helps to modify the HPA axis, reduce the physiological response to stress (Schaefer et al., 2001) and create an indirect muscle protein sparing effect to conserve muscle quality (Schaefer et al., 1992; 1997). Feed supplements, especially vitamins and minerals help to overcome the losses (Wotton and Hewitt, 1999), and create a calming effect before, during, and after transport (Broom et al., 2004). This would help improve the health and welfare of animals during transport.

Other propositions also include resting of animals for prolonged periods to help them recover the losses before slaughter. The use of mobile slaughter houses has been suggested to avoid transport of species that are especially susceptible to stress, particularly if the meat is to be sold to quality conscious market sections (Briese, 1996; Humane Slaughter Association, 2004).

Therefore, it is evident that evaluation of stress response, and experimenting methods to alleviate stress in emus will be quite challenging.

1.7 Objectives of the study and hypotheses

The overall objective of this thesis was to assess the welfare of emus during their handling, and transport to suggest methods to improve the same; and specifically

Chapter 2

To determine and assess the prevailing practices in the handling, transport, and slaughtering of emus with respect to their welfare.

To identify the problems faced by emu farmers during transport of emus.

Chapter 3

To determine the baseline concentrations of the selected physiological variables in adult emus.

To establish reference intervals for these variables in adult emus.

Chapter 4

To examine the effect of transport for six hours on the body temperature, body weight, selected hematological, enzymatic, biochemical, and hormone levels.

Transportation leads to loss of body weight (loss of body mass): This was determined from the change in body weight at three levels of transport- pre-transport, post-transport and at slaughter.

Transportation leads to dehydration: This was evaluated based on changes in PCV, total protein, albumin, and creatinine levels.

Transportation leads to metabolic imbalances: This was measured based on changes in body temperature (before and after transport), serum uric acid, triglycerides, plasma total protein and albumin, creatinine and blood glucose levels.

Transportation leads to changes in immune status: This was assessed with respect to changes in the total leukocyte count, differential leucocytes count and H/L ratio.

To determine the efficacy of special diets in reducing loss of body weight and changes in hematological, enzymatic, biochemical and hormone variables during transport.

Handling and transportation leads to visible physical injuries and malaise: This was estimated by recording the injuries (bruises, aberrations, feather loss, and broken bones), mortality and the number of non-ambulatory emus during and after transport.

Handling and transportation lead to physical injuries which may not be externally visible: This was assessed by examining the changes in the serum CK, AST and ALT levels from pre-transport levels.

Lairage helps to reduce the stress of transport: This was evaluated based on the changes in behavior, biochemical, hematological, enzyme variables from immediately after transport to slaughter.

Sex affects the variables examined and the physiological responses during stress: This was evaluated with respect to changes in the above mentioned variables recorded between the two genders of emus.

Chapter 5

To assess the meat quality characteristics of emus considering the influence of atmospheric temperature, gender and supplement treatment.

Transportation stress leads to changes in the yield and quality of meat: This was measured by evaluating the quality characteristics of meat such as color at 24 hours post transport, muscle pH at 45 minutes and 24 hours, fat quality, incidence of blood splashes, bruises, other injuries and dead on arrival rates.

Chapter 6

To identify and describe the behavioral repertoires of adult domestic emus.

Chapter 7

To assess the changes in behavior of emus during handling and transport from that normally seen under farm conditions.

Handling leads to fear and behavioral changes: This was evaluated based on the ease of handling, time taken to control and load emus, ease of movement, events during loading, unloading, during transport and at the processing plant. The behavior of emus in their group setting, before and after the shipping was compared. Variables evaluated included serum corticosterone levels, and event scores for emus during handling.

To suggest welfare measures to improve the economics of emu production.

1.8 Arrangement of chapters

The findings from my study are arranged in this thesis as follows:

The review of relevant literature is covered in Chapter 1. The current status of emu industry is reviewed in Chapter 2. As a preliminary step towards investigating the current practices adopted during the transport of emus in Canada, a survey was conducted among the transporters, emu farmers, and slaughter house personnel. Analyses of their response helped me to assess the prevailing situation in the emu industry, identify problems (discussed in Chapter 2) and undertake further research to mitigate them. Information gathered from this study was utilized in designing a six hour transport study to determine the physiological response in adult male and female emus.

To evaluate the stress experienced by emus, it was decided to assess the changes in body weight; body temperature; metabolic, hematological, electrolyte variables; and behavioral mechanisms. It is essential to consider the basal levels of these physiological variables and their fluctuations over time (European Commission, 2002) to evaluate stress and interpret welfare. As information on the hematological, enzymatic and biochemical variables was limited, it was necessary to establish baseline values for adult emus in Canada (discussed in Chapter 3). The changes in these variables from pre- to post-transport and then after lairage indicated the impact of the stressors of transport on emus. The effect of a nutritional supplement (Nutricharge) administered pre- and post- transport in mitigating stress in emus was also assessed. The results from this study formed the basis for the two transport experiments conducted on emus (Chapter 4). The stress related changes in metabolism and injuries during transport could impair the post-mortem muscle metabolism and affect meat quality. Evaluating processing yields and meat quality (Chapter 5) will not only benefit emus, but also the producers and consumers.

Information on the behavior of domestic emus in a farm setting was sparse and hence a study was undertaken to determine their major behavior and time budgets (Chapter 6). This provided the basis for interpreting the behavior of emus during and after transport. The relationship between handling times, stress hormones, physiological variables and behavior was used to evaluate stress in emus. These behavioral changes can be used as non-invasive indicators of stress in emus (discussed in Chapter 7). The general discussion, conclusions and recommendations are presented in Chapter 8.

2. A SURVEY OF THE KNOWLEDGE, ATTITUDE, AND PRACTICES OF EMU INDUSTRY PERSONNEL, WITH A FOCUS ON THE WELFARE MEASURES ADOPTED DURING TRANSPORT

2.1 Introduction

The production of emus is a relatively young industry. In Australia, emus were recognized as an agricultural commodity in only 1987 (Sales, 2009), although their commercial production started in the 1970's (Krauss et al., 2002). The trends in emu industry from the 1990s show numerous ups and downs. During the period from 1990-95, it moved through an industrial life cycle pattern, characteristic of a "bubble or fad industry", showing an unpredictable expansion, quick maturation, followed by a rapid decline (Turvey and Sparling, 2002). After the collapse in 1996-97, the industry remained in low ebb (see below).

Commercial emu farming in North America (NA), started in the late 1980s (Gillespie et al., 1997; Thompson, 1997), and at the industry's peak, there were more than one million emus in the US (Dingle, 1997). Statistics show that there were more than 5,200 emu farms and 48,200 emus in the year 2001, which dropped to 3,600 and 28,300, respectively by 2007 (United States Department of Agriculture (USDA), Census of Agriculture, 2007). Current unofficial statistics report around one million emus in the US (USDA, 2011), indicating a considerable increase in their population in the country over the last six years (Todd, 2012). The American Emu Association (http://aea-emu.org/; 2012) is currently experiencing a revived interest in the production of this bird in the US.

The status of the emu industry in Canada has not shown any progression in population in the past decade (Statistics Canada, 2008). According to census data, there were approximately 58,875 emus and rheas (probably there were more emus than rheas) on 910 farms in Canada in 1996. This plummeted to 14,451 emus and rheas on 483 farms in 2001, which further declined to 4,000 birds on 182 farms by the year 2006 (Statistics Canada, 2008). These numbers are likely to have dropped further, as the 2011 Census of Agriculture in Canada registered a 10.3 % decline in the number of farms and 10.1% decline in the number of farm operators from those in 2006 (Statistics Canada, 2012). Current number of emus and emu farms in Canada is unknown.

Emu farming started in India in the late 1990s and there were more than 0.6 million emus in the year 2008, in as many as1000 farms with capacities ranging from 4 to 10,000 birds (Narahari et al., 2008). The industry in India is largely integrated, but there are independent emu producers, who own more than 1000 emus (Gujral, 2009). Unofficial estimates suggest that there are currently around 2 million emus in India. The industry in India slowed down by mid 2012, but efforts are ongoing to prevent a breakdown, similar to that in the 1990s (G. Srikanth, Emu Producers Association, India, personal communication).

As the industry starts to mature, animal welfare concerns become increasingly important. Consumers demand animal products that are produced in an ethical and humane manner. Currently there are no guidelines in place on the handling and transport of emus in India or Canada. No studies have been conducted among the emu industry personnel on this interesting and sensitive topic. It is also important to congregate the know-how within the industry, gained through practice; and propagate need based information to newcomers (Demiryurek et al., 2008).

The objective of the current study was to review production practices used in the emu industry in Canada, US and India, in order to identify potential welfare concerns and suggest methods to mitigate the same. The current practices in North America and India will be compared to those in the guidelines already prevalent in Australia (PIMC, 2006; AHA, 2012). This would help to fine tune the practices in the industry, and improve the welfare of emus. This information was collected by conducting a survey of emu industry personnel on their current practices associated with the management, handling, transport, and processing of emus. The following specific research objectives were identified and served as a basis for the development of the survey:

(1) How are emus prepared for shipping?

(2) What are the conditions under which emus are transported?

(3) How are emus handled and how do they respond?

(4) What problems have been encountered before, after and during transport and what are the possible solutions to these problems?

(5) Which areas in emu handling, transport and slaughter can be improved?

2.2 Materials and methods

The Behavioral Ethics Research Board, University of British Columbia, Vancouver provided approval for this project (permit # H10-0052).

2.2.1 Design of questionnaire

Knowledge, attitude and practice (KAP) surveys are used to assess the understanding, values and belief systems of farmers in order to examine agricultural practices that may be acceptable, or need to be modified, or perhaps discontinued (Adhikarya, 1994). The KAP survey developed (Appendix A) was administered to the respondents from emu industry for the documentation of local expertise, and to identify knowledge gaps, in order to suggest ways to resolve them.

2.2.2 Respondents and their recruitment

A purposive sampling procedure (Hardon et al., 2004) was adopted to collect responses from the identified group of experienced industry personnel in Canada, US (together hereby designated as North America; NA) and India. The sampling frame included emu producers, shippers, processors, and emu farm managers who were actively involved in the emu industry in the last five years; with not less than 6 emus. Multiple methods (online, e-mail and in person) of data collection were used to administer the questionnaire and random sampling was not possible. A new version of the questionnaire was developed to accommodate each of these methods of data collection.

Canadian and US respondents were targeted using in- person administered survey, mail survey, and online survey. The printed questionnaire with an introductory letter and consent form (Appendix B) was directly distributed to emu producers in Canada and the members of the American Emu Association (http://aea-emu.org/), attending their annual meeting. Questionnaires were also mailed out to respondents referred by participants ("snow balling" technique; Atkinson and Flint, 2001). In addition to this, e-mail invites and requests with a link to the questionnaire (http://www.surveypool.com) were sent out to personnel involved in the emu industry. All respondents contacted by e-mail were sent reminders. The first reminder was sent two weeks after the first e-mail, while a second reminder followed two weeks thereafter.

Respondents from India were targeted using online survey with invites provided on producer website. Links to the survey was posted in the Indian emu producers' website: "Emu Farmers in India" (http://www.linkedin.com/groups?home=&gid=2911302&trk=anet_ug_hm), and individual invites were also sent to them by e-mail. The respondents from India are only those members of the group, who had internet access. Validity of the respondents in NA was verified from their demographic details. The validity of respondents from India was verified through the emu producers' association.

Out of the 89 questionnaires sent out and distributed in NA, 41 responses were received back. Eight respondents were excluded from analysis, because they had either never reared emus in the last five years or had only one or two emus (kept as pets). The e-survey, sent out to 52 respondents was answered by 16, but only 15 of them were usable. Therefore, this study reports the results obtained from 48 respondents, 35 from NA and 13 from India (Table 2.1). The surveys were followed up with in-depth interviews with eight of the respondents who showed willingness for the same (Appendix C).

The purposive sampling technique with multiple methods of administration was effective in eliciting a good response rate from this unique group. A sample size of 35 emu industry personnel from NA, where the population size is estimated to be well below hundred seemed to be a reasonable representation. Size of the emu industry in India is unknown, and I could contact only those members who had internet access. For this reason, the sample size is small and not representative of the group in India.

Table 2.1. Methods of administration of survey to respondents.

Method of survey administration	North America	India
Access to e-link	2	13
Snowballing	2	
E-mail	15	
Direct administration at meetings	16	
Total	35	13

2.2.3 Data analysis

As there were very few female participants (eight) and most of them were involved in the emu business as a family, sex of the producer was not considered in the analysis. The data from the questionnaire was tabulated, and descriptive statistics were calculated using Statistica (Statsoft10 Inc.). Data on general management practices were pooled from all respondents, regardless of country of origin, while data on farm categories and welfare practices were described on a regional basis i.e. separately for NA (Canada and US) and India. Information from open ended questions were summarized and presented.

2.3 Results and discussion

A summary of the responses of emu producers and other associated industry personnel, who participated in the survey, their attitudes and awareness on emu husbandry, welfare aspects, is presented. Only completed questions were included in the descriptive statistics. As shown in Table 2.1, about 50 % of the surveys were collected from group meetings and it is likely that the discussions within the group could have affected their responses.

2.3.1 Socio-demographic characteristics

Out of the 48 respondents, 83 % were producers and shippers or processors. The rest included a shipper, two farm managers, one emu-slaughter plant worker, and three people who market emu products. The socio-demographic characteristics of respondents from NA and India are presented in Table 2.2. Most of respondents from NA were above 50 years of age (average 56 ± 2 years), self-employed and involved in emu rearing on part-time basis only. The participants from India were younger (average 36 ± 2 years old) and involved in the industry either part-time or on full-time basis. While 14 % of the respondents had advanced education (masters/ doctoral), 35 % were university graduates, indicating that the respondents were highly educated.

Table 2.2. Socio-demographic characteristics of the respondents.

Variable	Categories	North America (n = 35)	India (n = 13)
Age groups	25-44	5	11
	45-59	17	2
	60-80	13	0
Family size	1-2	14	4
	3-5	17	7
	Above 6	4	0
Number of family members involved in emu industry	1-2	28	12
	3-5	6	1
Job type	Primary	3	5
	Part-time	18	4
	Others	5	1
Employment status	Employed for wages	5	2
	Self-employed	13	6
	Professional	0	4
	Other	1	0
Business type	Sole proprietorship	8	10
	Family business	14	3
	Others	4	0
Education	High school graduate	4	2
	College	16	7
	University graduates	10	4

2.3.2 Emu husbandry practices

2.3.2.1 Size of operation

Sixty-four percent of the respondents owned fewer than 50 emus (Figure 2.1), out of which, 33 % owned fewer than 20 emus. Only 15 % had 50-100 emus, and the rest owned more than 100 emus. A country-wise split up (Figure 2.1) indicated that farms of all sizes exist in the US, while there was only one large farm in Canada. While all the respondents had breeders, 82 % of them had birds ready for markets and 71 % had juveniles. Region-wise distribution of flock types and stock sizes depicted in Table 2.3 showed that with an average Indian producer, there was comparatively higher number of emus in each category.

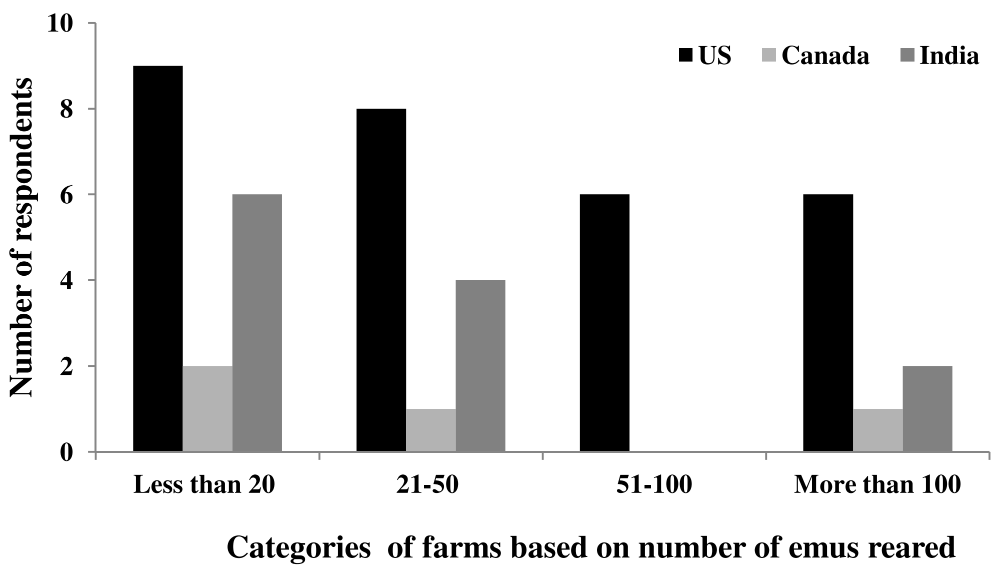

Figure 2.1. Categories of emu farms based on size of operation (number of emus reared).

The benchmark annual production criterion for optimum performance in the emu industry has been suggested to be 40 slaughter birds per breeder pair (Gillespie et al., 1997). Yet, most of the operators surveyed in this study were functioning well below this ratio, possibly because of the uncertainties in this industry. Furthermore, majority of the respondents stated that their size of operation is smaller than what would have been ideal.

Table 2.3. The mean stock size maintained by emu producers from North America and India.

Type of emus	North America			India		
	n	Mean ± S.E	Range	n	Mean ± S.E	Range
Breeders	30	30 ± 11	2-300	12	105 ± 32	4-400
Marketable birds[1]	23	114 ± 63	0-1400	10	256 ± 143	3-1200
Juveniles[2]	23	94 ± 61	0-1400	8	352 ± 229	6-1900

[1]Ready for slaughter.
[2]Less than 6 months of age.

2.3.2.2 Experience

Many respondents (50 %), especially from NA had more than 10 years experience in the emu industry (Figure 2.2), while 25 % had less than 2 years' experience (mostly from India). The expertise that comes from experience is expected to contribute positively towards their knowhow on emu management.

2.3.2.3 Breeding

Emus are considered to be predominantly monogamous and stable pair formation is common during breeding season (Blache et al., 2000). Eighty percent of the respondents found that the best male: female ratio for breeding emus is 1:1, while 15 % said that it could be 1:2. Having equal number of males and females is believed to reduce aggression during breeding season considerably. Few respondents (5 %) favored colony breeding, which has been suggested to reduce production costs considerably (Gillespie et al., 1997). Most respondents (81 %) had hatched emu eggs, typically using artificial incubation (76 %). Some respondents relied on either natural incubation or hatching (8 %), or used both natural and artificial methods to hatch emu eggs (8 %). Forty-six percent of the respondents had purchased emu chicks younger than 3 months' age, while 32 % had purchased yearlings and 27 % had purchased older emus (> 1 year of age). This indicated that the "breeders' market trend", considered detrimental to the industry (Turvey and Sparling, 2002), is prevalent even today.

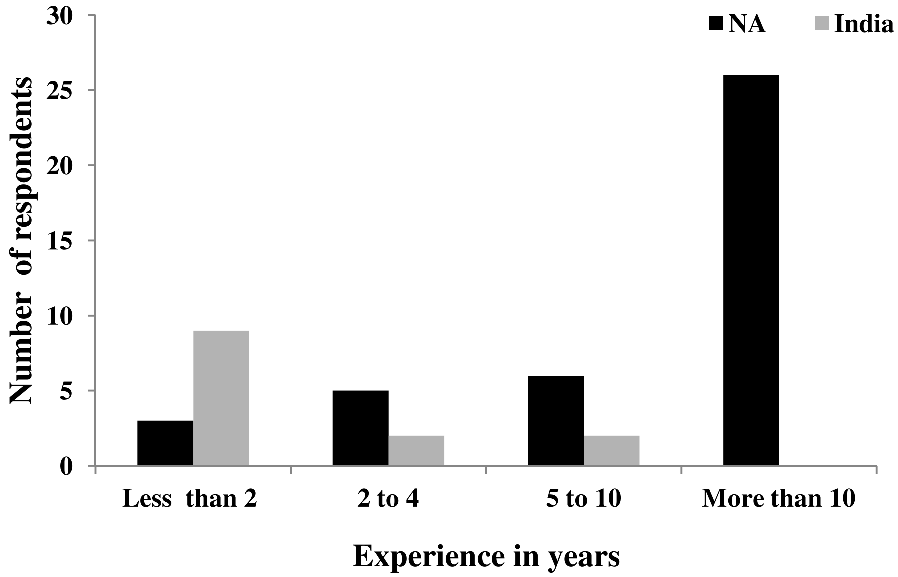

Figure 2.2. Region-wise categorization of respondents based on their experience.

2.3.2.4 Provision for feed and water

Most respondents manually add the feed to feeding troughs, and only 6 % used automated feeding systems. Most producers (65 %) relied on commercial pelleted feed, while others mixed their own feed. This shift to on-farm feed preparation by some producers is expected to help minimize feed costs (Scott et al., 2009), but may also be due to the non-availability of commercial feed, specific for emus.

Findings from this study indicated that most of the emu producers in NA either provided feed *ad libitum* or fed their birds once a day (Figure 2.3). Majority of the respondents from India provided feed on an *ad libitum* basis. Water was provided *ad libitum* to the emus in many farms (53 %), although there were producers who gave water just once (40 %) or twice (6 %) daily. It is extremely important that emus have access to good quality feed and clean water at all times (Department of Primary Industries and Water (DPIW), 2008), to ensure their well-being. Adequate back-up systems should be ensured in case, there is a breakdown (Harding and Rivers, 2008). In addition to this, feeders and waterers should be checked at least once daily.

2.3.2.5 Systems of rearing

Emus are kept in farms under conditions varying from close confinement to extensive rearing (Thompson, 1997; DPIW, 2008). In this study, approximately 40 % of the respondents raised emus in free range system (kept outdoors in a fenced-off pasture), while 37 % reared emus under semi-intensive system (kept under a shelter at night with limited access to pasture during day). Five of the farms were certified organic, whereas two of them were organic producers, though not certified. It is interesting to note that emu producers in NA have switched to an organic production system, recognizing the changing trends in farming (Berry, 2011) and to make use of the new opportunity.

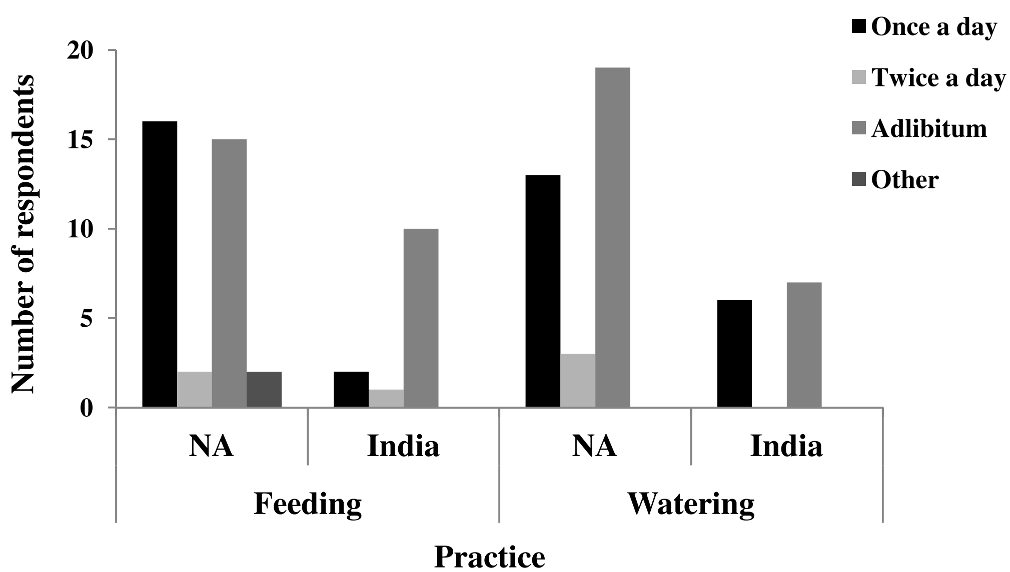

Figure 2.3. Frequency of providing feed and water to emus.

2.3.3 Transport of emus

2.3.3.1 Preparation for transport

The Australian animal welfare guidelines for the transport of livestock (AHA, 2012) stipulate that adult emus need to be fed till 24 hours before transport, while chicks must be fed every 12 hours. The maximum permissible period of water withdrawal for adults and chicks is 36 and 24 hours, respectively (AHA, 2012). The code of practice for poultry transport in Canada also states that water should not be withdrawn till loading (Canadian Agri-Food Research

Council (CARC), 2001). Feed and water withdrawal is practiced to avoid slippery floors during transport and to minimize contamination of the slaughter floor from gut contents. However, in this study, majority of the respondents did not withdraw either water (83 %) or feed (77 %) prior to transport. Only 10 % of the respondents provided fodder to emus in the transporting truck, though 80 % didn't. Approximately 60 % of the respondents did not provide feed to emus after reaching the processing plant, however approximately 25 % did provide fodder.

Respondents indicated that generally, emus do not eat or drink while being shipped and would continue to stay off-feed for a couple of days after transport. Therefore, it is necessary to provide water till loading and again after unloading, so that the wellbeing of emus is not affected. Providing feed and water till the start of transport has a settling effect on the animal (Chambers and Grandin, 2001) and is currently practiced in many species including ratites (A. L. Schaefer, Agriculture and Agri-Food Canada, Lacombe, Alberta, Canada, personal communication). This would imply that the respondents are quite knowledgeable about the recent changes in practices, which would help improve the welfare of food animals.

2.3.3.2 Handling and loading

More than half of the respondents said that emus rarely attacked handlers, while 41 % said they never attacked. Another 43 % of the respondents admitted that emus might attack each other while in the truck and the same percent of the respondents had observed such incidents while loading too. Emus resist being caught by kicking, running, jumping and have a difficult temperament. Emus usually don't attack people, but they can turn quickly or jump over; knocking down the handlers, especially when they panic. Therefore handling of emus is not easy (Bewg and Kent, 2008). Only 20 % of the respondents had heard emus vocalize while being handled, while 60 % had not heard this. Respondents have also found few emus vocalize during loading and also in the early part of the transport.

The different events noticed by the respondents during handling and transport of emus are presented in Figure 2.4. Emus are very difficult to be driven in a set direction and for this the use of mobile barriers in laneways is found helpful (Bewg and Kent, 2008). Although majority of the respondents (85 %) had experience in loading emus into vehicles, most found this procedure to be 'fairly to very difficult' (Figure 2.5). Though 84 % of the respondents stated that emus resisted being rounded up, the majority (85 %) of them did not use any tools or equipments, including hoods or collars to control emus during this procedure. Hooding to calm down does not work well with emus and unlike the case in ostriches, made them apprehensive (Crowther, 2002). They also observed that emus can be loaded easily at night, and that day time handling is very stressful, particularly under warm weather conditions (Bewg and Kent, 2008). Respondents were concerned about the fact that emus stressed by long chases, especially under warm weather conditions, collapsed and died.

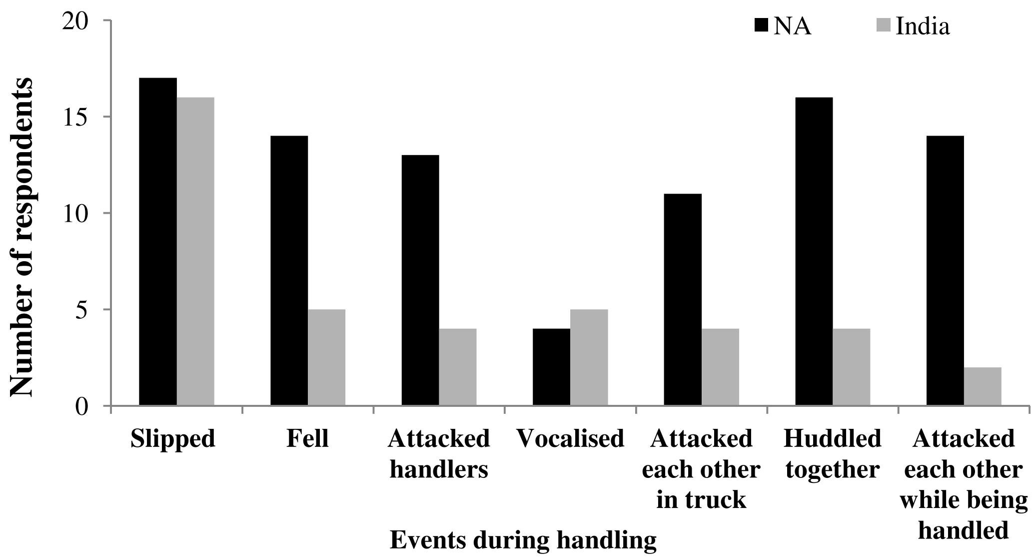

Figure 2.4. Events reported by respondents during handling and loading of emus.

Ramps were used by some handlers, but others lift and place emus into the truck. A non-slip ramp kept at 25° angle or less, as recommended for other livestock species may be used to load emus (Grandin, 2010b; AHA, 2012). Emus need to be trained beforehand for herding, use of yards and ramps to avoid stress during loading (PIMC, 2006). More than three-fourths of the respondents agreed that emus slipped while being loaded, whereas two-third agreed that few emus fell down too. These are areas of concern, as there is the likelihood of emus getting injured. Most injuries to ratites are related to activities associated with their handling and transport (Glatz and Miao, 2008). Injuries can be minimized by catching emus carefully and this is even more important as injured birds are extremely susceptible to transportation stress (CARC, 2001).

Transporters also need to be aware of the methods of emergency slaughter of emus mentioned in the "livestock transport emergency guide" (OHTG, 2007).

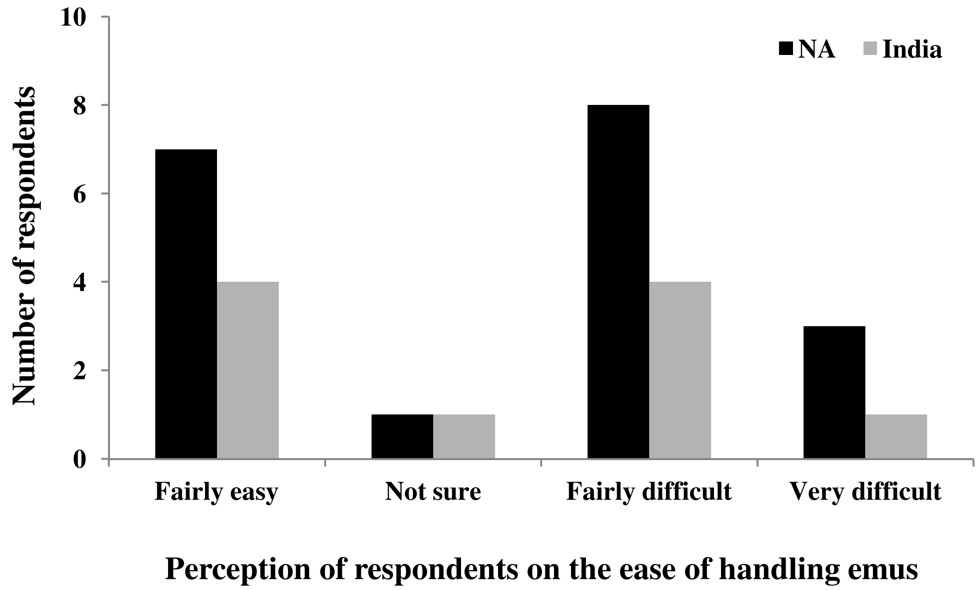

Figure 2.5. Ease of loading emus into vehicles as perceived by the respondents.

2.3.3.3 Precautions during handling

Precautions taken by the respondents during the handling of emus included being cautious at all times and using a flag to herd. While one person catches the emu, another person should be in charge of the door to the compartment, guiding them in. Therefore, at least three people may be needed to handle and load emus efficiently. As emus struggle quite strongly when first caught, holding them longer can be hard work (Bewg and Kent, 2008). A respondent observed that "*staying calm or backing off if the emus are very excited; knowing when to grab and when to let go comes only from experience*". This highlights the fact that only experienced people should handle emus or else, handlers need to be trained. They should wear protective clothing such as heavy boots, chaps, and gloves. Handlers need to follow instructions and be reminded of probable dangers at each step. Respondents were of the opinion that declawing emus would help prevent injuries from sharp claws. However, this is not without welfare concern, as improperly done declawing would result in severe pain, even affecting locomotion (Glatz, 2005). Declawing should be done with the administration of anaesthetics, proper analgesic precautions; and before the emus are five days of age (PIMC, 2006).

When respondents were asked in regard to the handling of emus, they indicated that emus should be handled as gently as possible because of their panicky nature. Emus are to be carefully caught from behind and slowly walked by holding at the base of the wings. Usually, calmer birds are loaded first, while trouble makers are loaded in the end. Emus are very inquisitive birds and might try to poke their head out into spaces if any. Hence, it is important to ensure that there are no gaps or crevices in the compartment, where their head or neck might get caught. There should be no projections or sharp surfaces inside the compartment, as they might cause bruises (CARC, 2001; Chambers and Grandin, 2001). Having spring loaded doors on the trucks would make loading easier. Air suspension (spring) trucks with minimal vibration are considered superior for the transport of emus (PIMC, 2006; Glatz and Miao, 2008).

2.3.3.4 Vehicles for transport

Two-third of the respondents shipped emus for slaughter in their own vehicles. The distances travelled varied from 6 to 965 km. In NA, emus were shipped a mean distance of 115 ± 50 km (median distance of 56 km), while in India, emus were shipped 269 ± 110 km (median distance of 125 km). In Canada and US, livestock are typically shipped 1,200 to more than 1,600 km before slaughter (Greger, 2007; Gonzalez et al., 2012). Trailers are the most common type of vehicle used by the respondents to ship emus (Figure 2.6).

