EFFECTS OF PRE-SLAUGHTER HANDLING, TRANSPORTATION, AND NUTRIENT SUPPLEMENTATION ON OSTRICH WELFARE AND PRODUCT QUALITY

by

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Abstract

Ostriches (*Struthio camelus*) are the largest living birds with only two toes on each of two long feet that support a heavy body mass. This special anatomical feature creates problems for transporting ostriches. However, little research has been done to examine ostrich welfare during handling and transportation and how this relates to product quality. The main goal of this dissertation research was to find ways of improving ostrich welfare during pre-slaughter handling and transport, which would also contribute to increased product quality and decreased product losses. To achieve this goal, three related research projects were conducted.

For the first research project, a producer survey was conducted in Canada and USA. From the survey results, I identified current ostrich pre-slaughter handling and transport norms (e.g., long transportation), and also potential welfare issues in the current ostrich pre-slaughter transport practices.

Based on the identified potential welfare issues from the survey, an experiment (with 24 birds) was conducted to study effects of pre-transport handling on stress responses of ostriches. The results showed that the pre-transport handling process is stressful for ostriches and should be minimized. During this research, an immobile sitting behaviour was identified as a behavioural stress response which could be used to identify the fearful birds during handling to minimize losses.

For the third research project, three transport trials (with 45 birds) were conducted to investigate the effects of pre-transport nutrient supplementation and transport duration on ostrich welfare. Results indicated that birds transported for a longer time had more weight losses, and male birds which did not receive the nutrient supplement lost more weight. Therefore, the present shipping condition of long distance transportation is detrimental to ostrich welfare with significant losses incurred by producers.

Specific ratite transport guidelines have not been developed in Canada or USA. Therefore, transport welfare guidelines from other countries were reviewed to find applicable guidelines to remedy the identified welfare issues, and research studies were carried out to find solutions for remaining issues. The information gathered will be provided to policy-making bodies to develop Codes of Practice for ostrich transportation in Canada and USA.
Preface

A version of Chapter 2 has been submitted for publication as: “Bejaei M and Cheng KM, A comparative study of current ostrich handling and transport practices through surveys and literature review for the improvement of ostrich welfare and product quality”. In this research, I developed the main objectives for the research, conducted the literature review and the review of the transport standards/guidelines from six different countries, developed the research methods, designed the survey questionnaire, completed the ethics application, conducted the surveys and interviews, gathered data from producers, analyzed results, and prepared the manuscript for publication. As the supervisor of this PhD research, Dr. Cheng, the co-author, provided editorial changes to the questionnaire and the manuscript, and provided valuable feedback on all aspects of the research. The project received research ethics board approval from UBC under certificate number: #H11-02380.

A version of Chapter 3 has been submitted for publication as: “Bejaei M and Cheng KM, Effects of pre-transport handling stress on physiological and behavioural responses of ostriches”. In this research, I developed the main objectives for the research, conducted a literature review, designed the experiment, prepared the animal care application, conducted the behavioural and body physical damage study, helped with the blood sampling, conducted the lab work, analyzed the data, and prepared the manuscript for publication. Dr. Cheng, the co-author, supervised the research, edited the manuscript, helped with the interpretation of the behavioural study results, and provided valuable feedback. Dr. Darin Bennett helped with animal care application, ordering/organizing equipment required for the transport trials and travel arrangements, helped with handling and blood sample collection process, and acted as a supervisory committee member. Dr. Valerie LeMay (supervisory committee member) provided advice on the experiment, data analyses, and provided feedback on the manuscript. Dr. Allan Schaefer (Agriculture and Agri-Food Canada, Lacombe Research Centre Scientist) and Dr. David Fraser provided valuable feedback as the supervisory committee members. The project received UBC Animal Care approval under certificate number: #A11-0110.

A version of Chapter 4 has been submitted for publication as: “Bejaei M, Bennett DC, Schaefer AL and Cheng KM, Effects of pre-transport nutrient supplementation and transport
duration on post-transport blood biochemistry, body weight and welfare of ostriches”. In this research, I developed the main objectives of the research, conducted the literature review, designed the experiment, completed the animal care application, recorded data during sampling, helped with the sampling process, conducted lab work and meat quality assessment tests, analyzed the resulting data, and prepared the manuscript for publication. The co-authors were supervisory committee members. Dr. Bennett helped with the animal care application, ordered and organized equipment required for the transport trials and travel arrangements, helped with the handling and blood sample collection, and provided feedback on the manuscript. Dr. Schaefer provided the nutrient supplement used in the three trials, and helped with the sample collection process, meat quality assessment tests and hematology tests. As supervisor of the PhD dissertation, Dr. Cheng supervised the research, edited the manuscript drafts, and provided valuable feedback. Dr. LeMay provided advice on the experiment, data analyses and provided feedback on the manuscript. The project received UBC Animal Care approval under certificate numbers: #A11-0110, and #A12-0028.

Presentations and publications related to this thesis

- **Bejaei M and Cheng KM** 2013 *A holistic approach to assess ostrich welfare during handling and transport practices*. Poultry Science Association Meeting, San Diego, California, USA
- **Bejaei M and Cheng KM** 2013 *Transportation of ostriches*. Animal Transportation Association (ATA) 39th Annual Conference. Las Vegas, Nevada, USA (*Invited speaker*)
- **Bejaei M, Bennett DC, Schaefer AL and Cheng KM** 2013 *Effect of transport duration and nutrient supplementation on post-transport blood biochemistry and live weight loss in ostriches*. Animal Transportation Association (ATA) 39th Annual Conference. Las Vegas, Nevada, USA
- **Bejaei M, Bennett DC and Cheng KM** 2013 *Effect of time spent in the pre-transport holding pen on physiological and behavioural stress responses of ostriches*. Animal Transportation Association (ATA) 39th Annual Conference. Las Vegas, Nevada, USA
- **Bejaei M** 2012 *Welfare of ostriches during handling, transportation and slaughter*. Iowa State University, Animal Science Department, Ames, Iowa, USA (*Invited speaker*)
• **Bejaei M and Cheng KM** 2012 *Ostrich handling, transportation and slaughter*. American Ostrich Association Meeting, Des Moines, Iowa, USA (*Invited speaker*)

• **Bejaei M and Cheng KM** 2012 *A survey of ostrich producers, handlers and processors for developing indicators to assess welfare related management and processing practices in ostrich production*. Poultry Science Association Meeting, Athens, Georgia, USA (*Winner of the Graduate Student Research Paper Certificate of Excellence in the Behavior and Well-Being section*)

• **Bejaei M, Bennett DC, Schaefer AL and Cheng KM** 2012 *Effects of transport and nutrient supplementation on hematology, blood biochemistry and live weight loss in adult ostriches*. Poultry Science Association Meeting, Athens, Georgia, USA

• **Bejaei M, Bennett DC, Schaefer AL and Cheng KM** 2012 *Influence of pre-transport handling and holding time on pre-transport hematological stress indicators and post-transport body condition of ostriches*. Poultry Science Association Meeting, Athens, Georgia, USA

• **Bejaei M, Bennett DC, Schaefer AL and Cheng KM** 2012 *Environmental stress factors affecting adult ostrich behaviour during shipment*. North American International Society for Applied Ethology (NA-ISAE), Banff, Alberta, Canada

• **Sunderland M and Bejaei M** 2012 Taking ostrich farming to the next level, *World Poultry* 28(01) (*Invited paper*)

• **Bejaei M, Bennett DC, Schaefer AL and Cheng KM** 2011 *Ratite research at the University of British Columbia, Canada*. American Ostrich Association Meeting, Huston, Texas, USA
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1 Introduction

Ostriches (*Struthio camelus*) are the largest living birds in the world and are endemic to Africa (Davies & Bamford 2002). Ostrich farming started in North America in 1980s (Deeming 1999 p 7). A unique anatomical feature of ostriches, the largest living birds with only two toes on each of two long feet that support a heavy body mass, makes their handling and transportation problems different from other livestock. Transportation of ostriches to a registered processing plant is necessary if the producers want to sell the meat through the retail sector, and transportation is identified as one of the main factors affecting ostrich welfare (Mitchell 1999; Wotton & Hewitt 1999). However, little research has been conducted on ostrich handling and transportation. Also, Codes of Practice specific for the transport of ratites have not been developed in Canada or USA.

Various stakeholders (policy makers, experts and producers) may have conflicting opinions regarding the treatment of animals. As a result, the lack of established standards and guidelines may cause decreased welfare. Because of the complexity of the welfare issues, I conducted my research on the impacts of pre-slaughter handling and transport practices on the welfare of ostriches using a holistic research approach involving the following steps: i) current ostrich farming practices were considered by conducting a producer survey to identify current potential welfare issues in ostrich handling and transport; ii) established standards and guidelines from other countries of the world were reviewed; iii) the effects of the identified potential welfare problems on the behavioural and physiological stress responses were examined; and iv) the product quality of ostriches under different handling and transport practices was assessed (See Figure 1-1).

Several factors may affect pre-slaughter welfare of ostriches and the quality of the products obtained from these birds (e.g., pre-slaughter handling process, transport duration, and feed and water withdrawal duration). However, very little research has been done to determine the effects of these factors on the welfare and product quality of ostriches. The research conducted in this study contributes to the welfare of ostriches by investigating current ostrich handling and transport practices in Canada and USA, using experiments to study the effects of the identified welfare problems on the stress responses and product quality of ostriches, and
studying possible mitigation of stresses via nutrient supplementation and shorter transport durations. Collectively with the review of transport welfare guidelines from other countries, this research contributes towards helping related organizations (e.g., National Farm Animal Care Council of Canada) to develop Codes of Practice for ostrich transportation and handling in Canada and USA.

An overview of ostrich farming history, farm products and wild ostrich behaviour is given at the beginning of this chapter. Then, definitions of stress and stressors are provided based on various published articles. Animal behavioural and physiological stress responses and different stress assessment methods are also described in this chapter. Specific pre-slaughter handling and transport stressors which affect the animal response to transport stress, impact production factors and behavioural/physiological stress responses of animals are then identified, and the methods that can be implemented to alleviate the transport stress of animals are described. The last section of the chapter describes the main goal of this research and specific objectives of each research chapter.

1.1 Ostriches

As noted earlier, ostriches are the largest living birds. They have a heavy body mass (above 85 kg) on two long feet and stand over 2 m in height. They are flightless birds and the only extant bird with two toes on each foot (Schaller et al. 2011).

1.1.1 Classification

Domain: Eukarya
Kingdom: Animalia
Phylum: Chordata
Class: Aves
Order: Struthioniformes
Family: Struthionidae
Genus: Struthio
Species: Struthio camelus

Five ostrich subspecies have been recognized based on their geographical distribution, difference in the bare skin color, bald patch form on the crown, presence of white feathers at the neck, structure and size of the egg shell (Davies & Bamford 2002):
- *Struthio camelus camelus* Linnaeus (Red-Neck ostrich)
- *Struthio camelus molybdophanes* Reichenow (Somali ostrich)
- *Struthio camelus massaicus* Neumann (Masai ostrich)
- *Struthio camelus australis* Gurney (Zimbabwean Blue-Neck ostrich)
- *Struthio camelus syriacus* (extinct since 1960s)

Most ostrich farms in Canada and USA are using developed crossbreeds of these subspecies. For example, South African Black ostrich, *Struthio camelus* var. *domesticus*, was developed by the cross-breeding of *S. c. australis*, *S. c. camelus* and *S. c. syriacus*.

1.1.2 Ostrich behaviour in natural environment

In natural habitat, ostriches live in social groups of mixed age and gender, headed by an adult male or a ‘major’ female. During the breeding season, however, they can be seen in small groups, in pairs or solitary (Deeming & Bubier 1999). Ostriches usually avoid close contact with other species (Deeming & Bubier 1999). They are diurnal, and adult ostriches are mostly vegetarian consuming 5-6 kg of fresh vegetation daily (Deeming & Bubier 1999).

1.1.3 Ostrich farming history

Ostrich farming for the production of feathers started in South Africa in the early 1860s; however, the demand for ostrich feathers diminished because of the global depression after World War I and the ostrich farming industry collapsed (Deeming 1999 p 6). The cooperative of South African ostrich farmers (the Klein Karoo Landboukoöperasie (KKLK)) started to rebuild the ostrich market in 1945, and at the beginning, their goal was ostrich leather as the primary product and then feathers for the secondary product. Meat has become an important ostrich product since 1980s (Deeming 1999 p 6-7). Ostriches were exported from African countries to
Israel and North America in 1980s, and ostrich farming has expanded to more than 50 countries since 1980s (Deeming 1999 p 7).

1.1.4 Ostrich farming products

Meat, skin, fat, eggs, feather, chicks and breeders are world-wide ostrich farming products. The slaughter age for the South African Black ostrich is 12 to 14 months, whereas the slaughter age for the Red-Neck and Zimbabwean Blue-Neck ostriches is 10 to 12 months (Balog & Almeida Paz 2007). Ostrich live weight includes 60% carcass (including 35% lean meat, 15% bone, and 9% fat) and 40% non-carcass (including 1% head, 6.5% blood, 3% feet, 7.5% hide, 1.5% wings, 4% offal, and 17.5% viscera) (Shanawany 1999). Hoffman et al. (2007) reported that the live weight of South African Black ostriches (84.9 ± 9.2 kg) was lower than Zimbabwean Blue-Neck ostriches (100.9 ± 4.2 kg) at 14 months of age.

1.2 Stress in animals

In this section, the main physiological stress response systems (sympathetic nervous system (SNS) and hypothalamus-pituitary-adrenal cortex axis (HPA)) are first discussed. Then, common stress assessment methods and a comparison between the stress response systems of poultry and mammals are presented. Finally, the potential impacts of accepting a definition for stress and selecting specific stress assessment indicators are discussed.

1.2.1 Definition of stress

Most animal physiology and welfare researchers have considered a link between animal welfare and stress biology (Moberg 1987; Barnett & Hemsworth 1990; Moberg 2000; Fraser 2008). Lack of stress can be a potential indicator of good animal welfare, but there are different definitions of stress and there are several biochemical indicators which could be measured to assess stress (Möstl & Palme 2002).

Initial studies on stress biology were conducted by Cannon who defined the fight or flight response system, or SNS response, which activates catecholamine release (Cannon 1929). Selye (1978) later identified the general adaptation syndrome (GAS) which involves the HPA response. He called the non-specific response of the body to any threat as stress; however he
named the pleasant activation of HPA as eustress or good stress and undesirable activation of stress as distress (Selye 1978). Many researchers have used corticosteroid concentrations to determine stress levels in various species since Selye’s findings.

Barnett & Hemsworth (1990) proposed a threshold for the HPA response to measure the effect of the stress on the welfare of animals. They suggested that if the concentration of free corticosteroids in the blood plasma in stressed animals was 40% higher than that of control animals, the animal may show detrimental consequences and their welfare was threatened. Reduced welfare may have a metabolic cost for animals or negatively impact on body functions such as immune system activities or on production by influencing the growth rate, pregnancy rate and sexual behaviour (Barnett & Hemsworth 1990).

Rushen (1991) questioned the accuracy of the use of corticosteroids for the measurement of different stressors and the 40% threshold determined by Barnett and Hemsworth (1990), because of the high variation in the secretion of corticosteroids and many unanswered questions about them. Welfare of animals might be at risk (e.g., difficulty in adapting to a condition) without showing detrimental consequences such as immunosuppression or effect on production.

Moberg (1987) proposed that pre-pathological indicators (i.e., changes in an animal’s biological functions) which have a potential to cause a pathological state should be used to measure stress and wellbeing of the animal. According to Moberg’s (2000) definition, stress is a biological response caused by any threatening factor on homeostasis of an individual, and if stressors really threaten the animal’s health, subsequently the animal will experience distress.

Fraser (2008) argued that if we accept Barnett & Hemsworth’s (1990) and Moberg’s (1987) proposals for animal welfare assessment, we would not be able to recognize welfare problems when there is no change in the biological function of animals as a result of stress. Fraser (2008) questioned the direct link which scientists have made between most stress responses and the welfare status of animals. He argued that the lack of an SNS or HPA response does not automatically mean a good welfare condition for animals, and on the other hand, activation of SNS or HPA response does not mean poor welfare condition because they may be activated in response to natural demands of animals (e.g., mating).
1.2.2 Stressors

Moberg (2000) defined stressors as factors that threaten the homeostasis of an individual (e.g., severe feed restriction, high and low environmental temperatures, fear and frustration, and noise and road transportation). Möstl & Palme (2002) also defined a stressor as an environmental stimulus which causes an imbalance of homeostasis.

Disease, nutrition, behavioural abnormalities, climate, and transportation are the main factors which can alter farm ostrich welfare (Mitchell 1999). Schaefer et al. (1996) stated that withholding feed, mixing unfamiliar animals, fighting, transport, weather condition and handling were the main antemortem stressors.

Schaefer et al. (1988), Warriss (1990) and Grandin (1997) considered stress factors in two categories: psychological stress factors, and physical stress factors. Grandin (1997) mentioned that fear is a very strong stressor which causes psychological stress. Fear is a common stressor in the animal kingdom which helps animals to avoid predators and other dangers. Grandin (1997) also reported novelty as one of the strong stressors because, in the wild, novelty is an important signal of possible threats. She indicated that the reason for high variation in an animal’s response to stressors (e.g., handling, transportation, contact with people, or exposure to novelty) is the variation in their psychological stress levels. Isolation is also one of the stressors related to handling of animals; it can increase the cortisol levels and result in physiological stress responses in animals (Grandin 1997).

Rosales (1994) categorized poultry production stressors as: physical stressors (e.g., catching, injections, transport, immobilization); physiological stressors (e.g., rapid growth, sexual maturation process); climatic stressors (e.g., extreme cold and heat, high humidity); nutritional stressors (e.g., deficiency of nutrients, feed intake problems); environmental stressors (e.g., wet litter, bright light, bad ventilation); psychological stressors (e.g., fear, rough handling); and social stressors (e.g., overcrowding, poor body weight uniformity).

1.3 Animal responses to stressors

Animals show behavioural and hormonal responses to stressors and there is a strong intimately interrelated correlation between the two (Dantzer & Mormède 1983).
Moberg (1987) proposed that an animal’s responses to stressful events occur in different stages. In the first stage, an animal recognizes a threat to its homeostasis via its central nervous system. At this stage, the animal has a perception of stressors. In the second stage, the animal responds to stressors by organizing its biological defence in its central nervous system, using stress response systems (behavioural and physiological) and by showing changes in biological function. In the third stage, the animal shows the consequences of stress which include change in biological function, pre-pathological state (e.g., suppression of immune system), and development of pathology (e.g., vulnerability to an infectious disease).

Grandin (1997) considered a complex interaction of genetics and prior experience as factors which affect the degree to which animals can respond to stressors, and she discussed three different response systems:

- **Behavioural reactions:** Different factors such as an animal’s genetics, previous experience, seasonal and environmental factors can affect their behavioural reactions.
- **Immediate stress responses:** The animal may activate the sympathetic nervous system (SNS), which Cannon (1929) called the fight or flight reaction.
- **Hypothalamus-pituitary-adrenal cortex (HPA) axis:** This response was introduced by Selye (1978).

Barnett & Hemsworth (1990) reported that animals may show a predominant behavioural response to a stressor (e.g., withdrawing from a dangerous stressor) which may be accompanied by short-term physiological responses (e.g., increase in heart rate or respiration). They indicated that if the animal is not able to solve the threatening situation by behavioural responses and short-term physiological changes then chronic physiological responses will be activated. The long-term stress responses affect the prevalence and severity of different health problems such as suppression of the immune system, hypertension, arteriosclerosis and development of gastric ulcers. Observation of behavioural responses is the first and most important component of stress response recognition (e.g., monitoring lameness in animals) (Barnett & Hemsworth 1990).
Pre-slaughter stressors (e.g., mixing unfamiliar animals, extreme weather, loading, transport, feed and water withdrawal, unloading and lairage duration) activate both behavioural and physiological response systems (Schaefer et al. 2001). Activation of these systems causes biochemical changes in an animal’s body during the pre-slaughter period that could prevail in the carcasses after slaughter (e.g., dehydration, protein catabolism and energy depletion which affect the meat quality); these negatively impact animal welfare as well as production yield and quality (Schaefer et al. 2001).

In a review paper, Rosales (1994) listed the impacts of stress in poultry as: increased corticosterone, insulin and glucagon concentrations; higher metabolic rate and increased resting energy expenditure; higher usage of glucose as an energy source; increased free fatty acids in plasma (less usage); hypoglycemia (more glucose utilization); lower growth and more muscle degradation; release of acute-phase cytokines; damage in the growth of cartilage and bone; redistribution of trace minerals; production of specific stress proteins; lower voluntary feed intake; higher body temperature; and immunosuppression.

Early life events and handling experience also affect an animal’s future physiological and behaviour stress response (Moberg & Wood 1982). During an open field test, lambs reared in isolation showed different behavioural response (e.g., withdrawn behavior or avoiding interaction, lower vocalization and less movement) compared to lambs reared with ewes or peers; however, there was no difference in their weight gain and plasma cortisol levels (Moberg & Wood 1982).

**1.3.1 Sympathetic nervous system and adrenal medulla (SNS)**

As noted earlier, SNS and HPA are the two major physiological stress response systems (Sapolsky et al. 2000; Romero & Butler 2007). The SNS response activation occurs immediately after stress. The SNS system is known as the fight or flight response system and it helps animals to avoid a threatening situation (stressor) or to fight the stress agent. It affects a variety of biological systems in an animal’s body including the gastrointestinal system, the cardiovascular system, and the release of catecholamines from the adrenal medulla (Cannon 1929).

The sympathetic nervous system rapidly releases catecholamines (norepinephrine and epinephrine) when faced with stressors, and stimulates release of catecholamines from the
adrenal medulla (Sapolsky et al. 2000; Romero & Butler 2007). Catecholamines can activate the breakdown of glycogen in the liver, and they can cause important metabolic changes by increasing lipolysis, gluconeogenesis and glycogenolysis (Romero & Butler 2007).

The SNS shows a rapid and specific response to different stressors and indicators related to this response system have been used to measure stress (e.g., measurements of heart rate, respiration rate, blood pressure and secretion of catecholamines) (Moberg 1987). Digital data loggers can be used to measure heart rate, respiration rate and body temperature without introducing additional stress to an animal.