2.3.3.4.1 Conditions inside the vehicle compartment

It is recommended that the vehicle for transporting emus have compartments with partitions, and be at least 1.6 m tall, to provide the recommended 20 cm space above the level of the back of the tallest emu, while standing erect (PIMC, 2006; DPIW, 2008; AHA, 2012). Most respondents (70 %) used trucks with partitioned compartments. The height of the truck used by one-third of the respondents was only as tall as the emu. It is important that vehicles with tall compartments are used, to ensure the well-being of emus. Australian guidelines on emu transport stipulate that vehicles be fully enclosed and the compartments completely darkened during shipping (Bewg and Kent, 2008; DPIW, 2008). Given that ratites are calmer when there is no bright light and ostriches were found to have lower heart rate in dark compartments with sufficient space to sit down (Crowther et al., 2003), it was interesting to note that few of the respondents (12 %) transported emus in darkened compartments. Respondents preferred to transport ratites at night as darker compartments reduced panic responses and made them sit down and rest (Crowther, 2002; Black and Glatz, 2011). All the respondents in this study preferred to transport emus either at night or early morning, as emus would not settle down during day time.

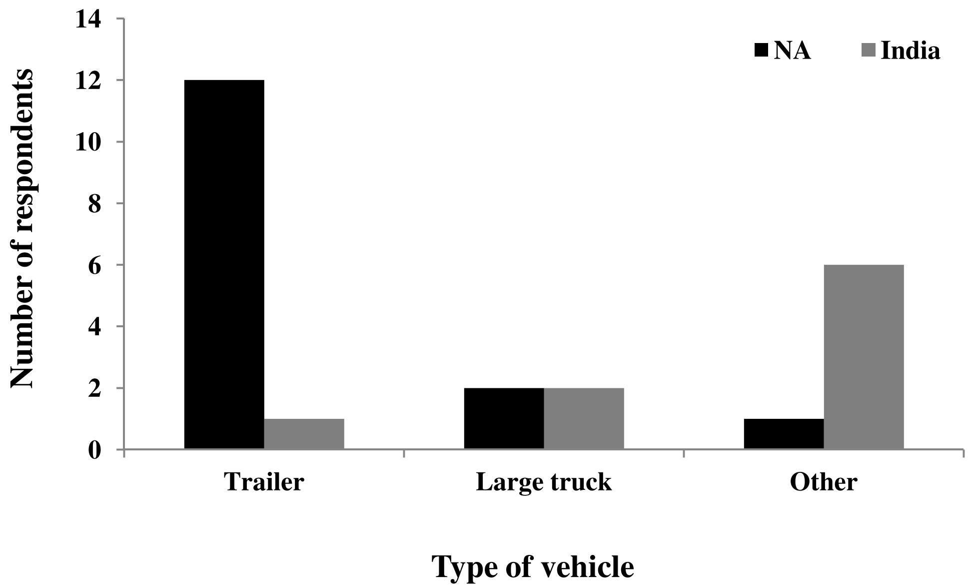

Figure 2.6. Type of vehicle used by respondents for transporting emus.

Most respondents used fairly to well-ventilated trucks for transport. Open trucks should never be used for emus (PIMC, 2006), though even under extremely cold weather, minimum level of ventilation is considered necessary for animal transport (Grandin, 2010a). Under warm weather conditions, ventilation scoops or fans in the roof would help reduce the build-up of heat (MacNamara et al., 2003). Shippers opined that emus may be kept in partitioned compartments inside the vehicle, which are devoid of any unfamiliar structures. Strange objects even in the pathway would frighten them, leading to bolting, trampling, hurting themselves or even death. Once loaded, there should be no further delay for the start of the journey. Rest stops except for the purpose of inspection should be avoided, as that would upset the emus (AHA, 2012).

The use of bruise-free material for padding in larger vehicles and providing sufficient space inside transport crates can reduce stress levels (Glatz and Miao, 2008). Two-thirds of the respondents provided some bedding on the floor of compartments of trucks/ trailers used to transport emus. Among the materials used as bedding, straw and hay were most popular (Figure 2.7). The bedding material should provide a firm footing and absorb moisture from droppings (Glatz and Miao, 2008; Harding and Rivers, 2008). Emus are bipedal and find it more difficult to balance themselves than other livestock, when the vehicle is in motion. Provision of non-slip, textured footing surfaces suitable to the species is essential, and non-compliance will be considered violation of the Health of Animals Regulations, Government of Canada, 2012 (CFIA, 2013c). Changes in thickness of bedding may be necessary under extremes of air temperature (Grandin, 2010a). Further research is needed to identify the appropriate material and thickness of bedding to be provided for emus during transport under varying weather conditions.

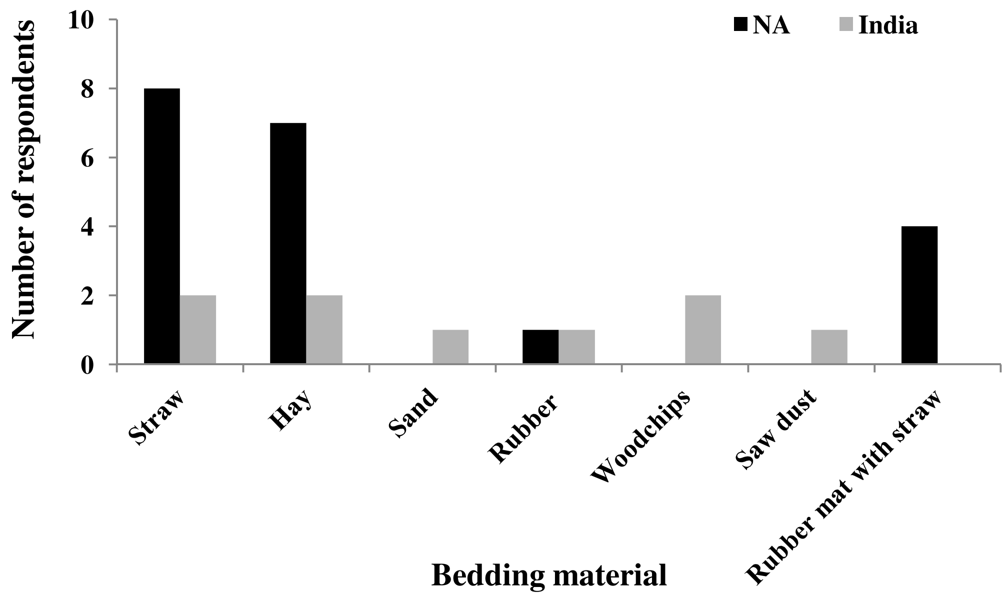

Figure 2.7. Preference of respondents for materials to be used as bedding in the truck compartments.

Most of the respondents preferred to transport emus in small batches (<20) as they found this more manageable. Respondents would ship up to 40 emus in a single load. The space recommended for an adult emus is 0.41/ m^2 and those for emus less than a year old is 0.38/ m^2

(Department of Local Government and Regional Development (DLGRD), 2003), which should be increased when the emus are heavier. A stocking density of 2 adult emus/ m^2 was followed by the respondents in general, which is as per the recommendations in the Australian guidelines (PIMC, 2006; DPIW, 2008). However, it is doubtful whether this spacing would be appropriate for adult emus above 50 kg in weight. It is necessary that the stocking density be modified, based on the size of the emu (AHA, 2012).

Approximately half of the respondents have driven the truck used to ship their emus. In Canada, the driver of the vehicle and all transporters are responsible for the care and safety of the birds transported (CARC, 2001). Maintaining a constant speed and avoiding unnecessary breaking would help to reduce injuries during transport (Cockram et al., 2004). Only 15 % of the respondents imposed speed restrictions on the truck drivers. All the respondents shipped male and female emus together, and most do not mix unfamiliar emus. Fighting is common in large groups of emus during transport, particularly when unfamiliar birds are shipped together (Glatz and Miao, 2008) and therefore should be avoided. When ordinary trucks rather than environmentally controlled ones are used, the time of transport should be adjusted according to weather conditions.

2.3.3.5 Problems encountered during transport of emus

When asked about the problems encountered during transport, respondents stressed that every precaution should be taken to avoid injuries to both emus and their handlers. One major processor from US said that both too low and too high space allocation could lead to injuries. Too little space would lead to emus climbing up on each other, leading to trampling and injuries. Another producer also mentioned a case where the emus legs were taped together to prevent climbing. Such practices would compromise the welfare of emus, and should be avoided. When few emus are shipped in large containers, they have more space to move around. Continuous walking inside the trucks could make them exhausted and also increase the chances of falling down and getting injured.

Emus tend to lose lot of feathers when handled, and one producer said that it is unlikely that emus transported for long distances in a group, arrive at the destination without feather loss. This is especially true for birds not declawed. Unless one is careful while unloading, there is the risk of emus jumping fences and escaping. So also, when there is too much light at the processing plant, they are difficult to be walked into the pens from the truck and they resist entering the facility. Water should be made available upon arrival at the destination, even if they might refuse to drink.

2.3.3.5.1 Body weight loss

Just 38 % of the respondents said that they had noticed reduction in the body weight (shrinkage) of the emu's consequent to transport, whereas 14 % hadn't. Half of the respondents were not aware of such losses. When one-fourth of the respondents opined that the losses could be less than 3 %, while 13 % said that it could be 4-6 %. Only one person felt that it could go up to 9 %. Reduction in body weight is dependent on the distance of transport, yet 56 % respondents were unable to assess the extent of such losses. Few of the respondents were aware of the fact that stress would amplify loss of body weight (Figure 2.8). This indicated the need to educate emu producers about the impact of loss of body weight on the welfare of animals transported; meat quality, and associated economic losses.

2.3.4 Suggestions to improve the welfare of emus during transport

The suggestions from respondents for improving emu transport included the following:

- It is easy to handle and load emus when they are confined in a small area of the pen.
- Only birds with clipped claws (toe nails) should be transported.
- Transport should be done in small groups and for short distances only.
- Thick bedding should be provided on the floor of the compartment.
- Ability to monitor events inside the truck is important to ensure welfare of emus.
- Water should be provided during long distance (more than 12 hours) transport.
- Modern livestock transporters (air spring suspension trucks) are needed.
- Better designed chutes and ramps, transporting trailers (skid proof) are needed.
- Appropriate stocking density should be followed, which is neither too high nor too low.
- Transporters and producers should be educated on the best ways to transport emus.
- Easier catching, loading and unloading techniques are required to reduce handling.

This list indicated that the experienced respondents were quite knowledgeable about the requirements for humane transport of emus.

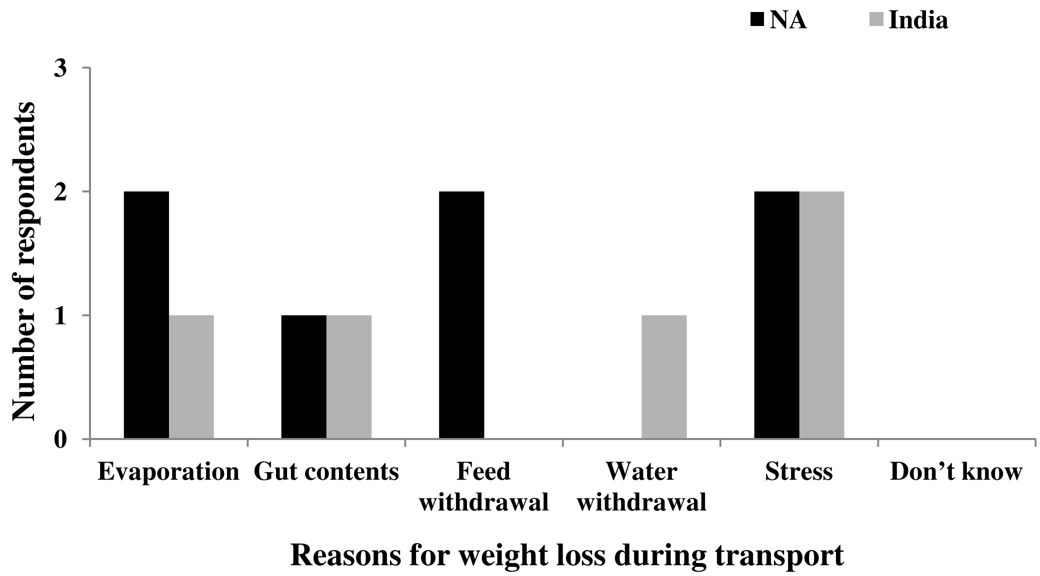

Figure 2.8. Reasons for body weight loss after transport as expressed by respondents.

2.3.5 Processing of emus

A major problem faced by emu producers is the difficulty in accessing processing facilities. This has been identified as a major factor curbing commercial farming of many game and specialty species (Scott et al., 2009). Approximately half of the respondents indicated that they slaughter and process emus on their own farm. The other half sends their birds to an abattoir for processing, either hiring shippers (27 %) or transporting emus by themselves (24 %). Almost all respondents expressed interest in using a mobile slaughter unit but they also indicated that this option was currently unavailable to them (89 %), and some worried about the cost of this service (4 %). The advantage of this facility is that the meat from such slaughter would be provincially inspected (Galbraith, 2011). Other reasons for using this facility included the absence of transportation stress, humane slaughter and cost savings on transport (Figure 2.9). An inspected mobile slaughter unit can therefore improve ante-mortem animal welfare considerably, but refrigerated storage of meat will certainly be a challenge (Galbraith, 2011). Two respondents were not interested in using a mobile slaughter unit, as they either had their own processing facility, or had access to a slaughter plant less than 10 km from their farm.

Respondents differed in what they considered the ideal age for slaughtering emus. While half of the respondents thought that less than two years of age was best, 31 % felt that it should be after the emus had completed at least one breeding season (i.e. between 2-3 years of age). Another 16 % considered that it should be between 3-5 years of age. Opinions varied depending on whether emus are slaughtered to supply the market for fresh meat (younger birds) or for their fat to supply the oil market (older birds). For a majority of the respondents (69 %), financial reasons (such as maximum fat yield, reduction of feed cost, maintaining the stock size) were the driving factors behind the decision to slaughter. Availability of slaughter plant, helpers, demand for meat, and the need for space to accommodate the new hatches were also important factors. Many respondents slaughtered emus in the fall (49 %) or in the summer (17 %), while 26 % slaughtered emus throughout the year. The fact that more slaughters were done in October would mean that emus are being transported during a period where they have maximal fat deposits.

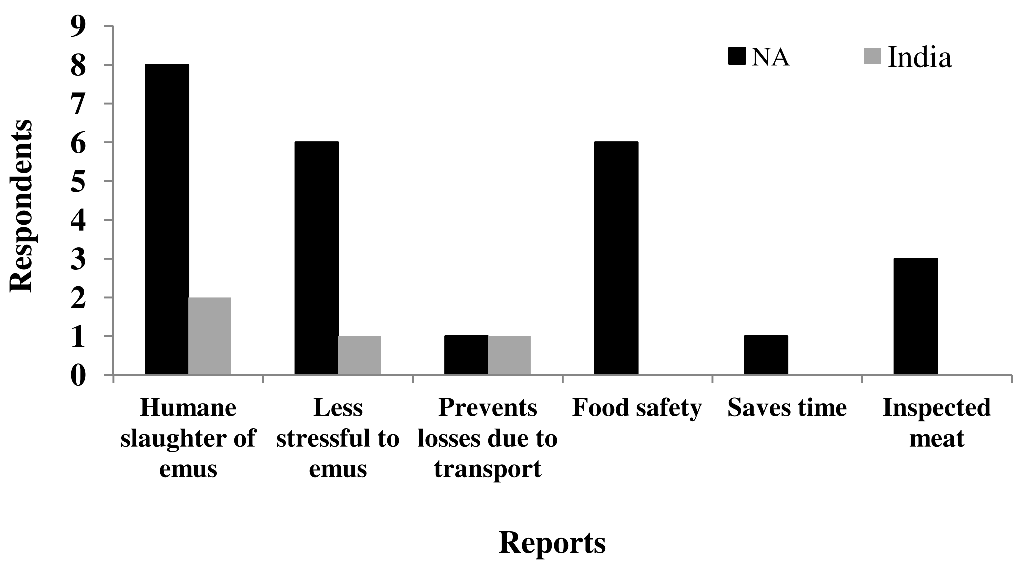

Figure 2.9. Reasons prompting respondents to use mobile slaughter units.

2.3.6 Product handling

About half of the respondents sold their products directly to consumers (Figure 2.10), whereas, 18 % sold through intermediaries. Emu fat was processed and sold as oil by 85 % of the respondents, while 55 % sold it as fat. While three-fourth of the respondents kept emu meat for personal use, 21 % sold it as pet food. The rest were sold from farm gate and also processed into products such as salami, pepperoni and jerky. Only 6 % of the respondents either tanned the hide or sold it to tanneries; most (61 %) disposed or composted it. Only 17 % of the respondents were using emu feathers, the rest either disposed or composted it. These findings indicate the lack of demand for feathers and hide. The market for emu products is sporadic and limited (Gillespie et al., 1997) and this is one of the reasons behind the poor performance of this industry. Steps are needed to create awareness on emu products and thus improve their marketability.

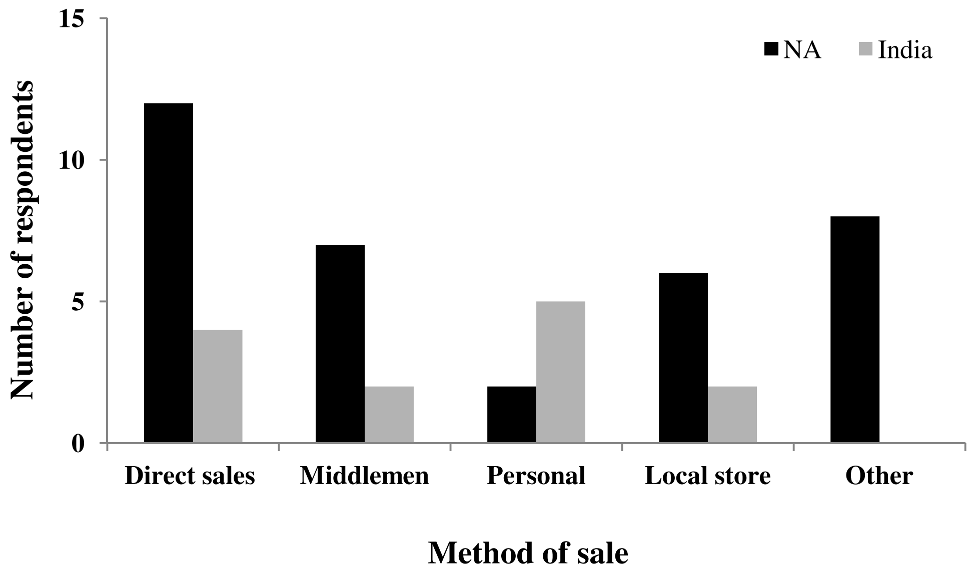

Figure 2.10. Methods of sales and utilization of emu products.

2.3.7 Perception of respondents on status of emu industry

When asked about their level of satisfaction regarding emu transport procedures, 41 % of the respondents were contented, 33 % were unsatisfied and 26 % were unsure of their response. The perception of respondents (region-wise) towards the status and prospects of the emu industry is provided in Table 2.4. Just one-third of the respondents were satisfied about the status of the industry in their own country, while there were 45 % who were satisfied about the status of the industry, globally. More than half of the respondents in both regions were not satisfied about the future prospects of the industry both in their country and globally. A good majority of the respondents (75 %) were unaware of any current research on emus undertaken in their country with government support. Yet, they (93 %) were optimistic that such efforts would help strengthen the emu industry.

Table 2.4. Perception of respondents (number) towards future prospects of emu industry.

Statement	Region	Reasonably good	Satisfactory	Unsure	Moderately unsatisfactory	Poor
How would you describe the current status of the emu industry in your country?	NA	4	8	5	11	4
	India	1	2	0	7	3
How would you describe the current status of the emu industry globally?	NA	4	8	11	8	-
	India	1	6	-	5	-
What do you feel are the future prospects for the emu industry in your country?	NA	6	7	1	8	10
	India	-	1	1	1	3
What do you feel are the future prospects of the emu industry globally?	NA	6	5	9	5	6
	India	-	2	1	3	7

2.4 Conclusions

This study gathered information on the management practices in emu industry, especially those associated with the transport, slaughter, and processing of emus. The results are mostly based on the findings from the emu industry personnel in North America. Due to the non-availability of processing facilities half of the respondents slaughtered emus in their own farms. Approximately half of the respondents transported their emus to processing plants. Most of them did not withdraw feed or water before transport. Emus are frequently transported for slaughter during fall, which is a period when their fat deposits are maximal. The current transport conditions are such that the welfare of emus is likely to be compromised. Respondents were concerned about the well-being of emus during handling and transport, as stressed emus would collapse and die. Emus are panicky in nature and difficult to manage.

Most of the respondents believed that the conditions of transport need to be improved further. The specific needs of the industry that could be identified include better chutes and ramps, well designed trucks for transport, trained handlers and processors, mobile slaughter houses and nearer slaughter facilities. The overall outlook of the respondents on the future of this industry is not very encouraging. However, respondents were optimistic that scientific research would help improve the current status of the emu industry.

3. HEMATOLOGICAL AND SERUM BIOCHEMICAL PROFILE OF FARM EMUS AT THE ONSET OF THEIR BREEDING SEASON[1]

3.1 Introduction

In North America, emus are transported for slaughter mostly at the beginning of their breeding season (fall months), when they have maximal fat depots. At this time of the year, the physiological status of emus could be altered as emus reduce their metabolic rate and go off feed and water until incubation is completed (Kent and Bewg, 2010). To assess stress, it is a usual practice to establish baseline values and then evaluate the changes over time in the variables that are considered to be indices of stress and metabolic homeostasis (hematology, serum biochemistry, enzymes, and body temperature and weight). There is very little information available on the hematology and serum biochemistry values of adult emus, especially during the pre-breeding and breeding season. Previous studies have either dealt with younger emus, involving comparatively smaller sample sizes and not considered the effect of sex on these variables.

The objective of this study was therefore to establish baseline values and reference intervals for selected variables (see Section 1.4.8), which are stress experienced by emus during likely to be affected by transport. Another objective of the current study was to see how emus prepare for the demands of the breeding season with respect to the selected blood variables. This can be achieved by comparing these results with published data on avian species, in which only females incubate the eggs and brood the young, and with species in which both parents share the responsibility. Clinical signs of diseases in poultry and ratites may be very subtle and confusing (Black and Glatz, 2011). Hence for more accurate diagnosis, it is important to establish standard values for the various blood variables and interpret them according to the age, sex, physiological states and stress levels (Fudge, 2003). Blood profiling is also used to detect subclinical, clinical, metabolic conditions, incorrect feeding practices and also the welfare of animals.

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[1]A version of Chapter 3 was previously published as Menon, D. G., D. C. Bennett, A. L. Schaefer, and K. M. Cheng. 2013. Hematological and serum biochemical profile of farm emus *(Dromaius novaehollandiae)* at the onset of their breeding season. Poult. Sci. 92:935–944.

Table 3.1. Comparison of the hematological and serum biochemical values reported in emus.

		Source							
	Units	Okotie-Eboh et al. (1992)	Costa et al. (1993)	Andreasen et al. (1997)	AAZV-ISIS (1996)	Fudge (2003)	Kumar et al. (2009)		This study[1]
Location		Texas, USA	Western Australia, Australia	Oregon, USA	Emus in Zoos, USA	California Avian Lab, USA	Hyderabad, India		Saskatchewan Canada
Sample size		33 (18♀, 15♂)[2]	10	10	26 from 8 institutions	not given	6	6	46 (19♀, 27♂)[2]
Age		1-4 years	12-18 months	adults	1-4 years	not given	4 years		4-6 years
Sex		mixed	not given	not given	not given	not given	♀[2]	♂[2]	mixed
WBC	x 10^9/L				15.2	14.9	14.1	18.1	12.2
Heterophils	%				71	78.8	32	30	62
Lymphocytes	%				22	20	66	67	32
Monocytes	%				4	0.1	1.0	1.0	4.4
Basophils	%				2.1	0.2	3.0	1.6	0.5
Eosinophils	%				1.0	1.1	1.8	1.8	1.1
PCV	%				42	47	43	46	51

	Units	Source							
		Okotie-Eboh et al. (1992)	Costa et al. (1993)	Andreasen et al. (1997)	AAZV-ISIS (1996)	Fudge (2003)	Kumar et al. (2009)		This study[1]
Creatinine	µMol/L	17.7			27	21	16.8	20.3	12
Glucose	mMol/L	8.7	15.9	9.9	9.9	11.8			9.5
Total protein	g/L	42	45	40	40	39	47	40	50
Albumin (A)	g/L	25	22	22	22	17	32	29	28
Globulin (G)	g/L	17	17	18	18	22	15	11	23
A/G ratio		1.5	1.0	1.2	1.2	0.8	2.1	2.6	1.2
Total cholesterol	mMol/L	9.2	2.7		8.8	3.2	1.7	2.2	4.8
Triglycerides	mMol/L	3.7			1.1		11.4	2.8	4.3
Uric acid	µMol/L	280	632	488	321	375			167
Sodium	mMol/L				146				146
Potassium	mMol/L				3.6				4.1
Chloride	mMol/L				106				105
Bicarbonates	mMol/L								24

	Units	Source							
		Okotie-Eboh et al. (1992)	Costa et al. (1993)	Andreasen et al. (1997)	AAZV-ISIS (1996)	Fudge (2003)	Kumar et al. (2009)		This study[1]
Calcium	mMol/L		2.9	2.9	2.5	2.3	3.2	3.1	4.0
Phosphorus	mMol/L		1.8	1.8	1.9	1.8			2.1
Urea	mMol/L	0.3-1.5							0.8
ALT	IU/L	16			14		0	0	17
AST	IU/L	104	151	292	221	227	16	38	179
CK	IU/L	264	223		675	306			314
GGT	IU/L	4		3	9				4.9
Amylase	IU/L								446

[1]Separate values for each sex are provided in subsequent tables.

[2]♂- Males; ♀- Females.

Most previously reported studies are from small sample sizes and the mean values reported show wide variation, while the reference intervals were not reported (Table 3.1).

Reference interval (RI) is the "interval between the lower and upper limit of the analyte for 95% of the population" (Clinical and Laboratory Standards Institute, 2012). RIs reflect variations more accurately than reference ranges and are more appropriate for diagnostic purposes (Hochleithner, 1994). The objective of the present study was to provide the baseline values and RIs, following the international recommendations (Geffre et al., 2011), for the hematological, metabolic and enzymatic components in adult emus at the onset of their breeding season.

3.2 Materials and methods

3.2.1 Ethical approval

This study was carried out with the approval of the University of British Columbia Animal Care Committee, under permits # A10-0183 and # A10-0184.

3.2.2 Experimental birds

Blood samples were collected from 46 adult birds (four to six years old) kept in colonies in an emu farm located at Saskatchewan, Canada, over a two-year period in the fall season (November). The emus were raised under semi-intensive system with a night shelter and free access to outdoor space. They were fed *ad libitum* a balanced formulated diet, containing barley, alfalfa and canola. They had *ad libitum* access to drinking water and the natural forages in the outdoor pen.

3.2.3 Health status

All the emus were checked for their general health including the conditions of head, eyes, nostrils, beak, neck, legs, wings, and the body. The level of alertness, mobility and abnormalities in gait, if any, were noted and findings from apparently healthy emus alone are reported. The sexes were determined by cloacal palpation (Samour et al., 1984). The body weights (Western SK-130-300 Vet Scale, Excell Precision Co, Ltd, Taiwan) and cloacal temperatures (Digital thermometer; Medline Industries, Mundelein, Illinois, US) were also recorded.

3.2.4 Blood collection and processing

Emus were restrained using procedures recommended by Minnaar and Minnaar (1997). The handler would calmly approach the emu, catch it from the side or behind, and hold the bird by the base of both wings using both hands. Emus may struggle initially, but would quickly settle down and were then guided by the handler to the sampling area. The jugular vein on right side is the preferred site for blood sampling in emus (Stewart, 1984; Fowler, 1991), especially on the right side. All the samples were collected in less than three minutes of restraint and in the morning hours.

From each emu, 5 mL of blood was collected into lithium heparin (anticoagulant) BD vacutainer® (Becton Drive, Franklin Lakes, NJ, US) plus blood collection tubes (whole blood for hematological assay) and 5 mL of blood into serum separator tubes (BD vacutainer® plus) for the serum chemistry components and total electrolytes. All samples were kept on ice or refrigerated till lab analysis. The samples were assayed for glucose, cholesterol, triglycerides, creatinine, urea, uric acid, ALT, AST, CK, gamma-glutamyltransferase (GGT) and amylase using a multi-channel auto analyser (Siemens, Dimension RXL, Tarrytown, NY, US; See Appendix D for assay techniques). The anion gap was calculated as the difference between the sums of bicarbonates and chloride from the sums of sodium and potassium (Lolekha et al., 2001) and it is used to identify acid base imbalances.

Blood smears were made at the time of blood collection to avoid morphological changes to blood cells due to storage (Blue-McLendon and Green, 2010) to estimate the differential cell counts and total WBC count manually (Fudge, 2000). The PCV was determined using the standard microhematocrit method with centrifugation at 12,000 G for five minutes at 4^{o}C (Campbell, 1994). H/L ratios were calculated from the stained blood smears.

3.2.5 Statistical analysis

Descriptive statistics such as mean, standard error, and tests for normality (Shapiro Wilk Test), kurtosis, and skewness for all the variables were carried out using Statistica 10 (Statsoft.Inc, Tulsa, Oklahoma, US). The outliers were identified and eliminated using Horns algorithm by establishing limits (Tukey inter quartile fences) as suggested by Friedrichs et al. (2011). The data were analyzed using GLM procedure to determine the effect of sex and years (experiments) on the variables with body weight as a covariate. Levene’s test was used to determine whether the data were homogeneous. When there was no difference between the replicates, analysis was done using ANOVA for normally distributed data and specific between-sex differences were evaluated using a Tukey's test. When the variables were not normally distributed, transformations were done to normalize the data; or else non-parametric tests such as the Kruskal-Wallis one-way ANOVA and Dunn's test were carried out. The means ± standard error are presented after standardizing for the variation in body weight, with an overall significance level (α) = 0.05. The recommended procedure for constructing RIs has been reviewed by Jones and Barker (2008). Although a sample size of 120 is often suggested, a minimum of 40 samples is necessary for making good estimates of reference intervals from species where larger sample sizes are difficult to obtain (Friedrichs et al., 2011). Reference value advisor program (Geffre et al., 2011) was used to compute RIs with body weight as a covariate. Reference value advisor computes "reference intervals using the standard and robust methods with and without generalized Box-Cox data transformation, while allowing the study of the effect of continuous covariates". It also "assesses normality using the Anderson-Darling test and outliers with Tukey and Dixon-Reed tests; displays scatter plots, histograms and Q1-Q3 plots for testing normality; and provides suggestions based on international recommendations" (Geffre et al., 2011).

3.3 Results and discussion

Review of the literature showed very few studies on the hematological and serum biochemical variables of emus. These references either provide data for emus with age not specified (Stewart, 1984; Okotie-Eboh et al., 1992; AAZV-ISIS, 1996; Fudge, 2003), or are from emus of ages up to 24 months (Costa et al., 1993; Patodkar et al., 2008). A comparison of the sample sizes, sex-differences and values previously reported are given in Table 3.1. This study is perhaps the first attempt to create RIs for analytes of adult healthy emus in the beginning of their breeding season, using a fairly large sample size.

3.3.1 Body temperature

The body temperature of emus is lower than that in other poultry (Maloney and Dawson, 1993). Females' body temperature (n = 19; $37.4^{o} \pm 0.9^{o}$ C) was not significantly different than that of males (n = 27; $37.0^{o} \pm 1.2^{o}$ C). In contrast, Maloney and Dawson (1993; 1994) found that females had significantly higher body temperature and basal metabolic rate than males in both the summer and winter months. In their study, females were also significantly heavier than males in the two seasons; the body temperatures varied from 37^{o} to 38.5^{o} C, but the age of emus was not specified. There are many factors (thermister vs. thermometer for measurement; time of the year when temperature was taken; age of the emus) that may affect the difference in this data set but perhaps the important one would be that there was no significant difference between the body weights of males and females (Table 3.2) and hence they may have similar basal metabolic rate (Maloney and Dawson, 1993) and body temperature at the time of this study.

3.3.2 Body weight

The body weight of emus recorded during November in the two experiments (Table 3.2) was similar. Female emus started to accumulate fat earlier (between April and June) than males, and their fat accumulation rate would slow down after June. Males start to accumulate fat later and have maximum fat gain between June and August (Kim et al., 2012). By November, males had more accumulated fat than females (Kim et al., 2012). This may relate to the fact that in November, the females were already mobilizing the stored fat for egg laying, whereas the males were still storing fat to be used as the energy source during incubation. With the breeding season progressing, male emus gradually reduce their metabolic rate and go off feed and water until incubation is completed (Kent and Bewg, 2010). It will be interesting to examine the seasonal changes in these variables that are more related to fat deposition (Musa et al., 2007), to determine whether there was sexual dimorphism in their temporal profile. Unfortunately, I could collect data at one time point only.

3.3.3 Hematology

The staining properties of avian leukocytes have been discussed in detail by Campbell (2004). All the samples examined had adequate level of thrombocytes, which was confirmed by examining the blood smears wherein, 2-3 per field under the oil immersion objective of the microscope was considered to be the required number (Campbell, 1994; Blue-McLendon and Green, 2010). The total WBC count, differential counts, H/L ratios and hematocrit as well as the type of RIs for both sexes are reported in Table 3.3.

Table 3.2. Sexes, ages, sample size and body weights (Kg) of emus in the study[1]

		Bodyweight					
		Females			Males		
Experiment	Age (years)	n	Mean ± S.E	Range	n	Mean ± S.E	Range
1	4-5	10	42.6 ± 1.5	36-52	14	42.6 ± 1.3	37-53
2	5-6	9	45.0 ± 1.6	41-48	13	45.6 ± 1.3	38-57

[1]No significant difference between the body weights of males and females in the two experiments.

These values were within the normal ranges for ratites (Fudge, 2000). There was no significant difference between the sexes for any of these variables. No sex difference in these variables was also reported in lesser rheas (*Pterocnemia pennata*; Reissig et al., 2002), red-legged partridges (*Alectoris rufa*; Rodriguez et al., 2004) and bar-breasted upland geese (*Chloephaga picta leucoptera*; Gladbach et al., 2010). Rheas are similar to emus in that it is the male which incubates (Fernandez and Reboreda, 1998), while in the red-legged partridges, both sexes incubate. The upland geese are a non-migratory species that breed in harsh weather conditions and while it is only the female which incubates eggs, both parents brood the young. However, sexual dimorphism with respect to hematological variables during breeding season has been reported in great tits (*Parus major*; Kilgas et al., 2006), mallard ducks (*Anas platyrhynchos*; Olsen, 1994), Canada geese (*Branta canadensis*; Olsen, 1994), and zebra finches (*Taeniopygia guttata*; Wagner et al., 2008), which are species where in only the female incubates eggs.

The results reflected the differences in the challenges faced by male and female emus with respect to the challenges imposed by the breeding season (Hõrak et al., 1998; Wagner et al., 2008). The emus are similar to rheas (another ratite) and upland geese as both sexes are affected by the challenges of the breeding season, and hence these variables did not differ significantly between sexes.

Table 3.3. Mean hematological variables in male and female emus with reference intervals and previously reported ranges[1].

Variables	Units	Mean ± SE Females (n = 19)	Mean ± SE Males (n = 26)	Reference interval[2] Sexes combined (n = 45)	Previously reported range[3]
WBC Count	$x10^9$/L	12.2 ± 0.7	12.2 ± 0.6	7 - 19[4]	8 - 21
Heterophils	%	61.5 ± 1.9	63.2 ± 1.6	41 - 77[5]	54 - 88
Lymphocytes	%	33.7 ± 1.7	31.8 ± 1.4	17 - 48[5]	10 - 44
Monocytes	%	4.8 ± 0.6	4.0 ± 0.5	0 - 6[5]	0 - 1
Eosinophils	%	1.3 ± 0.4	0.9 ± 0.2	0 - 4[6]	0 - 6
Basophils	%	0.5 ± 0.2	0.5 ± 0.1	0 - 4[5]	0 - 1
PCV	%	52.1 ± 1.8	50.3 ± 1.4	38 - 60[5]	Not given
H/L Ratio		1.9 ± 0.2	2.2 ± 0.1	0.8 - 3.3[5]	Not given

[1]No significant difference between the sexes for any of the variables.
[2]Calculated by "Reference value advisor".
[3]Fudge (2003).
[4]Standard Box-Cox transformed data.
[5]Nonparametric reference intervals using a bootstrap method.
[6]Robust method with a Box-Cox transformation.

In stress hemograms, leukocytosis could be accompanied by heterophilia and lymphopenia (Post et al., 2003; Blue-McLendon and Green, 2010). Differential cell counts would help identify pathological conditions and infections (Campbell, 1994). Heterophils play the role of neutrophils in emus and are the most abundant blood cells. The H/L ratio is used as an indicator of stress in birds (Vleck et al., 2000; Post et al., 2003; Singh et al., 2009; Onenc, 2010; Prieto and Campo, 2010). The previously reported ranges for hematological variables by Fudge (2003; Table 3.3) reveal comparatively higher heterophil counts than this study, probably due to the difference in handling stress. While all the references reported more heterophils than lymphocytes in emus, one study (Kumar et al., 2009) reported otherwise (Table 3.1) for reasons not mentioned. Such reversals are possible in viral and chronic bacterial infections (Campbell, 1994). Eosinophils were comparatively higher in number in emus when compared to ostriches and their role in ratites is said to be unclear (Campbell, 1994; Blue-McLendon and Green, 2010). Basophilia if seen in emus could be associated with stress (Maxwell, 1993). Slightly elevated hematocrit when compared to the normal values (PCV) in this study may be due to dehydration (Minka and Ayo, 2008; 2009) and also an age effect (Palomeque et al., 1991; Reissig et al., 2002). However, hematocrit values were found to decrease with the progress of laying season under the influence of estrogen in female zebra finches (Wagner et al., 2008), while higher levels were seen in male guinea fowls (*Numida meleagris*) than females (Nalubamba et al., 2010). This could be indicative of the hematological changes playing a role in shaping the costs of egg production in females (Wagner et al., 2008). Still, changes are possible with the progress of the breeding season. The results are not capable of demonstrating any effect of sex-role reversal as I have collected samples only at the beginning of the breeding season. Never-the-less, it is important to keep in mind that changes in hematocrit alone are not a reliable indicator of any health condition (Fair et al., 2007).

3.3.4 Metabolites

Blood metabolites, such as glucose, protein, albumin and triglycerides, are indicative of the nutritional status in general (Krautwald-Junghanns, 2004). There were significant differences between the experiments with respect to glucose ($P < 0.001$), creatinine ($P < 0.001$) and uric acid ($P = 0.01$) levels, probably due to the difference in their nutritional plane. The difference between sexes with respect to globulin concentrations was significant ($P = 0.007$) only in the second Experiment i.e. 28.15 ± 1.1 g/L (females) and 20.86 ± 0.9 g/L (males). The concentration of major blood metabolites in both sexes and the RIs are given in Table 3.4.

The ratio between urea and uric acid and that between uric acid and creatinine for emus are reported for the first time. When compared to previous studies, there is a widening of ranges for total protein, globulin, and cholesterol, while narrowing down of that for uric acid (Table 3.4) as an effect of age (Moniello et al., 2006) and the imminent breeding season (Garcia et al., 2010). The glucose concentrations were similar to those reported by most of the authors (Table 3.1), but Costa et al. (1993) reported almost double the values. Such elevations in glucose are possible as a result of handling stress, due to the release of GCs (poultry; Siegel, 1995). The blood samples from these emus were collected at the time of the year when they have considerably lower feed intake (daily intake of less than 300g per emu), which may also explain the relatively lower glucose values.

There were higher total protein, albumin and globulin concentrations in these emus when compared to the previous reports (Table 3.1) which are from younger emus, except Kumar et al. (2009), who reported values similar to the adult emus. This further indicated the influence of age on serum protein concentrations (Costa et al., 1993; Krautwald-Junghanns, 2004). The higher values could also be the effect of hydration status (as the emus had considerably lowered their water intake), higher globulin concentrations at the onset of the breeding season, or age (Hochleithner 1994; Krautwald-Junghanns, 2004).

The mean albumin concentrations reported are slightly higher due to the lower hydration levels as evidenced also by the higher PCV values. The significantly higher globulin values seen in female emus could be due to the developing follicles (Lumeij, 1987b; Harr, 2005) or enhanced investment in humoral immune defence as found in great tits (Hõrak et al., 1998) in anticipation of the forthcoming breeding season. Higher total protein and globulin concentrations were also reported in adult and female red-legged partridges (Rodriguez et al., 2004), breeding yellow-legged gulls (*Larus michahellis*) than the non-breeding ones (Garcia et al., 2010), and female bronze turkeys (*Meleagris gallapavo*; Schmidt et al., 2010).

The total cholesterol concentrations observed were similar to those reported by other authors (Table 3.1). Lipemia causes serum samples to turn cloudy and this has been reported in emus of both sexes during the spring and summer seasons in Australia as well as laying females (Van Cleeff et al., 2003). None of the serum samples tested showed signs of lipemia, probably as the emus had stopped fat deposition and also not started laying eggs. The absence of difference between males and females with respect to cholesterol concentrations could also be due to the fact that both sexes prepare for their respective roles in breeding. However, sex and breeding season influences leading to higher cholesterol concentrations in females have been reported in common terns (*Sterna hirundo*; Bauch et al., 2010); red-legged partridges (Rodriguez et al., 2004) and great tits (Hõrak et al., 1998) due to the influence of estrogen (Bittner, 2008).

Table 3.4. Mean serum metabolite concentrations in male and female emus with reference intervals and previously reported ranges.

Variables	Units	Mean ± SE[1]		Reference interval[2]	Previously reported ranges[3]
		Females (n = 19)	Males (n = 26)	Sexes combined (n = 41)	
Glucose	mMol/L	9.6. ± 0.3[a]	9.3 ± 0.3[a]	6 - 13[4]	6 - 11
Creatinine ***	µMol/L	13.5 ± 1.0[a]	8.7 ± 0.8[b]	5 - 26[5]	9 - 36
Total protein**	g/L	52.8 ± 1.0[a]	48.1 ± 0.8[b]	38 - 58[5]	31 - 53
Albumin (A)**	g/L	29.9 ± 0.6[a]	27.5 ± 0.5[b]	21 - 34[5]	19 - 32
Globulin (G)***	g/L	26.2 ± 0.7[a]	21.6 ± 0.6[b]	17 - 29[6]	12 - 21
A/G ratio		1.2 ± 0.1[b]	1.3 ± 0.1[a]	0.8 - 1.5[4]	Not given
Cholesterol	mMol/L	4.5 ± 0.5[a]	4.5 ± 0.4[a]	3 - 14[7]	1 - 4.3
Triglycerides***	mMol/L	7.8 ± 1.0[a]	1.8 ± 0.8[b]	0 - 22[5]	0 - 17
Uric acid	µMol/L	172 ± 16[a]	168 ± 14[a]	69 - 342[5]	42 - 517
Urea	mMol/L	0.7 ± 0.1[a]	0.8 ± 0.1[a]	0.2 - 1.7[7]	0.3 - 1.5
Urea/ uric acid ratio		5.1 ± 1.0[a]	3.8 ± 0.7[a]	1.2 - 10.0[7]	Not given
Uric acid /creatinine ratio		16.7 ± 3.0[a]	17.4 ± 2.7[a]	12 - 29.0[7]	Not given

[1]Means within a row lacking a common superscript differ significantly at [ab]$P < 0.05$; $^{**}P \leq 0.01$, $^{***}P < 0.001$.
[2]Calculated by “Reference value advisor”.
[3]Okotie-Eboh et al. (1992).
[4]Standard untransformed.
[5]Nonparametric reference intervals using a bootstrap method.
[6]Standard Box-Cox transformed data.
[7]Robust method with a Box-Cox transformation.

In terns and partridges, both sexes are involved in incubation, but in tits, only females incubate, though both sexes raise the chicks. These changes might be occurring with the progress of the breeding season in emus but unfortunately I could not collect blood samples during the different stages of breeding. Triglyceride concentrations vary depending on sex, season and nutritional status (Hochleithner, 1994). The significantly higher concentrations of triglycerides in female emus (Table 3.4) could be associated with the breeding season as reported by Kumar et al. (2009). Triglyceride levels of breeding female red-legged partridges (Rodriguez et al., 2004) and yellow-legged gulls (Garcia et al., 2010) are also greater than males.

The creatinine concentrations observed by us were comparatively lower than those reported by all other authors (Table 3.1) due to the older age (Hosten, 1990; Rodriguez et al., 2004) and resultant lower muscle turnover in adult emus when compared to yearlings. Creatinine concentrations could also vary depending on the protein level in diet (Palomeque et al., 1991) and as such are of limited importance in clinical diagnosis (Hochleithner, 1994; Krautwald-Junghanns, 2004). Female emus have higher muscle mass than males (Menon et al., 2012) which may account for their significantly higher creatinine concentrations but there is no conclusive evidence as in quails (females are heavier), higher creatinine concentrations were not reported (Scholtz et al., 2009). However, higher creatinine concentrations in breeding female chukar partridges (*Alectoris chukar*) have been reported, though males are heavier (Nafizi et al., 2011) in this species. So though this could be under the influence of hormones and metabolic changes, the exact reason for the difference in muscle turnover between sexes remains unclear.

Uric acid is the major end product of protein metabolism in birds and its concentrations can be used to detect starvation (Krautwald-Junghanns, 2004; McDonald, 2006), dehydration and renal diseases (Hochleithner, 1994) in conjunction with other analytes, such as creatinine. The lower uric acid concentrations possibly reflect the protein levels in the diet (Palomeque et al., 1991; Bovera et al., 2007). The emu was found to have limited ability to produce concentrated urine even though its water requirements are low (Dawson et al., 1985). Urea concentrations can be highly increased in dehydration in birds (Hochleithner, 1994) and are reported to be useful in diagnosing renal failure in pigeons (Lumeij, 1987a). Uric acid/ creatinine ratio and urea/ uric acid ratio (Table 3.4) are considered to be reliable indicators of renal function and dehydration (Lumeij, 1987a; Hochleithner, 1994). There was no difference between sexes in emus in the blood uric acid concentrations, as also reported in red-legged partridges (Rodriguez et al., 2004), common terns (Bauch et al., 2010), and Japanese quail (*Coturnix japonica*; Scholtz et al., 2009), suggesting that this might not be a variable affected by sex.

3.3.5 Enzymes

The enzyme activities and reference intervals in emus measured in this study are given in Table 3.5 and there was no significant difference between the sexes for any of the variables. Some other studies have reported sex effect on CK activities, which was higher in males than in female flamingos (*Phoenicopterus ruber*; Eren, 2006); while higher ALT activities were reported in bronze turkey females (Schmidt et al., 2010) during the breeding season. There is lack of information on the status of these variables during the pre-breeding stage.

ALT is ubiquitous and not specific to any organ function in avian species and hence may not be very useful in disease diagnosis (Krautwald-Junghanns, 2004), while changes in AST (associated with muscle and liver function), together with CK (indicative of the changes to skeletal, cardiac muscles and brain tissue) can be used to identify liver damage (Hochleithner, 1994). The wide variation in values reported in different studies (Table 3.1) could be due to the differences in the handling procedure and resultant muscular trauma (Palomeque et al., 1991; Baird et al., 2012). This emphasises the need to minimize handling if we are looking for feedback free samples. More than ten times increase in AST and fifteen time increases in CK activities from the baseline are suggestive of rhabdomyolysis in ratites (Paterson, 2007).

Amylase enzyme secreted by the pancreas is important in normal digestion of carbohydrates and abnormal increases may be noticed with diseases of pancreas (Krautwald-Junghanns, 2004; Harr, 2005). The concentration of GGT, which is important in nitrogen metabolism and found within liver cells (Hochleithner, 1994), was similar to that reported in ostriches (Bovera et al., 2007). Its level in egg laying ducks were found to be ten times the normal values, while double the normal values were found in moulting drakes (Olsen, 1994) but I did not find any difference between sexes in emus at this stage of the breeding season.

3.3.6 Electrolytes and minerals

The values for calcium, phosphorus and the other electrolytes are presented in Table 3.6 and were comparable to those reported for emus in AAZV-ISIS (1996). The serum potassium concentrations observed were elevated probably on account of storage (Zaki and Majid, 2011) and or hemolysis (Sawant et al., 2007). The anion gap used to identify acid base imbalances is within the reported range for avian species (Harr, 2005).

Table 3.5. Mean serum enzyme activities (IU/L) in male and female emus with reference intervals and previously reported ranges[1].

Variables	Mean ± SE		Reference interval[2]	Previously reported ranges[3]
	Females (n = 19)	Males (n = 26)	Sexes combined (n = 45)	
Alanine aminotransferase	16.2 ± 1.8	16.9 ± 1.3	4 - 36[4]	7 - 24
Aspartate aminotransferase	177 ± 7	181 ± 7	98 - 230[4]	57 - 151
Creatine kinase	335 ± 24	300 ± 23	95 - 588[5]	0 - 603
GGT Gamma-glutamyltransferase	4.3 ± 0.8	5.3 ± 0.6	1 - 15[5]	0 - 11
Amylase	447 ± 43	444 ± 48	366 - 554[5]	Not given

[1]No significant difference between the sexes for any of the variables.
[2]Calculated by "Reference value advisor".
[3]Okotie-Eboh et al. (1992).
[4]Standard Box-Cox transformed data.
[5]Robust method with a Box-Cox transformation.

Serum calcium concentrations were significantly higher in female emus than males as also reported by Kumar et al. (2009), but their ratio was similar to those reported by Andreasen et al. (1997). Significantly higher concentrations of serum calcium, phosphorus or both have been reported in breeding females of other species such as flamingos (Eren et al., 2006), yellow-legged gulls (Garcia et al., 2010), Egyptian goose (*Alopochen aegyptiacus*; El-Hanoun et al., 2012) as well as emus (Okotie-Eboh et al., 1992) and this could be a physiological feature (Krautwald-Junghanns, 2004; Harr, 2005) during ovulation. Similarly, adult ostriches were found to have higher calcium and phosphorus concentrations (Moniello et al., 2006) than yearlings. So in this respect, emus are no different from other species.

Table 3.6. Mean serum electrolyte concentrations (mMol/L) in male and female emus with reference intervals and previously reported ranges.

Variables	Mean ± SE[1]		Reference interval[2]	Previously reported ranges[3]
	Females (n = 18)	Males (n = 26)	Sexes combined (n = 41)	
Sodium	146 ± 1.2	147 ± 0.8	137 - 155	137 - 157
Potassium	4.2 ± 0.5	4.1 ± 0.3	2 - 6	2.3 - 5.8
Chloride	104.3 ± 0.8	104.8 ± 0.7	97 - 111	100 - 115
Bicarbonate	24.6 ± 1.0	23.4 ± 0.8	11 - 30	Not given
Calcium	4.6 ± 0.3[A]	3.5 ± 0.3[B]	2 - 7	2.2 - 2.9
Phosphorus	2.3 ± 1.1	2.1 ± 0.6	1 - 3	1.0 - 2.8
Anion gap	11.9 ± 1.2	13.4 ± 0.4	7 - 18	Not given

[1]Means within a row lacking a common superscript differ significantly at [A,B] P <0.001.
[2]Calculated by “Reference value advisor” is Robust method with a Box-Cox transformation.
[3]AAZV-ISIS, 1996.

Findings from this study showed the effect of sex on the selected analytes in emus at the beginning of their breeding season, which has not been previously reported. Adult emus in this study were found to have markedly higher values for total protein, and hematocrit from those reported for young emus in other studies (Table 3.1). Females had distinctly different values from males for analytes such as total protein, globulin, and triglycerides, which could be the reason for the wide variation found across the Table 3.1, where studies did not consider the sex effect. Elevated calcium concentrations in females could be noticed during the breeding season as in most avian species, though the calcium phosphorus ratios were unaffected. Similar to the findings in emus, during the pre-breeding period, both male and female mallard ducks were found to have significantly higher concentrations of albumin, GGT, and calcium concentrations than non-reproductive birds (Olsen, 1994), while the total protein and globulin concentrations increased progressively with the breeding season in female bronze turkeys (Schmidt et al., 2010).

Some of the previous studies in emus did not show variation between sexes probably because of the difference in the age of the birds and season during which these studies were conducted, and some of them also do not mention the health status of the subjects. So it is important to consider the effect of season, physiological status, sex, age and body weight in addition to nutrition while making interpretations of the blood biochemical profile in emus.

3.4 Conclusions

Blood profiling is helpful in detecting health issues, parasitism, subclinical metabolic conditions, erroneous feeding practices and the welfare of emus. Emus in general have lower body temperatures than other poultry. The findings from this study showed that at the beginning of the breeding season, concentrations of protein and hematocrit are higher, similar to the finding in most other avian species. Among metabolites, serum total protein, serum globulin, and triglyceride concentrations were significantly higher in females along with higher creatinine and blood calcium concentrations. The glucose and cholesterol concentrations which are related to the nutritional plane, as well as the phosphorus concentrations were similar in both sexes. This study also revealed similarity in the hematological and enzyme profile of male and female emus close to the breeding season due to the equally challenging roles they play in the breeding process.

4. TRANSPORTATION STRESS AND THE INCIDENCE OF EXERTIONAL RHABDOMYOLYSIS IN EMUS[1]

4.1 Introduction

Many emu farms are located in areas lacking slaughtering facilities that can process these birds (Michael, 2000; Stevens, 2012). This necessitates the transport of emus, frequently over long distances, to suitable facilities. Prolonged shipment often results in exposure to stressful and adverse conditions (Mitchell and Kettlewell, 2009) causing physiological changes, injury and mortality, and welfare concerns (Grandin, 1999; Smith et al., 2004).

Many critical points in the shipping process of traditional poultry and livestock species have been examined (Smith et al., 2004; Huff et al., 2008; Piccione et al., 2005), but emus have received little attention. Emus differ from other poultry in their behavioral and physiological requirements (Glatz and Miao, 2008; Black and Glatz, 2011), and hence strategies to minimize welfare problems specific to this species need to be examined. Emus like other ratites, are highly susceptible to stress and are prone to exertional rhabdomyolysis (Tully et al., 1996; Smith et al., 2005; Minka and Ayo, 2008). Several studies have found that nutrient (electrolyte, dextrose, and amino acids) therapy administered pre- or post-transport helped to mitigate the physiological effects of transport stress (dehydration, electrolyte imbalance, energy deficits) in food animals (Schaefer et al., 1995; Smith and Wilson, 1999; Olanrewaju et al., 2007; Arp et al., 2011).
The objectives of this study were

(1) to examine the physiological response of adult male and female emus to transport using selected indices of stress and metabolic homeostasis (changes in hematology, serum biochemistry, enzymes, and body temperature and weight), and

(2) to examine the efficacy of a nutrient supplement to mitigate the physiological responses to transport stress, in emus. Information gathered from this study will aid the development of guidelines to minimize shipping stress in emus.

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[1]A version of Chapter 4 has been submitted for publication as
Menon, D. G., D. C. Bennett, A. L. Schaefer, and K. M. Cheng. 2013. Transportation stress and the incidence of exertional rhabdomyolysis in emus *(Dromaius novaehollandiae)*.

4.2 Experiment 1

4.2.1 Materials and methods

This study was conducted in collaboration with an emu producer in Saskatchewan, Canada. One shipping Experiment was conducted in each of two consecutive years, in late autumn (November), just prior to the start of the emus' breeding season.

4.2.2 Experimental birds

Twenty four 4-6 year old emus (14 males, 10 females) were used. They were fed a diet containing barley, alfalfa and canola, and had access to the natural forages in the outdoor pen. Drinking water was available *ad libitum*. Sex of emus was determined by cloacal palpation (Samour et al., 1984) (confirmed after slaughter) and their general health and body condition was noted before transport.

4.2.3 Handling

See Section 3.2.4 for details on sample collection. Emus may struggle initially, but would quickly settle down. The bird was then guided by the handler to the sampling area, the transport trailer, or the stunning box.

4.2.4 Nutrient supplement treatments

Emus were allocated to one of three treatment groups: (1) nutrient supplement given both pre- and post-transport (S-S; 4 males and 4 females), (2) nutrient supplement given only pre-transport (S-W; 4 males and 3 females), and (3) no nutrient supplement given (Control, W-W; 5 males and 4 females). The nutrient supplement (Nutri-charge®; Appendix E developed at Agriculture and Agri-Food Canada, U.S. *Patents* 5505968 and 5728675, Lacombe, Alberta) contained electrolytes, an energy source (dextrose) and amino acids (Schaefer et al., 1997) and was added to the emu's diet, in 1:1 ratio (Kg), two days prior to transport. Nutrient supplement was also provided to the S-S emus post-transport in the diet.

4.2.5 Transport

Emus were randomly loaded, one by one, into one of the two compartments of a modified horse trailer at 7 am with a stocking density of two emus/m^2, and transported for six hours (485km). The compartment had natural lighting and the floor was provided with a thick bedding of hay. Loading and unloading accounted for an additional hour of confinement in the compartment. Emus were observed during the transport using a back-up night vision video camera that was connected to a monitor in the truck cab. Upon arrival at the slaughter facility (see below), the emus were unloaded and kept at lairage with access to feed and water, for 15 hours prior to slaughter.

The mean outdoor temperature prior to transport was 2^{o}C, but on the day of transport it was between 8^{o} and 17^{o}C. The temperature in the trailer ranged from 11^{o} to 22^{o}C (EasyLog EL-USB Data Logger, Springside Drive Akron, OH, US). The relative humidity varied from 45-89%.

4.2.6 Lairage and slaughter

After transport, emus were rested at the lairage for 15 hours, with access to feed and water *ad libitum*, prior to slaughter. The emus were slaughtered in a federally inspected research abattoir of Agriculture and Agri-Food Canada in Lacombe, and were subjected to percussion stunning (penetrating type captive bolt stun pistol- Cash Special 22Cal, Talford St.Works, Ashton, Birmingham, London, UK) and exsanguinated by a complete ventral cut.

4.2.7 Sample collection and processing

Two days prior to transport, all emus were weighed, bled from the jugular vein, their body temperature (cloacal) measured, their general health status and body condition were assessed (see Section 3.2.3.) to ascertain the pre-transport values of the variables assessed. Immediately after transport, emus were reweighed, their cloacal temperature measured, and their general condition was assessed. In Experiment 2, the emus were also bled from the jugular vein at this time. In Experiment 1, emus showed signs of thermal stress (open beak breathing, arching of neck (retrocollis), and bobbing of head) during unloading from the trailer. It was decided not to stress them further and I did not collect blood samples at this time point. Emus were again weighed just prior to slaughter (after 15 hours of lairage), and trunk blood was collected during exsanguination. See Section 3.2.4 for sample collection and processing protocol.

Injuries and feather loss were separately classified using a six point scale (Table 4.1) based on Bilcik and Keeling (1999). The number of emus with fractured bones, and dislocations were also determined at slaughter.

Table 4.1. Scoring of body conditions based on injuries and feather loss[1].

Injuries		Extent of feather loss	
Description	Score	Description	Score
No bruises/ wounds	0	No loss (0%)	0
Few bruises (≤ 5)	1	Very mild (10 %)	1
Several bruises (≥6)	2	Mild (20%)	2
Few to several small wounds	3	Moderate (50%)	3
Few big wounds	4	Extensive (75%)	4
Several big wounds	5	Complete (100%)	5

[1]Table adapted from Bilcik and Keeling (1999).

4.2.8 Statistical analysis

Three index scores were developed for each of the variables:

(1) Changes during transport (TS) = post-transport value - pre-transport value,

(2) Changes during resting at lairage (LS) = post-transport value - at slaughter value, and

(3) Overall changes (OS) = at slaughter value - pre-transport value.

A positive index score would indicate an increase, while a negative score would indicate a decrease in the corresponding variable. GLM procedure of Statistica (Statsoft 10 Inc.) was used to determine the effect of supplement, sex and their interactions on TS, LS and OS, with pre-transport body weight as a covariate. The variables evaluated were tested for normality (Shapiro-Wilk test) and equal variance. The variables AST, CK, corticosterone, and bicarbonate were subjected to power transformation, while heterophil count, H/L ratio and calcium were subjected to square root transformation to meet the assumptions. The results are reported as Least Square means with their standard errors.

The statistical model being:

$Y_{ijk} = \mu + S_i + N_j + SN_{ij} + B_k + E_{ijk}$

where, Y is the TS, LS, or OS of one of the variables; S = effect of sex (i = male, female); N = effect of nutrient supplement (j= S-S, S-W, W-W); SN = interaction between sex and supplement; B = covariate pre-transport body weight; E = experimental error. When the main effects or interaction were significant, the Bonferroni t-test was used for mean separation. The level of significance was set at $P \leq 0.05$. Chi square test of independence was done to compare the frequency distributions of injury, feather loss and total injury scores between the experiments and further confirmed by Monte Carlo method with 5000 simulations.

The experiment was a randomized complete block design with factorial arrangement of the main effects of sex and nutritional treatments, blocked by the year. The whole plots were sex and nutritional treatments and their interaction and the time periods of observation were the sub plots. Analysis was carried out as a split plot design using SAS (edition 9.2; SAS Institute Inc., 2007) for determining the effect of transport on all variables and all analyses on glucose, creatinine, triglyceride, ALT, uric acid and, uric acid /creatinine ratio (variables with significant pre-transport differences between the treatment groups). The variables were tested for normality (Shapiro-Wilk test) and equal variance (Bartlett's test of sphericity). Where necessary, power transformations were done, but the least square mean and standard error of the mean (SE) values are presented.

The statistical model being:

$$Y_{ijkl} = \mu + S_i + N_j + SN_{ij} + O_{k(ij)} + R_l + RS_{il} + RN_{jl} + RSN_{ijl} + OR_{kl(ij)}$$

where, S = effect of sex (i = male, female); N = effect of nutrient supplement (j = S-S, S-W, W-W); SN_{ij} = 2-way interaction; $O_{k(ij)}$ is the sampling error term; R = time of measurement (l = pre-transport, post-transport, at slaughter); RS and RN are the two way interactions; and RSN is the three way interaction. These effects were tested with the second sampling error term $OR_{kl(ij)}$. Post-hoc mean separation was done using Students t-test with Bonferroni's t-test adjustment. Pearson correlation analysis was carried out to determine whether a relationship existed between selected parameters.

4.2.9 Results

4.2.9.1 Effect of transport and resting in lairage

4.2.9.1.1 Body weight

Post-transport body weight was significantly ($P < 0.001$) lower (42.1 ± 1.0 kg) than the pre-transport values (44.7 ± 1.0 kg). There was a significant ($P < 0.001$) increase in slaughter body weight (42.6 ± 1.0 kg) from post-transport values. However, slaughter body weight was still significantly ($P<0.001$) lower than pre-transport values.

4.2.9.1.2 Body temperature

The body temperature of emus significantly ($P < 0.001$) increased from 37.0^{o} pre-transport to $39.6^{o}C$ after transport

4.2.9.1.3 Metabolites (Table 4.2)

The concentrations of glucose ($P<0.001$), and creatinine ($P<0.001$) increased significantly from the pre-transport to slaughter. The ratio between UA and creatinine reduced significantly ($P<0.001$) from pre-transport to slaughter. The TP, albumin, globulin, cholesterol and UA concentrations were not significantly affected by transport. The pre-transport values for these variables were 52.1 ± 0.9 g/L, 28.7 ± 0.5 g/L, 23.5 ± 0.6 g/L, 4.4 ± 0.3 mMol/L and 162 ± 12 μMol/L, respectively.

4.2.9.1.4 Enzymes (Table 4.3)

Serum ALT ($P<0.001$), AST ($P<0.001$) and CK ($P<0.01$) increased significantly from the pre-transport to slaughter.

4.2.9.1.5 Corticosteroids (Table 4.4)

The plasma cortisol concentration was less than 1pg/mL. There was a significant ($P<0.001$) increase in corticosterone (CORT) from pre-transport to slaughter.

Table 4.2 Blood metabolites changes after transport and lairage.

	Experiment 1*		Experiment 2*		
	Pre-transport	At slaughter	Pre-transport	post-transport	At slaughter
Glucose (mMol/L)	10.1 ± 0.4[a]	14.3 ± 0.4[b]	8.5 ± 0.5[c]	12.5 ± 0.5[a]	10.6 ±0.5[b]
TG[1] (mMol/L)	3.8 ± 0.6[a]	2.5 ± 0.6[a]	5.4 ± 0.7[a]	2.3 ± 0.7[b]	1.3 ± 0.7[b]
Creatinine[2] (µMol/L)	10.0 ± 1.0[b]	26.0 ± 1.0[a]	4.3 ± 1.3[b]	7.3 ± 1.3[b]	28.4 ± 1.3[a]
UA/Creatinine Ratio[2]	19.5 ± 2.4[a]	8.4 ± 2.4[b]	32.1 ± 3.6[a]	25.9 ± 3.6[b]	6.4 ± 2.9[c]

*Within each experiment, means for each metabolite followed by different superscripts were significantly (glucose: P<0.001; TG: P<0.01; Creatinine: P<0.001; UA/Creatinine ratio: P<0.01) different.
[1]Data were square-root transformed to meet assumptions of normality and equal variance.
[2]Data were power ($X^{0.1}$) transformed to meet assumptions of equal variance.

Table 4.3 Blood enzymes changes after transport and lairage.

	Experiment 1* (N =24)		Experiment 2* (N = 18)		
	Pre-transport	At slaughter	Pre-transport	post-transport	At slaughter
ALT[1] (IU/L)	18.7 ± 9.7[b]	84.5 ± 10.7[a]	12.8 ± 12.0[b]	29.0 ± 12.0[b]	109.9 ± 12.0[a]
AST[1] (IU/L)	206 ± 119[b]	1479 ± 121[a]	187 ± 145[b]	469 ± 145[b]	1665 ± 151[a]
CK[1] (IU/L)	235 ± 1229[b]	8899 ± 1246[a]	689 ± 1744[c]	3836 ± 1500[b]	16420 ± 1564[a]

* Within each experiment, means for each enzyme followed by different superscripts were significantly (ALT: P<0.001; AST: P<0.001; CK: P<0.001) different.
[1]Data were power ($X^{0.1}$) transformed.

Table 4.4 Plasma corticosterone changes after transport and lairage.

	Experiment 1* (N =24)		Experiment 2* (N = 18)		
	Pre-transport	At slaughter	Pre-transport	post-transport	At slaughter
CORT[1] (ng/mL)	9.5 ± 2.3^{b}	33.9 ± 3.6^{a}	5.3 ± 1.0^{a}	9.2 ± 1.2^{a}	9.6 ± 1.3^{a}

* Within each experiment, means followed by different superscripts were significantly (P<0.001) different.
[1]Data were power ($X^{0.1}$) transformed.