1.3.2 Hypothalamus-Pituitary-Adrenal Cortex axis (HPA) response system

The HPA response system takes a few minutes to become activated after an animal is confronted by a stressor. Multiple stressors can activate the HPA response system (Selye 1978). Most researchers relate the secretion of hormones in the HPA system directly to the wellbeing of animals and they have used HPA hormones (corticotropin releasing hormone (CRH), adrenocorticotropin hormone (ACTH), corticosteroids, cortisol and corticosterone) to assess stress (Romero & Butler 2007).

Activation of the HPA system is a long-term stress response compared to activation of the SNS and it helps animals to respond the environmental change (Sapolsky et al. 2000). A stressor stimulates the hypothalamus to first release CRH, then CRH stimulates the secretion of ACTH from the anterior pituitary (adenohypophysis), and, finally, ACTH stimulates the adrenal cortex to release corticosteroids (or glucocorticosteroids; primarily corticosterone in birds and cortisol in mammalians) (Sapolsky et al. 2000; Meisenberg & Simmons 2006; Romero & Butler 2007).

Glucocorticosteroids (GSs) affect the metabolism of glucose by increasing plasma glucose concentrations, activating gluconeogenesis from amino acids and increasing excretion of uric acid (Elrom 2000). GSs activate the synthesis of fatty acids and increase the saturated:unsaturated fatty acid ratio, and as a result of HPA activation, the carcass will show glycogen depletion and decline in muscle protein and fat (Elrom 2000).

Activation of the HPA system increases secretion of glucocorticoids, reduces secretion of insulin (i.e. reduced glucose storage as glycogen and fat), increases glycogenolysis and
production of glucose, reduces secretion of sexual hormones and sensitivity of testes and ovaries to sexual hormones, and decreases the secretion of growth hormones (Elrom 2000; Sapolsky et al. 2000; Romero & Butler 2007). Increased levels of corticosteroids reduce skeletal calcification in growing animals and cause osteoporosis in adults; this, in turn, increases the blood calcium levels (Elrom 2000). The hypothalamus has corticosteroid receptors which suppress secretion of CRH via activation of a negative feedback mechanism (Elrom 2000).

1.4 Stress assessment methods

A combination of different stress response systems (behavioural, SNS or HPA response systems) should be used to identify the effects of stressors; a single indicator may not provide an accurate measurement of responses to different stressors since each stressor shows specific characteristics as well as some non-specific characteristics (Dantzer & Mormède 1983; Moberg 1987; Moberg 2000). This is not compatible with Selye’s (1978) argument regarding the non-specific nature of the HPA response (Moberg 1987). Also, an inter-animal variability in biological stress responses commonly occurs, because the biological stress response of an animal depends on various factors including its genetics, previous experience, and physiological state (Moberg 1987). Moberg emphasized the importance of considering the relationship between the measured stress indicator and its impact on the wellbeing of an animal before measuring the indicator or interpreting its results. However, there are other welfare researchers who questioned Moberg’s suggestion, because some stressful situations may not directly show an impact on the wellbeing of an animal in spite of resulting in uncomfortable conditions for the animal (Fraser 2008).

Elrom (2000) suggested that chronic stress could be measured via end-organ response (e.g., heterophil and lymphocytes responses or lymphoid organ regression, the heterophil:lymphocyte ratio). End-organ responses are better indicators for chronic stress than measuring plasma hormone concentrations (e.g., CRH, ACTH, catecholamines and corticosteroids). However, plasma hormone concentrations are better indicators of acute stress response.

Rosales (1994) mentioned that each stressor (e.g., feed withdrawal, transportation, fear, extreme temperatures) causes a specific leucocytic response. Nevertheless, he concluded that cortisol levels or heterophil:lymphocyte ratios are not the best stress indicators in poultry.
Rosales (1994 p 200) suggested that more reliable stress indicators are “measurements of the suppression of the immune system determined by counts and proportions of leukocytes, different immunological functions, and size of the lymphoid organs”.

Overall, to assess the stress response of animals, various stress response indicators could be selected from behavioural or physiological response systems. In the following subsections, a summary of some of the common stress assessment indicators are given.

1.4.1 Behavioural observation

An animal’s abnormal behaviours can be used as an indicator of stress (Barnett & Hemsworth 1990). For example, changes in vocalization or locomotion relative to normal behavioural responses can be used to indicate stress. However, the relationship between the measured behavioural response and its effect on the welfare of the animal should be identified (Moberg 1987).

1.4.2 Production and pathology records

Flock records such as body weight gain/loss, mortality and stocking density could be used to identify birds raised under stressful conditions (Rosales 1994).

1.4.3 Catecholamines

Norepinephrine (noradrenalin) and epinephrine (adrenalin) are released from the sympathetic nervous system (SNS) and adrenal medulla (Sapolsky et al. 2000; Romero & Butler 2007). They could be measured directly in blood samples taken immediately after the initiation of the stress. Blood samples must be processed immediately because the half-life of catecholamines is very short. Therefore, it is not easy to use catecholamines as stress indicators (Elrom 2000).

1.4.4 Heart rate, respiration rate, blood pressure and body temperature

SNS activation affects heart rate, respiration rate, blood pressure, and body temperature; therefore, these physiological measures could be used as measures of stress responses. There is a positive relationship between heart rate and cortisol levels (Grandin 1997). However, heart rate is not only controlled by the SNS but it is also controlled by the parasympathetic nervous system.
Therefore, some researchers have indicated that heart rate is not a reliable indicator of SNS activation. Instead, beat-to-beat variability (heart rate variability which is a temporal distance between successive beat-to-beat intervals) may be a better measure than heart rate (Hagen et al. 2005).

Digital thermometers or infrared thermography (IRT) could be used to measure body temperature as a non-invasive method to measure stress response (Schaefer et al. 1988). Body temperature at slaughter (which is a species-specific response) also affects meat quality. This could be used as a stress indicator as noted by Schaefer et al. (2001), although they noted that, in cattle, the skin temperature increased during stress, whereas in pigs it decreased or increased.

1.4.5 Corticosteroids

Corticosteroids (especially glucocorticoids) are steroid hormones released from adrenal cortex because of secretion of ACTH (Romero & Butler 2007). The primary glucocorticoid in most mammals is cortisol and in birds it is corticosterone. Most researchers have used blood corticosteroids to measure stress; however, their secretions do not happen during all stressors and they have a circadian rhythm in several species such as pigs, cattle and horses (Möstl & Palme 2002).

Cortisol may be a useful indicator of acute stresses (e.g., castration), but it is a time-dependent measurement which reaches its peak in 10 to 20 min in blood (Grandin 1997). Cortisol concentration increases in blood a few minutes after exposure to a stressful events and it often drops to its baselines 1 h after the end of the stressful event (Dantzer & Mormède 1983; Mounier et al. 2006). Sampling time affects the cortisol concentration in plasma (Grandin 1997).

Cortisol levels are very variable and “absolute comparisons should not be made between studies” (Grandin 1997 p 253). Therefore, some researchers suggest that cortisol concentration should not be used as the only stress indicator. Genetics is one of the important influential factors and there is high variety in the corticosteroid levels between species and within species (between individuals) (Dantzer & Mormède 1983). An animal’s previous experiences also affect the levels of cortisol secretion. Cortisol concentrations should be measured immediately after exposure to a stressful event (Mounier et al. 2006).
Moberg (1987) indicated that measuring HPA hormones is not the most accurate method to assess stress because an adrenal cortisol response does not occur in all stress responses. There are other factors such as sampling method, season, diurnal, temperature, gender and several physiological conditions of the animal that may affect the secretion of corticosteroids.

Cortisol can be found in two forms in blood, free or bound to corticosteroid-binding globulins (CBG), and 80-90% of the total cortisol is bound cortisol (Elrom 2000). Free cortisol is able to enter different cells and fluids in the body. During stress, free plasma cortisol concentrations increase (Mormède et al. 2007). Researchers have different opinions regarding the measurements of free or total corticosteroids as stress indicators. Barnett & Hemsworth (1990) suggested measurement of free corticosteroids is a more reliable indicator of stress than total corticosteroid; however, most researchers usually measure total corticosteroids in blood. The ratio between free and total corticosteroids is not the same for different species (Mormède et al. 2007).

The sampling method affects the concentrations of glucocorticoids. In wild animals, blood samples must be taken within 2-3 minutes after capturing the animal to assess the baseline concentrations of glucocorticoids (Mormède et al. 2007). Multiple blood sampling is also a stressor by itself. It can increase infection risk and inflammatory response, and can affect the results of the experiment. Use of catheters for multiple blood sampling may overcome the problem.

To minimize the effects of blood sampling techniques on corticosteroid levels, some researchers have used remote blood sampling devices (e.g., Ingram et al. (1994) used this technique for blood sampling of deer). Others have implemented non-invasive sampling methods such as determining corticoid metabolites in urine, saliva or milk (Möstl & Palme 2002; Palme et al. 2005). Saliva free cortisol contains unbound and active steroids which can be measured successfully in some species as HPA activation indicators, and it is positively correlated to serum cortisol (Cook et al. 1996). Nevertheless, there are limitations for the application of these discussed methods. For example, milk samples can only be obtained from lactating animals, and gathering saliva or urine samples requires some manipulation of animals.

Möstl & Palme (2002) suggested that a better non-invasive sampling method to measure adrenocortical activity is using fecal sampling which does not have the limitations of saliva, milk
and blood sampling methods. Fecal samples can be collected easily and they are more stable with minimum storage requirements compared to blood and saliva samples. However, this method has its own limitations. For example, feed intake may cause variation in steroid concentration in fecal matter, there should be multiple samplings to be able to find secretion peaks, and there is a high variation in different species regarding the optimal sampling time. High inter-animal variations of cortisol metabolite concentrations in both plasma and fecal samples have been noted, along with substantial differences between species in the amount of cortisol metabolites excreted via urine or feces. Further, the concentration peak varies between species; for example, the peak could be about 12 h after activation of physiological stress response in sheep, 24 h after in ponies and 48 h later in pigs because of different intestinal passage times among species (Möstl & Palme 2002).

1.4.6 Measuring multiple steroid hormones

Instead of relying on the measurement of only one steroid hormone (cortisol or corticosterone), multiple steroids could be measured from a small amount of sample using new techniques which are validated for some species (Koren et al. 2012).

1.4.7 Immune system related indicators

Moberg (2000) mentioned that long-term stress decreases the number of lymphocyte cells and causes the atrophy of lymphoid organs. Immunosuppression occurs as a result of an increase in circulating glucocorticoids because of stress and this can increase infectious disease susceptibility (Stanger et al. 2005).

Stress decreases the number of lymphocytes but increases the number of heterophils in chicken blood samples (Gross & Siegel 1983). The heterophil:lymphocyte ratio (H:L ratio) is a better indicator of chronic stress response compared to plasma corticosteroid concentrations because other physiological factors can also affect corticosteroid concentrations in blood (Gross & Siegel 1983). Nevertheless, there are other factors (e.g., social rank of animals) which may affect the H:L ratio. Therefore, behavioural measurements should accompany the H:L ratio measurement to have more reliable indicators of stress (Gross & Siegel 1983). Stress increases H:L ratios and results in an increase in bacterial disease resistance and decrease in viral disease resistance (Elrom 2000a). Chronic stress and release of high levels of corticosteroids in blood
result in a decline in lymphocytes circulation, thymus involution, reduction in spleen mass and peripheral lymph nodes, and general immunosuppression (Elrom 2000a).

Rosales (1994) reported that different stressors induce different leucocytic responses; as a result, measuring the heterophil:lymphocyte ratio or corticosteroid level does not always indicate precise stress levels in poultry. He stated that better methods to measure the stress level in poultry are immune system suppression measurements via counts and proportions of different immunological functions, leukocytes, and size of lymphoid organs.

1.4.8 Creatine phosphokinase (CPK)

Warriss et al. (1994) reported that levels of plasma creatine phosphokinase (CPK) and lactate are useful indicators of handling-stress in pigs. They found a strong correlation between the sound level of squealing pigs in commercial abattoirs and CPK activity.

Warriss (2010d p 68) mentioned that when muscles need more adenosine triphosphate (ATP), the Lohman reaction occurs (i.e. creatine phosphate (CP) + adenosine diphosphate (ADP) ↔ creatine (C) + ATP) to allow the continued contraction of muscles. CPK catalyses this reaction and is abundant in muscles, and when the pH is neutral, the reaction moves to right (if the CPK is available), but during the recovery period the reaction moves to the left (Warriss 2010 p 68).

1.5 Stress responses of mammals vs. poultry

In mammals, the hypothalamus releases peptide hormones directly into anterior pituitary gland via nerve fibres. However, in birds, the hypothalamus is not directly connected to the anterior pituitary gland and hormones are released into a blood capillary system which passes through the anterior pituitary (Skadhauge & Dawson 1999).

Avian lymphocytes can release ACTH (Adrenocorticotropic hormone) if stimulated by an antigen in the presence of a corticosteroid releasing hormone (CRH or CRF) (Elrom 2000a). However, in mammals, the anterior pituitary gland produces ACTH after section of CRH by the hypothalamus.
As noted earlier, the primary corticosteroid in avian species is corticosterone (Rosales 1994); in mammals primary corticosteroid hormone is cortisol; however, their functions are similar.

In mammals, the link between different anatomical parts involved in an HPA response system is essential to having a baseline concentration of corticosteroids, but, in birds, the link between the hypothalamus-pituitary and the pituitary-adrenal is not necessary for the production of baseline levels of corticosteroids (Skadhauge & Dawson 1999). The pituitary gland and adrenal gland activations are not completely under the hypothalamic control in birds. In avian brains, there might be other areas in addition to the anterior pituitary that produce ACTH or an ACTH-like substance (Elrom 2000a).

1.6 Relationship between stress definition and stress measurement methods

Depending upon the definition of stress, different measures of stress responses can be used. Cannon’s (1929) fight or flight response activation (i.e., of the SNS) as a definition of stress could result in choosing catecholamines (epinephrine or norepinephrine) or the impact of catecholamine release on body (e.g., heart rate variation or respiratory rate) as stress indicators. Selye (1978) considered any activation of the HPA response system as the definition of stress. If we accept his definition, one of the following hormones should be measured to indicate stress levels: CRH, ACTH, or glucocorticoids (such as cortisol or corticosterone) or their metabolites. We can also select measurements related to the function of the HPA hormones such as glucose, glycogen, lactate, creatine phosphokinase or uric acid. Barnett & Hemsworth (1990) suggested measuring free corticosteroids instead of total corticosteroids. Based on Moberg’s (1987) definition of stress, measures that indicate a pre-pathological state should be used. According to his definition, researchers should only select those factors which affect the wellbeing of an animal directly. Using this definition, the impact of stress on an animal’s immune system would be measured, but other indicators that are not directly connected to animal wellbeing should not be measured. Stress could be caused by both physical and psychological factors (as also mentioned by Schaefer et al. 1988; Warriss 1990; Grandin 1997), and based on the suggestion of Dantzer & Mormède (1983), Gross & Siegel (1983) and Grandin (1997), we should measure a combination of behavioural and physiological factors to assess stress.
In addition to stress definitions, there are other factors that can affect the selection of the stress assessment method including: the type of stressor, the time exposed to stressor(s), climate factors, other physiological factors, species-specific characteristics, and the sampling method. If we believe that invasive stress measurement methods may affect the results and may threaten welfare of animals, then non-invasive stress response assessment indicators such as infrared thermography and fecal corticosteroid metabolites would be preferred.

1.7 Pre-slaughter handling and transportation problems of farm animals

In Canada, average numbers of animals per farm have increased while the number of farms has decreased (Statistics Canada 2008). Figure 1-2 shows the average number of ostriches per farm and number of farms in Canada from 1991 to 2006. Having fewer farms means farms are more widely distributed since Canada is vast. At the same time, farmers must transport their animals to an inspected slaughterhouse to be able to sell the products to the food retail sector. As a result, most farms are far from inspected slaughterhouses.

Pre-slaughter transportation of animals impacts animal welfare, causes health problems, bruises, injuries, weight and carcass losses, and affects the quality of products (Schaefer et al. 1997b; Buckham Sporer et al. 2008; Warriss 2010b) and may cause behavioral or physiological stress responses in animals (Grandin 1997; Schaefer et al. 2001). Northcutt (2001) found that almost all (90-95%) bruises of broilers happened during the last 12 hours before slaughter.

There has been a growing concern regarding animal welfare at different stages of the animal production process. Animal welfare concerns affect demand for specific food products. Therefore, it is economically important for producers and processors to consider Codes of Practice recommendations which assure consumers on the level of care for animals and on animal welfare during production, transportation and pre-slaughter practices. As previously stated, no Codes of Practice have been developed for ratite transportation in Canada and USA.

In each stage of pre-slaughter handling and transport, there are a number of factors which affect the wellbeing of animals. Some of the stress factors which can cause antemortem stress in livestock are: feed and water withdrawal, catching, loading, handling, the microclimate inside the vehicle, mixing unfamiliar animals, breaking social bounds, the transport duration, exposure to
unfamiliar environments, unloading, the holding time in lairage before slaughter, the stunning method, and the slaughter method. Further, genetic factors and prior experiences of animals affect the level of stress that they experience during antemortem handling and transport (Grandin 1997). Antemortem handling and transport affect blood pH and glycogen storage in muscles, and increase heterophil:lymphocyte ratio, total leukocyte count, haemoglobin and urine osmolality (Schaefer et al. 1997b).

Animals are exposed to different stressors during their pre-slaughter handling and transport in a commercial farming system. Problems related to different stages of animal pre-slaughter handling and transportation are described in the following sections.

### 1.7.1 Feed and water withdrawal

Feed withdrawal usually starts a few hours before transportation, continues until the end of the procedure, and animals usually do not receive any nutritional supplement before slaughter in commercial production systems (Schaefer et al. 2006, 1997b). Jones et al. (1988) noted that fasting stress alone did not reduce muscle glycogen stores significantly, but combined fasting, transportation and mixing stress factors could result in glycogen depletion, higher meat pH and dark, firm, dry (DFD) meat problems in cattle. Schaefer et al. (1997b) indicated that feed withdrawal alone results in weight loss in cattle, and, if this stress is combined with handling and transport stress, animals will experience higher weight losses. Cole (1995) reported a 9.9% body weight loss in un-transported sheep because of three days feed deprivation, with 80% of their weight loss was due to body water loss. In a study by Warriss (1985; as cited in Warriss 2010b), pigs lost 0.2% h\(^{-1}\) of live weight and 0.1% h\(^{-1}\) of carcass weight when feed withdrawal started, and 1.4% of carcass weight could be lost in pigs during the overnight holding in the lairage (1 kg meat per 90 kg pig). Chickens could lose 0.2 - 0.3% h\(^{-1}\) of their live weight because of feed withdrawal (Warriss 2010b).

There have been conflicting results regarding feeding before transport and even inside the lairage, and some research and food safety regulations suggest a few hours of pre-transport feed deprivation. Mormède et al. (1982) reported that serum glucose levels were still low a week after transportation, which means that feeding rations could not help the animals to recover from the energy deficit caused by long-term transport. However, Mounier et al. (2006) emphasized the importance of feeding bulls up to their loading time, because higher energy diets helped animals
to prevent potential glycogen depletion during transportation. Schaefer et al. (1996) developed a nutritional complex (Nutri-change, US patents 5505968 and 5728675) for the pre-slaughter period of livestock to help them cope with transport stress. Schaefer et al. (2001) found that antemortem feed deprivation depleted tissue energy, increased lactate and nitrogen concentrations in plasma, increased proteolytic enzymes and protein catabolism, and increased epinephrine, lipolysis and free fatty acid concentrations in plasma.

Northcutt (2001) reported that feed withdrawal (8 to 12 h for broilers and 6 to 12 h for turkeys) was necessary to reduce carcass fecal contamination because of a zero-tolerance to carcass fecal contamination according to Pathogen Reduction/Hazard Analysis and Critical Control Point System (HACCP) regulations of the US Department of Agriculture. She mentioned that short feed withdrawal (< 6 to 7 h for broilers, and < 4 to 5 h for turkeys) increased the risk of carcass contamination with intestinal material and resulted in a higher cost of the processing process. Long feed withdrawal (> 13 to 14 h) decreased the intestinal strength of broilers (almost 10%) and increased the bile contamination of the carcass (Northcutt 2001). Warriss (2010b) suggested feed withdrawal at least 4 h (and maximum 12-18 h) before slaughter for pigs. He mentioned that this time is required to prevent higher mortality rate of fed pigs during transport. He suggested 10 h feed withdrawal for poultry to prevent contamination of the carcass by feces potentially containing Salmonella and Campylobacter; however, he mentioned that a longer fasting period could cause Salmonella and Campylobacter contaminations as well. Schaefer et al. (2001) indicated that the commercial method of withdrawing feed and water before slaughter was started by cattle buyers who were trying to control carcass dressing percentage variations caused by variation in gastrointestinal fluid content. Overall, reducing the risk of carcass contamination by the gastrointestinal tract content has been mentioned as the reason for feed and water withdrawal, but experiments have shown that feed provision after transport in lairage did not increase the occurrence of gastrointestinal tract puncture during post-slaughter processing (Schaefer et al. 2001).

There are also controversial results regarding the effects of water depletion before slaughter. In most cases, water withdrawal starts a few hours before transport and continues until the holding area in lairage where animals receive water before slaughter (Schaefer et al. 1997b). Schaefer et al. mentioned that animals should not be kept off water during the transport process.
because handling and transport stress causes dehydration in animals because of higher respiration rates, ruminal and urinary water losses, and sweating. Economic costs of dehydration are high because dehydration results in live and carcass weight loss, organ weight loss and meat quality downgrade (Schaefer et al. 1997b). However, there are other reports regarding the ineffectiveness of the water withdrawal on the homeostasis of animals. For example, Parrott et al. (1996) reported that 48 h feed and water withdrawal did not affect cortisol secretion in sheep. They also found that plasma osmolality was in water balance after 48 h water withholding in sheep when they had access to feed. On the other hand, Fisher et al. (2010) reported that higher dehydration in sheep transported for 30 h and 48 h increased their total protein concentration in plasma compared to sheep transported for 12 h. Fisher et al. (2010) indicated that fatigue, dehydration and metabolic compromise are more influential problems in the long transportation of livestock compared to handling and novelty stress factors. Mormède et al. (1982) also reported the presence of acute dehydration in young cattle after a long journey and an increase in their chloride concentrations and plasma proteins after arrival.