4.2.9.1.6 Hematology (Table 4.5)

The H/L ratio increased significantly (P < 0.05) from pre-transport (2.2 ± 0.2) to slaughter (3.2 ± 0.4). The counts of monocytes, basophils and eosinophils in blood were not significantly affected by transport. The pre-transport values for these variables were 4.5 ± 0.4%, 0.4 ± 0.1%, and 0.6 ± 0.2%, respectively.

4.2.9.1.7 Electrolytes

Serum calcium, phosphorus and sodium were not significantly affected by transport, and their respective pre-transport values were 4.4 ± 0.3 mMol/L, 2.7 ± 0.3 mMol/L and 146.9 ± 1.6 mMol/L.

4.2.9.1.8 Behavioral observations

During transport some emus lost their balance, slipped and fell. These birds then tended to sit down frequently (at least once in every 15-20 min) for the rest of the journey and were trampled on by other birds. Observed behavior during transport included rubbing neck and sides of body against the walls of the compartment, open-beak breathing, tachypnea, gular flutter, increased bobbing and arching of the neck. During lairage, the emus drank water, but declined to feed.

Table 4.5 Hematological parameters changes after transport and lairage.

	Experiment 1* (N =24)		Experiment 2* (N = 18)		
	Pre-transport	At slaughter	Pre-transport	post-transport	At slaughter
WBC counts[1]	12.3 ± 0.7[a]	13.2 ± 0.9[a]	12.9 ± 1.2[b]	14.3 ± 0.4[b]	17.3 ± 1.4[a]
Heterophil counts[2, 3]	62.9 ± 1.7[a]	65.7 ± 2.0[a]	64.6 ± 2.9[b]	87.6 ± 1.1[a]	88.4 ± 1.2[a]
Lymphocyte counts[2, 3]	30.7 ± 1.3[a]	27.5 ± 1.7[a]	30.9 ± 3.0[a]	7.4 ± 1.0[b]	8.6 ± 1.0[b]
H/L Ratio[2]	2.2 ± 0.2[b]	3.2 ± 0.4[a]	2.6 ± 0.4[b]	14.3 ± 2.1[a]	14.4 ± 2.6[a]

* Within each experiment, means for each hematological parameter followed by different superscripts were significantly (WBC: $P<0.01$; Heterophil: $P<0.001$; Lymphocyte: $P<0.001$; H/L ratio (Experiment 1): $P<0.05$; H/L ratio (Experiment 2): $P< 0.001$) different.
[1]Counts x 10^9/L.
[2]Data were square-root transformed.
[3]Percent of total WBC.

4.2.9.1.9 Injuries

None of the birds had any injuries before transport. After unloading, I found that more than half of the birds had sustained bruises and minor wounds. Most of the injuries sustained were on either side, close to the mid central line on the back and on the belly, and on both sides of the legs. Four emus had serious injuries on the thigh and leg. None of the emus had bruises on the head, broken necks, dislocated or fractured bones when I examined the carcasses after slaughtering. The feather losses were mostly from the neck and back. Two-thirds of the emus (16) transported showed open-beak breathing and tachypnea during the latter part of the journey and even after unloading.

4.2.9.2 Effect of nutrient supplement

Nutrient supplement did not have any significant effect on any of the variables evaluated. However, it was observed that the feed consumption of emus during the pre-transport period was minimum and the emus might not have taken in the planned amount of supplement.

4.3 Pilot study

The objective of the pilot study was to see if the nutrient supplement, when actually consumed by the birds, would be effective in boosting the resistance to transportation stress. The pilot study was carried out in May, when the emus were consuming large amount of feed to regain body weight ready for the next breeding season. Eight emus (4 males and 4 females) maintained at the animal facility on university campus was divided into 2 groups of 4 birds each with balanced sex ratio. The treatment group was fed the nutrient supplemented diet as in Experiment 1, as well as with the supplement dissolved in their drinking water (9 g/litre) for 3 days prior to transport. The control group was fed the regular diet and drinking water *ad libitum*. The birds were shipped to an emu farm in Duncan, British Columbia, which is about a 6-hour journey (via truck and ferry) from the university campus. The birds were weighed and blood samples taken before and after the transport. Results indicated that while supplementation had no effect on post-transport body weight, the emus receiving the nutrient supplement had significantly reduced serum AST and CK values than the control birds at the end of the journey.

4.4 Experiment 2

Emus reduce their feed intake considerably during the breeding season and lose a significant portion of their body weight during the process (Davies, 2002). In Experiment 1, the emus were shipped at the earlier part of their breeding season and they have already reduced their feed intake. Putting the nutrient supplement in their feed may therefore not be effective in preparing them for the shipment. The day of shipment happened to be an unexpectedly warm day, and the emus were suffering from heat stress at the end of the journey. I was not able to obtain post-transport blood samples and missed an important time point to separate the effect of transportation from the whole transportation/slaughter process. A second experiment was therefore carried out in the following year to further assess the effect of transportation and nutrient supplementation. This experiment was approved by the University of British Columbia Animal Care Committee, under permits # A10-0184.

4.4.1 Material and methods

This experiment was conducted in collaboration with the same emu producer in Saskatchewan. The shipping Experiment was also conducted in late autumn, as in Experiment 1. Only procedures and conditions that were different from Experiment 1 are described below.

4.4.2 Experimental birds

Twenty eight emus (10 males and 8 females) were used.

4.4.3 Nutrient supplement treatments

As in Experiment 1, emus were allocated to one of three treatment groups: (1) S-S; 4 males and 2 females, (2) S-W; 3 males and 3 females, and (3) W-W; 3 males and 3 females. In order to standardize the amount of intake, the nutrient supplement was administered (65 g in 200 mL water) orally to individual birds in the treatment groups (S-S, S-W) using a modified calf feeder, while birds in the control group (W-W) received a placebo of same volume (200 mL) of water immediately prior to transport. This is the optimal one-time feeding volume for emus (Minnaar and Minnaar, 1997) with the completely miscible amount of the supplement. Nutrient supplement was provided to the S-S emus post-transport in drinking water in the same dosage amount as in the pilot study.

4.4.4 Transport

On the day of transport, outdoor temperature was between -5^{o} to -1^{o}C. The temperature inside the trailer (loaded with birds) ranged from 0^{o} to 17.5^{o}C. Immediately after transport, emus were reweighed, their cloacal temperature measured, and their general condition was re-assessed. Blood samples were also taken at this time (2 pm to 4 pm).

4.4.5 Results

4.4.5.1 Effect of transport and resting in lairage

4.4.5.1.1 Body weight

The pre-transport body weight (45.8 ± 0.8 kg) was significantly ($P < 0.001$) reduced after transport (44.4 ± 0.8 kg) but then increased significantly ($P < 0.01$) during resting at lairage (45.2 ± 0.8 kg). The body weight at slaughter was still significantly ($P < 0.01$) lower than the pre-transport body weight.

4.4.5.1.2 Body temperature

The body temperature of emus increased significantly ($P < 0.001$) from 37.2^{o} pre-transport to 38.9^{o}C post-transport.

4.4.5.1.3 Metabolites (Table 4.2)

There were significant correlations of pre-transport body weight with glucose ($r = 0.4$; $P < 0.03$) and cholesterol ($r = 0.38$; $P = 0.04$). Both serum glucose and triglycerides concentration increased significantly ($P < 0.001$) after transport and then decreased significantly ($P < 0.003$, $P < 0.001$, respectively) after resting in lairage. Though the post-transport creatinine concentration was not significantly higher than the pre-transport value, the slaughter value was significantly ($P < 0.001$) higher than the pre-transport value. The UA/creatinine ratio was significantly ($P < 0.01$) decreased from pre-transport to post- transport and further lowered ($P < 0.01$) during lairage.

4.4.5.1.4 Enzymes (Table 4.3)

Serum ALT and AST did not increase significantly after transport, but increased significantly ($P<0.001$) after lairage. There was a significant ($P < 0.001$) increase in CK activity ($P < 0.001$) after transport, with a further significant ($P < 0.001$) increase after lairage.

4.4.5.1.5 Corticosterone (Table 4.4)

There were no significant changes in CORT from pre-transport to post-transport and from post-transport to slaughter.

4.4.5.1.6 Hematology (Table 4.5)

There was no significant change in WBC counts from pre-transport (12.9 ± 1.2 10^9/L) to post-transport (14.3 ± 0.4 10^9/L) but a significant (P <0.01) increase from post-transport to slaughter (17.3 ± 1.4 10^9/L). The heterophil counts increased significantly (P < 0.001) from 64.6 ± 2.9 % (pre-transport) to 87.6 ± 1.1 % after transport and remained unchanged (88.4 ± 1.2 %) at slaughter. The corresponding decrease in lymphocyte counts from 30.9 ± 3.0 % (pre-transport) to 7.4 ± 1.0 % (post-slaughter) and 8.6 ± 1.0 % (at slaughter) was also significant (P < 0.001). The H/L ratio significantly (P < 0.001) increased from 2.6 ± 0.4 pre-transport to 14.3 ± 2.1 post-transport and remained high (14.4 ± 2.6) at slaughter. The PCV and monocytes, basophils and eosinophils counts were not significantly affected by transport. The pre-transport values for these variables were 55.7 ± 0.4 %, 4.5 ± 0.4 %, 0.4 ± 0.1 %, and 0.6 ± 0.2 %, respectively.

4.4.5.1.7 Electrolytes

Serum calcium, phosphorous and sodium were not significantly affected by transport, and their respective pre-transport values were 4.1 ± 0.4 mMol/L, 2.7 ± 0.3 mMol/L and 146.9 ± 1.6 mMol/L. Serum chloride concentration increased significantly (P < 0.001) from 104.8 ± 0.8 mMol/L pre-transport to 110.1 ± 0.6 mMol/L post-transport and then remained unchanged (109.8 ± 0.6 mMol/L) while resting at lairage. Chloride concentration at slaughter was significantly (P < 0.001) higher than the pre-transport values. Serum bicarbonate significantly (P < 0.001) decreased from 24.5 ± 0.9 mMol/L pre-transport to 21.4 ± 0.9 mMol/L post-transport and then increased significantly (P < 0.001) during lairage to 26.4 ± 0.9 mMol/L. As a result, the pre-transport and the slaughter bicarbonate values were not significantly different.

4.4.5.1.8 Injuries

None of the birds had any injuries before transport. After unloading, I found some birds with bruises and minor wounds but the number was less than in Experiment 1. None of the emus had serious (category 4-5) injuries. Only 6 emus transported showed open-beak breathing and tachypnea during the latter part of the journey.

4.4.5.2 Effect of nutrient supplement

The supplement treatment did not significantly affect the body weight of emus during transport (TS). However, during lairage, the S-S emus (1.5 ± 0.2 kg) regained significantly (P < 0.001) more body weight than the emus in the SW (0.6 ± 0.2 kg) and W-W groups (0.4 ± 0.2 kg).

The TS and OS levels of plasma AST activity were significantly (P < 0.05) lower in the S-S group than the W-W group (Table 4.6). Orthogonal comparison of emus receiving supplement (S-S and S-W) with the control (W-W) confirmed that nutrient supplement treatment resulted in significantly (P = 0.02) lower LS values for AST activity.

Table 4.6 Effect of nutrient supplement on plasma Aspartate aminotransferase activity (IU/L) of emus transported for 6 hours.

	Nutrient Supplement*		
AST activity[1, 2, 3]	W-W	S-W	S-S
Pre-transport	214 ± 25[a]	209 ± 25[a]	173 ± 25[a]
TS (N = 18)	521 ± 83[a]	290 ± 92[ab]	192 ± 92[b]
LS (N = 18)	1911 ± 628[a]	1715 ± 628[a]	618 ± 669[a]
OS (N = 42)	1724 ± 649[a]	1207 ± 649[ab]	840 ± 691[b]

*W-W = Control; no nutrient supplement given. S-W = nutrient supplement given only pre-transport. S-S = nutrient supplement given both pre- and post-transport.
[1] TS = Changes during transport (post-transport value - pre-transport value). LS = Changes during lairage (post-transport value - at slaughter value). OS = Overall changes (at slaughter value - pre-transport value). A positive value would indicate an increase, while a negative value would indicate a decrease in AST activity.
[2] Means within a row followed by different superscripts were significantly (P<0.05) different.
[3] Data were power ($X^{0.1}$) transformed.

The TS levels of plasma CK activity were significantly (P = 0.008) lower in the S-S group than the S-W or W-W groups (Table 4.7). Orthogonal comparison of emus receiving supplement (S-S and S-W) with the control (W-W) confirmed that nutrient supplement treatment resulted in significantly (P = 0.001) lower LS and OS values for CK activity.

An orthogonal comparison of emus receiving supplement (S-S and S-W) with the control (W-W) confirmed that nutrient supplement treatment resulted in significantly (P = 0.04) lower TS values for ALT activity (52 ± 22 IU/L, 142 ± 31 IU/L, respectively).

The overall increase in plasma bicarbonate concentrations at slaughter (OS) was significantly (P<0.01) lower in the emus in S-S group (1.0 ± 1.7 mMol/L), when compared to those in S-W and W-W groups (both with 1.8 ± 1.8 mMol/L). None of the hematological parameters were significantly affected by supplement treatment.

Table 4.7 Effect of nutrient supplement on plasma creatine kinase activity (IU/L) of emus transported for 6 hours.

	Nutrient Supplement*		
CK activity[1, 2, 3]	W-W	S-W	S-S
Pre-transport	340 ± 40[a]	278 ± 40[a]	330 ± 42[a]
TS (N = 18)	4104 ± 708[a]	4037 ± 708[a]	1731 ± 754[b]
LS (N = 18)	17437 ± 4346[a]	16405 ± 4349[a]	7893 ± 4462[b]
OS (N = 42)	12433 ± 2409[a]	12377 ± 2493[a]	5326 ± 2493[b]

*W-W = Control; no nutrient supplement given. S-W = nutrient supplement given only pre-transport. S-S = nutrient supplement given both pre- and post-transport.
[1] TS = Changes during transport (post-transport value - pre-transport value). LS = Changes during lairage (post-transport value - at slaughter value). OS = Overall changes (at slaughter value - pre-transport value). A positive value would indicate an increase, while a negative value would indicate a decrease in AST activity.
[2] Means within a row followed by different superscripts were significantly (P<0.05) different.
[3] Data were power ($X^{0.1}$) transformed.

4.4.5.3 Sexual dimorphism

Our preliminary analysis indicated that there was no significant interaction between Sex and Experiment. Data from Experiments 1 and 2 were therefore pooled to have a better assessment of sex differences. Females had significantly higher pre-transport concentrations of total protein, albumin, globulin, TG and creatinine than males (Table 4.8). While females had significantly higher plasma TG than males at pre-transport, their TG decreased significantly (P<

0.001) from pre-transport to slaughter. There was no significant difference between the sexes in plasma TG level at slaughter (Table 4.9). There was no significant sex difference in any of the hematological or enzyme parameters. Among plasma electrolytes, females had significantly ($P < 0.001$) higher pre-transport calcium concentrations than males, but they also had significantly ($P < 0.05$) greater reduction in calcium concentration (OS levels; -1.1 ± 0.3, -0.1 ± 0.3 mMol/L, respectively) than males during transport and lairage.

Table 4.8 Sexual dimorphism of pre-transport blood parameters in emus at the beginning of the breeding season.

		Pre-transport values (n = 42)		
		Males	Females	P value
TP[1]	(g/L)	49.9 ± 0.9	55.9 ± 1.1	P<0.001
Albumin[1]	(g/L)	27.9 ± 0.5	30.0 ± 0.7	P<0.05
Globulin [1]	(g/L)	21.9 ± 0.7	25.9 ± 0.8	P<0.001
A/G ratio		1.29 ± 0.04	1.17 ± 0.04	P<0.05
TG[2]	(mMol/L)	2.0 ± 0.8	6.9 ± 0.9	P<0.001
Creatinine[3]	(mMol/L)	6.1 ± 0.9	8.8 ± 1.0	P<0.001
Calcium[2]	(mMol/L)	3.4 ± 0.3	5.7 ± 0.4	P<0.001

[1]One outlier eliminated.
[2] Data were square-root transformed.
[3] Data were power ($X^{0.1}$) transformed.

4.4.5.4 Relationship among variables

The following correlations between variables were significant ($P< 0.05$). The injury scores were positively correlated to the blood glucose concentrations at slaughter (r = 0.53). The total injury scores were positively correlated to ALT activity after transport (r = 0.59), and the blood glucose concentrations at slaughter (r = 0.64). The increase in body temp (TS) was positively correlated to the following variables measured after transport: body weight (r = 0.54), plasma creatinine concentrations (r = 0.71), creatinine/UA ratio (r = 0.56), A/G ratio (r = 0.50), sodium (r

= 0.47). The ALT activity at slaughter was positively correlated with plasma UA concentrations (r = 0.47) and negatively to TP (r = -0.51) and albumin concentrations (r = - 0.54) at slaughter. The plasma CK activity at slaughter was highly correlated with AST activity (r = 0.85) and UA concentrations (r = 0.65) at slaughter. Blood glucose (r = 0.4; P <0.03) was significantly correlated with pre-transport body weight.

Table 4.9 Sexual dimorphism in serum triglyceride concentration (mMol/L) changes in response to handling and transport[*].

Plasma TG[1]	Males (N =10)	Females (N = 8)
Pre-transport	1.8 ± 0.8^{b}	8.4 ± 1.2^{a}
Post-transport	1.3 ± 0.8^{b}	2.8 ± 0.4^{ab}
At slaughter	0.6 ± 0.9^{b}	1.5 ± 0.3^{b}

[*]Means followed by different superscripts were significantly (P<0.001) different.
[1]Data were square-root transformed.

4.4.6 Discussion

Emu farms are often located in areas lacking slaughtering facilities that can process these birds, which necessitates their transportation to suitable facilities. During shipment emus are exposed to potentially stressful and adverse conditions, which can significantly impact their welfare. However, the welfare of emus during transportation has received little or no attention. This study is the first to examine the physiological responses of emus to transport. I also assessed the efficacy of using a nutrient supplement to alleviate the effects of the stress of transport.

The handling and sampling procedures used in this study could be stressful to emus, so every effort was made to minimize handling. The two experiments differed principally with respect to the weather conditions during transport (warm weather in Experiment 1 and cool weather in Experiment 2), and the mode of administration of the nutrient supplement pre-transport (added to the diet in Experiment 1 and tube-fed to individual emus using a modified calf feeder in Experiment 2). Temperature during transport was likely a major stressor to the emus, as a greater proportion of birds in Experiment 1 showed signs of heat stress (open-beak breathing, tachypnea, gular flutter) than in Experiment 2, and the birds were more agitated. Furthermore, the emus lost more body weight during transport in Experiment 1 than in Experiment 2, but regained similar amounts during resting in lairage. I did not collect blood samples from the emus after unloading from transport in Experiment 1, as I did not want to stress them further. Thus I cannot fully differentiate between the effects of transport and lairage separately in Experiment 1.

4.4.6.1 Effect of transport and lairage

Animals face many stressors during transport, including catching and handling, loading and unloading, novel environment, vibrations and jarring in the transport compartment, and exposure to extremes of meteorological and microclimate conditions (Wotton and Hewitt, 1999; Minka and Ayo, 2008). These factors stimulate the release of stress hormones (Sapolsky et al., 2000), which in turn cause changes in biochemical variables such as glucose and TG, in an effort to maintain homeostasis (Remage-Healey and Romero, 2001; Minka and Ayo, 2008; Huff et al., 2008; Voslarova et al., 2011).

4.4.6.1.1 Metabolites

In this study, blood glucose concentrations were elevated during transport (Table 4.2) probably by the actions of catecholamines and GCs (Nijdam et al., 2005; Bedanova et al., 2007; Zhang et al., 2009; Vazquez-Galindo et al., 2012). Emus with greater injury scores had significant greater increases in blood glucose concentrations as a part of the stress response. TGs are the major energy source in birds (Smith et al, 1999) and their decrease after transport was indicative of nutritional stress from feed withdrawal and gluconeogenesis (Remage-Healey and Romero, 2001; Huff et al., 2008). This reduction continued till slaughter, indicating the reliance on TGs as an energy source (Delezie et al., 2007), as the emus did not eat while in lairage.

Plasma creatinine concentration did not change during transportation, but was significantly greater at slaughter in both experiments. Increased creatinine concentration is indicative of either muscle protein turnover (Hochleithner, 1994), dehydration (Harr, 2002), or renal dysfunction (Giovannini et al., 2006). UA is increased in protein catabolism. PCV and TP are indicators of blood volume and should be high in animals suffering dehydration. Since there were no significant changes in UA, PVC and TP in my study, I could rule out dehydration as a cause for increased plasma creatinine (Hochleithner, 1994). Handling and transport causes muscle damage and lysis of muscle cell walls (Wotton and Hewitt., 1999; Petracci et al., 2010), releasing myoglobin pigments which accumulate in the renal tubules, resulting in kidney dysfunction

(Perelman, 1999; Huerta-Alardín et al., 2005) and elevated creatinine concentrations. Furthermore, the UA/creatinine ratio was significantly reduced after transport in Experiment 2, suggesting mild degree of renal dysfunction in the emus (Lumeij, 1987; Hochleithner, 1994).

4.4.6.1.2 Enzymes

The CK activities in ratites are generally higher than in other birds (Doneley, 2006). The activities of the enzymes AST, ALT, and CK in plasma increase significantly following handling, loading and transportation in many species (Scope et al., 2002; Kannan et al., 2003; Maria et al., 2004; Huff et al., 2008; Baird et al., 2012) due to increased permeability of muscle cell membranes (Williams and Thorne, 1996). In this study, their concentrations were significantly elevated at slaughter indicating physical stress and muscle damage in the emus after transport and lairage. Highly elevated CK activities occur in exertional rhabdomyolysis (Veenstra et al., 1994; Huerta-Alardín et al., 2005; Walid, 2008; Linares et al., 2009), a condition to which ratites are susceptible (Tully et al., 1996; Smith et al., 2005; Minka and Ayo, 2008) (see discussion below).

4.4.6.1.3 Corticosterone

CORT is the major stress hormone in ratites (Wolmarans, 2011; Lèche et al., 2013). CORT concentrations should be interpreted with respect to the pre-transport values and other variables, as wide variations are possible due to the pulsatile release (Romero and Reed, 2008). Increased CORT concentrations after transport have been reported in chickens (Nijdam et al., 2005; Delezie et al., 2007; Zhang et al., 2009), ostriches (Mitchell et al, 1996; Wolmarans, 2011), and rheas (Lèche et al., 2013). In this study, CORT concentrations of emus were significantly increased at slaughter in Experiment 1, likely reflecting the increased stress experienced by these birds during transport under warm weather conditions (Giloh et al., 2012). As stated above, a greater proportion of emus in Experiment 1 were heat stressed and agitated, but as I did not collect blood samples from the emus in Experiment 1 after unloading, I could not confirm elevated plasma CORT concentrations in these birds during transport. Plasma CORT level was elevated 40-fold in rheas after transport for 30 min (Leche et al., 2013), but only two-fold in ostriches after transport for 4.5 hours (Mitchell, 1999). This may indicate that, in ratites, elevation of CORT peaks early at the beginning of the transport process and shows large temporal variation. Elevated CORT concentrations were not observed after 6 hours of transport in Experiment 2. I might have missed the early peak of elevation when the emus were bled after unloading. Alternatively, I can interpret that the birds in Experiment 2 were less stressed than the birds in Experiment 1.

4.4.6.1.4 Body weight

Weight loss during transport may be due to the loss of intestinal contents, electrolyte imbalances, dehydration and/or loss of tissue mass (Richardson, 2005; Schwartzkopf-Genswein et al., 2007; Minka and Ayo, 2011). The decrease in body weight in Experiment 2 presumably reflects the loss of intestinal contents (Orlic et al., 2007) as the birds showed no signs of dehydration. The body weight loss of emus during transport in Experiment 1 nearly doubled that in Experiment 2 and likely reflects higher evaporative losses and dehydration (Vošmerová et al., 2010) in addition to the loss of intestinal contents. In both experiments, emus recovered similar amounts of body weight while resting at lairage. Weight loss is detrimental to producers, since it affects the quality and quantity of the meat and results in economic loss.

4.4.6.1.5 Body temperature

Body temperature increases during transport may be due to metabolic imbalances in response to stress (Elrom, 2000; Warriss et al., 2005). This increase reflects a major transport welfare issue of ratites (Piccione et al., 2005; Crowther et al., 2003; Minka and Ayo, 2008), particularly in emus, with large subcutaneous fat deposits (Kim et al., 2012) that insulate and prevent dissipation of the heat load. In this study, the increase in body temperature was correlated to both the size of the emu. The increase in body temperature after transport was high in Experiment 1 probably due to higher ambient temperature and relative humidity (Kettlewell et al., 2003; Yalcin et al., 2004).

4.4.6.1.6 Hematology

The elevation of total WBC and heterophil counts of emus after transport in Experiment 2 may be due to spleenic and bone marrow responses (Minka and Ayo, 2008; Wolmarans, 2011). Inflammation following injuries during handling and transport has also been reported in ostriches (Davis et al., 2008; Wolmarans, 2011). The increase in H/L ratio of emus was significant only in Experiment 2. This may reflect increased stress level due to the additional handling of the emus for tube feeding of supplement and additional post-transport blood sampling in Experiment 2. The H/L ratio has been used as an indicator of chronic stress in poultry (Smith et al., 2004;

Bedanova et al., 2007; Singh et al., 2009; Gross and Siegel, 2012). Our results suggest that H/L ratio, in combination with other indicators, can be used to assess handling and transport stress in emus (Scope et al., 2002; Moneva et al., 2009) as in the case of ostriches (Kamau et al., 2002; Minka and Ayo, 2008; 2011).

4.4.6.1.7 Electrolytes

Dehydration during transport causes increases in plasma electrolyte concentrations (Bilgili et al., 2006; Gonzalez et al., 2007 Vazquez-Galindo et al., 2012). With the exception of plasma chlorides, electrolyte concentrations were unchanged at slaughter, thus confirming that the emus in Experiment 2 were not suffering from dehydration. Bicarbonate concentrations often decrease during heat stress, due to the loss of carbon dioxide with rapid, deep respiration (Debut et al., 2005). In our study, the decrease in plasma bicarbonates during transport returned to pre-transport concentrations at slaughter, confirming the benefit of resting emus at lairage after transport.

Resting emus at lairage was beneficial as evidenced by the marginal regaining of body weight, and the lowering of glucose, WBC, heterophil counts and improvements in bicarbonate.

4.4.6.1.8 Behavioral observations

Open-beak breathing, tachypnea (rapid breathing), and gular flutter observed in the emus have also been reported in transported ostriches (Wotton and Hewitt, 1999), and are associated with heat stress. Heavy panting in emus results in arterial hypoxemia, which affects cell functions and vitality (Jones et al., 1983). These behaviors were more conspicuous in emus transported during warm weather due to the build-up of humidity and temperature inside the compartment (Piccione et al., 2005). The significantly higher hyperthermia in these emus further confirms the allostatic load on them. There were also more severe injuries (category 4-5, Table 1) in Experiment 1 (Figure 1), as more emus sat down (probably due to exhaustion) and were trampled.

4.4.6.1.9 Injuries

Injuries during transport were a result of trampling by other birds (Wotton and Hewitt, 1999; Glatz, 2011; Meyer et al., 2002), and jarring against the compartment walls. Feather losses were caused by handling, rubbing against each other or rough surfaces (Wotton and Hewitt, 1999; Orlic et al., 2007) and possibly stress molting (Moller et al., 2006). This revealed the need for better handling techniques and provision of smooth walls inside the truck compartments while transporting emus.

4.4.6.2 Efficacy of nutrient supplements in alleviating stress

In beef cattle and ostriches, the hypotonic concentrations of electrolytes, energy source (dextrose), and amino acids in the nutrient supplement (Schaefer et al., 1995; 1997; 2001; Arp et al., 2011) provided a protective effect on hepatic and muscle cell walls. Our findings (from Experiment 2) confirmed the need for administering nutrient supplements pre- and post-transport to reduce gluconeogenesis and glycogenolysis (Schaefer et al., 2001). The supplement when given before and after transport was successful in reducing muscle damage, resulting in significantly less leakage of the enzymes AST and CK. Our findings also showed that supplementing both pre- and post-transport was more effective than pre-transport treatment alone in alleviating stress in emus.

Emus are usually shipped to the processing plant at the beginning of the breeding season, when they have the thickest layer of subcutaneous fat. Unfortunately that is the same time when they have reduced their feed intake. Nutrient supplement has to be administered individually by gavaging but this may increase handling stress. Administering the nutrient supplement via drinking water may be a viable compromise.

4.4.6.3 Rhabdomyolysis

The overall clinical indicators of transport stress in emus from our study included hyperthermia, increased CORT concentrations, metabolic imbalances (increased glucose concentration, decrease TG concentration , increased WBC count and H/L ratio), cell damages (elevated ALT, AST and CK activities) and renal dysfunction (decreased UA/creatinine ratio). These clinical signs are suggestive of exertional rhabdomyolysis or capture myopathy, a condition to which ratites are highly susceptible (Tully et al., 1996; Smith et al., 2005; Minka and Ayo, 2008). Clinical findings of exertional rhabdomyolysis may range from asymptomatic increase in the CK activity, to paralysis and death. Very high increases (>10x) in CK activity (Nicholson et al., 2000;Hofle et al., 2004; Paterson, 2007) and reduced blood UA/creatinine ratio are pathognomonic to exertional rhabdomyolysis (Walid, 2008; Linares et al., 2009). Pathogenesis of this condition is by striated muscle damage (Baird et al., 2012), leading to leakage of cell contents into the blood stream (Paterson, 2007), increased AST and CK activities

(Harr, 2005; Marco et al., 2006), heterophilic leukocytosis (Smith et al., 2005), hyperthermia, hyperglycemia (Nicholson et al., 2000), electrolyte imbalances, acute renal failure, shock and intravascular coagulation (Huerta-Alardín et al., 2005; Linares et al., 2009; Baird et al., 2012) resulting in brain damage (Wotton and Hewitt, 1999). Pursuit, catching, restraint and transportation have been reported to cause this condition in a variety of birds, including red-legged partridges (*Alectoris rufa*; Hofle et al., 2004), eastern wild turkeys (*Meleagris gallopavo silvestris;* Nicholson et al., 2000), little bustards (*Tetrax tetrax*; Marco et al., 2006; Ponjoan et al., 2008), and ratites (Tully et al., 1996; Smith et al., 2005).

Cases of rhabdomyolysis were confirmed by examination of leg and thigh muscles of the transported emus (Menon et al., in prep). Hence this study confirmed the rhabdomyolytic changes occurring in emus during handling and transport. Immediate care should be provided to non-ambulatory emus, to ensure their welfare. Predisposition to exertional rhabdomyolysis warrants careful handling and transporting of emus. This again confirms the need for improved conditions inside the truck and minimal handling to avoid physical injuries. This issue should be of serious concerns to the producers as injuries and rhabdomolysis will cause down-grade of fat and meat.

4.4.7 Conclusions and recommendations

Emus in this study were transported for six hours and experienced elevated body temperature, weight loss, metabolic imbalances, and altered immune status, in addition to injuries. The clinical picture is suggestive of muscle injury and point to the incidence of exertional rhabdomyolysis. Resting overnight after transport helped improve the welfare of emus to some extent and hence is recommended. Administration of electrolytes and nutrients supplements to emus prior to and after transport helped to alleviate the stress imposed on them. Emus should be transported during night or during cooler weather to avoid thermal stress. Improved trailers or air sprung trucks to minimize vibrations, ramps and chutes to facilitate easier loading and unloading are needed. Better loading and unloading techniques to avoid injuries; combined with minimal and careful handling to avoid instances of exertional rhabdomyolysis is also recommended.

5. CARCASS YIELDS AND MEAT QUALITY CHARACTERISTICS OF ADULT EMUS TRANSPORTED FOR SIX HOURS BEFORE SLAUGHTER

5.1 Introduction

In North America, the age at which emus are slaughtered depends on which market (fresh meat verses oil) the producer primarily supplies into. Typically yearlings are used to supply the market for fresh meat, while older emus that have completed at least one breeding season are slaughtered for their fat to supply the oil market (see Chapter 2). Because of lack of promotion and lack of quality standards, the meat from older emus is sometimes sold as pet food or processed into value added products such as salami, pepperoni and sausages. Previous studies have reported carcass yields and meat quality of younger emus (Berge et al., 1997; Dingle, 1997; Frapple, et al., 1997; Sales et al., 1999; Blake and Hess, 2004), but adult birds have received little attention.

Many emu farms are located in areas lacking slaughtering facilities that can process these birds (Michael, 2000). Emu producers often ship their flocks to slaughter plants at distant locations in order to get emus slaughtered at an inspected abattoir. Prolonged shipment exposes animals to stressful and adverse conditions (Mitchell and Kettlewell, 2009) which can result in physiological changes, and injuries (Smith et al., 2004), often adversely affecting meat quality (Ali et al., 2008). Emus are highly susceptible to transport stress (See Chapter 4), and the effect of transport on emu carcass yields and meat quality has not been reported.

Several studies have found that nutrient (electrolyte, dextrose, and amino acids) therapy (pre- or post-transport) helped to mitigate transport stress and improve meat quality (Schaefer et al., 1995; Onenc, 2010). Providing emus with an oral nutrient supplement before and after transport was effective in protecting them against muscle damage and allowed for a greater recovery of body weight losses while resting in lairage. Hence, this study was undertaken to determine

(1) the effects of transport stress, and

(2) the impact of a nutrient supplement on the carcass yields and meat quality of adult emus.