Feed and water withdrawal are stressful for animals and dehydration increases the adrenocortical stress response (Schaefer et al. 2001). It can take several weeks for dehydrated livestock to recover from dehydration. As a result, water access immediately after transport in lairage does not result in the animal regaining tissue moisture (Schaefer et al. 2001).

1.7.2 Gathering or harvesting and mixing unfamiliar animals

Harvesting could be a very stressful stage in the poultry pre-slaughter handling process (Elrom 2000b). Gathering animals may result in fear, stress and injuries (Northcutt 2001).

Mixing animals from different pens, ages, and backgrounds could cause stress for animals (Schaefer et al. 1988). There is a strong hierarchy between most animals in their pens. When groups of animals from different pens are mixed together, the animals will try to establish new hierarchy inside the vehicle or lairage. They will fight and mount which may result in injuries and bruises (Warriss 2010a).

1.7.3 Loading and unloading

Fear or physiological stress levels of animals raised in an extensive production system were higher during loading and unloading compared to animals raised in an intensive production
system (Grandin 1997). María et al. (2004) reported that, based on their scoring system, loading was more stressful than unloading in cattle transportation. The average loading time in their research was 1-2 min per head and average unloading time was 0.72 min per head. Animals which were loaded quickly and without any problem had lower stress levels but there was no relationship between the ultimate pH of meat (pH 24 h after slaughter) and differences in loading stress scores (María et al. 2004). The stress levels (scores) of the loading and unloading were highly correlated. Fisher et al. (2010) indicated that psychological stress was higher during loading and initial stages of transportation of livestock. A further finding was that the presence of farm equipment to lead animals to a vehicle affects the loading-stress level (Mounier et al. 2006; Warriss 2010b).

When transportation time is longer, Gregory (2007) found that cattle have the opportunity to adapt to the environment inside the vehicle and this made the unloading more difficult. Mounier et al. (2006) found that unloading under a cold temperature condition or after a longer journey was more difficult than unloading in warmer temperature or shorter journey. Unloading was also more difficult if animals (bulls) were mixed immediately prior to loading into the vehicle (Mounier et al. 2006).

Mounier et al. (2006) reported that more difficult unloading occurred when farmers seldom spoke to their animals and easier unloading occurred when bulls were more disturbed during the journey. Therefore, based on their conclusion, unloading was more dependent on the truck condition, farmers’ attitudes, and the attributes of the journey itself.

1.7.4 Density in transport containers

Higher or lower densities inside a transport unit or holding pen in the lairage can increase the chance of injuries and bruises (Warriss 2010a). Because of the pressure of other animals inside an over-crowded transport unit, animals may not be able to stand and may fall. Alternatively, animals may stand on top of sitting animals and the result could be death or badly injured animals. Overcrowding also increases the temperature and humidity of the vehicle and can cause heat stress in animals. In poultry transport where often up to 6000 birds are transported in one trailer, overcrowding can cause high temperatures inside the trailer especially for the birds located in the middle of transport boxes (Northcutt 2001; Warriss 2010a).
1.7.5 Unfamiliar environment, and microclimate inside the transport vehicle and slaughterhouse

The novelty of being inside a transport unit, lairage and slaughterhouse is stressful for animals (Grandin 1997). Parrott et al. (1996) reported that the novelty of being in a new chamber increased the cortisol and prolactin release in sheep.

Livestock are very sensitive to environmental changes during transport and handling. Extreme temperatures, high humidity, light, noise, wind and other environmental factors can increase the stress level of animals (Schaefer et al. 1997b).

1.7.6 Transport duration and rest stops during transport

There are different reports regarding the impact of transport time on the production and welfare of animals. Fisher et al. (2010) reported increased body weight loss, higher creatine phosphokinase concentrations in sheep with increased transportation duration; however, the changes were still within clinical ranges. They reported that animals recovered from longer transportation stress (30 h and 48 h transport compared to 24 h transport) within 72 h after transport. They suggested that transporting sheep for 48 h does not compromise their welfare. Mormède et al. (1982) found that production of young cattle was not affected by transport duration; however, longer transportation increased the incidence of respiratory disease. On the other hand, Schaefer et al. (1988) reported that a longer transportation and longer fasting period in beef cattle increased the marketing stress level, blood bicarbonate (15% increase) and highly changed their acid-base balance compared to animals transported for a shorter time and with a shorter fasting period. Warriss (2010b) indicated that longer transport increased the dead-on-arrival rate.

In some reports and based on some transport guidelines, providing rest stops for animals in long transport has been recommended. However, Grandin (1997) reported negative effects of having too many rest stops in long-distance transportation where stress levels of calves were increased; she indicated that these were a threat for animal welfare and became stress stops instead of rest stops. She suggested that “legislating too many rest stops may be detrimental to welfare” of animals because of increased fear and stress in loading and unloading at rest stops, as well as the increased possibility of calves getting “infected with diseases at the rest stops” since
most of the calves were not vaccinated (Grandin 1997 p 252). However, she mentioned that fully vaccinated calves may benefit from frequent rest stops.

As a conclusion, I feel that rest, feed and water stops should be determined for each livestock species separately considering their specific psychological and physiological characteristics.

1.7.7 Waiting time inside the vehicle and holding time in lairage

To reduce stress, the waiting time inside the vehicle in the abattoir before unloading must be very short. For poultry especially, this time period should be very short because they are inside transport boxes (Northcutt 2001). Other livestock species are commonly kept in lairage overnight before slaughter. Some livestock species need recovery time (at least 17 h for bulls) in lairage to recover from handling and transport stress (Mounier et al. 2006). In some countries the accepted lairage time for mammals is 24 h before slaughter; in UK, it is a maximum of 48 h (Warriss 2010b). Holding pigs in lairage for 2 to 3 d before slaughter increased the rate of Salmonella contamination even if the pigs were fed (Hansen et al. as cited in Warriss 2010b). Warriss et al. (1988) reported that holding broilers in processing plants for more than 1 h increased the ultimate pH of the breast muscle from 5.78 to 5.84, because the breast muscle lost glycogen during the holding time and produced lower lactic acid after slaughter. However, mixing unfamiliar animals in lairage results in a more stressful environment as previously noted.

1.7.8 Stunning and slaughter methods

The stunning and slaughter methods affect carcass yield and meat quality of livestock. The correct application of an appropriate stunning method reduces the incidences of carcass bruising and bloodsplash (Warriss 2010c).

1.8 Factors affecting the ability of animals to manage pre-slaughter handling and transport stress

Abilities of animals to handle transportation stress depend on a number of factors including:
- **Prior experience**: Prior experience of rough handling will increase the stress level of animals in future handling compared to animals with gentle handling experiences (Grandin 1997). Tame animals are less stressed during handling than animals that are not used to people. For example, gentle contact with humans reduced pig and cattle fear responses to humans (Mounier et al. 2006), and early-life rearing conditions affected lambs’ modes of behavior to handling process in a novel environment (Moberg & Wood 1982).

- **Genetics**: Genetics affects an animal’s stress response (Grandin 1997; Buckham Sporer et al. 2008). Variation among species and individuals in their response to different stressors makes it difficult to interpret hormonal measurement results. For example, plasma cortisol concentration was higher in *Bos indicus* calves (32.60 ± 0.66 ng/ml) compared to *Bos taurus* calves (25.81 ± 0.76 ng/ml) in a 44-day study of baseline plasma cortisol levels (Zavy et al. 1992). Genetic factors (breed of animal) affected some physiological responses of pre-slaughter stress (e.g., cortisol levels); however, a consistent trend in differences between beef bull breeds’ stress responses has not been found (Buckham Sporer et al. 2008). Breeding and selection for a particular trial may affect other important characteristics of animals as well (Grandin 1997). Pigs with homozygote halothane gene are a good example of over-selection for a specific trait. The quality of the meat decreased in pigs with halothane gene; however, their lean meat production was higher (Pommier & Houde 1993).

- **Flight zone**: Grandin (1997) reported that animals show fear and an emotional behavioural response to the stressors present in their flight zone. Genetics and previous experience of animals affects their flight distance. Grandin (1997) reported that the presence of amygdala lesions could potentially be indicated by lack of flight reaction. Mounier et al. (2006) found that the flight zone of animals could be used to control them in handling, transportation and loading.

- **Social rank**: The social rank of an animal in the group affects its stress response (Grandin 1997). Animals with the social ranks of intermediate and submissive showed higher stress levels compared to dominant pigs (McGlone et al. 1993), especially when they were placed together in a new pen and experienced the same stressful situation (Dantzer & Mormède 1983).
• **Fear pheromones**: Fear pheromones can also affect the stress response studies. Vieuille-Thomas & Signoret (1992) found that urine of a stressed gilt affected the behaviour of other gilts while urine of an unstressed animal did not show any effect on the behaviour of other gilts. Grandin (1997) reported that the fear pheromone was scattered in 10 to 15 min.

• **Age and maturity**: Age and maturity affects the cortisol levels of animals, and sexually mature bulls had considerably lower cortisol concentrations compared to heifers, steers or cows (Grandin 1997).

• **Gender**: Gender is an influential factor in the level of stress response in animals (Grandin 1997). For example, females’ estrus status affects their glucocorticoids concentrations.

• **Sampling**: The sampling procedure could itself be a stress factor. Sampling should be done quickly since cortisol levels change quickly and the peak levels may not be measured. The invasiveness of the sampling method also affects the stress response (Dantzer & Mormède 1983; Mounier et al. 2006).

• **Ecological factors**: Ecological factors (e.g., season) can affect stress responses (Pierre Mormède et al. 2007).

• **Diet**: Diet can affect the metabolite concentrations in the fecal samples (e.g., by changing the food transit time) (Möstl & Palme 2002).
1.9 Impacts of pre-slaughter stress on production factors

Handling and transport stress factors decrease live and carcass weights, degrade meat quality, degrade animal wellbeing (Schaefer et al. 1997b), and can increase mortality, injuries and bruises. Therefore, economic costs of marketing stress will not only decrease the carcass yield but also will degrade meat quality (change in ultimate pH, water-holding capacity, color, texture and tenderness) (Schaefer et al. 2001).

1.9.1 Mortality

Higher temperature and/or longer transportation can increase mortality rates during transportation (Warriss 2010b). Warriss (2010b) reported the mortality rates of pigs (0.7%), broilers (0.19%), hens (0.2-0.5%) and sheep (0.02%) in UK from literature published between 1986 and 1994.

1.9.2 Bruises, hemorrhages and injuries

Bruises and injuries cause poor animal welfare and they also affect production. Bruises and injuries downgrading meat and skin quality (Warriss 2010b). As a way to ameliorate this downgrading of meat and skin, parts of carcasses with deficits should be trimmed resulting in carcass weight losses. These problems could occur because of: overstocking, understocking, mixing unfamiliar animals from different pens, mounting, fighting, long feed deprivation, chronic stress, distance traveled, stunning and slaughter (especially in poultry) (Warriss 2010b).

1.9.3 Broken bones in poultry

Broken bones are a common problem in culled hens and are dangerous for consumers. Gregory & Wilkins (1989) reported that 29% of live hens at the end of their production period in UK had broken bones mostly because of removing them from battery cages and handling them pre-slaughter. They reported that 98% of carcasses had broken bones at the end of the slaughter process mostly because of stunning, plucking and eviscerating processes.
1.9.4 Live weight and carcass weight losses

Cattle may lose 0.75% of their live weight per day during feed and water withdrawal; the weight loss also increasing by adding transportation stress (Schaefer et al. 1997b). Schaefer et al. also noted that a considerable proportion of weight loss was from carcass components; it was not from gastrointestinal tract fill which many considered as the main source of the transport weight loss. Fisher et al. (2010) reported that sheep showed body weight loss after transportation and level of creatine phosphokinase (muscle enzyme which is an indicator of muscle bruises and exertion) showed a slight increase immediately after transport.

Jones et al. (1988) reported that live weight loss of beef cattle was affected by transport duration and time off-feed. Beef cattle which were transported 640 km and were fasted 72 h lost more weight (53 g kg\(^{-1}\) or 25 kg) compared to beef cattle which were transported 320 km and were fasted 48 h (that lost 45 g kg\(^{-1}\) weight or 21 kg), and beef cattle which were transported only 3 km and were fasted 24 h (that lost 17 g kg\(^{-1}\) weight or 7 kg). Most of the weight loss for beef cattle in their research was from warm carcass weight and gutfill, and occurred during the initial stages of transportation and fasting; however, some of the weight loss was from moisture loss.

Live weight loss has an important economic impact on carcass yield (Northcutt 2001). Northcutt reported that the live shrink in broilers and turkeys depends on bird sex and age, grow-out house temperature, the bird’s eating pattern before feed withdrawal, and pre-slaughter holding conditions, and live shrink was not the same throughout transport duration (Northcutt 2001 p 13). Broilers and turkey lost 0.3 to 0.6% of their live weight per hour for the first 5 h of the feed withdrawal; however, after 5 h they lost 0.25 to 0.35% of their live weight per hour (live shrink in males was higher than females) (Northcutt 2001).

1.9.5 Meat quality degradation

It is well known that antemortem handling affects meat quality (Schaefer et al. 2001; Warriss 2010b). There are some controversial opinions regarding pre-slaughter stress and its impacts on the meat quality. Some believe that stressing an animal before slaughter can make its meat more tender (Gregory 2007). Others who have investigated the impacts of pre-slaughter stress on meat quality degradation and have reported that antemortem stress can cause problems
such as dark, firm and dry (DFD) meat, or pale, soft and exudative (PSE) meat, and can decrease the profitability of meat production (Bignon 1991; Mounier et al. 2006; Schaefer et al. 1996, 2001, 2006; Gregory 2007; Yue et al. 2010).

Antemortem stress depletes muscle glycogen, and intramuscular fat in cattle (Schaefer et al. 2001). Pre-slaughter glycogen depletion in cattle and hogs can result in dark, firm and dry (DFD) meat, and very rapid postmortem utilization of muscle glycogen when the muscle temperature is still high can result in pale, soft and exudative (PSE) meat conditions in swine and some other species (Schaefer et al. 2001; Warriss 2010b). However, Jones et al. (1988) reported that transport duration or fasting period of cattle did not affect the proportion of lean and fat in the carcass, and the meat quality factors (pH, moisture and fat content) were almost the same in their experiment, except that meat color and shear force both increased when transportation and fasting periods were longer (however the difference was not significant for color). Results of other studies (e.g., Mounier et al. 2006) have demonstrated an increase in the meat pH of transported beef cattle.

1.10 Effects of transportation stress on physiological reactions of animals

Transport stress could cause dehydration, negative energy balance, electrolyte imbalances, muscle glycogen depletion, and protein and fat catabolism (Schaefer et al. 2001). Transported beef cattle showed a 15% increase in blood lactate and a 10% decrease in the concentration of hydrogen-ion (i.e. higher pH) (Schaefer et al. 1988).

1.10.1 Effects of pre-slaughter stress on cortisol concentration

Transportation and handling procedures increase plasma cortisol concentrations (Schaefer et al. 1997b; Stanger et al. 2005). Fisher et al. (2010) reported that plasma cortisol increased in sheep at initial stages of transportation and, after a few hours, cortisol levels declined to basal values (after 12 h of journey in their experiment) because sheep became adapted to the environment inside the vehicle. Fisher et al. (2010) indicated that an increase in plasma cortisol concentration also caused hematological changes (e.g., a decrease in lymphocytes and an increase in neutrophil counts).
Buckham Sporer et al. (2008) reported that cortisol concentrations in bulls showed a 321% increase at 4.5 h post-transport (peak) compared to 24 h pre-transport measurement. This was followed by a decline until 14.25 h post-transport (less than basal level), reaching a baseline 24-48 h after transport. The plasma cortisol shows the immunosuppressive activity of glucocorticoids; it has anti-inflammatory and sometimes pro-inflammatory characteristics. Bruckham Sporer et al. (2008) found that the cortisol levels were higher in steers compared to bulls.

1.10.2 Effects of pre-slaughter stress on blood metabolites, hormones and enzymes

Schaefer et al. (1988) reported a reduction in blood bicarbonate, carbon dioxide, base excess and hydrogen-ion concentration measurements, and an increase in blood lactate, immediately before slaughter. Animals under stress realised higher cortisol concentration, causing heterophil:lymphocyte responses, had increased response of beta-endorphin and thyroxin hormones, had increased creatine phosphokinase, lactate dehydrogenase, and aspartate aminotransferase enzymes, had increased blood lactate concentration, ketones, and b-hydroxybutyric acid, and had a change in urea nitrogen concentrations in blood (Schaefer et al. 1997b).

Fisher et al. (2010) reported that increasing the transportation duration increased total protein, and reduced creatine phosphokinase and urine specific gravity until 48 hours after transportation. Based on research on transported beef bulls, Buckham Sporer et al. (2008) found a 7% reduction in albumin concentration 24 h after the beginning of the transport compared to 24 h before transport. They also found that the urea concentration was stable at the initial stage of transport and then it showed a 10% decrease at 48 h post-transport, coupled with this was an 11% decrease in total plasma protein concentrations at 24 h after transport (i.e. increased protein metabolism because of transportation stress), a 31% decrease in plasma creatine phosphokinase at 9.75 h after transport, and a 221% increase at 24 h post-transport. They mentioned that different factors such as duration of the transport or fasting condition of the animals may have affected the results.
1.10.3 Effects of pre-slaughter stress on immune status of animals

Immunosuppression occurs as a result of increased glucocorticoids circulation in stressed animals and this can increase the infectious disease susceptibility (Schaefer et al. 1997b; Stanger et al. 2005). Transportation stress may cause leukocytosis, lymphopenia, neutrophilia, eosinopenia, impaired leukocyte function and “proliferation and impaired antibody production” (Stanger et al. 2005).

After transportation, Schaefer et al. (1997b) found an increase in the packed cell volume (an indication of dehydration and splenic stress response system), a change in differential counts of white blood cells, and neutrophilia and leukocytosis accompanied by a reduction in blastogenesis and T lymphocyte numbers in cattle; however, there were no changes in immunoglobulin M or immunoglobulin G concentrations. Gross & Siegel (1983) found that stress decreased the number of lymphocytes but increased the number of heterophils (neutrophil) in chicken blood samples. Stress increased heterophil:lymphocyte ratios resulting in increased bacterial disease resistance and decreased viral disease resistance (Elrom 2000a). Moberg (2000) reported that chronic stress decreased the number of lymphocyte cells and caused the atrophy of lymphoid organs. Following 72 h transport of Bos indicus, Stanger et al. 2005 reported decreases in lymphocyte function and leukocyte numbers in transported steers (lymphocyte numbers remained the same), but animals recovered 6 d after the transport. Impaired lymphocyte proliferation and cytokine production could affect the immune responses to infectious agents post-transport (Stanger et al. 2005). Buckham Sporer et al. (2008) concluded that a combination of genetic and environmental factors can increase the cause and threat of bovine respiratory disease (BRD) in young beef cattle, and transportation stress can cause BRD.

1.10.4 Effects of pre-slaughter stress on body temperature

Transportation and fasting impacted mean infrared body heat loss and this was coincident with a darker meat color in beef cattle (Schaefer et al. 1988). Kenny & Tarrant (1987) reported an increase in the rectal temperature of steers after transport or after confinement on stationary truck and Warriss (1990) reported an increase in body temperature of cattle during pre-slaughter handling. Buckham Sporer et al. (2008) found that rectal temperature decreased after transportation in beef bulls.
The reason for this conflict in results could be related to difference in time and method of sampling (Schaefer et al. 1997b). Schaefer et al. (2001) explained that cattle body temperature increases at the initial stages of pre-slaughter handling and transport but, after a few hours, it shows a decrease. They explained that the heat production in an animal’s body is dependent on different factors such as transport duration, psychological stress experienced by animal and pre-slaughter feeding regimen.

Body temperature of animals at slaughter affects their meat quality (PSE or DFD meat results from hotter or colder body temperature at slaughter time). Thermography technology can be a useful tool in identifying these meat quality problems before slaughter (Schaefer et al. 2001). Schaefer et al. (1988) used infrared thermography technology (40 to 60% of produced body heat could be dissipated in the infrared energy wavelengths) and showed a negative correlation between the severity of the pre-slaughter stress and the loss of skin temperature. The temperature loss was compatible with glycogen store depletion and lower energy supply for heat production (Schaefer et al. 1997b).

1.10.5 Effects of pre-slaughter stress on heart rate

Pre-slaughter handling (loading and unloading) stressors increase the heart rate in Friesian steers (Kenny & Tarrant 1987; Warriss 1990; Schaefer et al. 1997b).

1.10.6 Effects of pre-slaughter stress on muscle glycogen concentration

Available muscle glycogen in cattle is usually lower than that of nonruminant animals (McVeigh & Tarrant 1982). During the transportation and handling procedure, animals usually are kept off-feed and they spend more energy to manage transportation stress. Therefore, their glycogen levels will be depleted during pre-slaughter stress. Lower glycogen in muscle post-slaughter means lower level of lactic acid production, higher ultimate meat pH, and production of dark, firm and dry (DFD) meat (Schaefer et al. 1997b).

1.10.7 Effects of pre-slaughter stress on electrolyte balance

Transportation and handling stress cause dehydration and induce a considerable change to the anion-cation balance in livestock and poultry ([Na$^+$ + K$^+$] - [Cl$^-$ + HCO$_3^-$]). The anion gap increases in stressed cattle because of an increase in bicarbonate levels, and cations decrease
because of a potassium depletion (Schaefer et al. 2001). Optimizing electrolytes can improve the performance of cattle and treat acidosis, viral diarrhea, heat shock and perspiration loss (Jones et al. 1988).

1.11 Behavioural reactions of animals to pre-slaughter stress

Animals’ previous experiences and genetics affect behavioural responses to transportation stress. Long transportation may also change the regular diurnal lying pattern; Fisher et al. (2010) showed that longer transportation duration decreased the lay down behaviour of sheep during the first 24 h after transport.