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[1]A version of Chapter 4 has been submitted for publication as
Menon, D. G., D. C. Bennett, B. Uttaro, A. L. Schaefer, and K. M. Cheng. 2013. Carcass yields and meat quality characteristics of adult emus (*Dromaius novaehollandiae*) transported for six hours before slaughter.

5.2 Materials and methods

This study was conducted in collaboration with a commercial emu producer in Saskatchewan, Canada and was approved by the University of British Columbia Animal Care Committee, under permits # A10-0183 and # A10-0184.

5.2.1 Experimental birds and management

See Sections 3.2.2, 4.2.2, and 4.4.2 for details on experimental birds and their management.

5.2.2 Nutrient supplement treatments

See Section 4.4.3 for details on nutrient supplement treatments.

5.2.3 Transport, lairage, slaughter and blood collection

See Sections 4.2.3, 4.2.5, 4.2.6 and 4.4.4 for details on transport, lairage and slaughter. The two experiments were under taken in the same trailer, for the same duration and same period of the year (first week of November). After resting at lairage for 15 hours, emus were weighed and slaughtered. Emus were subjected to percussion stunning (penetrating type captive bolt stun pistol- Cash Special 22Cal, Talford St.Works, Ashton, Birmingham, London, UK; Experiment 1) and decapitation (Experiment 2). They were immediately suspended by the feet and exsanguinated by a complete ventral cut across the neck, including carotid artery and jugular vein. In both experiments, trunk blood was collected into tubes containing heparin (BD vacutainer® plus) during exsanguinations for estimating selected analytes (see Sections 3.2.4 and 4.2.7 and). The carcass was plucked of feathers, eviscerated, and the subcutaneous fat over the back and the retroperitoneal (abdominal) fat removed and collected.

5.2.4 Carcass and meat quality measurements

Injuries were assessed using a six point scale based on the severity of bruising and number of wounds on the carcass (0 = none to 5 = severe; see Section 4.2.9.1.9.). The location of bruises on the carcass was recorded. The organ and fat weights were recorded. The loss in body weight at slaughter, dressing percentage, fat and organ yields (Appendix E) were calculated. Samples (200 g) of the *iliofibularis* (emu thigh fan) and *gastrocnemius medialis* (emu inside leg) muscles on the left side of the carcass were collected and stored overnight at 2°C.

The pH of the muscle samples were measured in triplicate at 45 minutes (pH_{45}) and 24 hours (pH_{24}) post-slaughter. An Accumet AP72 (Cole-Parmer Canada Inc. Buchan St., Montreal, QC, Canada) with temperature compensation probe (Mettler Toledo Spear tip Electrode, Mississauga, ON, Canada) was used.

For water holding capacity (drip loss; DL) estimation, weighed steaks (approximately 100 g) were placed in pairs on retail styrofoam tray with a dry-lock pad (Ultra ZAP Soakers, Paper-Pak Industries, La Verne, CA), overwrapped with oxygen permeable wrap, and stored at 2^{o}C. Drip loss was estimated after 24 hours (DL_{24h}) and 5 days of storage (DL_{5d}), and was expressed as a percentage of the initial sample weight.

Meat color was measured in triplicate at 24 hours post-slaughter, after the steaks were permitted to bloom for 30 min. A Minolta spectrophotometer (Model: CM2002, Illuminant C, 2 degree observer, 8 mm diameter viewing area, SCI, quartz glass cover; Advanced Test Equipment Corp, Roselle Street, San Diego) was used. The results were expressed in terms of lightness (L*), redness (a*) and yellowness (b*). The chroma [C*; $(a^{*2} + b^{*2})^{1/2}$] and hue angle [h^{o}; (TAN^{-1} b*/a*)] were calculated in the CIELAB color space model (De L'Eclairage-Cie, 1978). If a* <0, hue = 180 + TAN^{-1} (b*/a*). If a* >0 but b* <0, hue = 360° - TAN^{-1} (b*/a*) (Warriss, 2000). Thigh and leg muscle samples from seven selected birds (Experiment 1) were observed for visual color and texture (dryness) changes over 5 days of storage at 2^{o}C. Fat color was measured on a Hunterlab Labscan Spectrocolorimeter (Model LS/3685, Sunset Hills Road, Reston, Virginia) using CIELAB color space model (L*, a* and b*).

5.2.5 Statistical analysis

All the statistical analyses were carried out using SAS (edition 9.2; SAS Institute Inc., 2007). The residuals of variables were tested for normality using Shapiro-Wilk test, and Bartlett's test of sphericity for equal variance. Body weight loss, spleen weight, leg muscle color (L*, b*), and fat color were log transformed to meet the assumptions, but the mean non-transformed values ± standard error are presented. The significance level was set at $\alpha = 0.05$. Post-hoc mean separation was done using students t-test. The untransformed and transformed data were subsequently analysed using the following statistical model:

$$Y_{ijklm} = \mu + B_k + S_i + N_j + SN_{ij} + B(SN)_{k(ij)} + O_{l(ijk)} + R_{m(k)} + RS_{mi(k)} + RN_{mj(k)} + RSN_{mij(k)} + OR_{lm}(_{ijk})$$

where, B = effect of year (experiments; k = 2010, 2011); S = effect of sex (i = male, female); N = effect of nutrient supplement (j = S-S, S-W, W-W); SN = 2-way interaction; B(SN) = experimental error term to test the main effects and their interactions; $O_l(_{ijk})$ is the first sampling error term to test the block effect; R = effect of time of measurement (m = l = 45 minutes post-slaughter Experiment 1, 2 = 24 hours post-slaughter Experiment 1, 3 = 5 days post-slaughter in Experiment 1, 4 = 45 minutes post-slaughter Experiment 2, 5 = 24 hours post-slaughter in Experiment 2, and 6 = 5 days post-slaughter Experiment 2); RS and RN are the two way interactions nested within the year; and RSN is the three way interaction nested within the year. These four effects were tested with the second sampling error term $OR_{lm}(_{ijk})$. Post-hoc mean separation was done using Student's t-test (corrected for experiment wise error). Relationships between selected blood variables (See Chapter 4) and meat quality traits were determined using Pearson correlation analysis. The effect of injuries on meat quality characteristics was assessed by ANOVA after reclassifying injury scores into two categories: bruises only, and bruises and wounds.

5.3 Results

The nutrient supplement did not significantly affect meat quality, nor did it interact significantly with sex or length of storage.

5.3.1 Carcass yields

The carcass yields in both experiments from the two sexes are presented in Table 5.1. In both experiments, males had significantly more fat than females (12.43 ± 0.56 kg verses 9.50 ± 0.61 kg, P = 0.002). Hot carcass yield (excluding back and abdominal fat) and dressing percentage (including back fat) were significantly greater (P = 0.006 and P < 0.001, respectively) in Experiment 2 (48.80 ± 0.68 % and 74.58 ± 0.77 %, respectively) than in Experiment 1 (46.01 ± 0.58 % and 69.7 ± 0.59 %, respectively). There was no significant between sex difference in organs weights, and the mean weights (g) of heart, liver, kidney, spleen, and testes were 371 ± 37, 467 ± 71, 122 ± 24, 17± 1, and 72 ± 13, respectively. The fat yield was positively correlated to body weight at slaughter (r = 0.75, P = 0.05).

5.3.2 Injuries and carcass quality

None of the birds had any injuries before transport. See Section 4.2.9.1.9 for details on injuries in emus after transport. In Experiment 1, most of the emus appeared exhausted after transport, and two-thirds showed open mouth breathing, while in Experiment 2 only four birds showed this behavior. There were 6 and 3 emus with orange to red colored fat in the two experiments, respectively.

Table 5.1. Comparison between the carcass yields of male and female emus[1].

Variables	Weight (Kg)		
	Females (n = 18)	Males (n = 24)	P-value
Body weight (pre transport)	45.1 ± 1.1	45.5 ± 1.0	Not significant
Body weight (at slaughter)	43.8 ± 1.2	44.1 ± 1.0	Not significant
Back fat	7.3 ± 0.5	9.3 ± 0.4	<0.01
Abdominal fat[1]	2.3 ± 0.3	3.1 ± 0.2	0.05
Dressed carcass	21.3 ± 0.5	20.2 ± 0.4	Not significant
Meat yield	13.2 ± 0.3	12.5 ± 0.3	Not significant
Bone weight	8.0 ± 0.2	7.6 ± 0.2	Not significant
Gastro intestinal tract	3.4 ± 0.1	3.5 ± 0.1	Not significant

[1] Data subjected to power transformation to meet the assumption for equal variance.

5.3.3 Meat quality characteristics

5.3.3.1 pH

The pH_{45} and pH_{24} of both muscles were significantly higher in Experiment 1 than Experiment 2 (Table 5.2). The pH_{24} was significantly ($P < 0.05$) correlated with the pH_{45} in both leg ($r = 0.70$) and thigh ($r = 0.34$) muscles (Figure 5.1). The pH in thigh muscles decreased significantly during storage in both experiments. In leg muscles there was a significant reduction in pH only in Experiment 1.

Thigh muscle pH_{24} showed an inverse relationship with thigh L* (r = -0.43, P < 0.005) (Figure 5.2) but this was not significant in leg muscles. Thigh and leg muscle pH_{45} was significantly (P < 0.001 and P < 0.01, respectively) higher in emus with wounds than those with bruises alone (thigh: 6.84 ± 0.11 versus 6.39 ± 0.04; leg: 6.95 ± 0.11 versus 6.40 ± 0.04). Thigh muscle pH_{24} was significantly (P < 0.01) higher in emus with wounds than those with bruises alone (6.57 ± 0.17 versus 5.98 ± 0.05), but there was no significant difference between the categories with respect to leg muscle pH_{24}. Overall injury scores were positively correlated with thigh and leg muscle pH_{45} (r = 0.48 and r = 0.34, P < 0.05, respectively) and with thigh pH_{24} (r = 0.35, P < 0.05).

Table 5.2. Changes in pH in emu thigh and leg muscles during 45 minutes (pH_{45}) and 24 hours (pH_{24}) of storage[1].

Time of measurement		Muscle	
		Thigh	Leg
pH_{45}	Experiment 1	6.56 ± 0.04^{A}	6.77 ± 0.04^{A}
	Experiment 2	6.31 ± 0.05^{B}	6.02 ± 0.05^{C}
pH_{24}	Experiment 1	6.14 ± 0.05^{C}	6.38 ± 0.05^{B}
	Experiment 2	5.90 ± 0.09^{D}	5.97 ± 0.07^{C}

[1]Data subjected to power transformation to meet the assumption of equal variance.
$^{A–D}$Means with the same superscript within a column do not differ significantly P < 0.01.

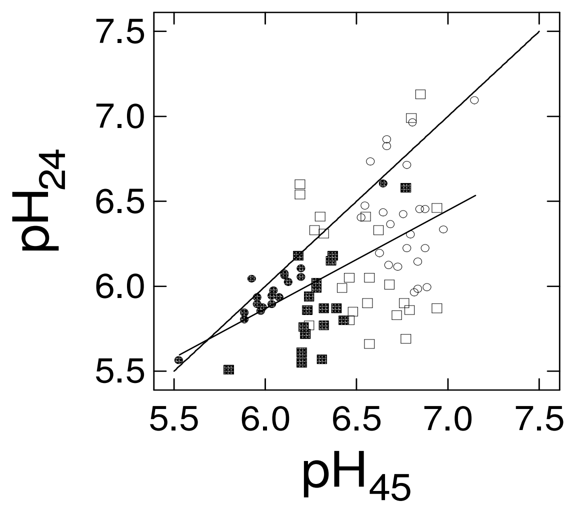

Figure 5.1. Relationship between pH of emu thigh (□) and leg (○) meat at 45 minutes (pH_{45}) and 24 hours (pH_{24}) post-slaughter ($r = 0.53$, $P < 0.03$).

Experiment 1 (open symbols), Experiment 2 (closed symbols).
Line of equality and regression line are plotted

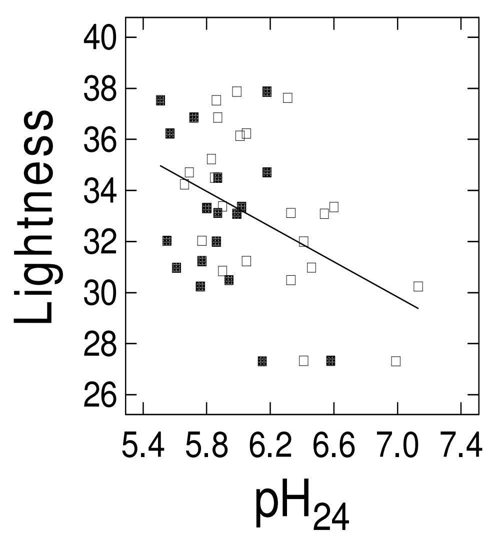

Figure 5.2. Relationship between lightness (L) and pH of emu thigh meat at 24 hours (pH_{24}) post-slaughter($r = -0.43$, $P < 0.005$).

Experiment 1 (open symbols), Experiment 2 (closed symbols).

5.3.3.2 Drip loss

The DL_{24} in Experiment 2 was significantly ($P < 0.01$) lower than that in Experiment 1, and storage significantly ($P < 0.001$) increased DL_{24} in both experiments (Figure 5.3). There was no significant difference between muscle DL_5, for either experiments. DL_5 in thigh muscle was significantly ($P = 0.001$) higher in Experiment 1 than Experiment 2. DL_{24} was correlated ($r = 0.66$, $P < 0.0001$) with the loss of body weight (%) at slaughter (Figure 5.4). Drip loss, pH, meat and fat color were not affected by sex and there were no significant interaction between sex and experiments.

5.3.3.3 Color

The color parameters of both thigh and leg muscles at 24 hours are given in Table 5.3. Lightness (L^*) ranged from 26 to 39 and did not vary significantly between muscles or experiments. The redness (a^*) values varied from 3 to 16 and yellowness (b^*) from -4.2 to 18.3. Redness (a^*) of the thigh muscles was significantly higher ($t = 2.88$, $P = 0.01$) in Experiment 1, while b^* was significantly higher ($t = -8.27$, $P = <0.0001$) in Experiment 2. In leg muscles, a^* was significantly higher ($t = 3.63$, $P = 0.003$) in Experiment 1, while b^* was significantly higher ($t = -4.17$, $P = 0.001$) in Experiment 2 than Experiment 1. The chroma (C^*) for thigh and leg meat was not significantly different between the two experiments. The hue angle (h^o) was significantly higher in Experiment 1 in both thigh ($t = 5.41$, $P < 0.001$) and leg meat ($t = -8.03$, $P < 0.001$). Both the thigh a^* and b^* ($r = 0.88$; $P < 0.05$) and leg a^* and b^* ($r = 0.88$, $P < 0.05$) showed high positive correlation.

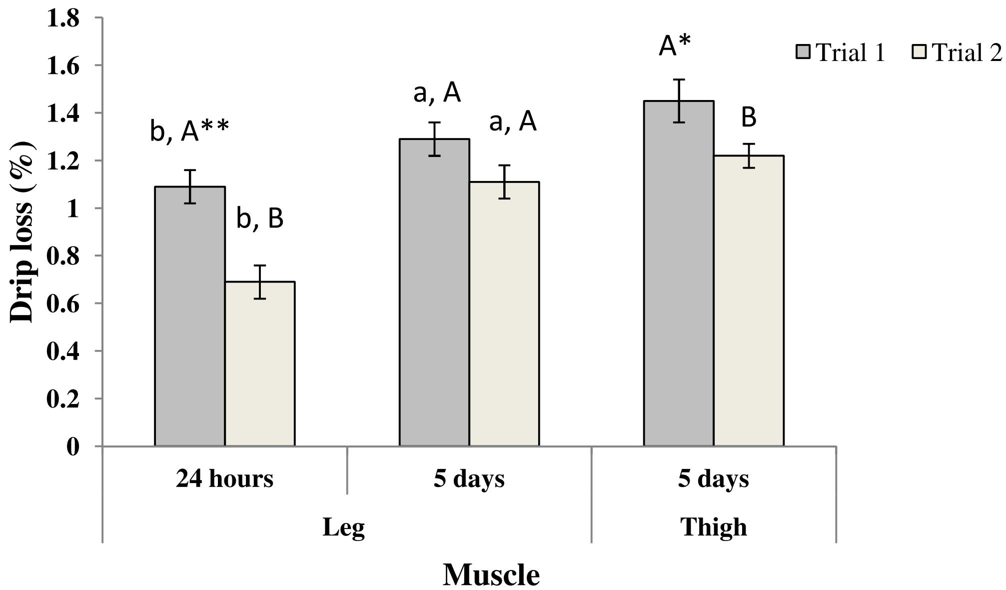

Figure 5.3. Changes in the drip loss (%) in emu meat at 24 hours (DL_{24}) and 5 days (DL_5) of storage[1,2].

[1]No significant difference between muscles within a Experiment at the same period of storage.
[2]Data subjected to power transformation to meet the assumption of equal variance.
[a–b]Means without the same superscript between time periods differ significantly (P < 0.001).
[A-B]Means between experiments without the same superscript in the same muscle differ significantly
* P < 0.01, **P < 0.001.

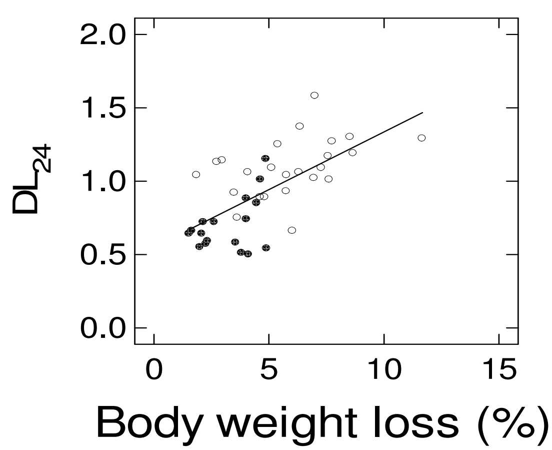

Figure 5.4. Relationship between 24 hours drip loss of emu leg meat (DL_{24}) and the loss of body weight (%) at slaughter (r = 0.66, P < 0.0001).

Experiment 1 (open symbols), Experiment 2 (closed symbols).

Table 5.3. Color L*a*b*, hue angle, and chroma recorded in emu leg and thigh meat at 24 hours of storage[1,2].

Color parameter	Thigh		Leg	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
L*	33.4 ± 0. 9	33.9 ± 1.2	33.2 ± 0. 6	31.4 ± 0.7
a*	11.94 ± 0.41^{a}	10.02 ± 0.53^{b**}	10.64 ± 0.55^{a}	7.38 ± 0.71^{b**}
b*	-0.40 ± 0.39^{b***}	4.85 ± 0.50^{a}	1.40 ± 0.85^{b**}	2.43 ± 1.11^{a}
Chroma (C*)	12.06 ± 0.46	11.74 ± 0.53	10.83 ± 0.83	9.22 ± 0.95
Hue angle (h^o)	202 ± 16^{a}	64 ± 18^{b***}	264 ± 12^{a}	77 ± 13^{b***}

[1] Comparisons are made between Experiment 1 and 2, within the muscle type only.
[2] Lightness (L*), redness (a*) and yellowness (b*), chroma [C*; $(a^{*2} + b^{*2})^{1/2}$] and hue angle [h^o; (TAN^{-1} b*/a*)].
[a-b] Means without the same superscript within a row in the same variable on the same muscle differ significantly **P < 0.01, ***P < 0.001.

The appearance of regular and defective types of emu meat is presented in Figure 5.5. Observation of changes in meat color and texture from day 1 to day 5 in Experiment 1 revealed that samples with mid-way pH_{24} (6.2 to 6.5) maintained color and kept well during storage (Figure 5.5A). The samples with highest pH_{24} (7-7.1) were darker and became drier with storage indicating "dark, firm and dry" (DFD) condition (Figure 5.5B). A sample from thigh muscle that was very light in color remained pale and friable during storage (Figure 5.5C). The samples with low pH_{24} (5.6 to 5.8) became darker (brownish) and unsightly by the fourth day of storage.

5.3.4 Fat color

The CIELAB color coordinates (L*, a* and b*) of back fat did not differ significantly between Experiments 1 and 2, and averaged L* = 68.41 ± 0.63, a* = 6.03 ± 0.42, and b* = 19.62 ± 0.55. Samples showing higher a* values (8-16) in fat was an indication of splashing of blood. There were 14 emus in Experiment 1 with splashing of blood in fat, whereas in Experiment 2, there were only 6 such cases.

5.3.5 Relationship between variables

The following relationships between variables were found to be significant at $P < 0.05$. The blood glucose concentration at slaughter was positively correlated to thigh muscle pH_{45} ($r = 0.32$), and to leg muscle pH_{45} ($r = 0.59$), pH_{24} ($r = 0.38$) and L* ($r = 0.44$). Similarly, plasma ALT concentration at slaughter was positively correlated to thigh muscle pH_{45} ($r = 0.32$), and to leg muscle pH_{45} ($r = 0.62$), pH_{24} ($r = 0.38$), DL_{24} ($r = 0.69$), and DL_5 ($r = 0.31$). The plasma AST concentration was positively correlated to thigh muscle pH_{24} ($r = 0.35$), and to leg muscle pH_{24} ($r = 0.32$). The H/L ratio was negatively correlated to DL_{24} ($r = -0.42$), to thigh and leg pH_{45} ($r = -0.36$ and $r = -0.55$), and to leg pH_{24} ($r = -0.41$).

5.4 Discussion

Literature on carcass yields and meat quality characteristics of emus is sparse, and are focused on young birds rather than adults. Utilizing the meat from this bird in addition to fat would improve the economics of their production considerably. This study is the first to examine carcass yields and meat quality characteristics of adult emus and will contribute to the existing knowledge base on emus.

5.4.1 Carcass yields

Adult emus in this study (Table 5.1) yielded more fat, but similar amounts of meat and bone, compared to younger birds as reported in other studies (Dingle, 1997; Sales et al., 1999; Blake and Hess, 2004). However, previous studies showed great variability in fat yields (from 12% to 28%; Mincham et al., 1998, Sales et al., 1999; Blake and Hess, 2004), likely reflecting seasonal changes in the size of their fat depots (Kim et al., 2012). Therefore, for producers to get better economic returns, it is essential to time the slaughter when the fat deposits are maximal (late August to early September in Canada; Bennett et al., unpublished data). Males had larger fat reserves than females (Table 5.1), probably to meet the requirements of a prolonged incubation period (up to 56 days; Mahato et al., 2006). Fat yields differed between experiments because the emus in Experiment 1 had already started to mobilize their fat reserves. Most of the females in this Experiment had developing follicles in ovaries, which were seen in only two females in Experiment 2. This indicated the dissimilarity in their preparedness for the breeding season.

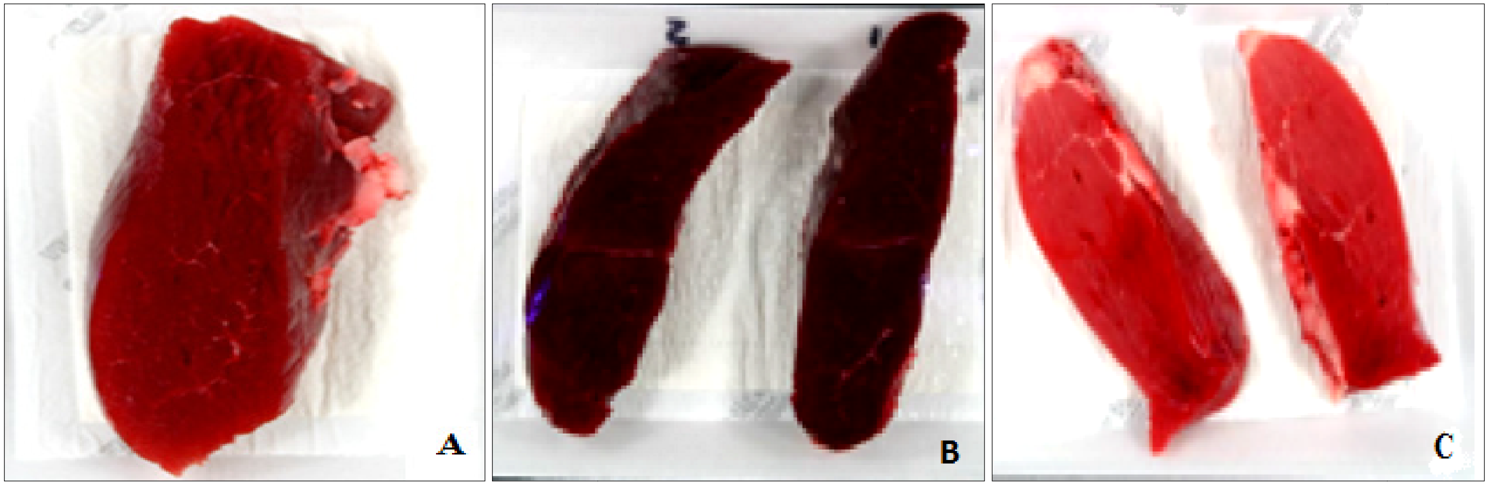

Figure 5.5. Appearance of regular and defective types of emu meat.

5.5A. Regular meat from the thigh of emu, pH_{24} = 6.5, L = 33.1, a* = 12.2, b*= -0.9,

5.5B. Dark Firm and Dry (DFD) meat from the thigh of emu, pH_{24} =7.1, L = 27.3, a* = 11.4, b* = -1.4

5.5C. Myopathy meat from the thigh of emu, pH_{24} = 7, L = 39.6, a* = 19.5, b*= 9.6

Loss of body weight during transport compromises the welfare of emus, alters meat quality and hence warrants attention. During post-mortem inspection, muscles with injuries will be trimmed and or discarded causing substantial economic loss (Schnetler, 2009).

5.4.2 Meat quality characteristics

Drip loss (DL), pH and color are major factors affecting the appearance and juiciness of meat, and thus consumer acceptance. None of the meat quality characteristics in this study were affected by sex, as previously reported in ostriches (Van Schalkwyk et al., 2005; Hoffman et al., 2009). The DL observed in emu meat was similar to that in hot deboned ostrich meat (Botha et al., 2007). The pH of emu meat (Table 5.2), like that of other ratites, is relatively high (Sales et al., 1999). An ultimate pH of less than 6 (Petrosky et al., 1997) can be reached within 3-4 hours post-slaughter (Berge et al., 1997), in birds not exposed to pre-slaughter stress.

The L* and a* values in the meat from the adult emus were comparable to those previously reported in young emus (Berge et al., 1997) and ostriches (Hoffman et al., 2008; Majewska et al., 2009; Polawska et al., 2011). The low b* coordinates (bluish coloration) of emu meat led to many samples with hue values in the fourth quadrant of the scale (Mclellan et al., 1995). The unique hue values were probably due to the high pH, high myoglobin, and less oxymyoglobin formation (Hernández et al., 2006), but I have not measured the latter two variables. Variation in b* values observed between experiments is due to the state of myoglobin in meat (Mancini and Hunt, 2005). The deoxygenated myoglobin of fresh meat is purple red in color. On exposure to air, blooming (oxygenation) of meat occurs resulting in the development of a bright red colored oxymyoglobin, or metmyoglobin, which is brown in color (Warriss, 2010). During storage for five days, most of the samples showed color changes, suggesting the instability of emu meat color.

Emus used in this study showed several behavioral and physiological changes, such as hyperthermia, increased corticosterone concentrations, metabolic imbalances and cell damage (elevated ALT, AST and CK activities) after transport (see Sections 4.2.9.1 and 4.4.5.1), indicating their susceptibility to stress. Transport also leads to dehydration, electrolyte imbalances (Schaefer et al., 1997; Vazquez-Galindo et al., 2013), and low glycogen concentrations in muscles (Hoffman et al., 2012). Low glycogen levels resulted in low lactic acid production during post-slaughter storage (Davids et al., 2010; Warriss, 2010), leading to high ultimate pH, low DL_{24} and darker colored meat (Hoffman et al., 2012), which are characteristic of DFD.

The decline in pH from 45 minutes post-slaughter to the ultimate pH is often used as a measure of pre-slaughter stress (Sales and Mellett, 1996; Berge et al., 1997; Van Schalkwyk et al., 2005; Hoffman et al., 2012). In this study, a wide range of pH was observed in emu meat (Table 5.2), indicating the variability in the physiological responses and susceptibility to stress. Meat samples with pH_{45} below 6.1 had only a slight drop in pH after storage for 24 hours (Figure 5.1). Most of the samples with $pH_{45} > 6.1$ showed a considerable reduction in pH, indicating lactic acid production. However, some meat samples with pH_{45} above 6.5 showed very little change or even an increase in pH during storage, indicating DFD properties (Figure 5.5B). These findings confirmed the intermediate to high ultimate pH in emu meat (Berge et al., 1997), similar to that in rheas (Filgueras et al., 2010).

An inverse relationship between L* and pH values of thigh muscles (Figure 5.2) as also observed in ostriches (Botha et al., 2007), further confirmed this tendency for DFD. High myoglobin content, low intramuscular fat and high ultimate pH are the factors that make emu meat more susceptible to DFD condition (Hoffman et al., 2012). Therefore, I would hypothesize that emu meat samples with pH_{24} around 7 were from stressed birds, while those with pH_{24} around 6 were from unstressed birds (Berge et al., 1997; Hoffman et al., 2012). Similar results have been reported in ostrich meat (Fasone et al., 2005; Van Schalkwyk et al., 2005; Hoffman et al., 2012).

Emus in both experiments were shipped for the same duration and distance in the same trailer, at the same time of the year. The only notable difference between experiments was with respect to weather conditions (see Sections 4.2.5 and 4.4.4). Transportation and exposure to heat stress leads to thermoregulatory problems and hyperthermia (Delezie et al., 2007). Emus transported in warm weather (Experiment 1) lost more weight and had higher body temperatures and plasma corticosterone concentrations (see Section 4.4.2.2 and 4.4.2.3), compared to emus transported under cool weather (Experiment 2). This would also imply that the effect of heat stress on meat was more profound in Experiment 1, in comparison to Experiment 2. The pH_{24} in the emu meat in Experiment 1 was significantly higher than Experiment 2 (Table 5.2), especially in thigh, suggesting DFD changes. The positive correlation between DL_{24} in leg meat and percentage loss of body weight at slaughter (Figure 5.4) indicated that loss of body weight after transport affects meat quality. The greater DL_{24} (Figure 5.1) and pH (Table 5.2) of both thigh and leg meat in Experiment 1 is likely the result of exposure to thermal stress (Petracci et al., 2001; Sandercock et al., 2001). However, after refrigerated storage there was no difference in DL_5 between experiments, as both underwent protein denaturation (Meat and Livestock Australia and the Australian Meat Processor Corporation, 2002). The significantly higher DL_5 in leg meat (Figure 5.3) is suggestive of its greater exposure to stress as previously observed in ostriches (Schaefer et al., 1995) and rheas (Filgueras et al., 2010).

Elevated plasma corticosterone concentrations during stress could damage muscle cell membranes and increase plasma CK activities (Sandercock et al., 2001; 2006). Emus with high stress levels (see Section 5.3.5) elevated blood glucose, ALT and AST) had higher pH_{45} in thigh muscles, further confirming that high pH corresponds to elevated stress levels (Fasone et al., 2005; Hoffman et al., 2012). However, the meat sample (Figure 5.5C) obtained from an emu with several injuries and feather loss, showed pale color and high pH. It did not keep well during storage. Such pale color in meat is associated with stress related myopathy (Wiklund et al., 1996; Hoffman, 2001) and confirmed the finding of exertional rhabdomyolysis in the emus after transport (see Section 4.4.6.3).

Muscle injuries due to handling and vibrations inside the truck compartment can result in altered cell wall permeability, proteolysis and lower pH (Warriss, 2010), leading to PSE meat. In this study, I observed two meat samples with low $pH_{24,}$ high DL and L* consistent with PSE, although this condition has not been described in ratites before. PSE meat is commonly seen in pigs and poultry (Tankson et al., 2001; Aksit et al., 2006; Bianchi et al., 2007). However it is not very common in red meat and warrants further research.

5.4.3 Fat color

Fat color is being reported for the first time in emus. Splashing of blood could be seen as spots in the fat layer of some emus and this was more prevalent in Experiment 1 than in Experiment 2, suggestive of the role of heat stress. Nevertheless, splashing of blood in fat was said to affect the fat texture (coarser) while rendering, result in lower oil yields from samples of similar weight without blood spots (Dwayne Harder, Emu Producer, Try Harder Farms, Saskatchewan, personal communication). So the reasons for splashing of blood into fat and its effect on oil quality will need to be investigated further.

Loss of body weight, bruises and injuries incurred during transport, besides compromising the welfare of emus, alters meat quality and hence warrants attention. Injuries could also increase the chance of bacterial contamination, leading to trims, discards and economic losses (Schnetler, 2009). Use of air sprung trucks with skid free flooring, and padded walls of the compartments would help to reduce injuries (Glatz and Miao, 2008) and thus improve the welfare of these birds.

5.5 Conclusions and recommendations

Transport of adult emus for six hours resulted in weight loss and physiological indications of stress, which ultimately impacted meat quality (high pH, tendency for DFD and poor color stability). These changes were greater after transport during warm weather conditions. Emus had injuries on thighs and legs after transport, and meat from emus with extensive injuries showed stress myopathy. Although administration of the oral nutrient supplement to emus prior to and after transport helped alleviate the stress imposed on them, this did not have any effect on meat quality characteristics. More research needs to be done to establish the appropriate composition and dosage of the oral supplement for this species. Loss of bodyweight, bruises and wounds consequent to transport compromise the welfare of emus and cause economic losses warranting interventions to mitigate the same.