Ostriches may show unusual behaviour during transportation such as head bobbing and long-time arching of the neck. Sudden stops or accelerations could also cause panic in the standing ostriches during transport (Mitchell 1999).

1.12 Methods to help animals to manage transportation stress

Various management practices have been proposed to reduce antemortem stress. Mounier et al. (2006) reported the importance of a proper loading facility at farms to reduce handling stress. Schaefer et al. (1988, 1996, 1997b, 2001, 2006) noted negative impacts of water and feed withdrawal on carcass yield, meat quality and animal welfare; they suggested application of nutritional supplements prior to transport and at the lairage to reduce the stress level of animals and to improve meat quality.

Pre-slaughter stress can cause dehydration, energy and ion depletion, and increased protein catabolism (Schaefer et al. 2001). In formulating a diet for pre-slaughter conditions of animals, physiological and behavioural responses to stress factors and animals’ requirements should be considered. Dietary micronutrients help stressed animals to compensate the excreted nutrients and reduced nutrient intake. Providing a single element or a single nutrient (e.g., water, vitamins, vaccines, fats, electrolyte mineral treatments or high energy treatments) has not been fully successful because stressed animals require different supplements to show normal behavioural and physiological stress responses (Schaefer et al. 2001). Provision of the following nutrients in the handling and transport nutrient supplement have been shown to improve animal
welfare and decrease losses: i) sodium, since kidneys excrete sodium to balance osmolality of plasma and prevent hyperosmolality during dehydration; ii) potassium, since, after excretion of sodium, the body loses significant concentration of potassium in feed-deprived animals; iii) magnesium, which has an important function as the neurotransmitter cofactor that affects response and resistance of animals to stress hormones, and it affects stress physiological response by modifying the HPA axis; iv) energy sources (e.g., glucose, triglycerides, and fat) to mitigate nitrogen excretion and weight loss; v) amino acids (e.g., leucine, glutamine, tyrosine and tryptophan) to regulate the physiological regulations of stress by acting as substrates for neurotransmitter, protein synthesis, a source of nitrogen or gluconeogenesis; and vi) trace nutrients, antioxidant and enzyme cofactors (e.g., providing B vitamins, vitamin A, zinc, copper, chromium, vitamin E and acid ascorbic in stressed animals) (Schaefer et al. 2001).

Northcutt (2001) indicated the importance of harvesting stress and waiting time in lairage in the ante-mortem stress levels in poultry. Grandin (1997) explained that novelty is one of the major stressors, and emphasized the importance of previous experience and proper handling in animal responses to pre-slaughter stresses. Many researchers reported the importance of transporting familiar animals and avoiding mixing ages and mixing unfamiliar animals during transportation (e.g., Grandin 1997; Mounier et al. 2006; Fisher et al. 2010).

Caretaker familiarity with the Codes of Practice recommendations, basic animal biology and stress behavioural responses of animals help to minimize many transportation problems (Grandin 1997; Mitchell 1999).

Other factors which could be used to help animals during handling and transport process are: an adequate farm facility (e.g., proper ramp for loading) (Warriss 2010b); familiarizing animal handlers with the basic anatomy physiology, behavioural response and requirements of animals; and the application of a good breeding system (Grandin 1997; Warriss 2010b).

1.13 Ostrich transportation problems

Adult ostriches can panic easily under pre-transport handling and capture stress conditions. They can run at high speed and collision with fences may cause serious injuries for the birds; they may injure their handlers as well (Mitchell 1999). To prevent some of these
behavioral reactions, frequent handling of young birds by professional caretakers has been recommended (Mitchell 1999 p 226; Hoffman & Lambrechts 2011 p 220).

Ostriches have the body size of mammals and the body shape of birds (their body weight is between 80 -130 kg). Because of their height and anatomical features, transportation is difficult. Vehicle motion causes long time postural instability for ostriches (Mitchell 1999). Further, ratites are not very tame animals; therefore their reaction to handling may be more aggressive than other livestock. Finally, ostriches are very sensitive to stimuli outside of the vehicle (e.g., light and cars) during transport (Mitchell 1999).

1.14 Dissertation objectives

The main goal of this research was to improve the welfare of ostriches during the pre-slaughter handling and transportation process and to improve their product quality by applying a holistic research approach and considering the North American farming conditions. The research model for this study (Figure 1-1) included gathering data from the industry to identify welfare issues related to current ostrich handling and transport, conducting transport trials to identify the physiological and behavioural stress responses of ostriches, and reviewing ratite welfare guidelines of other countries to provide applicable information for the development of the Codes of Practice for ostrich transport in Canada and USA. The null hypothesis of this study was that handling and transportation were not stressful for ostrich, and would not affect its product quality.

1.14.1 Objectives of Chapter 2

Because of the lack of information about ostrich farming and transportation in North America and lack of developed Codes of Practice for ratite transport in Canada and USA, the first objective of Chapter 2 was to identify current pre-slaughter handling and transport practices of the ostrich industry in Canada and USA, and to identify potential welfare issues based on the current practices. The second objective of this chapter was to identify welfare standards and guidelines related to ostrich pre-slaughter handling and transport from different countries and investigate their applicability to Canadian and American production systems. The null hypothesis of the study was that similar handling and transport guidelines/standards have been developed in
different countries based on scientific knowledge, and Canadian and USA producers apply common ratite transport standards during ostrich handling and transportation.

Results of this part of my research may contribute towards developing the Codes of Practice for pre-slaughter handling, transportation and slaughter of ostriches in Canada and USA.

1.14.2 Objectives of Chapter 3

There is limited published information about ostrich behaviour and welfare during the handling and transportation process. The main objective of Chapter 3 was to investigate the effects of the pre-transport handling stressors (based on the identified potential welfare issues from the producer survey) on ostrich behaviour and physiological responses. Furthermore, the intent was to identify a validated behavioural indicator that could be used by ostrich producers and handlers to identify stressed birds during pre-transport handling. Since producers do not have the resources and expertise to measure physiological stress responses, validated behavioural indicators are critical for identifying highly stressed birds. The null hypothesis of the study was that the pre-transport handling stressors will not affect the physiological and/or behavioural stress responses of ostriches.

Knowledge of pre-transport impacts on ostrich stress and availability of a validated behavioural indicator could be used to alter handling processes thereby decreasing potential losses and improving ostrich welfare.

1.14.3 Objectives of Chapter 4

Because of their unique body characteristics (heavy body mass on two long feet), transporting ostriches is different than other livestock species. Maintaining balance inside a moving vehicle is more stressful and is an energy-demanding process for ostriches and ratites in general. Considering the limited information available about the effects of nutrient supplementation and transport duration on the physiological stress responses of ostriches, the objectives of this study were: 1) To assess the effects of pre-transport nutrient supplementation on blood biochemistry changes and body weight loss in ostriches; and 2) To investigate the effects of transportation duration on blood biochemistry changes and body weight loss in ostriches. The null hypothesis of the study was that pre-slaughter nutrient supplementation
and/or transport duration would not affect the physiological stress responses and live weight of ostriches.

Results of this study may be utilized by the ostrich farming industry to improve the welfare of birds, reduce production losses and increase the economic sustainability of the farms.

1.14.4 Objectives of Chapter 5

Chapter 5 is the conclusion chapter and summarizes the findings from the three research chapters and includes recommendations and welfare implications based on the results of this study.
Figure 1-1 **A holistic research model to improve ostrich welfare and product quality.** Solid lines refer to the relationships discussed in this dissertation. Dashed lines refer to relationships considered in interpreting results and making suggestions, but were not investigated directly for the purposes of this dissertation.
Figure 1-2  A. Average number of ostriches per farm;
    B. Number of ostrich farms in Canada from 1991 to 2006 (Statistics Canada 2008)
2 Current ostrich handling and transport practices in Canada and USA, and comparison to practices established in other jurisdictions for the improvement of ostrich welfare and product quality

2.1 Introduction

The ostrich (*Struthio camelus*) is the largest living bird in the world and birds are farmed for meat, skin and fat. The North American ostrich industry is relatively young compared to the other livestock industries and locally-produced ostrich products have only been in the markets since the early 1980s (Deeming 1999).

Mitchell (1999) and Wotton & Hewitt (1999) identified transportation as one of the main factors affecting ostrich welfare. However, there is little published information about ostrich welfare during pre-slaughter handling and transport practices. In Canada, birds must be transported to a registered processing plant that can process ostriches if producers want to sell the meat through the retail sector. Because of the small size of the industry and the volume of the product, very few processing plants with abilities to process ostriches are currently available. Pre-slaughter practices are therefore critical for the welfare of birds and the economic sustainability of farms.

Pre-slaughter handling and transport practices can cause weight loss, mortality and poor welfare in animals, and product quality downgrading (Warriss 1990; Schaefer *et al.* 2001). In ostriches particularly, falling down during handling and transport has caused injuries and bruises and has resulted in meat and hide downgrading, poor animal welfare, and income loss (Hoffman & Lambrechts 2011 p 221). Ostriches are more prone to losses due to pre-slaughter handling and transport because of their unique anatomical features (heavy body mass on two long feet), and because they are less domesticated than other livestock species. Furthermore, the majority of livestock handlers in North America are not experienced in handling these birds.

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1 A version of this chapter has been submitted for publication. Bejaei M and Cheng KM, A comparative study of current ostrich handling and transport practices through surveys and literature review for the improvement of ostrich welfare and product quality.
As noted, there has been only limited research conducted on ostrich handling and transportation worldwide. Very little information is also available about the norms of the current ostrich farming industry in Canada and USA regarding potential pre-slaughter handling and transport welfare issues that may affect the wellbeing of the birds and their product quality. No specific Codes of Practice for the transport welfare of ratites have been established in Canada and USA; the lack of established standards and guidelines may result in improper actions from different stakeholders (policy makers, experts, producers and customers). Setting up clear and specific transport guidelines may also improve the economic sustainability of farms since animal welfare affects consumer behavior.

To provide a sound basis for the development of the ostrich transport Codes of Practice, we gathered information from three sources: 1) a survey of producers in Canada and USA concerning current practices by the industry; 2) a review of the literature for available scientific data; and 3) a survey of established standard and guidelines in other jurisdictions (Australia, European Union, New Zealand and South Africa). General livestock transport standards and guidelines were examined for Canada, USA and those countries in which there were no specific guidelines developed for the transport of ostriches.

The first objective of our study was to identify current pre-slaughter handling and transport practices of the ostrich industry in Canada and USA, and to identify potential welfare issues based on current practices. The second objective of this study was to identify welfare standards and guidelines related to ostrich pre-slaughter handling and transport from different countries and investigate their applicability to Canadian and American production systems. Results of this research will contribute towards developing the Codes of Practice for pre-slaughter handling, transportation and slaughter of ostriches in Canada and USA.

The null hypothesis of the study was that similar handling and transport guidelines/standards have been developed in different countries based on scientific knowledge, and that Canadian and USA producers comply with these transport guidelines in ostrich handling and transportation.
2.2 Methods

To design the producers’ survey, we conducted qualitative research using literature review, farm visits and one-on-one producer/expert interviews to identify potential key indicators which were required to be considered in the survey. We designed the questionnaire and conducted a survey of Canadian and American producers.

2.2.1 Producer survey

2.2.1.1 Identifying pre-slaughter handling and transport welfare indicators to design the questionnaire

Main ratite transportation problems were identified by one-on-one interviews with ratite producers from July 2010 to September 2011 (in Canada and USA). Site visits were conducted on two farms in Alberta, Canada, in September 2011 to identify indicators that should be considered in designing a producer survey.

Animal welfare assessment is a complex subject. Therefore, to be able to find the potential welfare issues using a producer survey, the UK Farm Animal Welfare Council (1979) five freedoms were selected as the global objectives of the research in defining the indicators based on Girardin et al. (1999) model to design the survey. Each one of the global objectives was defined using multiple measurable variables which were considered in designing the survey questions (see Figure 2-1). For example, for the global objective of freedom from hunger and thirst (considering the principal objective of the survey), the following variables were identified and were included in the survey: pre-transport feed/water withdrawal duration, access to feed/water during transport, access to pre/post nutrient supplement, and access to feed/water at lairage. Finally, the identified variables were transformed into questions which were included in the survey for ostrich producers. Pre-tests were conducted in September 2011 with three producers to modify the questionnaire before launching the survey.

The questionnaire (See Appendix A) had four sections and was designed according to Dillman (2007) guidelines. Nine general questions were for all respondents and then respondents were asked to answer the category of questions in production (37 questions), shipping (25 questions) and processing (20 questions) sections based on their activities in ostrich industry. An open-ended ‘other’ option was provided to the respondents to gather information from a wide
variety of the potential pre-slaughter handling and transport activities, and these responses were categorized (after conducting the survey) into related groups whenever possible. The University of British Columbia Behavioural Research Ethics Board Approval (#H11-02380) was obtained for conducting this survey.

2.2.1.2 Conducting the producer survey

Mail survey and internet survey methods were selected for this research because potential respondents were located in different provinces or states all over Canada and USA. We provided a printed copy of the invitation email, the questionnaire and an empty stamped envelope to the ostrich producers who attended the American Ostrich Association 2011 annual meeting in Texas, USA and the Canadian Ostrich Association 2012 annual meeting in Alberta, Canada. We also provided additional copies to the associations for mailing out to those members who did not attend the annual meetings. The online survey link was also provided to all respondents in the invitation email as the second option to complete the survey. Moreover, we identified other producers (who were not association members) based on their contact information published online and sent a printed copy of the questionnaire to them or invited them to complete our internet survey.

2.2.2 Reviewing ostrich transportation standards and guidelines

For the purposes of this project, pre-slaughter ratite handling and transport welfare standards/guidelines of Australia (Primary Industries Standing Committee (PISC) 2003; Animal Health Australia (AHA) 2012), European Union (SCECPAFP 1999; European Food Safety Authority (EFSA) 2004), New Zealand (Animal Welfare Advisory Committee (AWAC) 1998; National Animal Welfare Advisory Committee (NAWAC) 2011), and South African Ostrich Business Chamber (SAOBC 2011) were reviewed.

Specific ratite handling and transport guidelines have not been developed in Canada and USA. Therefore, general livestock transportation regulations and recommended Codes of Practice of Canada (Canadian Agri-Food Research Council (CARC) 2001; Minister of Justice 2013), and general livestock transport regulation of USA (Code of Federal Regulations 1906) were reviewed to provide applicable information for the development of specific ostrich (or ratite) transport guidelines in Canada and USA.
2.3 Results and discussion

Survey respondents’ characteristics and basic information about their farming practices are reported at the beginning of this section. Then, selected stages of ostrich handling and transport practices which could be transport related welfare issues are discussed based on the survey results, and applicable standards/guidelines from different countries.

2.3.1 Sample size of the survey, and strengths and weaknesses of the industry

We provided the questionnaire to 50 ostrich producers (40 in the USA and 10 in Canada). Overall, we had 39 respondents; 31 of the questionnaires were complete and eight producers answered only a few questions. Only a quarter of the survey respondents used internet survey option. Considering the size of the industry and number of ostrich association members in Canada and USA (which was less than 55 members in 2012), this is considered a good response rate. Figure 2-2 shows the geographical distribution of our survey respondents; there were multiple respondents from some provinces or states.

We asked the survey respondents to identify the strengths of the industry. Most ostrich producers considered unique characteristics of ostrich meat (as also discussed by Paleari et al. (1998) and Polawska et al. (2011)) as the main strength of the industry. Seventy percent of the respondents reported stronger demand than supply for their ostrich products (usually they do not have to look for customers and customers search for their products). Half of them indicated that land use efficiency is better in ostrich farming compared to other free-range livestock farming.

The survey respondents were also asked to identify the weaknesses of the ostrich industry in Canada and USA. They indicated the lack of a strong association as the main weakness of the industry. Transportation problems were the other important factor affecting economical sustainability of ostrich farms. The lack of quality standards, and high variation in the quality of products from different farms, lack of ostrich processing plants, lack of government support, and lack of research support were identified as the other weaknesses of the industry.

Based on the results of the survey, about half of the ostrich farming income on average was from the meat production. About 40 kg meat can be produced by an ostrich. Depending on the cut and the quality, a kg of ostrich meat can fetch the producer $10-20. The rest of their income from ostrich farming was from selling live chicks (20%), skin for making leather (13%);
13.5 ft² skin per ostrich which was sold for $7-20 per ft² by producers, depending on the quality of the skin and its processing (tanning) stage), infertile eggs (7%), fat (4%), fertile eggs (3%), and other products (e.g., processed food products, empty egg shells, and dietary supplements; about 3%). Ostrich producers are starting to enter the oil market. An ostrich can produce 200 oz of oil which can be sold for $5 per oz. Most of the producers were not aware of the high value of ostrich oil and were selling fat in bulk for about $5 per pound (about 15 lbs of fat can be harvested from each ostrich). Dollar values were reported by some producers based on their experience and estimation at the time of the survey.

2.3.2 Ostrich handling experience

Ninety-five percent of the respondents were at the age of 51 to 70 years old with more than 15 years of ostrich farming experience. The average age of the survey respondents was similar to the average age of farmers in the USA (USDA Census of Agriculture 2007) and Canada (Statistics Canada 2011). There are only a few young or new entrant ostrich producers every year in Canada and USA.

Our survey showed that having long production experience did not always result in proper ostrich handling and transport practices. The reasons could be related to the industry weaknesses identified by the producers (e.g., a lack of a strong association). Therefore, the lack of communication between producers to share their experiences and knowledge could have resulted in producers’ unfamiliarity with proper handling and transport practices which may cause welfare concerns. It was apparent from the survey results that for pre-slaughter handling and transport, the industry needs better trained and more knowledgeable handlers. However, because of the small size of the industry in Canada and USA, it is not easy to overcome this issue, unlike countries with a large and strong ostrich production industry (e.g. South Africa) which may have resources or government support to resolve these issues.

In general, handlers’ familiarity with basic animal biology and stress behaviour would help to minimize various transportation problems (Grandin 1997; Hoffman & Lambrechts 2011 p 219). Skillfulness and familiarity of the handlers with the anatomy, behaviour, welfare, humane handling, transport standards, health controls, and stress and disease signs of the transported animals is emphasized in animal transport guidelines in different countries (AWAC 1998 section (s) 4.3, 6.1; SCECPAFP 1999 article (a) 4.1; NAWAC 2011 Minimum standards (Ms) 1; AHA
SAOBC (2011) also recommends having well-trained handlers during the transport process, even inside the moving vehicle. In Canada and USA, handlers are not allowed to be inside the moving trailer with the livestock because of handler safety considerations.

SCECPAFP (1999 Appendix (App) 2) and SAOBC (2011 s 9.b) suggest having at least three experienced handlers to restrain an adult ostrich. It has been recommended that number of handlers should be restricted and birds should be handled by those handlers who the birds are familiar with, to reduce their stress levels (Wotton & Hewitt 1999; EFSA 2004 s 5.2.2).

Canadian animal transport guideline suggests that handlers should be familiar with the transported animal characteristics (CARC 2001 p 1). There are a few optional livestock transport training programs in Canada and USA, and some livestock transport companies may require training certificates from their employees (for example: a nationwide Poultry Handling and Transportation Quality Assurance (PHTQA) program in the USA (http://www.poultryhandling.org/), and in-class and online Canadian Livestock Transport (CLT) training programs (http://www.livestocktransport.ca/en/) for transport of main livestock species. However, handling and transport training programs have not been developed for ratites which are more precarious and require specific knowledge. Similar training courses could be developed for ratite transport in collaboration between government/university scientists and industry experts. Ratite producers could benefit from short-term online handling and transport training programs which could be funded by agricultural departments in each country to improve the safety of handlers, welfare of animals and the quality of products.

### 2.3.3 Familiarity of birds with the handling routine

Most producers surveyed observed their birds and spoke to them (100% and 80%, respectively); however, only about half of the producers regularly had physical contact with their birds. Considerable differences in transport practices exist among the producers which resulted in large variation in transport welfare conditions for birds. There were some producers who emphasized the importance of the regular handling of the birds (from the early stages of the production process) on the response of the birds to the handling and transport process, but there were also producers who do not know the importance of habituating birds to the handling practices. This could cause a potential welfare concern in transport in cases where birds are not used to the handling practices.
Familiarity with the handlers’ presence and routines throughout the production process is an important factor which affects livestock welfare during pre-slaughter handling and transport practices (Grandin 1997). Those ostriches which are in regular contact with their handlers get used to their handling style and may be less stressed when the handlers are around them during the transport process (Mitchell 1999 p 226; Hoffman & Lambrechts 2011 p 220). Birds which are not used to handling may cause injuries to themselves or their handlers when handled (Hoffman & Lambrechts 2011 p 214).

Ratites are more comfortable to be handled in a group and can be familiarized and habituated to human contact and handling procedures (including restraining methods) before transportation to reduce their stress levels (AWAC1998 s 4.1; SCECPAFP 1999 a 3.4; EFSA 2004 s 5.2.5; NAWAC 2011 Recommended best practice (Rbp) 5.1; AHA 2012 GB6.36;). SCECPAFP (1999 a 5.1) indicates that familiarizing birds with human contact and handling practices should start when they are chicks and should continue throughout production cycle.

There is no livestock handling guideline in Canada or USA concerning familiarizing ostrich chicks with handling, and there is no specific study comparing the stress responses of habituated and non-habituated birds. Researchers could investigate effects of familiarizing birds with handling practices from early stages of their life cycle on transport stress response and their meat/skin quality (as reported for other livestock species in Grandin (1997) and Mounier et al. (2006)). If the results of studies show significant benefits, the necessity of habituating birds to the handling process would be a good feature to be considered in the ratite handling and transport guidelines in Canada and USA.

2.3.4 Handling methods

The results of our survey indicate that ostriches were handled in a variety of ways in Canada and USA. Half of the respondent of our survey used hooding as a restraining method, 25% of them did not use any devices to assist in their handling of birds, 5% used a hook or crook, 5% used tranquillizers, and 15% applied other methods (e.g., livestock handling unit) to restrain their birds. To load birds into the trailer, only a few experienced producers mentioned that they use artificial lighting inside the trailer early in the morning pre-dawn to motivate the inquisitive ostriches to voluntarily go inside the trailer (from a pen close to the loading gate).
Most producers were not familiar with the pros and cons of each handling method and they chose a handling technique based on their previous experience and availability of equipment.

As noted earlier, there is no specific guideline regarding handling methods for ostriches in Canada or USA, even though handling ostriches has been shown to differ from handling other livestock. Considering the precarious nature of ostrich handling and the wide variety of techniques applied on different farms, some of the applied techniques could compromise handler safety and/or bird welfare.

The review of handling guidelines from other jurisdictions indicated the importance of considering the benefits and detriments of a handling method before applying the technique. For example, hooping is suggested as one of the safe restraining and handling methods for ostriches over 6 months of age, and hooded birds should be attended at all times (AWAC 1998 s 4.4.1; PISC 2003 s 7.2.1; SAOBC 2011 s 9.b; AHA 2012 GB6.34). EFSA (2004 s 5.2.9) and SAOBC (2011 s 9.b) emphasize that hoods must be removed a few minutes after applying, only necessary hooping is permitted (not as a regular procedure), and hoods should be removed soon after loading. EFSA (2004 s 5.1.8) also indicates that the disorientating effect of hooping could be stressful for ostriches. More research is needed to investigate the effects of hooping as a safe handling method.

EFSA (2004 s 5.1.6.) recommends using food to attract an ostrich into a narrow fenced-off area (with solid walls) which ends in a small triangular pen as one of the best methods to capture ostriches without causing high stress. However, this method is not practiced in Canada or USA. It has also been recommended that a triangular (‘V’ shape) crush (for adult ostrich treatment), or a shepherds crook (especially to capture and hood aggressive males in large camps) could be used for ostrich restraining (AWAC 1998 s 4.4.1; PISC 2003 s 7.2.1; SAOBC 2011).

A few ostrich producers in Canada and USA use hooks to restrain their birds. However, SCECPAFP (1999 App 2) and EFSA (2004 s 5.1.7, 5.2.7, 5.2.8) have prohibited restraining ratites by using hooks because it is a dangerous and stressful procedure which may cause neck and head trauma, trachea laceration or death.

Only a few ostrich producers used tranquillisers in Canada and USA to restrain the birds; however, using behavioural-modifying compounds for routine transport purposes should be
avoided based on the handling guidelines from different countries (SCECPAFP 1999 a 6; EFSA 2004 p 3; NAWAC 2011 Rbp 5.1.f; SAOBC 2011 s 9.b).

There is a need for a research project to compare the impact of different ostrich handling methods on the welfare and product quality of ostriches to be able to determine the best restraining practices that could be implemented in Canada and USA ostrich farming.

2.3.5 Mixing birds from different groups during handling and transport practices

Niney percent of producers transported their ostriches for processing, and 70% of them kept the birds in a pre-transport holding pen for various lengths of time (from 1 h to 18 h) before loading them into the trailer. Transportation and holding birds in a loading facility is a common practice in the ostrich industry in Canada and USA. Respondents of our survey also indicated that they have often kept birds of similar age or weight together in one holding pen or in a vehicle compartment but they have not considered sex or familiarity of the birds with each other when they were mixing birds in the pre-loading holding pen.

Keeping birds in a holding pen and mixing birds from different groups could cause welfare issues because the holding pen is a new environment for the birds, and novelty and pre-transport mixing of unfamiliar animals or animals from different groups alleviates the transportation stress and transport losses (Schaefer et al. 1988; Grandin 1997). Wotton and Hewitt (1999) also recommended that birds from different groups should not be mixed together. There is usually an established hierarchy among most animals in their home pens where each bird knows its position and there is minimum fighting. However, when unfamiliar animals are mixed together, they will start fighting to establish a hierarchy in the new environment (holding pen, vehicle or lairage), and that may result in injuries and bruises (Warriss 2010a).

Based on the transport guidelines from other countries, it has been suggested to consider different factors such as familiarity of ostriches when mixing them during handling and transport. For example, NAWAC (2011 Rbp 3.2) and EFSA (2004 s 5.1.3) recommend maintaining social groups of animals in the assembly and holding areas because slight changes in their social structure can cause stress-induced disorders (and aggression). Only animals which are raised together should be loaded in the same holding area, same vehicle compartment or same lairage pen (EFSA 2004 General conclusion (Gc) 1.2.8, 5.2.4).
Canadian and American ostrich producers did not consider the bird’s gender when assigning birds into different groups during pre-transport handling process. However, sex of the birds has been identified as an important factor in assigning birds into different compartments (SCECPAFP 1999 a 15.2; CARC 2001 s 4.3.2, 4.3.3, 4.5.1; Minister of Justice 2013 s 141.1).

Overall, in addition to age and weight that producers already consider when mixing birds during handling and transport process, considering the social familiarity of birds, their behavioural response and their sex when mixing them during handling and transport could improve welfare of birds and minimize losses.

2.3.6 Behavioural changes in ostriches during handling and transport practices

Producers identified fearfulness (26%), running (16%), vocalization (13%), kicking (9%), climbing on top of each other (10%), trampling (6%), stop feeding (3%), stop drinking (3%), and other responses (8%) as the behavioural changes observed during pre-transport handling practices. Six percent of the respondents did not notice any behavioural change. As for behavioural changes inside the trailer, producers observed fearfulness (20%), trampling (20%), climbing on top of each other (10%), compulsive repetitive movements (10%), kicking (5%), and vocalization (5%). About one third of respondents indicated that they had not noticed any behavioural changes of ostriches inside the trailer. However, we do not know whether these producers actually observed birds during transport.

Ostriches are diurnal birds (Deeming & Bubier 1999) and prefer to stand during transport when there is light inside the trailer (Mitchell and Kettlewell unpublished observations as cited in Mitchell 1999 p 224-225; Wotton and Hewitt 1999). However, birds will sit down in a dark trailer and they are calmer when transported at night (EFSA 2004 s 5.1.9; SAOBC 2011 s 9.b; AHA 2012 GB6.29, GB6.33) or when they are tired (SAOBC 2011 s 9).

There is little published literature about the behavioural changes of ostriches during handling and transport. If researchers could identify the stress behavioural responses of ostriches considering their physiological stress responses (as explained by Grandin 2010a), handlers could use those responses as signs of stress to identify birds which are highly stressed and transport them with additional care. Continuous monitoring of birds’ behaviours using infrared cameras could also be beneficial to track the behavioural changes during transport and intervene whenever necessary.
2.3.7 Feed and water withdrawal throughout handling and transport practices

In more than 90% of farms, birds had unlimited access to water but about half of the producers held birds off-feed at least a few hours before loading them into the trailer (or they did not provide the last pre-transport feed supplement to their birds). In some cases, birds were kept off-feed for as long as 2 d before transport because of the producers’ concerns regarding wet floor problems caused by ostrich droppings and the processor concerns about carcass contamination problems. Our results showed that producers have not adhered to a specific guideline when deciding about the feed-withdrawal duration.

Feed and water were not available inside the trailer (except for very long transport durations), and feed was not available at the processing plant (however water might be available in the processing plant). Therefore, birds were off-feed from assembly time until slaughter. Providing pre-transport nutrient supplements to the birds was also not practiced in the industry.

Pre-transport feed/water withdrawal may cause dehydration, meat quality degradation and compromised welfare (Jones et al. 1988; Schaefer et al. 1988; Warriss et al. 1993). In Canada, animals which will be transported for longer than 12 h must have access to feed and water within 5 h before loading (except chicks which could be kept off-feed for maximum 72 h post-hatch) (Minister of Justice 2013 s 138.2.b, 138.3). However, it has been recommended that animals which will be transported for more than 4 h should have access to feed within 24 h before loading (CARC 2001 s 2.3.2). There is no specific maximum pre-transport feed withdrawal duration reported in the USA animal transport related guidelines. The specific gastrointestinal characteristics of ostriches requires specific research to identify the maximum pre-transport and pre-slaughter feed withdrawal duration for these birds and general recommendations may not be the best applicable guidelines for ostriches.

There are also different standards and guidelines regarding the pre-transport availability of feed during pre-slaughter handling and transport practices in different countries. AHA (2008) implies maximum 24 h off-feed duration for young ratites, and maximum 36 h off-water for adult ratites (AHA 2012 SB6.1). SAOBC (2011 s 12.c) specified that feed should not be provided to the birds from 10 h before loading for a long transport (12 h or longer). NAWAC (2011 Rbp 5.1) indicated long off-feed periods should be avoided because long term feed withdrawal (more than 24 h) activates the fat reserves of animals (if they have less fat, they will
not be able to survive the long term pre-transport feed withdrawal). They also recommend providing pre-transport nutrient supplements especially for animals which may experience nutrient deficiencies in their pre-transport feed.

Mitchell (1999 p 226) suggested providing feed up to 4 h before transport (and accessibility of water until loading birds into the trailer) to minimize the risk of slipping and injury as a result of fecal contamination of the trailer floor. However, Glatz & Miao (2008) recommended availability of feed up to 8-12 h before transport. Pre- or post-transport nutrient supplementation has been used to rehydrate livestock, and to improve their welfare and product quality (Schaefer et al. 1997b, 2001, 2006; Arp et al. 2011). However, no specific research could be found on the assessment of the best feed withdrawal duration for ostriches, and because of the conflicting guidelines from different countries, research is needed to determine the best pre-transport off-feed period that could be recommended in Canadian and American ostrich farming.

2.3.8 Vehicle design

Producers usually use a closed-top modified livestock transport trailer or horse trailer (with a roof height above 2 m) to transport ostriches in the USA and Canada. Some of the respondents have set up partitions inside their trailer but some of them used a trailer with only one partition (which may result is mixing unfamiliar birds or higher densities and more losses). Only half of the respondents mentioned that they provide bedding material on the floor of the vehicle.

Most of the birds were being shipped during the day and most drivers started the transport during daytime. As a result, 90% of the respondents indicated that birds were exposed to natural light during transportation. Because of having open small side windows, less than half of the respondents had ventilation systems installed in the trailer. Only a few experienced producers emphasized the importance of providing dimly-lit space inside the trailer to calm the birds down. These producers mentioned that they have had fewer losses since they have darkened their trailer.

Trailer design significantly affects the welfare of birds and transport losses. Light inside the trailer also has a significant impact on the behaviour and restlessness of the birds during transportation and may result in injuries, bruises, and weight loss. Mitchell (1999 p 225) recommended that ostrich transport vehicle should have sufficient ventilation, closed sides, and
low light levels to isolate birds from outside noises and visual images. Having birds exposed to natural light and visual contact of objects outside of the transport vehicle is stressful for the ostriches and will result in birds standing during day light transport (Mitchell 1999 p 225; Crowther et al. 2003; Hoffman & Lambrechts 2011 p 222). Nevertheless, birds sit down at night or inside a dark vehicle (i.e. they have higher stability and less losses) and experience less shipping stress (Crowther et al. 2003).

Regardless of specific ostrich anatomy and significant differences between ostrich transport versus other livestock transport vehicle requirements, there are no Canadian or American ostrich transport vehicle design guidelines. Moreover, guidelines from other countries differ in their suggested requirements for ostrich (or ratite) transport vehicle design. Australian and European Union guidelines suggest transporting ratites in a fully enclosed vehicle with dimly-lit compartments (or at night) (EFSA 2004 s 5.2.11; AHA 2012 GB6.22). Use of air-sprung trucks in livestock transportation is also encouraged as they reduce the effect of road surface and the risk of birds slipping or falling during transport (AHA 2012 GB6.24). However, South African ostrich transport guidelines recommend using a vehicle which is specifically designed for ostrich transport and mandates having experienced handlers in each compartment during transport to accompany ostriches (SAOBC 2011 s 12.d, 12.f). This is also discussed in Hoffman & Lambrechts (2011 p 212-213) and they suggested that handlers should stand inside the moving vehicle to monitor birds and help them if required during transport to minimize losses. This design could not be implemented in North America because handlers must not stand inside a livestock trailer when the trailer is moving because of handler safety protection in the USA and Canada. Lack of specific information about the design of a proper trailer for the transport of ostriches based on the Canadian and American transport condition indicates the necessity for research in this area.

2.3.9 Bird density inside the vehicle

There was a wide variation in the densities of the birds transported in a vehicle (in a range of 0.3 m$^2$ to 0.8 m$^2$). Producers have not followed any specific guideline regarding the density of the birds inside the trailer. The loading density depended on the number of the birds to be sent to the processing plant and the size of the trailer that they have access to. A few of the survey respondents mentioned that higher densities had resulted in more losses.
Wotton and Hewitt (1999) emphasized the importance of providing enough space for each bird to sit down inside the trailer during transport to avoid trampling and injuries (if they are not transported in individual partitions). Overcrowding can cause trampling and injuries (CARC 2001 s 4.4.1). Providing enough space inside the trailer that animals can stand (in their natural position) inside the trailer without contacting a deck or roof is required in Canada (Minister of Justice 2013 s 142.a), and animals should be able to maintain their natural position without having contact with the vehicle roof or upper deck (CARC 2001 s 4.4.2). There is no specific density requirement suggested for ostrich transport in Canada or USA. There are also various density requirements suggested in different countries.

Australian guidelines recommend 0.41 m$^2$ floor space for 95 kg birds, and 0.48 m$^2$ floor spaces for over 110 kg weight (AHA 2012 GB6.15). In South Africa, it has been suggested to provide at least 0.5 m$^2$ space inside the vehicle per 80 kg of ostrich, and there should not be more than 12 adult ostriches in each compartment (SAOBC 2011 s 12.d). European Union recommends at least 0.75 m$^2$ space inside trailer per adult ostrich (EFSA 2004 s 5.2.13), and suggests transporting maximum 12 birds at the age of 3 to 18 months and not more than 7 adult ostriches in one compartment (EFSA 2004 s 5.1.15).

There has not been specific research conducted on determining the optimal density of ostriches inside the trailer. Hoffman & Lambrechts (2011 p 213) suggested at least 0.5 m$^2$ floor space per bird, and Mitchell (1995 p 225) recommended a stocking density of minimum 0.75 m$^2$ per adult ostrich, and mentioned that birds should be able to stand or sit during travel. More research is required to investigate the proper densities of birds inside the trailer.

2.3.10 Transport duration, and feed, water and rest stops

Survey results revealed that there is a wide variety of ostrich pre-slaughter transport durations in Canada and USA (less than an hour to 20 h of transport). A few extreme transport durations (more than 24 h) were also reported by the respondents. There were very little on-farm slaughtering in Canada and USA.

Thirty percent of respondents indicated having one stop every hour of drive; 13% monitor their birds once during every 2 h of drive and half of the respondents monitor birds once during every 3 h of drive. Installing infrared cameras could give the drivers the option of
monitoring livestock throughout transport; however, only two of the producers mentioned that they used trailers which had infrared cameras installed.

In most pre-slaughter transport, feed and water were not available inside the trailer for birds (except for extremely long transport durations), and birds were not unloaded for the purposes of access to feed/water and rest before reaching their destination.

Transport duration is an important factor affecting the welfare of animals during handling and transport process. CARC (2001 s 2.2.1.a, 2.1.8) indicated that longer transport duration increases the risk of injury or death in transported animals. In Canada, monogastric animals (including ratites) must not be confined for transport purposes more than 36 h without access to feed, water and rest (Minister of Justice 2013 s 148.1.a). After 36 h of transport, animals should be unloaded in a pen and have access to feed, water and rest for a minimum of 5 h before being loaded into the trailer again, and maximum feed withdrawal duration from the beginning of pre-transport handling until the end of lairage should not exceed 52 h for ruminant livestock (cattle, sheep and goats) and 40 h for pigs, poultry and horses (Minister of Justice 2013 s 148.4; CARC 2001 s 5.5.8.1).

Based on the Twenty-eight Hour Law in the USA (active since June 29, 1906), animals must be unloaded from the vehicle after 28 h confinement inside the vehicle (without considering loading and unloading duration) to have access to feed, water and rest for a minimum of 5 h and the law suggests avoiding extra stops (Code of Federal Regulations 1906). However, there is an exception for this law and that is when animals are being transported in a vehicle with access to feed and water and enough space to rest, they do not need to be unloaded (Code of Federal Regulations 1906). However, this law has not been closely enforced by US Department of Agriculture nor by the Department of Justice (Animal Welfare Institute nd).

Nutrient requirements of ostriches and their stress responses are different from other livestock species. However, there is no specific maximum transport duration suggested for ostriches in Canada and USA. Moreover, recommendations regarding the maximum transport duration of ostriches differ for different countries. Based on the Australian standards, adult ratites must be unloaded after 36 h of transport for a minimum 24 h access to feed, water, and rest before starting another journey (AHA 2012 SB6.5). Based on the European Council Standards, ratites must have access to feed at least once in 24 h of transport and water must be
provided at least once in 12 h of transport (SCECPAFP 1999; Council Regulation (EC) 2004 No 1/2005). EFSA (2004 s 5.2.17) recommends 8 h rest period (if possible, at night) after 24 h transport for adult ostriches (similar to mammals). However, based on the current existing evidence, EFSA (2004 s 5.2.3) recommends having maximum ratite transport duration between 8 to 12 h. Water must be provided within 6 h after water withdrawal and off-feed period for monogastrics must not exceed 24 h (except transport to the processing plant that animal will be slaughtered immediately) (NAWAC 2011 Ms 10), and mature animals should have rest stops once every 24 h (NAWAC 2011 Rbp 10.a). SAOBC (2001 s 12) recommends avoiding long transportation of ostriches. In South Africa, birds must be unloaded after 12 h transport for about 6 h to be watered and have rest (SAOBC 2011 s h).

Hoffman et al. (2012) reported lower meat quality and greater weight loss in ostriches transported for 5 h compared to 1 h transport duration. However, little published information is available on the effects of transport duration longer than 5 h which is a common practice in the USA and Canada. Reducing transport duration or using on-farm mobile slaughterhouses perhaps is a good solution for major ostrich transport problems as also suggested by Wotton & Hewitt (1999). EFSA (2004 Gc 1.2.4) also recommends on-farm slaughter of ratites (using mobile slaughterhouses) when birds are not habituated to humans and handling processes, and when transport would cause poor welfare for those birds.

Long distance transportation is detrimental to ostrich welfare and may cause significant losses (e.g. mortality, bruise and injury, and product quality degradation). More research is required to determine the effects of feed/water stop frequencies and maximum ostrich transport duration in Canada and USA. The effects of using on-farm slaughterhouse facilities on the stress levels of birds and their losses should also be investigated.

2.4 Conclusion and welfare implications

Handling and transport injuries, bruises and losses are common in ostrich farming, and they cause poor welfare and downgraded meat and hide losses. Ostrich transport is a common practice in the Canadian and American ostrich industries and transport duration is also longer in Canada and USA compared to the other countries because farms are located far from the processing plants. Physical characteristics of ostriches are also different from other livestock.
species nevertheless Canada and USA have not established specific transport guidelines for the handling and transportation of ostriches (or ratites). To improve the welfare of the birds during transport and to decrease losses based on our survey and review of the transport guidelines, we conclude that:

- Handlers’ familiarity with the birds’ behaviour, physiology, anatomy and requirements before handling could improve handler safety, welfare of the birds and decrease transport losses.
- In addition to the age and weight of the birds which are already being considered in mixing animals during handling and transport process, their social bonds, sex, behaviour and physical state could also be considered to improve transport condition.
- Long-term feed and water withdrawal is detrimental to ostrich welfare, and research is required to determine the maximum feed and water withdrawal duration during handling and transport of ostriches.
- The pros and cons of different handling methods could be communicated to producers so that they can choose the best handling practices.
- Research is required to identify validated stress behavioural responses which could be used to identify stressed birds before causing poor welfare or high losses.
- Guidelines of different countries and literature have suggested using an enclosed ostrich transport vehicle (with subdued light) to lower the stress levels of the birds.
- Overcrowding causes considerable losses, and research is required to determine the best density of birds inside the trailer.
- Long transportation is harmful for ostriches and may cause weight loss and product quality degradation. Research is needed to identify the maximum acceptable range of ostrich transport duration, and frequency and duration of rest (and feed/water) stops.
- Effects of implementing on-farm slaughtering (using mobile slaughter house) could be investigated to choose the best slaughter method to decrease transport losses.
Figure 2-1 Flow chart describing the process for developing questions for the survey to identify the pre-slaughter handling and transport potential welfare issues. Step 1: The UK Farm Animal Welfare Council (1979) five freedoms were selected as the global objectives of the research (based on Girardin et al. (1999) indicator development model); Step 2: Each one of the global objectives was defined using multiple measurable variables (only a few variables are shown here); Step 3: Each one of the specific objectives were integrated to one or more questions in the producer survey.
Figure 2-2 Geographical distribution of the producer survey respondents in Canada and USA using Google maps (There were multiple respondents from some of the provinces and states.)
3 Effects of pre-transport handling stress on physiological and behavioural responses of ostriches²

3.1 Introduction

Ostrich (*Struthio camelus*) production for meat, oil and leather is a relatively young industry in North America, and there is limited published information about ostrich behaviour and welfare during handling and transportation. Moreover, features of the ostrich anatomy, specifically a large body with two long feet and only two toes on each foot, may introduce unique problems compared with the transportation of other poultry and livestock species.

In Canada, ostrich producers must have their birds processed in a registered processing facility to be able to sell the meat to the retail sector. Although there are a few red-meat processing plants which have the facility to slaughter ostriches, very few of them are willing to interrupt regular operations and change the processing lines to process a few ostriches. Ostriches usually have to be transported to a processing plant far from the farm to be slaughtered. As a result, the transportation process is one of the main factors compromising ostrich welfare (Mitchell 1999; See also Chapter 4 of this dissertation).

The transportation process can be divided into five stages: pre-transport, loading, transportation, unloading, and post-transport. There are multiple factors which can affect the welfare of birds at each stage (Schaefer et al. 2001). Pre-transport handling is one of the major stress factors in livestock transportation (Knowles & Broom 1990) and can affect the welfare of slaughter animals and the quality and the quantity of products produced.