6. UNDERSTANDING THE BEHAVIOR OF EMUS – A MEANS TO IMPROVE THEIR MANAGEMENT AND WELFARE PART – I. MAJOR BEHAVIOR AND TIME BUDGETS OF ADULT DOMESTIC EMUS

6.1 Introduction

Among ratites, the behavior of ostriches has been widely researched into, while that of emus has received very little attention (Immelmann, 1960; Sales and Horbañczuk, 1998; Glatz, 2000; Patodkar et al., 2009). Current popularity of emu farming in the US (Todd, 2012) and India (Gujral, 2009), warrants the need to understand the behavior of these birds. It also become increasingly important to understand behaviour repertoires for improving husbandry practices and interpreting welfare implications (Csermely, 2007).

Studies conducted on the behavior of ostriches have identified behavior groups such as sitting, standing, pacing, walking, foraging, feeding (Deeming, 1998); inactive, locomotive, ingestive, ground pecking, object pecking, preening and aggression (hiss /beak gapes, threat display, chase, run-chase, chest ramming, kicking, mounting and trample; Meyer et al., 2002 Csermely et al., 2007).

To date, there have been very few published studies on the behavior of emus. Glatz (2001) described the behavior of emus under six major categories namely, inactive, ingestive, posture change, grooming, aggressive and locomotion, while looking at the effect of declawing. There are few studies conducted on particular behaviors of emus such as sleep (Immelmann, 1960), reproduction (Blache et al., 2000), vigilance (Boland, 2003) and an abstract on emu chick behaviors (Jennifer et al., 1997). The only study on adult emus (Sales and Horbañczuk, 1998) did not describe their behavior or conduct any statistical analysis, though it was conducted on a small sample size (five emus each) during the breeding (two years old) and non breeding (one year old) seasons in Australia and Britain. Therefore, it was necessary to determine the behavior repertoires and time budgets in adult domestic emus (above five years of age) in Canada to establish a list of common behaviors to be utilized for further studies on their welfare.

The qualitative assessment of behavior for welfare assessment purposes would include affective states like fear, aggression, frustration, content, pleasure, playfulness, interest, relaxation, inquisitiveness, agitation, friendliness, and anxiousness (Wemelsfelder et al., 2000; Haynes, 2008). Understanding the feelings in animals through behavioral observations is a vital step in improving their welfare (Watanabe, 2007). An animal's welfare is compromised when it is having difficulty in coping with its environment (Broom, 2006); when in pain, feeling fear, or when unable to control its interactions (Wiepkema, 1987). Changes in the repertoire and time budgets of poultry could indicate welfare issues (Sanotra and Weeks, 2004; Mollenhorst et al., 2005). Behavioral changes would encompass unusual, repetitive (stereotypic) actions as well as the absence of normal behavior of the species. Therefore the objectives of the current study were to:

(1) Identify and describe the behavior;

(2) Establish the time budgets and behavioral repertoires; and

(3) Determine the effect of time of the day, sex, and weather on activities of adult domestic emus.

This data would be utilized to build a knowledge-base on emu behavior for assessment of their welfare.

6.2 Materials and methods

This study was approved by the University of British Columbia Animal Care Committee, under permit # A10-0104.

6.2.1 Management

Four pairs of adult emus (> 5 years of age) were observed in the post breeding season, just after the cessation of egg laying (late spring, early summer). Emus were housed in a large outdoor pen (28 x 45 feet) containing natural forages, that was located at the large animal care facility of the University of British Columbia. Emus had *ad libitum* access to pelleted ratite feed (Otter Feeds) and drinking water. Each emu was identified according to the differences in coloration and markings on their body. The sex of the emus was determined by cloacal palpation (Samour et al., 1984) at the end of the study.

6.2.2 Observations

A blind was set up at a good vantage point outside the emu pen, one week prior to the study. The observer spent one hour in the blind every day for the emus to acclimatize to the observer getting in and out of the blind and also to refine the observation protocols. The emus were observed for 30-minute periods in the morning (07:00 am to 09:00 am) and late afternoon (3:00 pm to 5:00 pm), for 20 days prior to the start of the experiment to get a set of activities for constructing a behavior repertoire. The description of each behavior was also noted down (Table 6.1) and thus a data recording sheet was developed (Appendix G). A behavior that lasted for the whole observation interval (30 second) was classified as a state. Those behaviors that were shorter in duration (< 30 second) were classified as events (Martin and Bateson, 1993). During scoring, no distinction was made between states and events, and between single and multiple occurrences during the interval.

The main study was conducted over a 12 day period, during which the behavior of individual emus in their natural group setting was recorded for four 30-minute long observational sessions per day. Each session (30-minutes) was divided into sixty 30-second intervals. All the occurring events were noted down using one zero sampling technique (Smith, 1985). All behavior, which did not occur in a particular interval were scored zero, while those occurring were scored one (Martin and Bateson, 1993). During each observational session, two randomly selected emus were observed at the same time, such that eight emus were observed each day. There was a 30-minute break after each observation period. Each emu was observed for a minimum of 10 hours prior to the commencement of the study and then for six hours during the study. All of the activities of emus were recorded during the intervals and then the one zero scores for each behavior was calculated (Martin and Bateson, 1993). The routine management of these emus was not disrupted and therefore, how these emus reacted to their care-takers was also evaluated. As a follow up of this study, I also noted the unusual behavior in emus, consequent to regrouping into separate pens, and upon introduction into a new facility with unfamiliar emus (see Chapter 7).

The meteorological data including temperature, humidity and weather condition were also noted (www.climate.weatheroffice.gc.ca) during the different observation periods and categorized for analysis.

The weather conditions recorded under six categories were scored as follows.

1). Clear = 1, 2). Mainly Clear = 2, 3). Mostly Cloudy = 3, 4.) Cloudy = 4, 5). Rain Showers = 5 and 6.) Rain = 6.

The relative humidity in three categories was scored as follows:

1) Low = 47-60 %, 2). Medium = 61-71 % and 3). High =72-83 %.

The temperament of these emus inside the pen was noted down on all the 12 days during the time when regular handlers were inside the pen to provide feed or water. Their behavioral response to handlers was classified as friendly, docile, cautious, panicky and flighty (Table 6.1).

Table 6.1. Categories of temperament of emus towards regular handlers.

Categories[1]	Description
Friendly	Would walk towards the handler and stay close to the handler, head is kept down.
Docile (calm)	Would not mind having the handler in close proximity, though would not approach the handler. Head is kept down.
Cautious	Would move away from the handlers and stay at a distance watching the handler, Head is not always down.
Panicky	Would move away from handlers, keep a distance, head is kept high, and sometimes run away. May pace at a distance from the handler.
Flighty	Would bolt at the sight of handlers, running against fences.

[1]Adapted from Koolhaas et al. (2010).

6.2.3 Statistical analysis

The mean of each of the behavior for individual emus was calculated by adding the total observations (ones) and dividing by the total number of periods so as to produce the corresponding one zero scores. Then the time–activity budget for the morning and late afternoon sessions were calculated (Zinner et al., 1997) as Time budget = number of intervals for the behavior / total no of intervals X 100. Twenty-five behavior types listed (Table 6.1) were summarised into eight major categories. The main effects of time of the day and emus and their interaction on time budgets were tested using a two way ANOVA. For determining the effect of weather condition, and relative humidity on behavior, separate one way ANOVA or Kruskal-Wallis ANOVA on Ranks were done. The post-hoc tests done were Bonferroni test or Dunn's test respectively. Mann-Whitney U test was done to compare the differences in time budget between sexes. Wilcoxon matched pair test was done to compare the difference in time budget within an individual during the two time periods of the day. All results were interpreted at a significance level of 0.05 using Statistica 10 (Statsoft Inc, Tulsa, OK, USA). Spearman correlation coefficient (r_s) was used to determine the linear relationship between the continuous variables studied and also the association between categorical and continuous variables. To study the relationship between periods of the day and the continuous variables, Point Biserial correlation (r_{pb}) (XLstat Version 2012. 1. 01 New York, NY) was used. The results are presented as means with their standard errors

Table 6.2. List of behavior of emus and their descriptions.

No	Behavior	Description
I. INGESTIVE BEHAVIOR		
1	Eating	Consuming the concentrate feed.
2	Foraging	Searching for and consuming the fodder in the field.
3	Picking	Picking up feed /poking on ground/ pecking objects.
4	Voiding	Elimination of droppings.
II. DRINKING		
5	Drinking	Touching water surface with the beak with or without gulping.
III. GROOMING		
6	Preening	Self-preening with the beak.
7	Dust bathing	Rubs the neck or any portion of body against the ground, shaking both the wings either crouching or sitting.
8	Feather picking	Pecking at the feathers of other emus.
9	Body shake	Shaking body/wings while standing.
10	Head shake	Shaking the head.
IV. STANDING		
11	Standing	Stands with the neck in S shape and feathers flattened or straight.
V. LOCOMOTION		
12	Running	Running sprints (with or without the wing extended) but not chasing

No	Behavior	Description
		other emus.
13	Walking	Walking without the wings extended.
14	Parallel walking	Walking together with no attempt to attack each other.
15	Pacing	Walking parallel to and along the fence.
VI. SOCIALISATION		
16.	Aggression	Attacking other emus (contact between bodies by kicking / pushing).
17.	Threatening	Standing tip-toed, and making a hissing sound towards other emus.
18.	Chasing	Chasing an emu away with or without running after it, causing it to flee, retreat or react.
VII. VOCALIZATION		
19.	Drumming	Sound produced by the female emu (Davies, 2002; Doneley, 2006).
20	Grunting	Sound produced by the male emu (Davies, 2002; Doneley, 2006).
21.	Distress call	A sound (loud and high pitched) produced by emus of both sexes, other than the usual grunt / boom (drumming) sound.
VIII. RESTING		
22.	Sleeping	Standing/ sitting still with eyes closed (not handled or threatened by other emus).
23.	Sitting	Crouching / sitting with or without the head raised.
IX. OTHER BEHAVIOR		
24.	Yawning	Opening mouth briefly and closing it.
25.	Pecking fences	Pecking at the fence wire or post.

6.3 Results

6.3.1 Emu behaviors

6.3.1.1 Ingestive behavior

Ingestive behavior included feeding on concentrate feed pellets in the feed trough, foraging on insects and plant material, and picking up stones and ingesting them (Table 6.1). Refilling of feed troughs in the morning was found to stimulate the emus to eat the concentrate feed. The dominant emus (judged by how frequently they displaced other emus from the feeding trough) were the first to approach the feed trough. Other emus were found to wait for these emus to move away before approaching the feed trough. Emus walked across the pens, pausing in between to nib at the grasses and other foliage inside the pen. They were also found to pick up stones from the ground or just peck on the ground while grazing. They would also infrequently peck on the fences, wooden poles and other plants inside the pen while grazing.

6.3.1.2 Grooming behavior

Emus frequently preen feathers with their beaks and they do this while sitting, standing or walking. Their feathers become quite moist when exposed to rain.

6.3.1.3 Resting behavior

Emus were found to rest with their eyes closed while standing or in the sitting position. Emus sit on their hocks in the squatting position and they would eat, drink, preen and even forage from this position. They would also sit with just the ventral portion of the body touching the ground or with the whole length of the neck also in contact with the ground. Emus preferred to sit under the sun with their neck extended and even sleep at dusk and early in the morning in the sitting position.

6.3.1.4 Vocalizations

The vocalizations included “drums” by female and “grunts” by male emus (see Table 6.1). There was considerable variation in the loudness, duration and pitch of these calls among individuals.

6.3.1.5 Unusual behavior

Emus which were separated from conspecifics (see Section 7.2.3), continuously paced along the fences, without eating or foraging. Immediately after changing pens, emus were found to huddle (stay in groups in one corner) away from the emus already present in the pen. Another behavior observed after regrouping was increased frequency of pecking at the fences. Responses to handlers included running into fences, jumping, kicking, slipping, stopping (balking), falling, and sitting (disinclination to move).

6.3.1.6 Temperament

These categories were not mutually exclusive. From my observations, two of the emus were friendly and docile while three of them were cautious and docile. The other three were panicky. There were no flighty emus in this group. None of the emus were aggressive towards handlers.

6.3.1.7 Other findings

Emus drank by using their beak to scoop up the water, shaking their head while gulping and pausing in between. They liked to splash water on their body and would dip themselves in large drinking water troughs. Their activities were not generally interrupted by rain and I have never observed them seeking shelter when it was raining. Emus were startled by the slightest noise and then they would stop all their activities and became vigilant. Usually at least one of the emus kept watch while others were feeding.

6.3.2 Time budgets

From among all the behaviors observed, major categories identified were ingestive, drinking, standing, locomotion (walking, parallel walking and running grouped together), grooming (comfort movements; preening, dust bathing, feather picking, body shake and head shake grouped together), vocalization (grunting in males and booming in females), resting (sitting, sleeping), and other behaviors including voiding, yawning, unusual/ displacement behavior (pacing, huddling, pecking fences or poles) (Table 6.1). The mean total activity score (one zero scores) of an emu during a 30-minute observation period was found to be 120 ± 32. The atmospheric temperature during the period of study varied from 12.9^0 to 26.2^0C, while the relative humidity varied from 47 to 83 %. The time budgets (eight major behaviors) given in Figure 6.1 showed that emus spent most of their time walking, standing and eating.

The time budgets for the time periods (morning and late afternoon) are given in Table 6.3. The emus stood for significantly longer ($P = 0.02$) duration in noon than in the morning period and walked significantly ($P = 0.03$) less in the noon. There was significant between emu differences only with respect to other behaviors (including voiding, yawning, pacing, huddling, pecking) i.e., emu #2 verses emu #7 ($t = 3.4$, $P = 0.02$), emu #2 verses emu #4 ($t = 3.3$, $P = 0.03$). There was no significant difference between the sexes for any major behavior. There was no significant effect of weather condition or temperature on the time budgets.

6.3.3 Relationships among behaviors

The linear relationship between the following variables was significant ($P < 0.05$). Locomotion was positively correlated to eating ($r_s = 0.27$) and grooming ($r_s = 0.25$). Resting was negatively correlated to eating ($r_s = -0.32$), and vocalization ($r_s = -0.25$). The emus stood more when the relative humidity was less ($r_s = -0.26$). The variable standing and the period of the day were significantly associated ($r_{pb} = 0.26$) meaning that as the day progressed, emus spent more time standing.

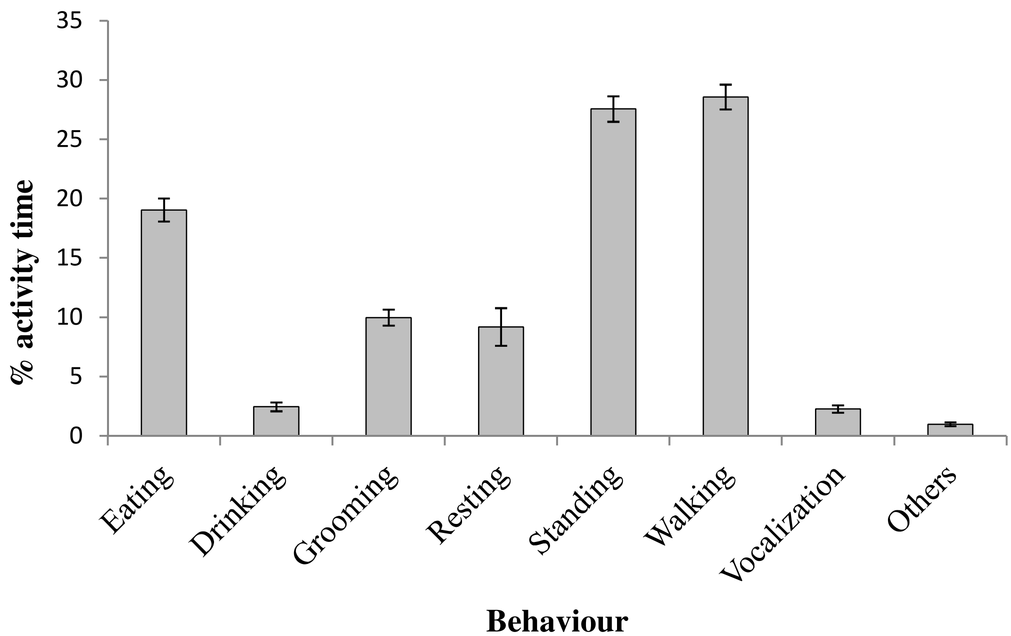

Figure 6.1. Time budgets for major eight behavior of emus.

Mean time budgets (%) for each behavior category with the standard errors are presented.

6.4 Discussion

A thorough understanding of the behavioral needs of emus is important to ensure better management and welfare. However, the behavior repertoires of emus have received very little attention (Sales and Horbañczuk, 1998). In this study, I have identified and described the behavior in adult emus in the post- breeding period. Examination of behavioral repertoires of emus is important to better understand the changes consequent to stress. One zero scores are correlated to both frequencies and durations of behavior (Altmann, 1974). Hence one-zero sampling is considered to be a good method to record events and intermittent behavior (Suen and Ary, 1984; Martin and Bateson, 1993). The shorter durations of the time intervals selected i.e. 30 seconds, helped to improve the reliability of the findings (Altmann, 1974).

Table 6.1. Relative humidity, temperature and time budgets for major behavior of emus during morning and late afternoon hours[1].

Variables	Morning	Noon	P-value
	Mean ± S.E	Mean ± S.E	
Relative humidity (%)	68.1 ± 1.0	56.9 ± 0.9	< 0.01
Temperature (^{o}C)	17.4 ± 0.4	17.3 ± 0.6	Not significant
Feeding	18.2 ± 1.3	19.9 ± 1.3	Not significant
Drinking	2.8 ± 0.5	2.1 ± 0.5	Not significant
Grooming	9.8 ± 0.0	10.2 ± 0.9	Not significant
Resting	10.2 ± 2.3	8.2 ± 2.3	Not significant
Locomotion	31.0 ± 1.5	26.1 ± 1.4	0.02
Standing	25.2 ± 1.5	29.9 ± 1.5	0.03
Vocalization	1.9 ± 0.4	2.6 ± 0.4	Not significant
Other behavior	0.9 ± 0.2	1.1 ± 0.2	Not significant

[1]Least square means ± standard error of the means are presented.

6.4.1 Behavior of emus

The major categories of behavior identified by us were similar to those observed in ostriches by Degen et al. (1989); Deeming (1998); Meyer et al. (2002); and Csermely et al. (2007). Incidental observation of behavioral responses is considered helpful in assessing the potential impacts of common managemental practices on animals (Stephens and Perry, 1990). Sitting with just the ventral portion of the body touching the ground or with the whole length of the neck also in contact with the ground seems to be a resting behavior (ostriches; Degen et al., 1989). The resting postures found in this study are similar to those observed in other studies on emus (Immelmann, 1960) and ostriches (Degen et al., 1989). Emus sleep in this position at dusk and also early in the morning (Immelmann, 1960), but none of these studies reported them standing with eyes closed. Emus which are on vigil and having curtailed night sleep were found to exhibit such “micro naps”, with either one or two eyes closed, similar to that reported in migratory birds (Fuchs et al., 2009; Mahan, 2009). Vigilance behavior in emus has been observed in small groups (Boland, 2003), similar to the one in this study. Similar to ostriches, these emus spend considerable amount of time standing alert rather than foraging or resting (McKeegan and Deeming, 1997).

Emus lack preen glands (Roots, 2006) and hence their feathers become quite moist when exposed to rain. Their preference to play in water has already been reported (Jennifer et al., 1997) and hence could be suggested as a means to enrich their pens. Thus providing shallow pools inside pens, especially in summer, would promote their welfare. The dominance shown by females was evident as some of them were always successful in displacing others from the feed trough. It has also been reported that the weaker, smaller and subordinate birds in the group are displaced at the feed bin by bigger, healthier and dominant emus (Rao et al., 2000). The aggressive behavior (not very common) of emus observed in this study is similar to that reported by Glatz (2001), and is comparable to that in ostriches (Meyer et al., 2002).

6.4.2 Time budgets

The findings on time budgets are comparable with those observed by Sales and Horbañczuk (1998) and Glatz (2001) in emus. Emus also seem to have similar behavior as that of other ratites such as ostriches (Degen et al., 1989; Williams et al., 1993; McKeegan and Deeming, 1997; Csermely et al., 2007) and rheas (Sales et al., 2000; de Azevedo et al., 2010). There was no significant effect of the time of the day on most activities of emus because there were similar weather conditions during the morning and noon hours. In this study, emus were found to stand more in the latter half of the day compared to morning as they had a more active foraging period in the morning. Similar temporal variation in activities has been reported in ostriches (McKeegan and Deeming, 1997; Paxton et al., 1997; Crowther et al., 2003). There was no significant effect of sex on the time budgets in emus, similar to the findings of Sales and Horbañczuk (1998) in emus and Deeming (1998) in ostriches, probably because the period of study was after the end of breeding season in Canada.

6.4.3 Relevance of this study in evaluating welfare of emus

Time budgets are often used as a diagnostic tool to assess adjustments made by animals to the behavior homeostasis (Breed and Moore, 2011) on exposure to stressors. To study the impact of transport related stress on emus, it is important to focus on the changes in normal behavior (absence), and identify unusual ones. Behavioral changes (Koene, n.d; King, 2003; Swaisgood, 2007) such as the development of stereotypic behaviour (Wiepkema, 1987; Weller and Bennett, 2001; Broom and Kirkden, 2004) and vocalization (Manteuffel et al., 2004) provide greater insight to the physical and emotional status of these birds and have to be studied in greater detail.

Emus are curious birds and spend considerable amount of their time in beak related activities including foraging, preening, pecking on the ground and other objects in the pen. Emus also pick up stones and grit while feeding, which is found to help them in digestion (Herd, 1985). In cases where there is inadequacy of substrates and forages inside the pen, they might have a very limited activity choice. At such times, it is likely that they spend more time pecking inanimate objects (Hughes and Grigor, 1996) as they experience boredom. However, separation of emus into groups led to fence pecking which in turn might be their method of expressing frustration.

Pacing has been reported as a predominant activity in ostriches (McKeegan and Deeming, 1997; Csermely et al., 2007), rheas (Sales et al., 2000) and even emus (Sales and Horbañczuk, 1998). It is also at times referred to as an abnormal behavior due to frustration and / or confinement (Degen et al., 1989), if done stereotypically (Sales and Horbañczuk, 1998). Hence it is important to consider the time budgets over a period of time to know the drive behind this behavior. Increase in displacement activities (Dantzer and Mormede, 1983) such as pacing is induced by confinement, movement restriction (Mason and Latham, 2004) and social reorganisation (Stephens and Perry, 1990). This is an indicator of stress in emus (Glatz, 2005; 2008), ostrich (Deeming, 1998; Mitchell, 1999) and chicken (Bracke, 2006; Sherwin, 2010), as well as a mechanism to overcome the stress (McMillan, 2009). Therefore this behavior is important while assessing the welfare of emus.

Huddling is the first line of response to a strange environment (Barnett and Hemsworth, 1990) and also an attempt to reduce heat loss from the body. So in emus, the focus should be on three behaviors namely, pacing, huddling and fence pecking while comparing time budgets.

Although I did notice aggressive behavior during this study, it was not common. Aggression in females is common during the breeding season (Blache et al., 2000; Doneley, 2006; Bewg and Kent, 2008), and otherwise there is very little hostility visible among emus. Because of this seasonal difference in temperament of emus in the breeding season, it is suggested that human interventions might add to the stress they already experience. Any attempt to interfere with the social orders through regrouping or transportation during this period might prove especially stressful.

Observations on the response of emus to handlers showed that training them or maintaining constant contact would help to reduce flighty responses, making handling easier. Thus stockmanship seems to have an important role in the welfare of emus. In overall, this study provided important information on the behavior and activities of emus in a farm setting in Canada.

6.4.4 New questions raised by this study for future research

A closer study of the different behavior of emus prior to and after handling could perhaps shed light into the individual variation in their response to stressful situations. Interpretations on unusual behavior should be made only after observing them for a considerable period of time. The time budgets of emus during breeding season might vary from what was observed in this study. However, it may be necessary to look into behavioral sequences and year round changes in repertoire patterns to get a better understanding of emu behavior. Though conducted on a very small sample size, this study provided vital information on the behavioral peculiarities of emus in captivity, which could be made use of in further studies.

6.5 Conclusions

This study collected valuable information on the different behavior as well as patterns thereof in adult domestic emus. Emu behaviors observed in this study were mostly unaffected by sex, except that they had different vocalizations. The behavior of male and female emus did not differ significantly probably as this study was not conducted during the breeding season. Time budgets before and after an intervention would indicate the stress experienced by them. The findings from this study could be utilized to build a knowledge-base on the relationship between behavior and welfare of emus.

7. UNDERSTANDING THE BEHAVIOR OF EMUS – A MEANS TO IMPROVE THEIR MANAGEMENT AND WELFARE PART- II. BEHAVIORAL CHANGES IN ADULT DOMESTIC EMUS DURING THEIR HANDLING AND TRANSPORT

7.1 Introduction

Farm animals are transported for several reasons such as sales and processing. Handling and transport are traumatic experiences exposing animals to a multitude of stressors (Minka and Ayo, 2009), such as dehydration, hunger, heat or cold stress, vibration, noise and transport vehicle conditions. Age, species, previous experiences, trained handlers, driving skills, and duration of the transport have been listed as factors affecting their responses to stressors (Broom, 2005). Animals try to adapt to stressors through a combination of behavioral, physiological, and metabolic mechanisms (Puvadolpirod and Thaxton, 2000), which are characteristic of the species (Koolhaas et al., 1999). Emus are highly susceptible to stress (see Chapter 4) and hence there is the need to identify non-invasive indicators of stress in these birds. Observation of behavioral responses will be helpful in assessing the potential impact of management practices on animals (Stephens and Perry, 1990).

One of the primary approach in animal handling is preventing or minimizing stress by habituating animals to human contact, handling procedures and transport. Another important challenge will be to manage stress, so that it does not progress to distress (King, 2010). If animal handlers are able to identify critical stress related behavior(s) in the species, actions could be modified earlier, so that the animal's welfare is not compromised. Measuring the reaction of emus to handlers would help to realize how they perceive people in general (Waiblinger et al., 2006). Correlating behavior to the changes in physiological and hormonal variables (Maria et al., 2004; Cockrem, 2007), would help in evaluating stress, non-invasively.

The objectives of this study were to:

(1) Record the behavioral responses of emus to handlers to identify temperament categories,
(2) Describe the events consequent to their rounding up, handling, loading, transport, unloading and while resting at lairage,
(3) Compare the time budgets of emus prior to and after transport to identify absence of usual behavior and/or the presence of unusual behavior,

(4) Determine the effect of handling time on selected physiological variables, and
(5) Identify potential behavioral indicators of stress in emus.

7.2 Materials and methods

All the experiments were carried out with protocols approved by the Animal Care Committee, University of British Columbia as per permits # A-10-0183 and # A-10-0184.

7.2.1 Emus and management

Forty-two, 4-6 year old emus were used in two experiments with 24 (14 males, 10 females) and 18 (10 males, 8 females) emus each, from Try Harder Farms, Denholm, Saskatchewan, Canada. See Section 3.2.2, 4.2.2 and 4.4.2 for their management.

7.2.2 Behavioral observations

Randomly selected emus (Experiment 1: 18; Experiment 2: 10) were observed pre- and post-transport, for 30-minute long observation periods, during which two randomly selected emus/ period were observed. The same behavior recording sheet developed in Chapter 6 (Appendix G) was used to record behavior. While scoring, no distinction was made between states and events, or between single and multiple occurrences during the interval. All behavioral occurrences were noted down using one zero sampling technique (Smith, 1985). All behavior, which did not occur in a particular interval were scored zero, while those occurring were scored one (Martin and Bateson, 1993).

Individual emus differed considerably with respect to their ease of handling. Based on the temperament groups classified in Section 6.3.1.6 (Table 6.2), I could identify temperament categories in emus, while they were handled for loading. Based on the degree of difficulty at which handlers could approach, catch, and manage (control) them, emus were categorized into three handling temperament categories, “calm and easy”, “slightly panicky”, and “flighty” (Table 7.1) for improved statistical power and better comparison of the physiological variables.

In Experiment 1, hourly inspections (four times) were conducted during the transport to note down behavior and injuries. In Experiment 2, all events during transport were observed using a camera equipped with LED night vision (Vehicle Rear Sight Waterproof Video Camera), connected to a monitor in the truck cab. The behavior of emus was observed for 2 hours immediately after unloading in both experiments. The behavior and incidents during handling and transport are described in Table 7.2.

7.2.3 Handling and transport

Emus were regrouped into separate pens in experiments 1 (5 days before transport), but not in Experiment 2 for administration of dietary supplement treatments. Emus were handled during pre- and post-transport blood sample collection and weighing, during loading and unloading, and when taken to the slaughter box. In addition to this, the emus were handled for administration of the supplement solution (hand-fed using a modified calf feeder; see Section 4.4.3). Duration of all the procedures (Appendix H) during transport, i.e. the loading time, unloading time and total handling time (sampling, loading, and unloading together) were recorded using a stop watch.

Unlike ostriches, attempts to hood emus made them panicky, and hence hooding was not done. Handlers held emus on either side of the body, at the base of both wings. They stood close behind the birds' body, holding firmly to steer them forward. This led to considerable struggling initially and vocalization, after which they calm down. For sample collection, the emus were held firmly on a bed of straw and straddled by a handler. While the second handler held the neck to raise the vein, the third handler did the venipuncture. All the samples were collected in less than three minutes restraint. See Section 4.2.3 and 4.4.4 for details on the conditions of transport.

7.2.4 Sample collection and slaughter

See Sections 3.2.4, 4.2.7 and for details on sample collection and processing. See Section 5.2.3 for details on slaughter.

7.2.5 Vocalizations

The vocalizations from emus during handling were recorded using a R5-Roland Wave Recorder (Roland US. Los Angeles, CA) connected to an external microphone (Beyerdynamics MCE 86), kept 10 cm away from the head of the emu. The recorded vocalizations were analyzed using the "Raven Lite Interactive" sound analysis software from Cornell Lab of Ornithology, Ithaca, NY, US (http://www.birds.cornell.edu/ raven).

Table 7.1. Categorization of emus according to the ease of handling (temperament).

Category	Handling temperament		
	Approachable	Catchable	Manageable
Calm (docile) and easy	The bird remains calm and does not move away when approached by handler.	Easy to catch.	No struggling when handled. Easy to walk.
Slightly panicky	Bird is calm, but keeps its distance when approached.	Somewhat difficult to catch.	Moderate struggling during handling. Easy to walk.
Flighty	Moves away from the handlers when approached.	Very difficult to catch.	Struggles a lot when handled. Difficult to walk.

Table 7.2. List and description of behavior during handling, loading and unloading of emus.

Behavioral events	Description
Aggressive posture towards handlers	Standing on the tip of toes with extended neck, to the full height of the bird. Feathers on the neck are ruffled (Samson, 1996).
Hissing	Making a hissing sound towards handlers and also other emus (Meyer et al., 2002).
Jumping	Jumping or jumping over fences/ handlers.
Kicking at handlers	Kicking (forward/ backward/ sideways) with one or both legs.
Distress call	A sound (loud and high pitched) produced by emus of both sexes, other than the usual grunt / boom (drumming) sound.
Arching or twisting of neck (retrocollis)	Head moving frequently with the twisting of neck, found to be associated with the involvement of central nervous system and also stress in ostriches (Payne, 1993).

Behavioral events	Description
Rolling	The bird rolls on ground while being handled.
Running	The bird runs around in the pen or and hits against fences.
Sitting	Sitting down on the floor when tried to be walked/ loaded.
Slipping	Less than 3/4th of the body touching ground while losing balance.
Stopping (balking)	Stands still, resisting any attempt to walk.
Struggling	Attempting to break free from the hold of the handler.
Trampling	A bird stands over sitting bird, steps on it with its feet on the sitting birds' back and sides.
Falling	More than 3/4th of the body touching ground while losing balance.
Frothing	Frothy materials noticed at the corner of the mouth.
Gular flutter	"Non-ventilatory expansion and compression of the buccal cavity, performed with the mouth open, associated with heat stress and is thought to contribute to evaporative cooling." (Brainerd, n.d.).
Head bobbing	Forward and backward movement of the head characteristic of birds (Ortega, 2005) found to be a sign of stress in ostriches (Mitchell, 1999).
Huddling	Body of an emu comes in contact with that of other group members while standing/ sitting (Meyer et al., 2002).
Open beak breathing	Difficulty in breathing (CFIA, 2013b)
Panting	Rapid breathing, gasping, tachypnea. (CFIA, 2013b)
Aggression towards other emus	Same aggressive posture as towards handlers and also trying to peck or kick.
Vocalizing	Grunting /drumming sound produced by male and female emus.

7.2.6 Statistical analysis

The total handling time (5-14 minutes) was divided into two categories (1) less than or equal to 8 minutes and (2) above 8 minutes. The effect of handling time on blood biochemical variables was analysed by ANOVA, using the GLM procedure. One way ANOVA was used to compare the temperament categories with respect to total protein, Albumin, ALT, AST, CK, corticosterone, uric acid, urea, blood glucose, H/L ratio, and WBC counts.

Time-activity budgets were calculated from the one zero sampling scores of behavior as: Time budget = number of intervals for the behavior / total no of intervals X 100 (Zinner et al., 1997). The time budget data was subjected to Blom transformation for proportions (Blom, 1958) and analysed by two-way ANOVA with Experiment and time period as main effects. Post-hoc mean separation was done with Bonferroni t-test. The events during handling were recorded using "all occurrences sampling" (Altmann, 1974). The events were scored according to their frequency or intensity, as given in Table 7.3. The proportion (%) of emus exhibiting each of the events was tabulated. Spearman correlation coefficient (r_S) was used to determine the linear relationship between the continuous variables studied.