‘Freedom to express normal behaviour’ and ‘freedom from fear or distress’ are two of the Farm Animal Welfare Council's (1979) required five freedoms which are necessary for the basic welfare of animals. To fulfill the five freedoms requirements, both physiological and behavioural

² A version of this chapter has been submitted for publication. Bejaei M and Cheng KM, Effects of pre-transport handling stress on physiological and behavioural responses of ostriches.
responses of animals can be studied to provide an accurate assessment of stress in livestock (Gross & Siegel 1983; Barnett & Hemsworth 1990; Grandin 1997; Mitchell & Kettlewell 1998). Assessing only physiological responses or only behavioural responses may not provide us with sufficient information about the welfare of animals. Behavioural and physiological stress responses might also be correlated (Dantzer & Mormède 1983; Dawkins 2003). However, most of the physiological stress responses require expensive and invasive samples to be taken from animals, whereas behavioural stress responses can be more easily observed and measured. These behavioral responses should be validated by measuring physiological responses of animals to confirm that they indicate stress before they are used by producers and handlers to identify highly stressed animals and poor welfare conditions (Grandin 2010a).

The main objective of this study was to investigate the effects of pre-transport handling stress on ostrich behaviour and physiological responses. Regular pre-transport handling stress is caused by many stressors such as restraining birds, hooding, separating them from their pen-mates, mixing them with unfamiliar birds, holding them in an unfamiliar pre-transport smaller size loading pen, novelty from their routine, and handlers’ presence. However, for the purpose of this study, pre-transport holding time was used as a handling-stage stressor in the statistical analysis because it is quantifiable and a common practice in the industry to transfer birds to a smaller-sized pre-transport loading facility closer to the loading gate and to keep the birds there for a few hours (or days) before transport to make loading easier.

Furthermore, the intent was to identify a behavioural indicator that could be validated and used by ostrich producers and handlers to identify stressed birds during pre-transport handling. Since producers do not have the resources and expertise to measure physiological stress responses, validated behavioural indicators are critical for identifying highly stressed birds. Knowledge of pre-transport impacts on ostrich stress and availability of a validated behavioural indicator could be used to alter handling processes thereby decreasing potential losses and improving ostrich welfare.

The null hypothesis of the study was that the pre-transport handling stressors will not affect the physiological and/or behavioural stress responses of ostriches. A specific hypothesis was also developed for each measured variable in this study.
3.2 Methods

3.2.1 Handling, sampling and transport process

This research was conducted utilizing routine transportation procedures by an ostrich farm in Alberta, Canada in October 2011 (ambient temperature 13.7 ± 0.2 °C, and relative humidity 62.8% ± 0.8). Twenty-four 2.5 year-old crossbred ostriches (12 males and 12 females) which were hatched and raised at the same farm under the same management and feeding program were monitored for the study. The day before transportation, 38 ostriches were transferred from their home pens to a smaller-sized holding pen (36 m$^2$) with access to feed and water (fourteen extra non-experimental same-age ostriches were also transferred to the holding pen to keep company with the experimental birds to reduce the stress of the last handled experimental birds). The experimental birds were then individually restrained, hooded and walked (approximately 12 min/bird) to a sampling pen (30 m$^2$, next to the holding pen but physically and visually isolated). The first experimental bird spent 13 min while the last one spent 279 min in the holding pen before being moved to the sampling pen.

In the sampling pen, pre-transport blood samples were taken (10 ml of blood into lithium heparin Vacutainer® Plus blood collection tubes) from the wing vein and birds were weighed. Upon completion of the sampling procedure, birds were released in a nearby larger familiar pen (physically, visually and audibly isolated from the holding and sampling pen) to reduce their stress levels. They were loaded the following morning and shipped to another farm 1100 km away (18 h of driving). The farm vehicle, a modified livestock transportation trailer with one deck divided into three compartments was used. The density of birds inside the trailer was about 0.5 m$^2$ per bird as recommended by Animal Health Australia (AHA) (2012) and South African Ostrich Business Chamber (2011). A post-transport blood sample was then obtained from each bird along with the bird weight around noon at the destination farm.

For both pre-transport and post-transport sampling procedures, birds were hooded and calmed. Ostriches are large and dangerous animals; they can cause considerable injuries to themselves and to their handlers. Therefore, routinely, three handlers handled the birds throughout the pre- and post-transport handling process. This research was conducted under the University of British Columbia (UBC) Animal Care guidelines (Certificate #A11-0110).
3.2.2 Behavioural observation

The behaviours of each bird were recorded at different stages of the handling and transport process. Monitoring behaviours started in the pre-transport holding pen 90 s before restraining each bird, continued throughout the handling procedure (i.e. blood sampling and weighing) in the sampling pen (about 5 min/bird), and ended 90 s after the sampling process. The observations were recorded by a single observer less than 4 m distance away guided by a modified ostrich behavioural response classification (Deeming & Bubier 1999; Minka & Ayo 2008) including standing, walking, running, sitting, eating, drinking, pecking at different objects (except food/water), falling/slipping, vocalization, fighting/aggression, kicking and jumping.

Some birds exhibited an immobile sitting behaviour with necks held stiffly upright and eyes open when handled in the sampling pen (note: not in the holding pen). For these birds, the responsiveness or irresponsiveness to the handling practices and environmental stimuli (e.g., removing hood) was also recorded during the handling process. These birds were also remaining in this posture and not responsive to the handlers’ initial attempt to get them up for more than 2 min.

A no-brand wired infrared camera was also used to observe the behaviour of birds inside the trailer during 18 h transport. The camera was installed at a height of 2 m from the deck to provide a view of the birds inside the trailer. The behaviour of the birds was recorded throughout transport.

3.2.3 Pre- and post-transport physical condition

Qualities of animal agriculture products are often affected by pre-slaughter handling and transport practices that can cause bruises and other physical damage (Engelbrecht et al. 2009; Glatz & Miao 2008; Warriss 2010a). In this study, each bird was examined for any physical damage before and after transport. Bruises were visually assessed by a single observer in terms of the severity of the bruise (slight, medium and heavy) and site of the bruise (neck, fore-chest, ribs, back, thigh, leg, foot, wing and tail area) based on a scale adopted from the Australian Carcass Bruises Scoring System (Anderson 1978). Underlying tissue bruises were not considered (Strappini et al. 2009) since monitoring was done on live birds. Severity and location of feather losses (neck, fore-chest, ribs, back, thigh, foot, wing and tail area), and swollen foot/wing problems were measured using a scale similar to the bruise assessment scale. Photographs were
also taken of the physical damage to each bird and these photographs were later used to reconfirm or adjust assigned scores.

### 3.2.4 Blood sample analysis

Blood samples were analyzed by a multichannel chemistry analyser (Olympus AU 5431, Olympus, Center Valley, PA) to determine the concentrations of metabolites (e.g., glucose, sodium), enzymes (aspartate aminotransferase, alanine aminotransferase and creatine phosphokinase). Blood smears were made immediately after blood sampling for WBC differential counts to calculate heterophil:lymphocyte (H:L) ratios. Packed cell volume (hematocrit) was measured by centrifuging blood in microhematocrit tubes. Plasma corticosterone concentration was determined by a Corticosterone ELISA kit (Enzo Life Science, Catalog No. ADI-900-097).

### 3.2.5 Statistical analysis

Observations and sampling procedures were conducted on individual birds in this study. All analyses were conducted using the PASW Statistics software (PASW Statistics Grad Pack 17.0, release 17.0.2., SPSS Inc., Chicago, IL) and a significance level of 0.05 was used for all tests. Also, all model assumptions were evaluated prior to interpreting model results. Because of logistic limitations, it was not possible to replicate the study. Advanced statistical models (Schank and Koehnle, 2009) were therefore used to obtain valid and reliable results.

To investigate the effects of the pre-transport handling stress, linear models were fitted using selected plasma metabolites as response (output) variables and pre-transport holding time (in min) as the predictor variable. Because of the possible effect of pre-transport body weight on the plasma metabolites, pre-transport body weight of the ostriches (in kg) was also included in the models resulting in the following equation:

**Equation 1:** \( (\text{Response variable})_i = \text{Intercept} + \beta_1 \text{(pre-transport weight)}_i + \beta_2 \text{(holding time)}_i + \epsilon_i \)

where the Intercept, \( \beta_1 \) and \( \beta_2 \) are parameters to be estimated; weight is in kg and time is in min; \( i \) is the observation (i.e., bird) number; and \( \epsilon_i \) is the residual error.

Each model was fitted using the GLM procedure. Initially, sex was also considered as a predictor variable. However, our preliminary results indicated that there were no differences in
response variables between males and females, similar to other ostrich studies (Levy et al. 1989; Hoffman et al. 2012). Therefore, sex was not included in the model. Also, the predictor variables were dropped from the model if not significant. As a further indicator of stress, the change in weight between pre- and post-transport was examined using Equation 1. One highly influential case was identified in the corticosterone concentration based on calculated Cook’s distance and standardized DFBeta and it was removed (Field 2005 p 165-166).

Recorded behaviours were summarized over all birds. As noted, five birds exhibited an immobile sitting behaviour which may indicate a pronounced stress response. Therefore, the pre- and post-transport plasma metabolite concentrations of birds were compared between birds that displayed this immobile sitting behaviour versus those that did not. For this purpose, a linear model was used (GLM procedure) to conduct a repeated measures analysis of variance (ANOVA), separating within subjects effects (i.e., within birds, sampling time at two times: pre- or post-transport) from between subjects effects (i.e., between birds, immobile sitting behavior in two levels: yes or no). For the plasma metabolite concentrations where the interaction between sampling time and immobile sitting behaviour was significant, marginal means and standard errors were calculated for combinations of the two factors. Also, in that case, two Least Squared Difference (LSD) post-hoc tests were used to test for differences between birds that displayed the immobile response versus those that did not for pre-transport measurements and for post-transport measurements, separately, using a Bonferroni adjustment to control the overall significance level. Where interactions were not significant, marginal means and standard errors were calculated for the main effects of sampling time (pre- or post-transport) and immobile sitting behaviour (yes or no) separately; since there were only two levels of each factor, no further tests were needed.

Finally, recorded physical damage data were summarized over all birds. These were also separated into birds that exhibited the immobile sitting behaviour versus those that did not. The Pearson correlation was used for two continuous variables and the phi correlation was used for two dichotomous variables to evaluate the relationships. The chi-square contingency table test was used to test the independence of two binomial variables.
3.3 Results

3.3.1 Behavioural response of ostriches during pre-transport handling

All birds were standing or walking inside the holding pen before being restrained and hooded. None was observed sitting, vocalizing, running, falling, jumping, kicking, fighting, feeding or drinking in the holding pen before the beginning of the handling procedure (food and water were available in the holding pen). When handlers approached a bird to restrain and hood it, the bird tried to escape by running inside the holding pen, jumping, or kicking the handlers. After being hooded, the birds calmed down (in less than 1 min) and were walked to the sampling pen.

The first 13 birds were hooded, calm and standing during the whole handling procedure in the sampling pen. Blood samples were taken immediately after bringing a bird to the sampling pen, and then the bird was walked (hooded) to a scale to measure its weight in the sampling pen. However, five birds (two females & three males) among the last 11 birds displayed an immobile sitting behaviour after bringing them to the sampling pen (Note: These birds were hooded and appeared to be calm). This behavioural response happened at the second half of the handling procedure and the hypothesis was that it was correlated to the handling stress. Results showed that time spent in the holding pen differed for birds that displayed the immobile sitting behaviour (217 ± 34 min) versus those that did not (136 ± 82 min) ($t_{16.73} = -3.35$, $P = 0.004$, t-test for unequal variances). The immobile sitting behaviour was independent from the sex of the birds ($\chi^2 = 0.25$, $P = 0.61$). Birds that exhibited the immobile sitting behaviour did not exhibit this behaviour again once they had resumed standing.

3.3.2 Effects of the pre-transport handling stress and the immobile sitting behaviour on physiological stress parameters

Of the 19 birds that had not displayed an immobile sitting behaviour during pre-transport handling, one died during transport because of being trampled by the other birds in the compartment, and of the five birds that displayed an immobile sitting behaviour during pre-transport handling one suffered a broken tendon during transport. Also, the post-transport blood sample could not be obtained within 3 min of capture from two birds (one from each behavioural response groups). Therefore, the pre-transport regression model was fitted using all 24 birds.
However, the repeated measures ANOVAs were obtained using a reduced set of 20 birds for the blood metabolites and 22 birds for the weight change.

### 3.3.2.1 Plasma aspartate aminotransferase (AST) levels

The pre-transport plasma AST levels were not affected by pre-transport body weights, and the model was refitted without pre-transport body weight. The relationship with pre-transport holding time was significant (Table 3-1, $F_{1,22} = 7.19, P = 0.014$). Using this model, birds which were kept for a longer time in the pre-transport holding pen (i.e. experienced higher handling stress) had higher pre-transport plasma AST levels, and each minute of pre-transport holding time increased the AST level by 0.32 IU/L (see Figure 3-1).

There was an interaction between immobile sitting behaviour (yes or no) and sampling time (pre- versus post-transport sampling) ($F_{1,18} = 11.85, P = 0.003$); therefore, the effects of the two factors on AST levels could not be separately interpreted. Pre-transport AST levels of immobile birds ($361 \pm 30$ IU/L) and non-immobile birds ($316 \pm 13$ IU/L) did not differ significantly ($F_{1,18} = 1.866, P = 0.189$), but birds that displayed an immobile sitting behaviour had higher post-transport AST levels ($2060 \pm 252$ IU/L) compared to non-immobile birds ($1077 \pm 106$ IU/L) ($F_{1,18} = 12.89, P = 0.002$) (see Figure 3-2).

### 3.3.2.2 Plasma alanine aminotransferase (ALT) levels

Both predictor variables (pre-transport body weight and pre-transport holding time variables) contributed to the model (Table 3-1, $F_{2,21} = 5.73, P = 0.010$); heavier birds had higher ALT levels, and birds which were kept in the pre-transport holding pen for a longer time also had higher ALT levels. As a result, each kg increase in body weight increased the ALT level by 0.098 IU/L if holding time was held constant, and each min longer pre-transport holding time increased the ALT level by 0.016 IU/L if body weight was held constant in the fitted model.

There was an interaction between immobile sitting behaviour and sampling time ($F_{1,18} = 13.80, P = 0.002$) on ALT levels. Pre-transport ALT levels were not different between immobilized birds ($11.0 \pm 1.7$ IU/L) and non-immobilized birds ($8.7 \pm 0.7$ IU/L) ($F_{1,18} = 1.616, P = 0.220$), but immobilized birds had higher post-transport ALT levels ($94.3 \pm 12.3$ IU/L) compared to the non-immobile birds ($42.4 \pm 5.2$ IU/L) ($F_{1,18} = 15.14, P = 0.001$).
3.3.2.3 **Plasma creatine phosphokinase (CPK) levels**

Pre-transport CPK levels were not affected by body weight nor by pre-transport holding time ($F_{2, 21} = 1.98, P = 0.163$). However, there were positive correlations between the pre-transport AST and CPK levels (controlling for the pre-transport holding time; $r = 0.44, P = 0.036$), and between the pre-transport holding time and the pre-transport plasma CPK level ($r = 0.38, P = 0.035$).

An interaction was found between immobile sitting behaviour and sampling time on the CPK levels ($F_{1, 18} = 18.37, P < 0.001$). Pre-transport CPK levels were not different between the immobilized birds ($4093 \pm 708$ IU/L) and non-immobilized birds ($2981 \pm 297$ IU/L) ($F_{1, 18} = 2.097, P = 0.165$), but immobilized birds had higher post-transport CPK levels ($20776 \pm 27578$ IU/L) compared to the non-immobile birds ($73220 \pm 11585$ IU/L) ($F_{1, 18} = 18.76, P < 0.001$) (see Figure 3-3).

3.3.2.4 **Plasma sodium concentration**

Pre-transport weight and holding time variables were both significant (Table 3-1, $F_{2, 22} = 6.82, P = 0.005$). Heavier birds had lower pre-transport plasma sodium concentrations. Each kg heavier in pre-transport body weight translated to 0.089 mmol/L less in pre-transport sodium concentration if the effect of the holding time was held constant in the fitted model. Keeping birds for a longer time in the pre-transport holding pen increased their pre-transport plasma sodium concentrations, and each min longer in the holding pen increased the concentration of the pre-transport plasma sodium by 0.019 mmol/L if the effect of the body weight was held constant in the fitted model (see Figure 3-4).

There was an interaction between the immobile response and the sampling time on sodium concentrations ($F_{1, 18} = 6.79, P = 0.018$). Pre-transport sodium concentrations were not different between the immobilized birds ($147 \pm 2$ mmol/L) and non-immobilized birds ($145 \pm 1$ mmol/L) ($F_{1, 18} = 0.572, P = 0.459$), but immobilized birds had lower post-transport sodium concentration ($145 \pm 1$ mmol/L) compared to non-immobilized birds ($149 \pm 0.5$ mmol/L) ($F_{1, 18} = 12.74, P = 0.002$).
Packed cell volume (PCV)

Pre-transport PCV was significantly affected by body weight and pre-transport holding time variables (Table 3-1, \(F_{2, 21} = 6.97, P = 0.005\)). Overall, each kg heavier in pre-transport body weight increased the pre-transport PCV about 0.099\% if the holding time was held constant, and each minute of pre-transport holding time increased the PCV by 0.013\% if pre-transport body weight was held constant in the fitted model (see Figure 3-5).

The mean pre-transport PCV was 43.6 ± 0.8 \%. Repeated measures ANOVA test did not detect any interactions or main effects for PCV.

Plasma glucose concentration

Differences in the pre-transport body weight of the ostriches and their pre-transport holding time did not significantly affect their pre-transport glucose concentrations (\(F_{2, 21} = 0.842, P = 0.445\)).

There was an interaction between the immobile response and the sampling time on the glucose concentrations (\(F_{1,18} = 5.78, P = 0.027\)). Pre-transport glucose concentration was not different between immobilized birds (11.0 ± 1.2 mmol/L) and non-immobilized birds (10.6 ± 0.5 mmol/L) (\(F_{1,18} = 0.084, P = 0.775\)), but immobile birds had higher post-transport glucose concentrations (20.6 ± 1.9 mmol/L) compared to the non-immobile birds (15.9 ± 0.8 mmol/L) (\(F_{1,18} = 5.10, P = 0.036\)) (see Figure 3-6).

Heterophil to lymphocyte ratios (H:L ratio)

Differences in body weight and pre-transport holding time did not affect pre-transport H:L ratios (\(F_{2, 21} = 0.421, P = 0.662\)).

No interaction was detected for H:L. In terms of main effects, the post-transport H:L ratios of all birds (9.1 ± 1.5) were higher than their pre-transport H:L ratios (2.9 ± 0.5) (\(F_{1,18} = 16.96, P = 0.001\)), and there was no difference between the post-transport H:L ratios of immobilized birds and non-immobile birds.

Plasma corticosterone concentration

Pre-transport holding time did not affect pre-transport plasma corticosterone concentrations and the model was refitted with the pre-transport body weight variable. Pre-
transport body weight affected the pre-transport plasma corticosterone concentration (Table 3-1, \( F_{1,22} = 10.546, P = 0.004 \)). Results of this analysis indicated that heavier birds had lower corticosterone concentrations, and each kg heavier weight resulted in 0.162 ng/ml lower corticosterone concentration.

The mean pre-transport plasma corticosterone concentration was 5.4 ± 1.1 ng/mL. No interactions or main effects were detected for plasma corticosterone concentration using the repeated measures ANOVA.

### 3.3.2.9 Body weight

Weight loss (in kg) was calculated by deducting post-transport weight from pre-transport weight for each of the 22 birds weighed. The pre-transport holding time did not affect the weight loss of birds and the model was refitted without pre-transport holding time. The relationship with pre-transport body weight was significant (Table 3-1, \( F_{1,20} = 5.201, P = 0.034 \)). Heavier birds lost more weight during the handling and transport process, and each kg heavier pre-transport weight results in 55 g greater weight loss.

No interaction was detected for body weight. In terms of main effects, post-transport body weight of all birds (74.8 ± 3.2 kg) was considerably lower than their pre-transport body weight (84.5 ± 3.4 kg) (\( F_{1,20} = 553.63, P < 0.001 \)), and there was no difference between the post-transport body weight of immobile birds and non-immobile birds.

### 3.3.3 Physical condition of ostriches during transport considering their pre-transport sitting behaviour

Body damage scores were assigned to all birds (24). None of the birds had bruises, swollen wing/foot or obvious feather losses before transport. However, after transport, numerous birds had one or more of these problems. Birds which displayed the pre-transport immobile sitting behaviour had more bruises (in their neck, back and wings) and feather losses (in their neck, back, fore-chest, ribs and wings) compared to the non-immobile birds (Table 3-2).

Observations of the behaviours of ostriches inside the trailer during transport showed that none of the birds displayed the immobile response. However, they would fall down due to sudden movements of the trailer or when pushed by other birds. When they fell down and failed to get back on their feet immediately, they would be trampled by other standing birds and suffer
loss of their back feathers. Results showed that all birds that displayed pre-transport immobile sitting behaviour had back feather loss (φ = 0.61, P < 0.01; Table 3-2).

There was no significant difference between the frequencies of post-transport swollen foot problems in two different behavioural groups. However, a swollen right wing problem was more common in birds that displayed pre-transport immobile behaviour (φ = 0.49, P < 0.05; Table 3-2).

3.4 Discussion

Handling and transport is an important part of ostrich production, and this manipulation is highly stressful for the birds. Producers usually keep their birds in a pre-transport holding pen before loading them into the trailer and no previous study was found that examined the effect of the pre-transport handling stress on the physiological and behavioural responses of ostriches. This study was conducted under real transportation conditions to identify welfare problems in the current handling and transport practices and to provide information that would help to improve the handling practices and welfare of the birds. In this study, we investigated the effects of the pre-transport handling (using holding time as the quantifiable handling stress factor) on the physiological and behavioural stress responses of ostriches. As well, we had hoped to find a validated behavioural response that could be used by producers and handlers to identify birds that are experiencing higher stress levels. Collectively, information on impacts of handling stress and a validated behavioural response indicator of stress would provide producers with information needed to alter procedures, thus reducing transportation stress and losses.

Even though there was no replication for the study, the use of mixed models and advanced statistical analyses allowed us to obtain valid and reliable results (Schank and Koehnle, 2009). Furthermore, results of the related studies on ratites and other livestock handling and transport were compared to confirm our findings. A surprising result of this study was that ostriches exhibited an immobile sitting behaviour. Ostriches show two natural behavioural responses when handlers approach them: they either avoid the handlers (especially if they are not used to handling) or they may approach the handlers because of their inquisitive nature (especially when they see shinny objects such as handlers’ eyeglasses) (Shanawany 1999). Minka & Ayo (2008) reported capture myopathy (i.e. “when an ostrich fell and refused to stand
on its own, even when helped”) as one of the observed behaviours during handling and loading as a result of extreme exertions during restraining. Capture myopathy (or exertional rhabdomyolysis) is well documented in handling of several species of wild or extensively raised mammals and birds, and is caused because of the muscle tissue breakdown as a result of exertion (Chalmers & Barrett 1977; Spraker et al. 1987; Marco et al. 2006). Capture myopathy could be seen in ostriches under high stress handling and transport condition, and it often causes death in a few hours or in 2 wks. (Wotton & Hewitt 1999). In our study, none of the immobile sitting birds died during handling or transport. A few weeks after the transport trial, we contacted the producers of the ostriches that we took our samples from during their transport process, and they reported that there was no mortality in the birds after transport. Moreover, the immobile sitting birds in our study were observed walking and standing after sampling and during transportation. Therefore, we conclude that our observed immobile sitting behaviour may not be a consequence of capture myopathy. However, the possibility that immobile sitting may cause very mild capture myopathy cannot be ruled out.

Samson (1996) also reported submission social behaviour and stargazing abnormal behaviour in ostriches raised in Canada. Submission behavioural response is explained as running away from an aggressive bird or falling on the ground without defending (Samson, 1996). The immobile sitting birds in our study did not fall or were not running away, they were sitting with the neck held stiffly upright, therefore the observed behaviour in our study is not a submission behaviour. Behavioural stargazing also was not the behaviour that we observed based on Samson’s (1996) definition that “a bird will continually lift its head up and back to the extent that it eventually touches its spine”.

Grandin (2010b p 78) explained that some species “raise their head up high” as a sign of fear to search for predators. In the observed immobile sitting behaviour in our study, the head of the birds was up high and their eyes were open. These could be signs of fear.

An immobilization response under threatening situations when an animal is not able to escape or fight has been termed tonic immobility (Hoagland 1927, 1928). When animals are incapable of escaping from predators, they will go into the tonic immobility stage and may appear dead. This can increase the probability of survival because most predators have difficulty detecting immobilized objects (Bracha et al. 2004; Miyatake et al. 2004; Nesse 1999). Tonic
immobility has been recorded in many livestock species, wild animal species and humans in severe threatening situation or when they are restrained (Fraser 1960; Gehlbach 1970; Miyatake et al. 2004; Abrams et al. 2009). Grandin (2010b p 78) also reported tonic immobility response in Bos indicus cattle and American bison when animals “lie down and become immobile” as a sign of fear when animals are under handling stress which they cannot escape.

Tonic immobility has been identified as a fear response in chickens by Gallup (1978) and Jones (1987). Cashman et al. (1989) also concluded that, in broilers, pre-transport waiting time and transport duration (both) are positively correlated with the mean tonic immobility duration, and the tonic immobility response increased in the intense, prolonged or inescapable situations. The immobile sitting behaviour observed in our study could be a tonic immobility-like response because it was observed when the birds were restrained and were undergoing human handling in the sampling pen. The birds that exhibited the immobile sitting behaviour in our study experienced higher pre-transport handling stress, were kept in the pre-transport holding pen for longer durations, and had more experience with their pen-mates being handled and then disappearing. No study has reported tonic immobility responses in ostriches and there are a few differences between the observed immobile sitting behaviour in our study and the common tonic immobility response in chickens or quail. For example, the irresponsiveness of ostriches during the immobile sitting behaviour was stronger than the reported tonic immobility response in chickens. Therefore, we called the observed behaviour in our study ‘immobile sitting behaviour’ or ‘tonic immobility-like behavioural response.

In our study, we were not able to tease apart the effects of different pre-transport handling stress factors (e.g., capturing, separating pen-mates, novelty, and holding duration in the smaller size loading pen). We selected pre-transport holding time duration as the quantifiable factor to investigate the effects of pre-transport handling stress as a combination of different stressors in the real handling and transport practices in Canada. The main objective of the study is to improve the current ostrich handling practices which are being done without having any specific ratite handling and transport Codes of Practice in Canada and USA.

Few researchers to date have investigated the relationship between physiological stress responses of animals and their tonic immobility response. Only differences in the corticosterone concentrations and the H:L ratios have been investigated in birds showing tonic immobility

Results from our study indicate that higher pre-transport handling stress (or longer pre-transport holding time) increased pre-transport AST, ALT, sodium concentrations and PCV levels, and birds which were heavier also had higher levels of pre-transport ALT and PCV, and lower concentrations of sodium and corticosterone. Moreover, the immobile sitting behaviour was observed mostly in the birds which were kept in the pre-transport holding pen for a longer period, and immobile sitting birds had higher concentrations of post-transport AST, ALT, CPK and glucose. Birds with the pre-transport immobile sitting behaviour also had more physical damage and transport losses.

Most of the pre-transport blood biochemistry and hematology test results in our study were within the reference ranges reported by different studies: AST concentration (Verstappen et al. 2002; Moniello et al. 2005); ALT concentration (Van Heerden et al. 1985); CPK concentration (Verstappen et al. 2002); sodium concentration (Krautwald-Junghanns 2007); PCV (Krautwald-Junghanns 2007); glucose concentration (Verstappen et al. 2002). However, pre-transport H:L ratios reported by Mitchell et al. (1996) were higher than the H:L ratios measured at this study. Corticosterone concentration in this study was higher than that report by Mitchell et al. (1996).

AST is not a liver specific enzyme; it has a wide distribution in animals’ tissues (Krautwald-Junghanns 2007), and it is the most important enzyme indicator of liver disease. Nevertheless when an increase in the AST concentration is simultaneous with an increase in the CPK concentration, it is a sign of soft tissue damage/trauma (specially muscle) (Krautwald-Junghanns 2007) rather than liver damage. Janssen et al. (1989) also reported a very high correlation between AST and CPK activations during muscle damage in humans. In our study CPK and AST concentrations were also positively correlated which means the high AST concentration could be a sign of muscle damage.

ALT is ubiquitous and can be found in various cells in the body. In human, an increase in ALT concentration is usually interpreted as liver health problem. However, Nathwani et al. (2005) reported an increase in ALT concentration because of muscle injury, which was confirmed by increases in serum aspartate aminotransferase (AST), creatine phosphokinase
(CPK) and lactate dehydrogenase (LDH) concentrations. Mechanical stress and muscle damage can increase the leakage of the muscle enzymes (CPK, AST, ALT and LDH) into the bloodstream (Janssen et al. 1989).

Post-transport plasma glucose concentrations of immobile sitting birds were higher than that of non-sitting birds in our study. The only fuel for the central nervous system (CNS) and red blood cells (RBC) is glucose (Reed 2009). Adrenaline secretion as a physiological response to stress and exercise results in an increase in the cyclic adenosine-3',5'-monophosphate (cAMP) concentration and stimulation of the glycogen phosphorylase activity to provide more local fuel (glucose-6-phosphate) in the muscle (but the glycogen phosphorylase mechanism in liver is different and is stimulated by glucagon to control the glucose concentration in the whole body) (Reed 2009). When carbohydrate stores are depleted because of fasting, starvation or maximal activity, glycogenolysis and gluconeogenesis (i.e. synthesis of new glucose in liver or kidneys) will be promoted to provide glucose for the CNS and red blood cells using glucogenic amino acids, oxaloacetate, glycerol and lactate (Reed 2009). These would likely be the causes for glucose concentrations increase after the handling and transport process especially in the immobile sitting birds in our study.

Sodium concentration increased when livestock were held for a longer period in the pre-transport holding pen. Schaefer et al. (2001) reported an increase in the sodium concentration during livestock handling and transportation because of the dehydration which increases osmolality of the blood and the plasma sodium concentrations. Gray et al. (1988) also reported an increase in the sodium concentration because of water deprivation in ostriches and a return to stable concentration after rehydration.

Although birds had access to feed and water in the pre-transport holding pen, they did not drink, likely because they were kept under the novel and stressful situation in higher densities. PCV may increase during severe dehydration because of reduced plasma volume and fluid loss (Randolph et al. 2010). Schaefer et al. (1997b) also reported an increase in the PCV during handling and transport process in cattle, similar to the results of our study.

H:L ratios increased (as a result of both increased number of heterophils and decreased number of lymphocytes) in both immobile sitting and non-sitting birds alike after transport probably because H:L ratio is an indicator of the chronic stress response (Gross and Siegel
Potential challenges should be considered while using H:L ratio as a stress indicator (Davis et al. 2008). There have been different reports regarding changes in the H:L ratio when also considering the tonic immobility response of animals (e.g. Jones (1989) with Brown Leghorn pullets; Zulkifli et al. (2000) with market age broiler chickens).

Pre-transport corticostrone concentrations in our study were higher (5.4 ± 1.1 ng/mL) than those of reported by Mitchell et al. (1996) (4.9 ± 2.9 ng/mL). Mitchell et al. (1996) transported their ostriches for 4.5 h and after transportation corticosterone concentrations increased by 75%. Lèche et al. (2013) also reported a dramatic increase (40 times) in the corticoestrone concentration of greater rhea (a member of the ratite family) compared to that of its pre-transport hormon concentraton after a 30-min transport. However, in our 18-hour transport study the post-transport corticoestrone concentration was higher (6.9 ± 2.1 ng/mL) but not significantly different from the pre-transport hormone level. The difference between our study versus Mitchell et al. (1996) and Lèche et al. (2013) studies could be because of the difference in the transport duration in these three studies and the variability in the glucocorticords concentrations in different stages of the stressful event (Dantzer & Mormède 1983; Mounier et al. 2006) and their circadian secretion rhythm (Möstl & Palme, 2002). Glucocorticoids are time dependent measurements which reach their peak in 10 to 20 min (Grandin, 1997) and sampling time affects cortisol concentrations in plasma. Mounier et al. (2006) indicated that plasma corticosteroids do not show stress level for events which occurred several hours ago.

The corticosterone concentrations of the ostriches which showed immobile sitting behaviour were compared to that of non-sitting birds to investigate the Hypothalamic-Pituitary-Adrenal (HPA) axis physiological stress response. There were no differences between the corticosterone concentrations in the two different behavioural groups in our study. Our results are consistent with those reported by Jones et al. (1994) and Richard et al. (2008) who found no significant differences in the corticosterone concentrations of Japanese quail (Coturnix japonica) selected for their short and long tonic immobility response and the control group. Also, Gudev et al. (2011) reported that the tonic immobility duration in chickens was not correlated to the corticosterone concentrations during catching and crating.

None of the ostriches of this study had bruises, swollen wings/feet or feather losses before transport. However, after transport, numerous birds had one or more of these problems
(Table 3-2). Other researchers have also reported bruises and injuries in livestock because of handling and transport (Schaefer et al. 1997b; Northcutt 2001; Grandin 2010a; Warriss 2010b). Post-transport physical damage in ostriches were more severe than observed in other livestock species (bruises measured on carcasses of US fed steers and heifers was 35.2% (Garcia et al. 2008)).

Higher post-transport plasma AST, ALT, CPK and glucose concentrations of immobile sitting birds compared to non-sitting birds in our study are probably due to the higher stress levels that immobile sitting birds experienced during the pre-transport handling process. They likely had earlier depletions of energy stores which resulted in problems in the muscle cell membrane integrity and higher energy demands to maintain postural stability during the transport process, more muscle injuries, more physical damages and poor welfare (Janssen et al. 1989; Warriss et al. 1994; Mitchell 1999; Schaefer et al. 2001; Nathwani et al. 2005). However, based on observed behaviour during transport, birds were not sitting voluntarily inside the trailer but rather fell after being pushed by other birds or because of sudden vehicle accelerations, stops or turns. Overall, immobile sitting birds were probably weaker, lost their balance more easily, and were less resistant to external forces (i.e., pressures from the other birds and trailer motions) and may have very mild capture myopathy. Therefore, they fell down more frequently, were trampled under the standing birds in the same compartment, and had more losses and injuries (i.e., losing the back feather and bruises on the back are indications of being trampled under the other birds). Therefore, mixing immobile sitting birds with stronger and less stressed birds in the same compartment may have resulted in higher rates of physical damage in immobile sitting birds.

Because of the results of other physiological stress indicators, immobile sitting behaviour could be considered as one of the behavioural fear indicator in ostriches. Also, immobile sitting behaviour could be used to identify the fearful birds during handling practices and to alter practices to minimize losses. Producers should closely monitor birds which show immobile sitting behaviour during the pre-loading handling stage and should not mix these birds with stronger birds during loading and transport to minimize loses.

Based on the dramatic increases in the concentrations of CK, AST and ALT enzymes and more physical damages after transport, we can conclude that, with the current industry handling
practices, transportation results in more losses and more muscle damage in ostriches. Researchers in this study did not manipulate the conditions of the current routine industry transport process to identify potential welfare issues. Higher stress levels of the birds and higher incidence of injuries also demonstrate the need for development of a Codes of Practice for the transportation of ratites in Canada. Ostrich transportation problems may also be different from the transportation problems of other species because of specific features of the ostrich anatomy.

Overall, based on the results of our study, we conclude that the pre-transport handling stress should be minimized for all birds. We also conclude that the immobile sitting behavioural response is an important indicator of high stress levels in ostriches, and can be used to identify stressed birds before loading and reduce losses.
Table 3-1 Multiple regression model results for pre-transport plasma metabolite concentrations and weight loss (as output variables) in relation to their pre-transport body weight (kg) and the holding time (min) as the predictor variables (n=24).

<table>
<thead>
<tr>
<th>Predictor variables</th>
<th>B (Coefficients)</th>
<th>Standard Errors</th>
<th>t-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma aspartate aminotransferase (AST) (IU/L)</td>
<td>Constant 279.910</td>
<td>20.479</td>
<td>13.668</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Holding time 0.318</td>
<td>0.119</td>
<td>2.681</td>
<td>0.014</td>
</tr>
<tr>
<td>Plasma alanine aminotransferase (ALT) (IU/L)</td>
<td>Constant -2.081</td>
<td>3.854</td>
<td>-0.540</td>
<td>0.595</td>
</tr>
<tr>
<td></td>
<td>Body weight 0.098</td>
<td>0.043</td>
<td>2.287</td>
<td>0.033</td>
</tr>
<tr>
<td></td>
<td>Holding time 0.015</td>
<td>0.006</td>
<td>2.401</td>
<td>0.026</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>Constant 150.752</td>
<td>3.687</td>
<td>40.890</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Body weight -0.089</td>
<td>0.041</td>
<td>-2.173</td>
<td>0.041</td>
</tr>
<tr>
<td></td>
<td>Holding time 0.019</td>
<td>0.006</td>
<td>3.070</td>
<td>0.006</td>
</tr>
<tr>
<td>Packed cell volume (PCV) (%)</td>
<td>Constant 32.415</td>
<td>3.205</td>
<td>10.115</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Body weight 0.099</td>
<td>0.036</td>
<td>2.757</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>Holding time 0.013</td>
<td>0.005</td>
<td>2.404</td>
<td>0.026</td>
</tr>
<tr>
<td>Corticosterone (ng/mL)</td>
<td>Constant 19.432</td>
<td>4.388</td>
<td>4.428</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Body weight -0.162</td>
<td>0.050</td>
<td>-3.247</td>
<td>0.004</td>
</tr>
<tr>
<td>Weight loss (kg)</td>
<td>Constant 5.153</td>
<td>2.095</td>
<td>2.459</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td>Body weight 0.055</td>
<td>0.024</td>
<td>2.280</td>
<td>0.034</td>
</tr>
</tbody>
</table>
Table 3-2 Percentage of birds with the post-transport bruises, feather losses or swollen wing/foot problems on different sites of their body separated into the two identified pre-transport behavioural response groups, and Phi correlation tests (i.e., correlation between the two dichotomous variables, body damage and the immobile sitting behaviour).

<table>
<thead>
<tr>
<th></th>
<th>Non-immobile sitting behaviour birds</th>
<th>Immobile sitting behaviour birds</th>
<th>Phi correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slight</td>
<td>Medium</td>
<td>Heavy</td>
</tr>
<tr>
<td>Bruises on the neck</td>
<td>5</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>Bruises on the back</td>
<td>5</td>
<td>5</td>
<td>60</td>
</tr>
<tr>
<td>Bruises on the fore-chest</td>
<td>10</td>
<td>5</td>
<td>40</td>
</tr>
<tr>
<td>Bruises on the ribs</td>
<td>5</td>
<td>16</td>
<td>40</td>
</tr>
<tr>
<td>Bruises on the thighs</td>
<td>5</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>Bruises on the wings</td>
<td>5</td>
<td>21</td>
<td>10</td>
</tr>
<tr>
<td>Bruises on the legs</td>
<td>16</td>
<td>5</td>
<td>40</td>
</tr>
<tr>
<td>Bruises on the tail</td>
<td>26</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td>Neck feather losses</td>
<td>20</td>
<td>20</td>
<td>0.41*</td>
</tr>
<tr>
<td>Back feather losses</td>
<td>16</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>Fore-chest feather losses</td>
<td>10</td>
<td>5</td>
<td>80</td>
</tr>
<tr>
<td>Ribs feather losses</td>
<td>16</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Thighs feather losses</td>
<td>10</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>Wings feather losses</td>
<td>32</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Tail feather losses</td>
<td>47</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Swollen left foot</td>
<td>5</td>
<td>21</td>
<td>5</td>
</tr>
<tr>
<td>Swollen right foot</td>
<td>16</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Swollen left wing</td>
<td>5</td>
<td>5</td>
<td>40</td>
</tr>
<tr>
<td>Swollen right wing</td>
<td>5</td>
<td>5</td>
<td>60</td>
</tr>
</tbody>
</table>

* P < 0.05, ** P < 0.01, *** P < 0.001
Figure 3-1 A scatter plot showing correlation between pre-transport plasma aspartate aminotransferase (AST) concentrations and time spent in the pre-transport holding pen for 24 ostriches (each dot shows the result for a bird, and the solid line shows the regression line)
Interaction: $F_{1,18} = 11.85$  
$P = 0.003$

Post-transport post-hoc:  
$F_{1,18} = 12.89$  
$P = 0.002$

Figure 3-2 Pre- and post-transport plasma aspartate aminotransferase (AST) concentrations of ostriches considering their pre-transport immobile sitting behaviour
Figure 3-3 Pre- and post-transport plasma creatine phosphokinase (CPK) concentrations of ostriches considering their pre-transport immobile sitting behaviour

Interaction:
$F_{1,18} = 18.37$
$P < 0.001$

Post-transport post-hoc:
$F_{1,18} = 18.76$
$P < 0.001$
Figure 3-4 A scatter plot showing semi-partial correlation between pre-transport plasma sodium concentrations (adjusted for the pre-transport weight) and time spent in the pre-transport holding pen for 24 ostriches (each dot represents a single bird, and the solid line shows the regression line)

$r = 0.56$

$P < 0.01$
Figure 3-5 A scatter plot showing semi-partial correlation between pre-transport packed cell volume (PCV adjusted for the pre-transport weight) and time spent in the pre-transport holding pen for 24 ostriches (each dot represents a single bird, and the solid line shows the regression line)

$r = 0.46$

$P < 0.05$
Figure 3-6 Comparing pre- and post-transport plasma glucose concentrations of ostriches showing immobile sitting behaviour with those that did not

Interaction:
$F_{1,18} = 5.78$
$P = 0.036$

Post-transport post-hoc:
$F_{1,18} = 5.10$
$P = 0.036$
4 Effects of pre-transport nutrient supplementation and transport duration on the post-transport blood biochemistry, body weight and welfare of ostriches

4.1 Introduction

Ostriches (Struthio camelus) are the largest living birds. They are flightless and the only extant bird with two toes on each foot (Schaller et al. 2011). Because of their unique body characteristics (heavy body mass on two long feet), transporting ostriches is different than other livestock species. Maintaining balance inside a moving vehicle is more stressful and an energy-demanding process for ostriches and ratites in general.

Ostrich farming is a relatively new industry in North America (Deeming 1999). Ostrich farming in the USA and Canada occurs in a number of different states/provinces, but most farms are located far from processing plants. Transportation of ostriches to an inspected processing plant is inevitable if producers want to sell the meat to the food retail sector. At the same time, most local processing plants do not process ostriches because of a lack of expertise or proper facilities. A further complication is that the small number of ostriches that producers can send to the market annually means that it may not be economically efficient for the processing plants to interrupt their regular operation and prepare the processing equipment for slaughtering ostriches. Therefore, ostrich producers in North America may have to ship live birds over long distances to find a suitable processing facility.

The pre-slaughter transport process can be divided into five stages: pre-transport handling, loading, transport, unloading, and lairage. Multiple stressors affect the welfare of livestock at each stage, including: pre-transport feed and water withdrawal (Jones et al. 1988; Schaefer et al. 1988; Warriss et al. 1988), pre-transport holding time and handling practices (Grandin 1997; Northcutt 2001), type of loading gate/ramp (Mounier et al. 2006; Hoffman &

3 A version of this chapter has been submitted for publication. Bejaei M, Bennett DC, Schaefer AL and Cheng KM, Effects of pre-transport nutrient supplementation and transport duration on the post-transport blood biochemistry, body weight and welfare of ostriches.
Lambrechts 2011), microclimate conditions inside the vehicle (Mitchell & Kettlewell 1998), mixing of unfamiliar animals (Grandin 1997; Mounier et al. 2006), transport duration (Fisher et al. 2010), waiting time in lairage before unloading and unloading practices (Northcutt 2001; Warriss 2010a), holding time in lairage before slaughter (Warriss 2003), and stunning and slaughter methods (Grandin 2010a). These factors can cause poor welfare, health problems, bruises, injuries, weight loss, product quality degradation, and even mortality which affect the economic sustainability of farms (Warriss 1990; Schaefer et al. 2001).