All analyses were performed using Statistica version 10 (Statsoft, Inc. OK, USA). The significance level was set at $\alpha = 0.05$. Data are presented as least square (LS) means ± standard error of the mean (SEM).

7.3 Results

7.3.1 Observations during handling

The pre-transport concentrations of the measured blood analytes did not differ significantly between the temperament categories. The body temperature measured after transport and the concentration of analytes measured at slaughter in the temperament categories are presented in Table 7.4.

Table 7.3. Events observed during the handling of emus and their scoring[1].

Events	Scores 0	1	2	3
Kick, Fall, Sit, Balk, Slip	Nil	Once	Twice	More than two times
Vocalize, Aggression, Jump, Run	Nil	Mild[2]	Moderate[3]	Severe[4]

[1]Total event scores were calculated as the sum of scores for each of the above listed events occurring during the handling of individual emus.
[2]Shows the behavior for less than half of the handling time.
[3]Shows the behavior of more than half of the handling time, but not throughout.
[4]Shows the behavior throughout the handling period.

Table 7.4. Comparison of the temperament categories in emus with respect to selected variables estimated at slaughter.

Variables	Units	Calm and easy (n = 17)	Slightly panicky (n = 11)	Flighty (n = 14)	P-value
Body temperature[1]	^{o}C	38.3±0.1^{b}	38.4±0.2^{b}	38.9±0.2^{a}	0.017
Total protein	g/L	56.2± 2.8^{a}	52.7±1.1^{ab}	48.0±1.5^{b}	0.031
Albumin	g/L	28.3±0.7	28.6±0.8	26.1±0.9	0.070
Heterophil/lymphocyte ratio		4.5±2.5^{b}	15.1±3.1^{a}	8.3±1.0^{ab}	0.033
Corticosterone	ng/mL	31.3±5.5^{a}	10.0±2.1^{b}	31.4±4.8^{a}	0.008
Aspartate aminotransferase	IU/L	1851±377	1934±326	2234±1029	0.073
Mean event scores[2]		3.0±0.5^{b}	4.0±0.6^{ab}	4.9±0.5^{a}	0.040

[a-b]Means within the same row, without similar superscript differ significantly.
[1]Measured after transport.
[2]Measured during handling.

The emus which were in the "flighty" category had significantly ($P < 0.05$) higher body temperature after transport than the emus in the other two categories. The emus in the "calm and easy" category had significantly ($P < 0.05$) higher serum total protein concentrations than the "flighty" category. The H/L ratio in the emus in the category "slightly panicky" was significantly ($P < 0.05$) higher than that in the "calm and easy", while the "flighty" emus had H/L ratio not significantly different from the other two groups. Plasma corticosterone concentrations were significantly ($P < 0.01$) lower in the emus in the "slightly panicky" category than the other two categories. Emus in the "flighty" category tended to have lower serum albumin ($P = 0.070$) concentrations and higher AST activity ($P = 0.073$). Blood glucose, plasma uric acid concentrations, WBC counts, PCV and the activities of enzymes ALT, and CK did not differ significantly among temperament categories.

The mean handling time during loading did not differ significantly between the two experiments (9.1 ± 0.4 and 8.5 ± 0.4 minutes respectively), or among temperament categories. Handling time was significantly correlated to plasma corticosterone ($r_s = 0.32$, $P < 0.05$), ALT ($r_s = 0.34$, $P < 0.05$), AST ($r_s = 0.55$, $P < 0.05$) and CK ($r_s = 0.58$, $P < 0.05$) values at slaughter. Emus handled for more than 8 minutes had significantly ($P<0.05$) higher concentrations of blood glucose, WBC counts and plasma corticosterone, urea, as well as ALT, and CK activities (Table 7.5).

Among the nine events observed during loading (Figure 7.1), jumping and running were the most predominant (more than 40 % of the emus) ones. The total event scores were correlated with the increase in corticosterone concentrations in Experiment 1 ($r_s = 0.65$, $P<0.01$), but not in Experiment 2 ($r_s = 0.17$; $P<0.43$). The event scores varied from 1 to 10 in these emus with a mean score of 4.3 ± 1. The mean event scores for the emus in the three temperament categories are given in Table 7.4. The distress calls (loud, high pitched sound) made by emus while being handled were recorded and sonograms were created. The distress calls and normal drums in a female emu, and the distress calls and normal grunts in a male emus are presented (Figure 7.2).

Table 7.5. Effect of total handling time on biochemical variables of emus estimated at slaughter.

Variables	Units	Total handling time[1] ≤ 8 minutes (n = 14)	> 8 minutes (n = 28)	P-value
Corticosterone	ng/mL	15.1 ± 4.7	29.3 ± 3.4	0.020
Glucose	mMol/L	10.1 ± 1.0	12.7 ± 0.4	0.030
Aspartate aminotransferase	IU/L	1022 ± 577	2429 ± 422	0.050
Creatine kinase	IU/L	6705 ± 2249	15143 ± 1642	0.004
White blood cell count	$X10^9$/L	17.2 ± 1.2	13.4 ± 0.9	0.010
Urea	mMol/L	0.84 ± 0.10	1.29 ± 0.09	0.003

[1]Total handling time includes time for sampling, loading, and unloading.

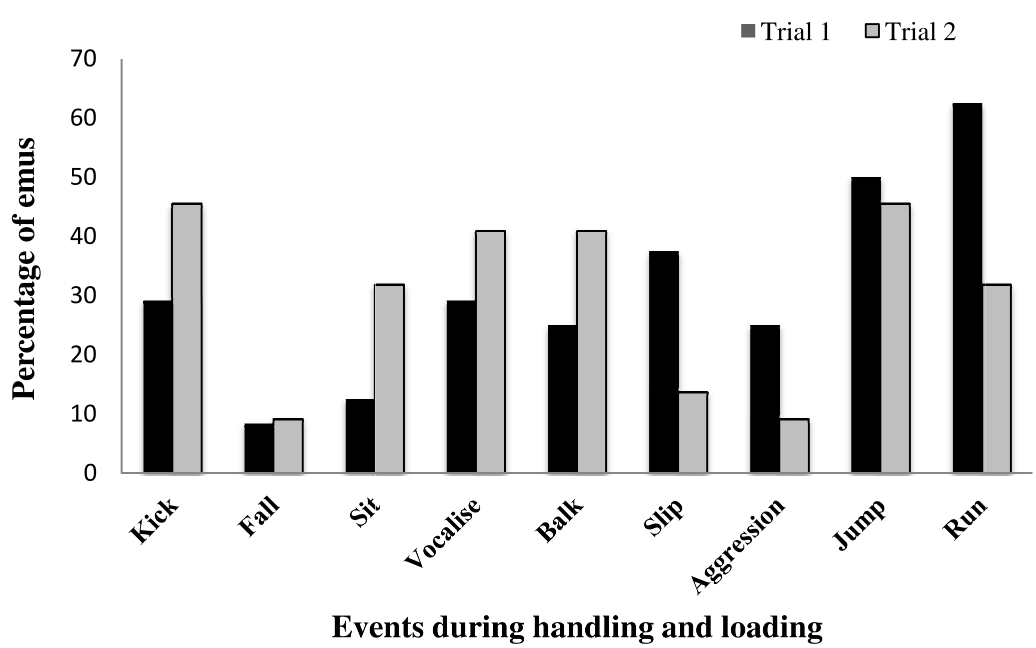

Figure 7.1. Proportion of emus in showing each of the events during handling.

7.3.2 Observations during transport

Immediately after loading, emus were found to huddle at a corner of the compartment. Later, they started to walk around, inspecting the corners of the compartment. They would often rub their neck and body against the side walls and partitions, which resulted in loss of feathers and abrasions on the neck and belly. Panting was observed in the emus in Experiment 1 at three hours from the commencement of the transport. Injuries were noticed in two birds after four hours of transport. At that point, more than half of the emus showed open beak breathing and panting, while three emus showed gular flutter (Table 7.2). These behaviors were not seen at the same period of observation in Experiment 2. For most of the duration of the transport, the emus kept standing. During transport, two emus lost balance, slipped and fell. These birds then tended to sit down frequently (at least once in every 15-20 minutes) for the rest of the transport. They were trampled by other emus, lost considerable amount of feathers, and sustained bruises on back and rump.

7.3.3 Observations after transport

Emus in both experiments drank water, but did not feed for the 15 hour period in lairage after unloading. In both experiments, emus huddled together for about 1-2 hours after unloading and later, some of them started pacing. In Experiment 1, the symptoms of heat stress were more obvious at unloading, with two-thirds of the emus showing open mouth breathing, panting, gular flutter and frothing at the corner of the beak. Two emus had moderate loss of feathers, while two others had extensive loss of feathers and injuries on the back and rump. Unusual behavior such as arching or twisting of neck (retrocollis), frequent head bobbing, could also be noticed in two of them (Table 7.2). Only four emus showed panting and open beak breathing in Experiment 2. In Experiment 1, all emus drank water after unloading, except the emus with extensive injuries and feather loss (see Sections 4.2.9.1.9 and .4.4.5.1.8). They continued to sit without feed and water, till slaughter.

7.3.4 Time budgets before and after transport

In Experiment 1, repeated pecking of the fences and pacing were seen in emus separated from their conspecifics, and it continued for up to five days. The major activities in these emus included feeding, standing, locomotion, resting, unusual behavior (continued pacing, pecking fences or poles, aggression and huddling), and grooming. The pre- and post- transport time budgets of emus are given in Table 7.6.

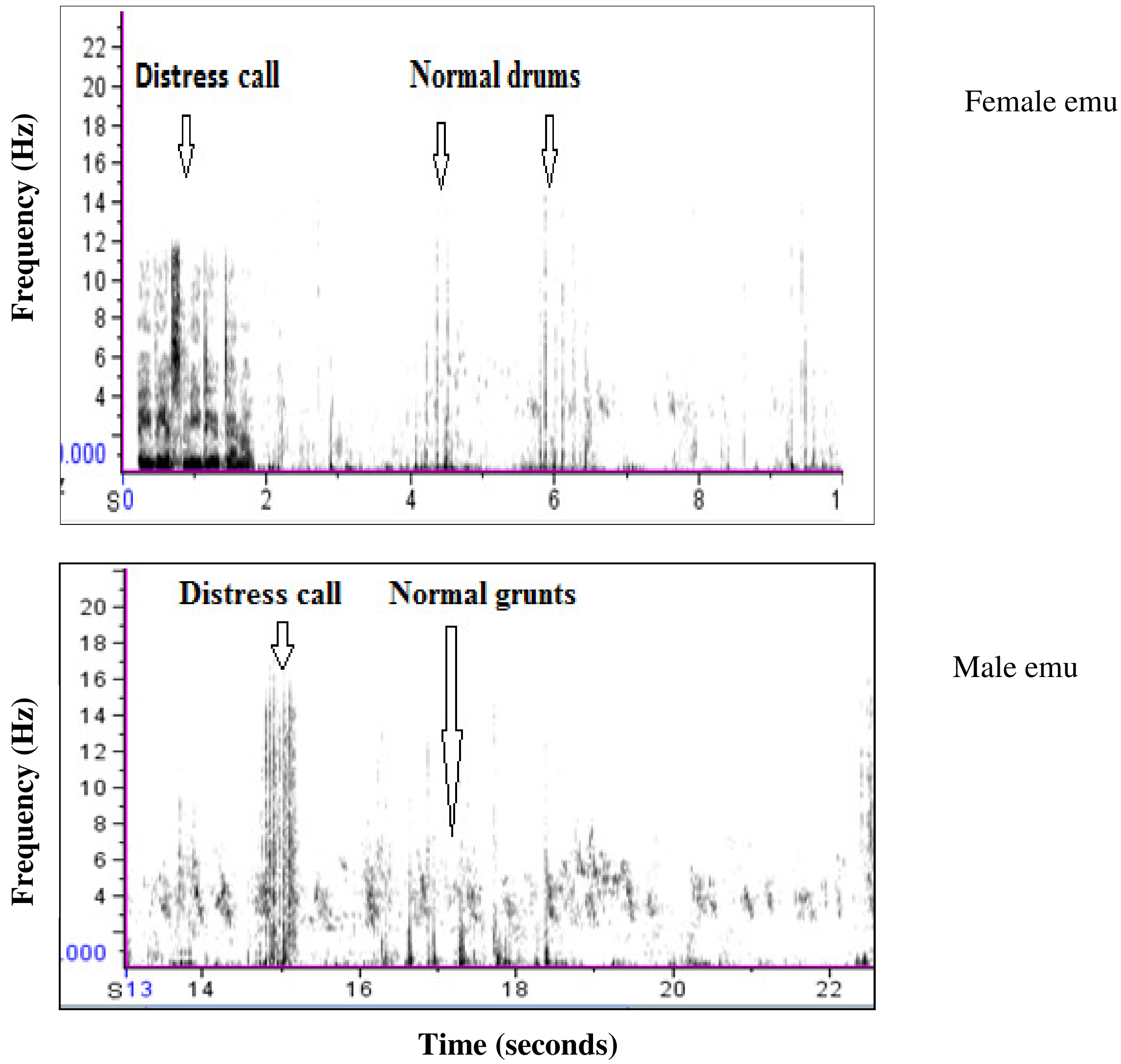

Figure 7.2. Sonogram of the distress calls and normal vocalizations made by female (top) and male emus (bottom).

There was a significant interaction between the time periods and experiments for the time budgets of the behavior fence pecking ($P < 0.01$), pacing ($P = 0.01$), and grooming ($P = 0.03$). Pacing along fences was observed even prior to transport, in Experiment 1 where there was pre-transport regrouping of emus, while it was not seen in Experiment 2.

Table 7.6. Pre- and post-transport time budgets (%) of emus (n = 28) in the two experiments.

Behavior	Experiment 1		Experiment 2		P-value
	Pre-transport	Post-transport	Pre-transport	Post-transport	
Fence pecking	13.1 ± 2.0^{a}	5.2 ± 2.0^{b}	0.4 ± 2.7^{c}	3.3 ± 2.7^{b}	0.010
Pacing	10.4 ± 5.9^{a}	5.2 ± 5.9^{b}	0	10.0 ± 7.9^{a}	0.010
Grooming	3.2 ± 1.4^{b}	0.8 ± 1.4^{b}	8.4 ± 1.9^{a}	0.3 ± 1.9^{b}	0.030
Huddling	0.5± 4.3^{c}	43.9 ± 4.3^{a}	0	23.2 ± 5.9^{b}	<0.001
Standing	33.5 ± 3.4^{b}	40.1 ± 3.4^{a}	46.1 ± 4.8^{b}	52.9 ± 4.8^{a}	<0.001

$^{a\text{-}c}$LS Means within the same row, without similar superscript differ significantly.

The pre-transport time budget for standing behavior was not significantly different between experiments. The post-transport budgets for standing behavior increased significantly($P < 0.001$) from pre-transport in both experiments, but the post-transport time budget was significantly ($P < 0.001$) higher in Experiment 2 than in Experiment 1. Huddling was not commonly noticed in these emus before transport in both experiments, but there was significant ($P < 0.001$) amount of huddling noticed after transport. The time budget for feeding behavior was significantly ($P < 0.001$) reduced in both experiments, from 7.9 ± 2.4 % to none. The time spent on locomotion was significantly ($P < 0.001$) reduced from pre- to post-transport in both experiments, from 32.5 ± 4.8 % to 6.7 ± 3.4 %. Aggression in female emus towards conspecifics was seen only in Experiment 1 (< 0.5%). Resting behavior (1.1 ± 4.5 %) was not significantly ($P > 0.05$) affected by transport.

7.3.5 Behavior at the stunning box

Emus did not vocalize or fall at the stunning box, but they did struggle and slip while being walked to and retained in the stunning box. It was difficult to aim the stunning gun on the emus' head as they constantly kept moving the neck. Hence, four emus had to be shot twice, while one emu thrice for proper stunning.

7.4 Discussion

This study identified the temperament categories, enlisted and described stress related behavior of adult emus (adapted from Koolhaas et al., 2010). Emus have been farmed intensively only since the early 1970s (Krauss et al., 2002), and hence they are less domesticated, compared with most other livestock. This makes their management quite challenging.

7.4.1 Behavior after regrouping

Usually birds spend most of their time in beak related tasks. Partitioning of pens with wire fences led to the separation of pairs, but permitted visual interactions, resulting in pecking of fences prior to transport. Emus are also more likely to spend more time pecking when there is limited choice of activities (Hughes and Grigor, 1996). In the group with just five days of separation (Experiment 1), time spent on fence pecking was significantly greater than that in the group (Experiment 2) with no separation, as the emus were experiencing the stress of disruption in social order. Confinement and boredom are two possible reasons suggested by Glatz (2000) for this behavior, especially in emus low in the pecking order. Pecking behavior has been reported to helped lower H/L ratio and corticosterone concentration in chicken (Freire et al., 2001), suggesting their relevance in overcoming stress.

Pacing was significantly greater in Experiment 1 than Experiment 2, due to separation from group members. Increased pacing is considered to help overcome anxiety in several species, especially the stress induced by confinement, frustration (Degen et al., 1989) and movement restriction (Stephens and Perry, 1990; Mason and Latham, 2004). This is an accepted indicator of stress in emus (Glatz, 2005), ostriches (Mitchell, 1999; Deeming, 1998), and chickens (Bracke, 2006; Sherwin, 2010). However, pacing has been reported to be part of the normal repertoire of ostriches (McKeegan and Deeming, 1997; Csermely et al., 2007) and rheas (Sales et al., 2000). Thus it is important to observe this behavior over a considerable period of time so as to differentiate between normal and displacement behaviors before drawing any conclusions. It may be inferred that significant increases in fence pecking and pacing could be associated with the stress experienced by the emus after disruption of their social order.

7.4.2 Observations during handling

The reaction to human presence inside their pens indicated the proactive and reactive strategies in emus that are triggered by the influence of the HPA and SA axis activities (Koolhaas et al., 1999). These strategies may vary among individuals, depending upon the type of the coping styles (Koolhaas et al., 2010) that predominates in the individual at that time. This in turn could have caused the variations in the temperament, and differences in the concentrations of corticosterone and metabolites in circulation.

Emu produce a low intensity drum when threatened or while defending territory to signal imminent dangers, (Davies, 2002) and this vocalization designated as “alarm call” by him is made less often by males than females. However, the vocalizations made by the emus in this study were heard only while being handled or restrained. Emus did not vocalize at the sight of handlers and after handling. Hence this particular vocalization appeared to be distress calls (Laiolo et al., 2004), which may be made with the intention to startle or distract the handler, attract attention towards the caller, or elicit mobbing behavior (Bergstrom and Lachmann, 2001; Neudorf and Sealy, 2002; Hollen and Radford, 2009). Analysis of the sonograms revealed the similarity of the distress call to drums (Figure 7.2). However, the sonogram patterns showed considerable variation even within the same sex and hence, more research is needed to interpret them in detail (Regosin, 2002). Nevertheless, this vocalization will be a helpful signal to detect situations which could be stressful to emus.

The behavior observed in emus during handling is probably their means to communicate to conspecifics about their particular affective states (Fraser, 2009). The observed events such as slipping, falling, running, bolting away, and hitting fences are the results of flight responses (Grandin, 2003; Maria et al., 2004). Sitting (cattle; Lanier and Grandin, 2000); rolling (cassowaries; Stewart, 1984), stopping (cattle, pigs, Grandin, 1997), and frothing at the corner of mouth (Lanier and Grandin, 2000) are also fear responses. Such signs of fear during procedures indicate compromised welfare (Downing and Bryden, 2002; Zulkifli and Azah, 2004). The defensive (fight responses) behaviors observed included hissing (also reported in ostriches; Meyer et al., 2002), and kicking forward, backward and laterally (Stewart, 1984; Siegal-Willott, 2007).

Unlike ostriches (Black and Glatz, 2011), hooding made the emus more excitable, and hence is not recommended (Shury, 2007). Holding the emus at the base of wings is risky, since excessive struggling could lead to fracture or dislocation. Handlers need to consider alternative methods of herding. Emus lack diaphragm and hence sternal movements along with expanding ribs are absolutely essential for respiration (Tully and Harrison, 1994). This could be inhibited when handlers straddle the emu for long periods, leading to the death of the bird, on account of hypoxemia (Shury, 2007; Siegal-Willott, 2007). Therefore careful handling of emus is extremely important.

7.4.3 Relationship between metabolites, corticosterone concentrations and behavior

The stress responses of emus (Table 7.4) varied among the handling temperament categories (Table 7.1). The "calm and easy" emus had high corticosterone concentrations, which was unexpected. This would indicate that emus in both "flighty" and "calm and easy" categories experienced considerable amount of stress during handling. The flight responses were very pronounced in the flighty emus, under the influence of predominant SA axis activity (Koolhaas et al., 1999), resulting in higher body temperature and lower serum total protein concentrations. Emus in the "calm and easy" category showed coping strategies with a more predominant parasympathetic activity, resulting in significantly lower H/L ratio. The fact that slightly panicky emus had lower corticosterone and a greater heterophilia is suggestive of the dissimilarity in the sensitivity of these two variables to stressors, implying the need to look into both of them to evaluate stress (Muller et al., 2011) in this species. The lower total protein concentrations in the "flighty" emus revealed that birds in this group were possibly in a negative energy balance after greater physical activity and stress related gluconeogenesis (Sporer et al., 2008). The trends for higher AST in this category were suggestive of greater muscle injuries (Huff et al., 2008).

Elevated corticosterone concentrations would adversely affect the production and reproductive efficiency of emus (Hemsworth and Coleman, 2010). Emus with flighty temperament seemed to be more susceptible to stress, and hence require special precautions while handling. For breeding purpose, emu producers eliminate birds with fractious behavior (flighty category), and select only those with even temperament (Dwayne Harder, Emu Producer, Try Harder Farms, Saskatchewan, personal communication).

The significant effect of handling time on serum biochemical variables further indicated that minimal handling of emus is necessary to reduce stress and also to get feedback free samples for diagnostic purposes. Similar observations were made in cattle by Voslarova et al. (2011). Handling and transport being stressful, led to significant increase in corticosterone and enzyme activities in emus, as seen in chicken (Manhiani et al., 2011) and ostriches (Wolmarans, 2011). The alterations in serum metabolite concentrations and immune responses are under the influence of corticosterone (Remage-Healey and Romero, 2001) and hence stress linked (Sporer et al., 2008; Minka and Ayo, 2008).

The total event scores were highly correlated to corticosterone concentrations at slaughter in Experiment 1 only, but not in Experiment 2, probably because of circadian variations. Considering the fact that secretion of corticosterone is pulsatile, showing diurnal and seasonal variations (reviewed by Palme et al., 2005), it should not be considered as the sole indicator of stress. A study on ostriches (Mitchell, 1999) observed a two time increase in corticosterone concentrations after 4.5 hours of transport, while rheas showed 40 times increase after a 30-minute transport (Leche et al., 2013). It is likely that the time elapsed from handling to sample collection would have affected the plasma corticosterone concentrations in the emus in this study.

7.4.4 Observations during transport

Continuous monitoring of the emus during transport is beneficial to identify emergencies and act accordingly, so that their welfare is not compromised. Non-ambulatory emus found during transport should be immediately attended to and if needed, humanely euthanized (OHTG, 2007).

A comparison between the behavior shown during and after transport revealed that warm weather could considerably affect the welfare of emus during transport. The proportion of emus showing stress related behaviors during and after transport was greater in Experiment 1, conducted under warm weather conditions. Panting or hyperventilation seen with the progress of transport is the effect of hyperthermia (Mosley and Gunkel, 2007) as the temperatures inside the compartment went up to 22^{o}C during transport. Gular flutter (Table 7.2) is a mechanism to combat thermal stress by increasing evaporative cooling in ostriches (Maloney and Dawson, 1993) and many other bird species (Brainerd, n.d). Open mouth breathing indicated the buildup of noxious gases and insufficient oxygen levels inside the compartment. This is another physical indicator of stress in some species (pigs; Ritter et al., 2009). Emus are considered to be “rare or occasional head-bobbing birds” (Ortega, 2005) and increase in this behavior is stress linked (Mitchell, 1999). In cattle, slipping and falling during transport is usually linked to driving events such as gear change, cornering and braking (Smith and Wilson, 1999). In the case of emus, in addition to being bipedal, constant walking inside the compartment seemed to be another reason for the loss of balance during transport. Placing of partitions and appropriate stocking density (two emus per m^2) inside the compartments would help to limit this behavior (Minka and Ayo, 2011a) and resultant injuries.

7.4.5 Observations after transport

Injured emus decline to eat and drink, and if this continues for long, it is advisable to tube feed them with electrolyte solutions (Samson, 1997). Since emus would drink after transport, it will be beneficial to supplement drinking water with anti-stress medications such as Nutri-charge that contain energy sources, electrolytes, and essential amino acids.

Comparing the activities of emus before and after transport seemed to be an efficient diagnostic tool to assess adjustments made to overcome stress (Breed and Moore, 2011), and to identify unusual behavior (Sanotra and Weeks, 2004; Mollenhorst et al., 2005). Emus introduced to novel environments, huddled for a considerable period, primarily as an attempt to reduce heat loss from their body (Downing and Bryden, 2002). Reduction in ongoing behavior such as feeding, grooming and locomotion after transport are also due to fear of unfamiliar places and objects (Cheng and Jefferson, 2008; DellaCosta et al., 2013).

Aggression in female emus is frequently seen during the breeding season (Blache et al., 2000), and is otherwise uncommon (Davies, 2002). This study was conducted immediately prior to the breeding season of emus, which explains the change in their temperament. Feeding and preening are important behavior affected by transport stress.

It is essential to reduce injuries during transport, as this would add on to the stress already experienced by the bird. Improved handling, restraining and stunning methods need to be designed for emus to ensure that their welfare is not compromised during slaughter. Emus need to be conditioned to handling and herding to reduce their panic responses. Darkened facilities may be used while emus are handled and the handlers should be trained to restrain them from behind, standing close to the body and legs to avoid being kicked (Bewg and Kent, 2008). This study revealed the role of stockmanship on ensuring the welfare of emus.

7.5 Conclusions and recommendations

Behavioral responses of emus while being handled and transported denoted the associated physiological responses to stress. corticosterone responses alone will not indicate the stress experienced by emus. Identification of such stress related behavior would aid to improve the management and welfare of emus. Handling time should be minimized while emus are restrained for sample collection. Analyzing time budgets would be helpful while assessing the welfare of emus. Training of emus to handling and herding would reduce their panic responses, minimize stress levels and avoid changes in biochemistry and enzyme activities. The use of mobile slaughter houses should be encouraged.

8. GENERAL DISCUSSION AND CONCLUSION

8.1 Discussion

This study congregated the knowledge on emu husbandry practices from experienced producers in the industry (Chapter 2). The survey was successful in identifying major problems faced by emu producers during the handling and transport of emus. The finding that many producers in NA slaughter emus only after they have completed one to two breeding seasons, confirmed the relevance of evaluating the stress responses in adult domestic emus. Long distance shipments (up to 600 km) of emus are always a cause of concern. Majority of the producers were not satisfied with the current methods of handling and transport of emus and expressed their concern over the lack of slaughter facilities and trained personnel to slaughter emu.

Handlers found night time handling and loading of emus easier than that during day time (Chapter 2). All the respondents preferred to transport emus either at night or early morning. This would mean that these birds were off feed and water since the evening of the previous day. They are further confined in the trailers for another 6-8 hours without feed and water. Emus would not eat or drink while being shipped and would remain off feed even for prolonged periods after stressful events. Therefore it is very likely that such emus would be in a negative energy balance at slaughter, unless they are provided electrolytes and energy sources at lairage. The meat from such birds will be more susceptible to defective conditions (Warriss, 2010). This necessitated access to water until loading (Glatz and Miao, 2008), nutritional interventions and sufficient lairage after transport to avoid the deleterious effects of transport stress.

Around half of the respondents slaughtered birds in fall, indicating that emus are transported during a period when their fat deposits are maximal (Chapter 2). This is an advantage in that producers get more fat off the bird (Kim et al., 2012) and thus better economic gains. However, this insulating cover of fat would make thermoregulation difficult in emus and render them susceptible to thermal stress (Glatz and Miao, 2008). This meant that adult emus transported immediately prior to their breeding season are likely to experience considerable amount of stress, and the transportation studies confirmed the same (Chapters 4, 5).

As a preliminary step to evaluate physiological responses to stress, the reference intervals for hematological, serum enzymes, metabolites, and electrolytes values in adult emus were estimated for the first time, using the “reference value advisor program” (Friedrichs et al., 2011).

These values could be utilized to assess the health status, metabolic diseases, nutritional deficiencies and thus the welfare of emus (Chapter 3). Based on these variables, this study revealed how male and female emus prepare themselves for the challenges of the breeding season.

The most challenging aspect was to collect samples with minimal handling. Though in literature, emus are described as generally docile (Patodkar et al., 2009), their panicky temperament made handling quite challenging. Emus would kick forward and sideways, which made it necessary that handlers stay at the back and close to their body. A minimum of three people were needed to control and collect samples from emus. Jugular vein on the right side was found to be the most suitable site for blood collection. Emu blood tended to hemolyse, which necessitated immediate preparation of smears to avoid morphological changes to blood cells.

There was no significant between-sex difference in body weights, body temperature, and all the hematological and enzyme variables measured, as both sexes prepare to play their challenging roles during the breeding season (Chapter 3). Female emus in the pre-breeding phase are similar to other avian species in that they had significantly higher concentrations of plasma calcium, phosphorus, triglycerides, serum total protein, albumin, and globulin concentrations than males. Emus are unique among domesticated birds in that they reduce their feed intake considerably, in the breeding season and rely on their fat reserves for nutrition. Therefore it is necessary that during the pre-breeding and breeding periods, their diet be fortified with all necessary nutrients so as to avoid deficiencies and associated metabolic diseases (Black and Glatz, 2011).

Change in selected indices of stress and metabolic homeostasis (hematology, serum biochemistry, enzymes, and body temperature and weight) were used to evaluate the physiological response to transport in emus (Chapter 4). This study revealed that transport of emus for six hours under the prevailing industry transport conditions resulted in weight loss, hyperthermia; metabolic and immune imbalances and loss in body weight (physiological indications of stress), injuries and feather loss. The activities of all three enzymes (ALT, AST and CK) increased significantly from pre-transport to slaughter due to muscle cell wall damages (muscle injuries). Resting for 15 hours at the lairage seemed to be beneficial to emus, as they partially recovered the body weight losses. However, it is a concern that the enzyme levels continued to increase even during this period (Chapter 4).

A major problem encountered by producers while transporting emus was that when stressed by long chases, emus collapsed and died. This study identified that the cause for non-ambulatory conditions and mortality in emus was exertional rhabdomyolysis (Chapter 4). Such incidences could compromise the welfare of emus considerably (Wotton and Hewitt, 1999). Incidence of rhabdomyolysis underlined the need for careful handling and transport. Two emus which had body temperature above 39.5^{o}C during handling, later showed clinical signs of rhabdomyolysis. Therefore any emu with such high body temperatures should not be handled any further.

To facilitate easier loading, emus should be moved into a narrower pen during rounding up. The fencing should be solid and taller than six feet, as emus are likely to jump that high and also climb fences. Emus do not respond well to hooding and hence it is not recommended. Handlers need to be careful while holding the wings as they are likely to dislocate or fracture under undue pressure. Any attempt to straddle should be done with utmost care as inhibiting the expansion of ribs could turn fatal for emus.

This underlines the need for trained handlers for emus during loading, and unloading. Improved trailers or air sprung trucks to minimize vibrations, ramps and chutes to facilitate easier loading and unloading need to be used (DPIW, 2008; Glatz and Miao, 2008). Minimal handling, better loading and unloading techniques to avoid injuries, padded interiors in truck compartments, and conditioning of emus to yarding are other preventive measures to be adopted (AHA, 2012; Wotton and Hewitt, 1999).

Thermal stress had a serious impact on emus as evidenced by significantly higher weight loss; increases in body temperature, plasma corticosterone and PCV than that in cool weather (Chapter 4). The behavioral indicators of stress such as open mouth breathing, panting, and gular flutter were more pronounced under warm weather. Therefore, it is important that emus are transported either during cooler hours of the day (night or early morning) or in thermoregulated trucks.

The fact that 40 % of the emus had bruises, while 21.4 % incurred small wounds during transport indicated compromised welfare of the bird and hence deserves serious attention. Such bruises and wounds resulted in significantly higher meat pH (Chapter 5). Meat from those birds with extensive injuries showed stress myopathy. Injuries could increase the chance of trims, discards and bacterial contamination. Incidence of defective conditions in meat would result in economic losses to the producer. Findings from the transport study further confirmed that heat stress resulted in greater insults on the body of the emus (Chapter 4) and also affected meat quality (high pH, tendency for DFD and poor color stability; Chapter 5).

Emus are bipedal with just three toes on each foot. Absence of a well-developed wing is another biological feature, which makes it hard for emus to balance themselves during transport. One third of the producers did not provide bedding on the trailer floor, even though it is a recommended practice (CFIA, 2013c). Without proper footing, there are high chances for them to slip and fall, during transport, leading to more injuries. Well lit interiors in the truck compartments probably encouraged walking in emus during transport. Walking led to slipping and falling, and increased the chances of trampling by other emus. Therefore darkening of compartments may be followed as a means to reduce walking of emus (DPIW, 2008). Another option would be to place partitions in the compartment and reduce the space allowances (Minka and Ayo, 2008). Continuous monitoring of events inside the compartments during transport would help early detection of injured birds and attend to them immediately.

Producers and shippers were concerned about injuries and mortality during transport. However, more than half of the respondents were unaware of the loss in body weight, which is associated with greater drip loss and resultant poor saleability of meat. This study points to the need for optimizing transport conditions of emus in NA to maintain meat quality. Use of air sprung trucks with skid free flooring, and padded walls of the compartments would help to reduce injuries (Glatz and Miao, 2008). In the warm weather, emus experienced significantly higher weight loss. The drip loss in meat after 24 hours storage was higher in emus which had greater live weight loss after transport, confirming the adverse effect of stress on meat quality.

The supplement treatment pre- and post-transport significantly reduced the leakage of enzymes from muscle cells, and stress hormone levels (Chapter 4). It also helped the emus regain losses in body weight. The key to effective usage of supplement lies in ensuring intake. From the Experiment 1, I realized that due to the reduced feed intake during the pre-breeding season, it will not be easy to ensure intake of the supplement if it was administered along with regular feed. A pilot study (Appendix I) was conducted in which the supplement was administered in both feed and drinking water to emus from three days prior to transport. The results revealed significantly lower enzyme levels in the treatment group after transport. Care was taken to maintain the concentration of the supplement to a level, where there won't be any change in color of the drinking water as emus might hesitate to drink it. This Experiment validated the assumption that emus would consume the supplement if administered in drinking water.

These results provided the encouragement to use the supplement in powder form in Experiment 2, where intake was ensured using a modified calf feeder (Chapter 4). However, this method is not without practical challenges. Tube feeding of emus would require trained handlers who can administer the same without causing aspiration. Two more handlers will be needed, one to hold the emu down, and the other to open the beak. The second task was more difficult as emu beaks have serrated edges and a pointed tip. Thick gloves may need to be worn to avoid injuries to handlers. Therefore administration of the supplement in drinking water prior to transport and after unloading seems to be a more practical solution to overcome the effect of stress on these birds if producers are unwilling to subject emus to an additional handling stress.

After transport, though the emus declined to take feed, but they did drink water. Therefore, it will be beneficial to administer electrolytes and other supplements in drinking water to compensate for the losses during transport. It would have been more informative, if there was a treatment group, which was given supplement only after transport, to confirm the advantage of the nutritional intervention in alleviating the effect of stress on emus. As administration of oral supplements did not have any effect on meat quality characteristics (Chapter 5), more research needs to be done to establish the appropriate composition and dosage for this species.

The processing yields and meat quality characteristics were determined from a large population of adult emus for the first time (Chapter 5). Male emus yielded significantly more fat, suggesting that slaughtering more males may be beneficial for producers interested in fat yields alone. Females in this study were not significantly heavier because males had put on more fat to meet the challenges of the prolonged incubation period. The months of September or October seemed to be an appropriate time to slaughter emus as after this period; they would reduce their feed intake and start to mobilize their fat depots.

This project provided the opportunity to understand the behavior of emus, and their responses to regular handlers (Chapters 6 and 7). The distress calls (vocalizations) during handling (visualized as sonograms for the first time) could be utilized in assessing the welfare of emus. Information on behavioral repertoires, time budgets, can be utilised to detect unusual behaviors. Huddling in a corner, isolating themselves from other emus, increased pecking at the fences, and pacing were also observed after interruption to their social structure. The time budgets for feeding, grooming, and locomotion were significantly reduced after transport. These behaviors seemed to be behavioral indicators of stress in emus. Identification of such stress related changes in activities of emus would be useful as indicator of stressful events. Early detection of such behavior would aid to improve the management and welfare of emus.

The plasma corticosterone concentrations were determined in this species for the first time (Chapter 4). However, the correlation between the corticosterone concentrations and event scores in Experiment 1 could not be replicated in Experiment 2 (Chapter 7), probably because of multiple factors that influence corticosterone concentrations. Hence I could not conclude that the event scores are reliable non-invasive indicators of stress in emus. Among the temperament categories identified, flighty emus showed highest corticosterone concentrations, while the calm and easy to handle emus showed high corticosterone and low H/L ratio, suggesting that these two indicators of stress in emus vary in their sensitivity to different types of stressors. The information on the activities of emus could be utilized to build a knowledge-base on the relationship between their behavior and welfare (Chapter 7).

Based on this study, the choice of variables for evaluating handling and transport stress in emus include glucose and triglyceride concentrations (blood biochemical variables); activities of all the three enzymes ALT, AST and CK; WBC counts, and H/L ratio (Chapter 4). While evaluating triglyceride concentrations, it may be borne in mind that females are likely to have higher concentrations than males in the pre-breeding season. Handling time should be considered while evaluating the concentrations of corticosterone, glucose, enzyme activities and white blood cell counts (Chapter 7).

All transportation involving emus should be as short as possible considering their increased susceptibility to stress (DLGRD, 2003). A suggestion from producers to overcome the effects of stress was to ship emus to farms near the processing plant or the processing plant itself well in advance so that the birds are rested over a considerable period of time before slaughter. Another option would be slaughtering emus on farm, but then the meat could not be sold to public. A third option would be making available mobile slaughter units (Galbraith, 2011). Though most producers are willing to use this facility, their non-availability and high cost make it a not so viable option to the producers. Therefore, if this facility could be made widely available and more affordable, there will be more producers willing to use it.

8.2 Conclusions drawn on the objectives presented in the introduction

Chapter 2: The study succeeded in documenting the prevailing practices in the handling, transport, and slaughtering of adult domestic emus. The major problems identified included lack of slaughter facilities; absence of trained personnel to handle and slaughter emus; and incidence of injuries and mortality during transport.

Chapter 3: The baseline concentrations and reference ranges for the physiological variables in emus were established. There was no significant between-sex difference in body weights, body temperature, and all the hematological and enzyme variables measured in this study, as both sexes face the similar challenges during the breeding season. Female emus are similar to other avian species as they had significantly higher concentrations of plasma calcium, phosphorus, triglycerides, and serum total protein, albumin, and globulin levels than males.

Chapter 4: Emus transported for six hours experienced elevated body temperature, loss of body weight, metabolic imbalances, altered immune status, and increased enzyme activities, in addition to injuries and feather loss. The clinical picture and post mortem examination revealed muscle injuries, and stress myopathy, confirming the incidence of exertional rhabdomyolysis. Resting overnight in lairage after transport helped the emus to recover the losses in body weight marginally and hence is recommended. Administration of electrolytes and nutrients supplements to emus prior to and after transport helped to reduce the effect of stress on them.

Chapter 5: Meat from emus transported for six hours showed incidence of defective conditions such as DFD and myopathy, which would result in economic losses to the producer. Meat quality characteristics were unaffected by sex and supplement treatments. The injuries incurred during transport had a significant influence on meat pH. In the warm weather, emus experienced significantly higher loss in body weight and their meat pH was high. The drip loss in meat after 24 hours storage was higher in emus which had greater live weight loss after transport, confirming the adverse effect of stress on meat quality.

Chapter 6: This study determined the behavior repertoires, and collected information on the behavioral peculiarities in adult domestic emus. Time budgets were mostly unaffected by sex, weather and relative humidity.

Chapter 7: The change in time budgets from before to after transport revealed the effect of the stress on emus, which could be utilized to build a knowledge-base on the relationship between behavior and welfare of emus.

8.3 Significance and contribution of the research reported in this thesis

- Baseline hematological variables and reference ranges for adult male and female emus were determined.
- Behavior and time budgets of domestic emus were determined.
- The physiological responses to handling and transport stress were described for the first time.
- Oral feeding of supplements was found to be effective in reducing the effect of transport stress in emus.
- Handling and transport stress was found to cause rhabdomyolysis in emus.
- Transport under warm weather led to increased loss in body weight and drip loss in meat.
- Handling time significantly affected biochemical and enzyme variables.
- The distress calls in emus were visualized as sonograms for the first time.
- The survey on knowledge, attitude and practices in the emu industry is the first one of its kind in North America.

8.4 Limitations of this study

This study was completed in two experiments and a pilot study, with a comparatively large sample size. However, the number of emus in each treatment group was small, and many analyses showed wide variability, making it difficult to get significant effects. The investigation of the physiological responses was limited to a transport study of six hour duration and one hour confinement only. The findings from this research were suggestive of the efficacy of the supplement in alleviating stress, but it should be noted that only one dosage of the supplement was evaluated. The study was limited to adult emus and hence it cannot be stated whether the older birds had increased susceptibility to exertional rhabdomyolysis. The transport studies were conducted during the pre-breeding season and the influence of this physiologically challenging phase is also included in their stress responses. The blood sampling from emus was done only at two stages of transport and hence it was not possible to differentiate between the stress of handling and that of transport.

The findings from this study will contribute to the data base on hematological, biochemical and enzyme variables in emus, which could be utilized to assess their health, nutritional status and thus improve their welfare. The results and suggestions could be utilized to improve the handling and transport of emus and other ratites.

8.5 Possible future research directions

This study revealed that emus experienced loss of body weight and energy deficits during transport. Administration of an oral supplement containing electrolytes, an energy source (dextrose) and amino acids helped to overcome the losses and improve the welfare of emus. The supplement though effective in reducing the leakage of enzymes, did not affect meat quality. Based on this study, one of the future directions for research would include investigating the appropriate dosing and composition of the nutritional supplement for emus.

The major problem identified during transport is the incidence of rhabdomyolysis, which could cause paralysis and death in emus. Therefore another important topic to look into will be the methods to reduce the incidence of rhabdomyolysis. Better loading and unloading techniques are important to reduce injuries and handling time. Therefore, it is also important to determine better methods of herding and handling emus so as to improve their welfare during transport. The use of mobile slaughter houses also needs to be encouraged.

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APPENDICES

Appendix A Emu industry survey – questionnaire

Avian Research Centre,
2357 Main Mall,
Vancouver, B. C., Canada, V6T 1Z4.

Dear Respondent

We are contacting you to request your help with an important project, "Exploring efficient ways of transporting and processing emus" with the objectives of not only minimizing work for the producers, minimizing stress and injuries to emus during transportation, but also improving consumer acceptance of emu products. This project is a collaboration between the Faculty of Land and Food Systems at The University of British Columbia and the Lacombe Research Centre, Agriculture and Agri-Food Canada. To start the project, we first need to collect information about the emu industry.

Therefore, we are asking you to complete this questionnaire focusing on the current management practices and concerns of the emu industry. Participation is voluntary and you may skip any question you are not comfortable answering. Any information collected through this questionnaire will be held in strict confidence and your anonymity is assured. You may withdraw from the study for any reason, at any time and any data that you have contributed will be deleted and the researcher will not have access to it.

<u>Instructions</u>

1. Please complete this questionnaire by putting a "√" against the most appropriate choice.
2. Make only a single choice unless otherwise stated.
3. Add additional information wherever necessary. Space is provided at the end of the survey.
4. Should you have any questions regarding this questionnaire or the project you can contact Deepa Raju Menon at xxx-xxx-xxxx. Email emustudyubc@gmail.com or my supervisor Prof Kim Cheng at xxx-xxx-xxxx Email xxxx@interchange.ubc.ca

QUESTIONNAIRE

KINDLY GO THROUGH THE FOLLOWING CATEGORIES AND SELECT THE ONE OR COMBINATION WHICH DESCRIBES YOU THE BEST AND FOLLOW THE INSTRUCTIONS GIVEN AGAINST EACH CATEGORY.

Category	**Select**	
Producer only, Producer and Processor, Producer and Shipper		If this is your category, go to Section A, Question 1 and complete all the sections.
Processor only, Processor and Shipper		If this is your category, go to Section B, Question 8
Shipper only		If this is your category go to Section C, Question 12
Other (please specify) _____________		If this is your category go to Section D, Question 40

	SECTION-A **THE FOLLOWING QUESTIONS ARE INTENDED TO COLLECT INFORMATION ABOUT THE MANAGEMENT PRACTICES ON YOUR EMU FARM.**
1	How long have you been working with emus? Less than 2 years 2-4 years 5- 10 years More than 10 years
2	2a. How many emus do you usually keep in an average year on your operation? (Answer this question with respect to the last two years of your operation) Less than 20 21-50 51-100 More than 100 2b. How many emus in each of the following categories do you keep in a regular year? Breeders: _______ Emus going for market this year:_______ Juveniles:_______ 2c. How does your current operation compare with your concept of an ideal size of your emu operation? Exactly same Bigger Smaller
3	How long have you been breeding emus? Never Less than 2 years 2-5 years More than 5 years
4	From your experience, what do you feel is the best male - female ratio for breeding emus? 1:2 1:1 Other (Specify) ______________________________

5	Do you hatch emu chicks for your operation? Yes No If yes : Artificial incubation Natural incubation
6	If you have purchased emus for your operation, what would be the average age at which you purchased them? (You may check more than one answer if applicable.) 0-3 months 4-12 months More than one year Breeders
7	Please answer the following questions based on the current management practices on your operation. 7a. How frequently do you usually feed your emus? Once a day Twice a day *Ad libitum* Other (Specify)_______________ 7b. What type of feed do you provide for your emus? (Check all the applicable options.) Commercial pelleted feed Other poultry feed Mix my own feed Fodder Organic Feed Other (Specify)_____________________________ 7c. How frequently do you usually provide water for your emus? Once a day Twice daily *Ad libitum* Other (Specify)________________ 7d. How do you raise your emus? Free range Semi-intensive Certified Organic Transitional Organic but not certified
	SECTION-B **THE FOLLOWING QUESTIONS ARE INTENDED TO COLLECT INFORMATION ON HOW EMUS ON YOUR OPERATION ARE BEING PROCESSED**.
8	Who usually processes your emus? You yourself Other (Specify) ____
9	Where do you process emus currently? (Check all the applicable options.) On farm Shipped by yourself to processing plant Shipped by others to processing plant I am the processor Other (Specify) ____

10	**10a**.Have you ever processed emus at a mobile on-farm slaughter facility? Yes No **10b**. If no, why not? Too expensive Not available Processing plant close by Other (Specify) ____________________ **10c.** Would you be interested in processing emus at a mobile on-farm slaughter facility if it is available? Yes No Reasons for your answer ____________________
11	Please answer the following questions based on your own experience on your own emu operation. **11a.** At what age are emus usually processed? 1-2 years 2-3 years 3-5 years 6 years or older **11b.** How important are the economic factors (e.g. cash flow requirements, price for oil, etc) in deciding the time to process emus? Very important Somewhat important Not sure Somewhat unimportant Not important I do not make that decision **11c.** What time of the year do you usually process emus? Spring Summer Fall Winter Throughout the year **11d.** Please specify the month(s) during which you generally process your emus? (Specify)____________________ **11e.** Please state any additional reasons which influence your decision to slaughter emus: ____________________

SECTION–C

THE FOLLOWING QUESTIONS ARE INTENDED TO GATHER INFORMATION ON HOW THE EMUS ON YOUR OPERATION ARE TRANSPORTED TO THE PROCESSING PLANT.

If you are a producer who processes emus on your own premises, please go to Question 22.

If you are only a processor, please go to Question 31.

12	When you ship emus to a processing plant, what is the maximum distance between the farm and the processing plant? _____________ Kilometers or _________________miles.
13	What type of vehicles do you use for shipping? (Check all the applicable options) Own Hired Other (Specify)________________ Trailer Large truck Pick-up truck
14	How many emus would you usually carry in the vehicle? Number:________________
15	Please answer the following questions regarding the conditions inside the vehicle(s) you have used to transport emus. **15a.** Are there partitions inside the animal compartment of the vehicle? Yes No Don't know **15b.** How tall is the roof of the animal compartment? As tall as a standing emu One foot above the head of a standing emu More than one foot taller No roof Don't know **15c.** How is the provision for light inside the compartment while transporting emus? Natural lighting alone Completely dark Don't know Other (Specify)___________________________________ **15d.** How is the provision for ventilation inside the compartment while transporting emus? Fairly ventilated Well ventilated Open vehicle Don't know **15e.** Are bedding / mats provided inside the compartment? Yes No Don't know **15f.** If yes, what is the bedding material commonly used? (Specify)______________________________________ **15g**. If yes, usually how thick is the bedding provided? Less than 1 inch 1-2 inches More than 2 inches Don't know **15h.** Do you define any speed restrictions for the vehicle that transport emus? Yes No **15i.** If yes, what is the speed limit? ___________ km/hour or ______________mph
16	Are male and female emus shipped together? Yes No Don't know

17	Are unfamiliar emus (can be from your own farm or other farms) being shipped together? Yes No Don't know
18	Do the emus have access to fodder while they are being shipped? Yes ☐ No ☐ Don't know ☐
19	Do the emus have access to fodder after reaching the processing plant? Yes ☐ No ☐ Don't know ☐
20	Do the emus have access to drinking water while they are being shipped? Yes ☐ No ☐ Don't know ☐
21	**21a.** Do the emus experience any loss in live weight (shrinkage) after shipping? Yes No Don't know **21b.** If yes, what approximately is the average percent of shrinkage (to the best of your knowledge)? 1-3% 4-6% 7-9% 10% and more Don't know **21c.** In your opinion, what might be the reason(s) for this shrinkage? ____ ____ ____
22	How long do you usually withhold <u>feed</u> from emus before shipping? If you slaughter emus at your farm, please check the period of feed withdrawal until the emus are slaughtered: Not withdrawn ☐ Less than 6 hours ☐ 6-12 hours ☐ More than 13 hours ☐ Don't know
23	How long do you withhold <u>water</u> before shipping? (This is time between the last time the emus were given water to drink and the time they were loaded). If you slaughter emus at your farm, please check the total period of water withdrawal until the emus are slaughtered: Not withdrawn ☐ Less than 6 hours ☐ 6-12 hours ☐ More than 13 hours ☐ Don't know

24	**24a.** Do you usually help load the emus onto the vehicle for shipping? Yes No **24b.** If yes, how easy do you find it to load emus onto a vehicle? Very easy Fairly easy Not sure Fairly Difficult Very difficult **24c.** Do the emus resist being rounded up and loaded onto the vehicle? All the time Most of the time Don't know Rarely Never
	24d. Do you use any tools (e.g. cattle prod, lassoes, tranquilizer, hooding, etc) to help you handle the emus? No ☐ Yes ☐ (Specify) ____________________ **24e.** How many emus slip (just lose balance, without falling) while being handled? None ☐ Just a few ☐ Many ☐ All ☐ Don't know ☐ **24f**. How many emus fall down (more than half of the body touching the ground) while being handled? None ☐ Just a few ☐ Many ☐ All ☐ Don't know ☐ **24g.** Do emus attack their handlers? No ☐ Rarely ☐ Frequently ☐ Always ☐ Don't know ☐
25	What precautions do you take to ensure the safety of the personnel handling emus? ____________________ ____________________
26	What precautions do you take to ensure the safety of emus during handling? ____________________ ____________________
27	Please state any other concerns you have with respect to the handling of emus? ____________________ ____________________
28	**28a.** Do the emus vocalize more when they are being transported? No ☐ Yes ☐ Don't know ☐ **28b**. If yes , please check all the applicable options

	While being loaded ☐ During early part of the transportation ☐ Vocalization by few emus ☐ During the later part of the transportation ☐ Vocalization by many emus ☐ **28c.** Do emus attack each other while being transported? No ☐ Rarely ☐ Frequently ☐ Always ☐ Don't know ☐ **28d.** Do emus huddle together while in the transport vehicle? No ☐ Rarely ☐ Frequently ☐ Always ☐ Don't know ☐ **28e.** Do emus attack each other while being handled? No ☐ Rarely ☐ Frequently ☐ Always ☐ Don't know ☐
29	Are you the driver of the vehicle or accompany the shipment to the processing plant? Yes ☐ No ☐
30	At what time of the day do you prefer to transport emus for processing? Night Day Early morning No preference
31	Please describe any problems encountered during the shipping of emus to the processing plant: ____ ____ ____ _____
32	How satisfied are you with the way your emus were transported? Extremely satisfied Moderately satisfied Somewhat satisfied Not sure Least satisfied Dissatisfied
33	How much do you agree with the following statement "The way emus are transported in this country can be further improved"? Strongly disagree Disagree Uncertain Agree Strongly agree
34	In your opinion, what needs to be done further to improve the conditions for transport of emus in your country ____

	SECTION–D **THE FOLLOWING QUESTIONS ARE INTENDED TO COLLECT INFORMATION REGARDING THE VARIOUS PRODUCTS AND BY-PRODUCTS FROM EMUS AND HOW THEY ARE BEING MARKETED.** **If you are only a shipper, go to SECTION –E**
35	How do you usually sell the products and by-products from the emus? (Please check all that apply) Sold from the farm to the consumers directly Sold through a middleman Kept for personal use Sold to a local store Other (Specify) ____
36	What is done with the emu fat harvested? (Please check all that apply) Processed and sold as oil Sold as fat Disposed/Composted Other (Specify) __________________________________
37	What is done with the meat from emus? (Please check all that apply) Sold as pet food Kept for personal use Sold for human consumption Disposed /Composted Other (Specify) ____________________
38	What is done with the hide obtained after processing the emus? You tan the hide Sold to tanneries Disposed /composted Other (Specify) __________
39	What is done with the feathers obtained after processing the emus? (Please check all that apply) Sold by yourself Sold to middlemen Disposed / Composted Other (Specify) ____________________________________
	SECTION–E **THIS SECTION IS INTENDED TO COLLECT INFORMATION ON YOUR VIEWS ABOUT THE CURRENT STATUS AND FUTURE PROSPECTS FOR EMU INDUSTRY IN YOUR COUNTRY AND GLOBALLY.**

40	How would you describe the way emus are generally transported in your country? Extremely good Satisfactory Moderately unsatisfactory Poor Unsure
41	Are you aware of any recent research efforts by university and government researchers to rejuvenate or promote the emu industry in your country? Yes No
42	In your opinion, how would the recent effort to scientifically validate the therapeutic properties of emu oil affect the current status of emu industry in your country? Very helpful Somewhat helpful No effect Not helpful Somewhat harmful
43	How would you describe the current status of the emu industry in your country? Reasonably good Satisfactory Moderately unsatisfactory Poor Unsure
44	How would you describe the current status of the emu industry globally? Reasonably good Satisfactory Moderately unsatisfactory Poor Unsure
45	What do you feel are the future prospects for the emu industry in your country? Reasonably good Satisfactory Moderately unsatisfactory Poor Unsure
46	What do you feel are the future prospects of the emu industry globally? Reasonably good Satisfactory Moderately unsatisfactory Poor Unsure
PLEASE ADD BELOW ANY ADDITIONAL COMMENTS THAT YOU MAY LIKE TO MAKE ON ANY OF THE TOPICS FROM THIS QUESTIONNAIRE	

SECTION–F **THE FOLLOWING QUESTIONS ARE INTENDED TO COLLECT DEMOGRAPHIC INFORMATION FROM THE RESPONDENTS. THIS INFORMATION IS BEING COLLECTED FOR STATISTICAL PURPOSES ONLY**.
1. What is your age __________
2. Gender Male Female
3a. Country of origin Canada US Other (Specify) __________
3b. Location of emu operation Canada US Other (Specify) __________
3c. State/Province __________________
4. Family size

Number of people	How many people including children live in your house including you?	How many people in your family are active in your emu operation?
1-2		
3-5		
6 and more		

5. How are you associated with the emu farm operation? (Please check the <u>one</u> option that best describes you) Primary job Part-time job Farm tourism Recreational purpose Other (Specify)________________________________
6. Employment Status Are you currently employed any job other than the emu operation? (Please check the <u>one</u> option that best describes this other employment) Employed for wages Self-employed Professional Other (Specify)________________________________
7. Beside the emu operation, what other type of farm business do you operate (Please specify) ____
8. Business Type Select the type of business you operate with respect to the emu farm (Select one or all that apply) Sole proprietorship Family business Partnership <u>without</u> a written agreement Partnership <u>with</u> a written agreement Non-family Corporation Cooperative Other (Specify)______________________________

9. Education Completed What is the highest grade or year of school you completed? Elementary High school graduate Some college or technical school University graduate Advance Degree -Masters or PhD
10. Annual Income What is your total annual on the farm income? (Please include your income from the emu operation also) up to $29,999 $30,000 to $59,999 $60,000 to $89,999 $90,000 to $119,999 $110,000 to $149,999 $150,000 and above

Thank you for completing this survey!

Please return this survey within seven days. If you have any other comments or any questions, please include them here. Once again, thank you. We really appreciate your help.

If you are a BC or Alberta resident, and willing to be interviewed as a follow up to this questionnaire we will be happy to come to your farm / processing plant or an alternate location of your choice. We may be contacted at emustudyubc@gmail.com or at xxx-xxx-xxxx.

Appendix B Survey consent form

Exploring efficient ways of transporting and processing emus: A survey of present management practices, attitudes, and preferences in the emu industry

Principal Investigator
XXXXXXXXXXXX

Research Team
XXXXXXXXXXXX

Study Objectives:
1. Minimizing work for the producers in rounding up emus for transportation.
2. Minimizing stress and injuries to emus during loading and transportation.
3. Improving consumer acceptance of emu products.

The data from this survey will be used as part of a PhD thesis completed within the Faculty of Land and Food Systems at The University of British Columbia. A free electronic copy of the thesis will be available upon its completion, from https://circle.ubc.ca/.
If you have any questions at any time about the survey or the procedures, you may contact us by email at: XXXXXXX@gmail.com>

or telephone XXX-XXX-XXXX

If you have any questions about your rights as a participant in this study, you may contact the Research Subject Information Line in the UBC Office of Research Services at XXX-XXX-XXXX or if long distance e-mail to <XXX@XXX.ubc.ca>

Please note the following:
•The adult, 19 years of age or older, who is involved with the emu industry should fill out the questionnaire
•The questionnaire should take about 20 minutes to complete
•Your participation in this study is completely voluntary: completion of the survey indicates your consent to participate in this research. There are no foreseeable risks associated with this project, but if you feel uncomfortable you may refuse to participate in full (and not return the questionnaire), or in part by skipping questions that you are not comfortable with.
•Your survey responses will be anonymous and data from this research will be reported only in the aggregate and for academic research purposes only. Only study team researchers will have access to survey data. While the survey is anonymous, it may be possible for some people to identify you by the content of your answers. Addresses sampled for this survey will be destroyed, resulting in no links to survey responses.
By completing and returning the survey, you are consenting to participate. Please keep a copy of this consent form for your records.

Version: August 5, 2010.

Appendix C Questionnaire -follow up interview

Questions for follow-up interview/discussion with the emu producers

How long have you been rearing emus?

Over the past five years have your operation expanded or collapsed at any point of time?

From your experience, what is the general temperament of emus?

Have you ever transported emus for processing?

If yes, where did you process them? Was their processing ever inspected?

What are the main problems encountered during the handling and transport of emus?

What are the possible solutions to the above mentioned problems?

Do you process emu fat? If yes, how do you do that?

What do you feel is the marketability of the emu oil?

Are you planning to get or do you have organic certification for your farm?

Have you ever processed emus in your farm? If so how do you generally process them?

Is there any demand for emu meat? What do you do with the meat obtained from emus?

Appendix D Assay techniques used in the autoanalyzer

1. Total protein (Modified biuret method)
2. Albumin (Bichromatic analysis)
3. Creatinine levels (Jaffe-Larsen method with picric acid)
4. Serum uric acid (Bichromatic method)
5. Serum urea (Urease method)
6. Serum triglyceride (Lipoprotein lipase method)
7. Serum glucose levels (Hexokinase method)
8. PCV (Microhematocrit or auto analyser)
9. Serum corticosterone levels (Enzo Corticosterone EIA Kit)
10. Serum creatine kinase (Rosalki method)
11. Serum aspartate amino transferase (Karmen method)
12. Serum alanine amino transferase levels (Wroblewski and LaDue method)
13. Corticosterone ELISA

A competitive immunoassay measuring optical density was used to calculate the concentration of Corticosterone. The kit used a polyclonal antibody to corticosterone to bind, in a competitive manner, to the standard or sample with corticosterone covalently attached to it. After a incubation at room temperature, the excess reagents were washed away and substrate was added. After a short incubation time the enzyme reaction is stopped and the yellow color generated is read on a microplate reader at 405 nm. The intensity of the bound yellow color was inversely proportional to the concentration of corticosterone in either standards or samples. The data obtained was analyzed utilizing a four parameter logistic curve fitting program immunoassay software package.

Appendix E Nutrient supplement mentioned in chapters 4 and 5

Composition of Nutri-Charge® (Stress conditioner), developed at Agriculture and Agri-Food Canada, US. Patents # 5505968 and 5728675, Lacombe, Alberta, Canada.

Nutricharge[1]	%
Crude protein	20
Crude fat	4.3
Crude fiber	14.3
Sodium	1
Magnesium	0.3
Potassium	1.7

[1]Ingredients: Corn, corn gluten meal, alfala, whey powder, skim milk powder

Composition of the powder form of Nutricharge

Ingredient	%
Methionine	1
Lysine	0.5
Tryptophan	0.3
Threonine	0.7
Mgso4	1.5
KCl	1.5
NaCl	3
KHco3	4
NaHCo3	4
Dextrose	20
Fat	2
Mollasses	4

Appendix F Definition of terms used in chapter 5

Dressing weight is the saleable (warm) carcass weight including lean, bone and fat (Harris et al., 1993) after the removal of head, feathers, skin, viscera genital organs, feet up to the carpal and tarsal joints (SCARM, 2003).

Dressing percentage is the weight of carcass expressed as a percentage of the live weight of the bird (Warriss, 2010).

Yield is the weight of each saleable product harvested from the bird after processing, often expressed as a percentage of live weight at slaughter (Pollock, 1997).

Appendix G   Behavior recording sheet used in chapters 6 and 7

Date	Time	RH	Weather	Emu #	Sex	Session

	Time (seconds)	**Eating**	Foraging	Picking	Drinking	Preening	Dust bathing	Feather picking	Body shake	Headshake	Running	Resting	Sleeping	Walking	Standing	Sitting	Pacing	Vocalizing	Running	Chasing	Aggression	Parallel walking	Threatening	Yawning	Pecking fences	Huddling	Voiding	Remarks
1	30																											
2	60																											
3	90																											
4	120																											
5	150																											
6	180																											
7	210																											
8	240																											
9	270																											
10	300																											
….	……																											
60	1800																											

Appendix H Description of procedures involved during handling of emus

Handling of emus: Started the moment the handler got hold of the emu, brought it under control, walked it to the weigh scale / brought it down on to the bed of straw.

Blood Sampling: Started the moment the attempts were made to disinfect the site of collection and collect samples from the jugular vein using vacutainers.

Loading: Started the moment from which the emu was caught, walked to the truck and over the ramp and placed inside the truck compartment.

Unloading: Started the moment the truck doors were opened and the emus were brought out and placed inside the lairage pens.

Total handling time: Sum of the time for handling, blood sampling, loading and unloading.

Appendix I Pilot study

Research notes

The eight emus in the South campus, UBC were divided into two groups (2 males and 2 females each), and one group (treatment) was given a special diet (1:1) ratio and then the special nutrient formula was supplemented in their drinking water (9 g/L) for 3 days prior to the transport. The groups were divided 2 weeks prior to the start of the experiment. The behavior of the birds was observed for 3 days prior to transport. Their feed intake (one week prior to the experiment) was on an average 300 g/day and this improved to little less than 700g per day two days prior to transport. The baseline values of hematological and blood biochemical variables were estimated 5 days prior to transport. On the day of transport the emus were weighed, loaded into a truck and transported for six hours from Vancouver to Duncan. The behavior changes and incidents during handling and transport were recorded. On reaching the new facility, blood samples were collected and weights recorded. The birds were housed in two separate pens and continued on the special diet and drinking water supplement for three more days and their behaviors were also recorded during this period.

At 60 hours after transport, blood samples were again collected and the weights were recorded. The data collected over three time periods was analyzed to determine the changes during transport and the probable recovery process in emus based on the behavioral, hematological, serum biochemical and hormonal changes.

The body weights were found to decrease after transport (due to a problem with the weighing scale the third set of data recorded is not usable). The emus remained off feed for at least 36 hours post transport and hence a further reduction was noticed in some emus.

The blood glucose levels remained unchanged immediately after transport, but further increased at 60 hour period. Creatinine levels were reduced significantly immediately after transport ($P < 0.05$) and then increased substantially but still not different from the after transport levels. Total protein and globulin levels remained unaffected while albumin levels were reduced significantly ($P < 0.05$) and continued to decrease even at 60 hours after transport.

Liver enzymes (ALT and AST) registered only moderate increase after transport, but the levels were further elevated ($P < 0.05$) when measured at the 60 hour period. The CK levels were significantly elevated after transport ($P = 0.006$) and then were moderately reduced but significantly higher ($P < 0.05$) than the pre-transport levels.

There was significantly ($P < 0.01$) lower CK level in the treatment group than the control while there was interaction between treatment and period in the AST levels. At the 60 hour period, the control group had significantly ($P < 0.05$) higher AST levels than all other levels and groups. Triglyceride, total protein, uric acid and PCV levels were lowered but this was not statistically significant.

The WBC counts were significantly increased ($P < 0.05$) immediately after transport but at 60 hours, the value was decreased and was not different from the other two time periods. Heterophil counts increased ($P = 0.006$), while lymphocyte count decreased significantly ($P < 0.05$) after transport; and remained unchanged even 60 hours thereafter. The H/L ratio showed increase after transport and this value was significantly lower in the treatment group when compared to the control ($P < 0.05$). The analyzed data revealed that supplementation of special diet and electrolytes resulted in significant reduction in the AST, CK and H/L ratio alone.

Intensity of injuries was found to affect the resuming of feed intake of emus after transport; they remained off feed for a minimum of 36 hours.