An important factor influencing the welfare of livestock during pre-slaughter handling and transport practices is the length of time that animals are kept off feed and/or water. Feed/water withdrawal may start a few hours before loading animals into a trailer and may continue until slaughter. Guidelines/standards vary among countries with regard to the livestock pre-transport feed and water withdrawal duration (Canadian Agri-Food Research Council 2011; Animal Health Australia 2012). Pre-transport feed and water withdrawal causes dehydration, physiological stress responses, meat quality degradation and compromised welfare (Jones et al. 1988; Schaefer et al. 1988; Warriss et al. 1993). Pre- or post-transport nutrient supplementation has been used to improve welfare of livestock and their product quality, and to rehydrate them and alleviate the effect of the antemortem feed/water withdrawal (Schaefer et al. 1997b, 2001, 2006; Arp et al. 2011). Handling, transport and time off-feed (18 – 24 h) caused exceedingly high weight losses (10 -17%) in ostriches which were transported only 3 km (Schaefer et al. 1995). Schaefer et al. (1995, 1997a) investigated the effect of pre- and post-transport electrolyte therapy in very short transport distance (3 km), and found that pre-transport electrolyte therapy reduced the percentage weight loss of ostriches. Nevertheless, the effects of pre-transport nutrient supplementation on a long duration of transportation are still unknown.

Transport duration is one of the important factors which affect the welfare of animals during handling and transport practices. Warriss et al. (1993) reported that longer transportation duration of broilers to a processing plant increased the osmolality and dehydration after unloading, and caused physiological responses and negatively affected meat quality. Hoffman et al. (2012) reported greater weight losses and lower meat quality in ostriches which were transported for 5 h compared to 1 h transportation duration. However, little research has been done on the effects of transport duration on the physiological stress responses of ostriches.
Moreover, no study was found in literature on the effects of transportation duration above 5 h on the physiological stress responses of ostriches, even though it is a common practice in North America (personal communication, Canadian Ostrich Association Meeting, March 10th 2012).

Considering the limited information available about the effects of nutrient supplementation and transport duration on the physiological stress responses of ostriches, the objectives of this study were:

- To assess the effects of pre-transport nutrient supplementation on blood biochemistry changes and body weight loss in ostriches; and
- To investigate the effects of transportation duration on blood biochemistry changes and body weight loss in ostriches.

The null hypothesis of the study was that pre-slaughter nutrient supplementation and/or transport duration would not affect the physiological stress responses and live weight of ostriches. A specific hypothesis was also defined for each measured variable in this study.

Results of this study may be utilized by the ostrich farming industry to improve the welfare of birds, reduce production losses and increase the economic sustainability of the farms.

4.2 Materials and methods

A total of 45 ostriches (between 1 to 2.5 yrs old, and with a pre-transport body weight of 85.1 ± 1.9 kg) were used in three transport trials (30 min, 7 h and 18 h of driving) conducted in the provinces of Alberta and British Columbia, Canada to study the effects of transport duration and nutrient supplementation on the physiological stress responses of ostriches under regular shipment practices and conditions used in the North American ostrich industry.

4.2.1 Nutrient supplementation

Nutrient supplementation was applied in all three transport trials. There were two treatment groups in each trial: control (n = 22) and nutrient supplemented (n = 23). All birds received 1 L of liquid by gavaging (individual tube feeding using a modified oral calf feeder tube). Control birds (birds which did not receive nutrient supplement are referred as control birds for the remainder of this paper) were each tube fed 1 L of water. The nutrient supplemented birds
each received 1 L of liquid supplement (providing 815 kcal/kg ME and 41 g crude protein) with the following ingredients combined:

- 200 g NUTRI-CHARG® TM (Registration #680152 and US Patent #5505968) prepared by Agriculture and Agri-Food Canada, Lacombe Research Centre, AB (including: dextrose, sodium bicarbonate, magnesium sulfate, potassium chloride) (Schaefer et al. 1996);
- 50 g whey protein(82% protein); and
- Water to yield 1 L of liquid supplement.

4.2.2 Three transport trials

4.2.2.1 Trial A (30 min driving)

In trial A, birds were shipped from a farm in British Columbia (BC) to a nearby processing plant (20 km, 30 min driving). The trial was conducted in early May, and during handling and transport procedure the temperature was 12.6 ± 0.7°C, and the relative humidity was 70.9 ± 3.9%. A modified livestock transport trailer was used in trial A.

Ten birds were used in this trial: five control birds (two females, three males) and five nutrient supplement birds (two females, three males). Birds in this trial experienced all pre-slaughter handling and transport practices in a short transport trial to make sure that the only major difference was the transport duration between three trials.

4.2.2.2 Trial B (7 h driving)

In trial B, birds were transported from a farm in east Alberta to a processing plant in south Alberta (550 km, 7 h driving). Trial B was conducted in July, and temperature during handling and transport was 18.1 ± 0.2°C, and relative humidity was 74.1 ± 0.3%. A livestock transport trailer was used in this trial.

Eleven birds were used in this trial: five control birds (two females, three males) and six nutrient supplement birds (two females, four males).

4.2.2.3 Trial C (18 h driving)

Birds in trial C were transported from east Alberta to east BC (1100 km, 18 h driving). The transportation was conducted in September, and temperature during handling and transport...
was 13.7 ± 0.2°C, and relative humidity was 62.8 ± 0.8%. A larger modified livestock transport trailer was used in this trial.

Twenty four birds were used in trial C: 12 control birds (six females, six males) and 12 nutrient supplement birds (six females, six males). A female control bird was dead on arrival and, and a female nutrient-supplement bird had a broken tendon on arrival. Therefore the post-transport body weight and blood samples could not be taken from these two birds. There were also two birds which were identified to be too weak for the second blood sampling and only their weights were recorded.

4.2.3 Handling and sampling practices

This research was conducted under the University of British Columbia Animal Care guidelines (Certificate A11-0110, and A12-0028). Feed and water were available to the birds ad libitum up to the timing of sampling and loading.

4.2.3.1 Density of ostriches inside trailer

There is no specific guidelines/standard in the USA or Canada regarding the density of ostriches inside a trailer. Therefore, guidelines from other countries were considered for the purpose of this research. Animal Health Australia (2012) has recommended 0.41 m² minimum space per 95 kg of ostrich, and South African Ostrich Business Chamber (2011) has recommended 0.5 m² minimum space per 80 kg of ostrich. Therefore, we considered about 0.5 m² minimum space allotted per bird in each of three trials, resulting in an almost equal number of birds for trials A and B, but about twice as many birds in Trial C since the modified trailer was also twice as large.

4.2.3.2 Sampling practices

Before and after transport, each bird was restrained by handlers who were familiar with the bird, hooded to keep the bird calm, walked to the sampling station, blood samples were taken immediately from a wing vein in each bird (10 ml blood was collected using lithium heparin tubes, BD Vacutainer), and finally the birds were weighed. The last stage of pre-transport handling included the nutrient supplementation by individual tube-feeding.
4.2.3.3 Blood sample analysis

Plasma concentrations of sodium, glucose, total protein, uric acid, creatine phosphokinase (CPK) and aspartate aminotransferase (AST) were measured by a multichannel chemistry analyser (Olympus AU 5431, Olympus, Center Valley, PA). Plasma corticosterone concentration was determined by Corticosterone ELISA kit (Enzo Life Science, Catalog No ADI-900-097).

4.2.3.4 Temperature and humidity data logger

A temperature and humidity data logger (EL-USB-2-LCD+ from DATAQ Instruments) was installed in the trailers at 1.5 m height to record ambient temperature and humidity during transport (once every five minutes).

4.2.4 Experimental design and statistical analysis

To study the effects of the pre-transport nutrient supplementation and transport duration on the physiological stress responses of ostriches, change values were calculated by deducting the pre-transport values from the post-transport values. Blood sampling and supplementation was conducted on each bird and each bird was an experimental unit in this study.

A generalized randomized block design (Kutner et al. 2005 p 906-909) was used in this study considering transport duration as a fixed-effects blocking factor (in three levels: 30-min, 7 h and 18 h), nutrient supplementation (in two levels: control and nutrient supplement) and sex of birds (in two levels: female and male) as two crossed fixed-effects factors, and with replicates (i.e. birds) of each treatment in each block. Change values for each physiological response and for weight were the response variables (Note: pre-transport body weight was not included in the model because there were no relationships between change values and the pre-transport body weight).

For each response variable, the general linear model procedure (GLM) in PASW Statistics software (PASW Statistics Grad Pack 17.0, release 17.0.2., SPSS Inc, Chicago, IL) was used to conduct the analysis of variance (ANOVA). The main effects of the transport duration, nutrient supplementation and sex, as well as all two-way and the single three-way interactions were included in the model. For all but the analysis of uric acid changes, the three-way interaction was not significant. For simplicity, it was then dropped from the model and pooled with the error, although this approach has been debated in the literature (Kutner et al. 2005 p
Model assumptions were checked prior to interpreting statistical tests and a significance level of 0.05 was used for each test. For multiple comparisons using least squares means, the significance level was divided by the number of post-hoc tests using a Bonferroni correction (Kutner et al. 2005 p 857). Standard errors are reported for each least square mean. Only significant results are reported in this chapter.

4.3 Results

4.3.1 Pre-transport (baseline) values

Pre-transport baseline values measured in this study were in reference ranges reported in other studies (Table 4-1).

4.3.2 Body weight loss

The least squares mean body weight loss of birds (here after just called means for the rest of the paper) was -5.3 ± 0.2 kg after transport. Body weight loss was affected by transport duration (F2,33 = 158.91, P < 0.001). Birds in trial C had the highest weight loss during transportation (-9.9 ± 0.3 kg) compared to birds in trials A and B. Birds in trial B had higher weight loss (-5.4 ± 0.4 kg) compared to trial A birds (-0.5 ± 0.4 kg).

There was an interaction between the nutrient supplementation and sex (F1,33) = 5.34, P = 0.03). Males which received nutrient supplement lost 1.2 kg less weight (-4.7 ± 0.4 kg) compared to the control males (-5.9 ± 0.4 kg) (F1,33 = 4.50, P = 0.04). Nutrient supplementation did not affect the weight loss in females (female control weight loss was -4.9 ± 0.5 kg, and female nutrient weight loss was -5.6 ± 0.5 kg) (F1,33) = 1.27, P = 0.27).

4.3.3 Plasma glucose concentration change

The mean post-transport plasma glucose concentrations of birds increased by 2.5 ± 0.4 mmol/L compared to their pre-transport glucose concentration. Plasma glucose change was affected by transport duration (F2,31 = 23.78, P < 0.001). Post-transport plasma glucose concentration increased in trial C birds (5.7 ± 0.5 mmol/L) more than trial A and B birds. Birds in trial B had also higher increases in their glucose concentrations (2.1 ± 0.7 mmol/L) compared to trial A birds which had a slight decrease in their plasma glucose concentrations (-0.3 ± 0.7 mmol/L).
Male control birds had higher increases in their post-transport glucose concentrations (3.5 ± 0.7 mmol/L) compared to female control birds (1.3 ± 0.8 mmol/L) ($F_{1,31} = 7.48, P = 0.01$).

**4.3.4 Plasma sodium concentration change**

The mean increase in the post-transport plasma sodium concentration of birds was 2.1 ± 0.6 mmol/L compared to their pre-transport sodium concentration. The sodium concentration change was not affected by transport duration ($F_{2,31} = 2.11, P = 0.14$).

Males which did not receive nutrient supplement (control) had higher increases in their post-transport sodium concentrations (3.4 ± 1.2 mmol/L) compared to males which received nutrient supplement (-0.29 ± 1.1 mmol/L) ($F_{1,31} = 5.24, P = 0.03$).

**4.3.5 Plasma total protein concentration change**

The mean total protein change showed a slight decrease (-0.3 ± 0.6 g/L) after transport. Transport duration affected the plasma total protein change, $F_{2,31} = 5.22, P = 0.01$. Trial A birds lost more plasma total protein after transport (-3.0 ± 1.1 g/L) compared to trial B birds (1.6 ± 1.1 g/L) and trial C birds (0.6 ± 0.8 g/L) which showed plasma total protein increases after transport (total protein changes of trial B and C birds were not significantly different).

**4.3.6 Plasma creatine phosphokinase (CPK) concentration change**

The mean post-transport CPK concentration increased by 38 266 ± 8376 IU/L compared to the pre-transport CPK concentration. Transport duration affected the CPK concentration change ($F_{2,31} = 12.03, P < 0.001$). Trial C birds had a higher increase in their CPK concentrations after transport (89 371 ± 11 235 IU/L) compared to trial B birds (23 471 ± 15 669 IU/L) and trial A birds (1955 ± 16 092 IU/L).

**4.3.7 Plasma aspartate aminotransferase (AST) concentration change**

The mean post-transport AST concentration was 371 ± 60 IU/L higher than the mean pre-transport AST concentration. The AST concentration change was affected by the transport duration ($F_{2,31} = 22.29, P < 0.001$). Trial C birds had the highest increase in their AST concentrations after transport (872 ± 80 IU/L) compared to that of trial B birds (166 ± 111 IU/L) and trial A birds (73 ± 114 IU/L).
4.3.8 Plasma uric acid concentration change

The mean plasma uric acid concentration increased after transport by 70 ± 18 μmol/L. There was a three way interaction among transport duration, nutrient supplementation and sex (F_{2,29} = 3.86, P = 0.03). Trial C control males had a higher increase in their post-transport plasma uric acid concentrations (334 ± 49 μmol/L) compared to that of trial A control males (1 ± 63 μmol/L), trial B control males (-62 ± 63 μmol/L), trial C nutrient males (168 ± 45 μmol/L), and trial C control females (136 ± 49 μmol/L). Trial C nutrient males (168 ± 45 μmol/L) and trial A nutrient males (127 ± 63 μmol/L) had higher increases in their post-transport uric acid concentrations compared to that of trial B nutrient males (-56 ± 55 μmol/L).

4.3.9 Plasma corticosterone concentration change

Post-transport corticosterone concentration was higher than the pre-transport corticosterone concentration by 3.1 ± 0.9 ng/mL. No effects of transport, nutrient supplementation, or sex were detected.

4.4 Discussion

Ostriches must be transported and slaughtered at an inspected processing plant in North America so that producers can sell the meat to the retail sector, and most ostrich farms are located far from processing plants. There is very limited information available about the effects of pre-transport nutrient supplementation and transport duration on the welfare of ostriches and their physiological stress responses. In this study, we investigated the effects of these two factors, nutrient supplementation and transport duration, on ostrich welfare and their post-transport blood biochemistry and body weight.

To provide producers with applicable results to practical farming conditions, all three trials were actual transportation by producers within Canada. The same handling protocol was applied in all three trials to minimize potential differences among trials. Temperature and humidity inside the trailer are important stress factors in livestock road transportation (Mitchell & Kettlewell 2008), and both were recorded in our three ostrich transport trials. Livestock Weather Safety Index was calculated for each trial based on NOAA National Weather Service chart (as cited in Grandin 2010c; University of Kentucky College of Agriculture nd), and three
calculated indices were less than 70 indicating that all three trials were conducted at the no-stress weather transport category. Statistical analyses were conducted on the calculated changes to minimize the effects of pre-transport body weight (which was not related to the change values) and potential differences among pre-transport measurements. While different transportation vehicles were used in the different trials, bird densities inside the trailers were the same.

Pre-transport blood biochemistry measurements in this study were compared to previous ostrich studies (Table 4-1) and all the parameters measured were within the reference ranges reported. This comparison confirmed that the experimental birds in this study were in normal physiological state.

In our study, birds which were transported for a longer period had simultaneous increases in their glucose, CPK, AST, uric acid and total protein concentrations, and these concurrent changes indicated dehydration, fasting (because of body’s tissues catabolism), or extensive trauma as reported by Krautwald-Junghanns (2007).

Plasma glucose concentrations increased after transport in the two longer transport trials (C and B). Mitchell (1999) also reported an increase in the glucose concentration for a shorter 4.5-hour transport. An increase in the plasma glucose concentration is one of the physiological stress indicators. Physiological stress results in adrenalin secretion, which then stimulates glycogen phosphorylase activity thereby providing more local fuel sources in the muscles (Reed 2009). When carbohydrate stores are depleted because of fasting or maximal activity, glycogenolysis and gluconeogenesis will occur providing glucose for the central nervous system and red blood cells (Reed 2009). Our results indicated that the longer transport duration increased the glucose concentration. This could be a sign of higher stress levels in birds which were transported for a longer period.

Control males had higher increases in plasma glucose concentrations than control females in our study. Male and female ostriches may show different behaviours: for example, males are more vigilant than females (Bertram 1980), and they stand, pace and walk more (McKeegan & Deeming 1997; Ross & Deeming 1998) compared to females that have higher frequencies of feeding and foraging (McKeegan & Deeming 1997; Ross & Deeming 1998). Therefore, during handling and transport, males are potentially more agitated and experience higher stress levels than females. Higher increases in their glucose concentration could be a sign of higher stress
levels in the control males. Males also lost more weight and they had higher uric acid and sodium concentration changes than females.

Plasma sodium concentration increased after transport in our study. Male control birds had higher increases in their post-transport sodium concentrations compared to males which received nutrient supplement. An increase in the sodium concentration of ostriches as a sign of dehydration was reported by Gray et al. (1988). Therefore, male control birds with higher sodium concentration were probably experiencing higher dehydration levels.

The longer transport trials (B and C) showed increases in total protein concentrations after transport compared to the shortest transport trial (A). Higher total protein concentration has also been reported as a sign of higher dehydration levels in animals (Krautwald-Junghanns 2007). Therefore, birds which were shipped for longer periods were probably experiencing higher dehydration levels.

CPK and AST concentrations were dramatically higher after transportation in our study; in particular, birds in the highest transport duration (trial C) had the greatest increase in their plasma enzyme concentrations (CPK and AST). When an increase in the AST concentration is concurrent with an increase in the CPK concentration, it is a sign of soft tissue damage or trauma (especially muscle damage) (Krautwald-Junghanns 2007) rather than a liver damage. Janssen et al. (1989) also reported a very high correlation between AST and CPK activations during muscle damage. The increase in the muscle enzymes in our study could be a sign of muscle injuries and similar results are reported by Janssen et al. (1989) and Nathwani et al. (2005) after extreme exercises or mechanical stress conditions. Also, Mitchell (1999) reported increased post-transport plasma CPK and AST concentrations in ostriches as a result of a change in the muscle cell membrane integrity because birds were standing during transport and had difficulties in maintaining their postural stability. Therefore, the dramatic increases in the post-transport CPK and AST concentrations in our study indicate substantial muscle damage especially in birds which were transported for 18 h.

Trial C control males (18 h driving) had higher uric acid concentration increases after transport compared to the uric acid changes of the control females and nutrient supplemented males at the same trial, and also compared to control males in the other shorter transport duration trials. Mitchell (1999) also reported an increase in the uric acid concentration of ostriches
because of handling and transport stress. An increase in the uric acid concentration may occur due to causes such as fasting (as a result of the catabolism of body tissues) or extensive trauma (Krautwald-Junghanns 2004).

In our study, birds which were transported for a longer period of time had simultaneous increases in glucose, CK, AST, uric acid and total protein concentrations, and these concurrent changes indicated dehydration, fasting (because of body’s tissues catabolism), or extensive trauma as reported by Krautwald-Junghanns (2007).

The corticosterone concentrations of the ostriches increased during transportation in our study and also in Mitchell’s (1999) ostrich transport trial. An increase in plasma corticosterone concentration is one of the physiological stress responses indicating the activation of the Hypothalamic-Pituitary-Adrenal (HPA) axis response (Selye 1978). The increase in post-transport corticosterone in our study could be a sign of birds experiencing stress as a result of transportation as most researchers have used blood glucocorticoids (e.g., cortisol in livestock and corticosterone in poultry) as indicators of physiological response to stress. However, the secretions of glucocorticoid do not happen in all stressor responses, and furthermore, they have a circadian rhythm in several species (Möstl & Palme 2002). There is also considerable variation in the glucocorticoids concentrations in plasma; these secretions are dependent on time of sampling, duration of exposure to stress factors, previous experience of animals, season, temperature and genetics (Dantzer & Mormède 1983; Grandin 1997; Mounier et al. 2006). Collectively, these are likely the underlying reasons that no effects of transport duration and nutrient supplementation on corticosterone changes were found in our study. As Moberg (1987) suggested, glucocorticoids should not be used as the only indicators of stress; other physiological stress indicators (e.g., glucose, CPK, AST, and weight loss) should also be considered in assessing livestock welfare.

One of the main factors affecting both bird welfare and economic sustainability of farms is the weight loss of birds during handling and transportation. It has been well-established that, in many livestock species, the longer the transportation time, the more severe is the weight loss (Jones et al. 1988; Fisher et al. 2010). Our study results were consistent with this expectation. As with our results, Hoffman et al. (2012) reported that the 5-hr transport duration in their study caused greater weight losses in ostriches than the 1-hour transport duration.
Nutrient supplementation was used in this ostrich transport study to investigate possible effects on the physiological stress responses. Nutrient supplementation decreased the weight loss of ostriches in male birds but no difference was noted for females. Schaefer et al. (1997b) reported that feed/water withdrawal increased weight loss due to transportation stress in cattle. They showed that a considerable proportion of weight loss was from carcass components rather than from the gastrointestinal tract content which many had considered the main source of the transport weight loss. Providing small quantities of essential nutrients pre- or post-transport may reduce dehydration of animals, improve their rehydration, reduce muscle protein catabolism, reduce depletion of glycogen from muscles, and improve product quality and the wellbeing of animals (Schaefer et al. 2001).

Schaefer et al. (1995, 1997a) provided an electrolyte supplement for ostriches in two short transport trials (3 km). They found that a form of solid pre-transport electrolyte supplement decreased the weight loss of ostriches (Schaefer et al. 1997a), but a post-transport liquid electrolyte supplement did not significantly affect the weight loss of ostriches (Schaefer et al. 1995). In our study, control males had higher weight losses compared to the nutrient-supplemented males indicating potential positive effects of nutrient supplementation. However, further research is needed regarding the best nutrient supplement formula for ostrich handling and transportation practices based on body requirements.

In addition to the weight loss, one of the other important factors which directly affects the welfare of birds and the economic viability of the farms is the deaths on arrival after transport. Birds in Trials A and B did not have any mortality or any signs of post-transport physical injuries whereas one bird (female bird from the control treatment group) died in Trial C. Another bird from Trial C (a female bird from the nutrient supplement treatment group) had a broken tendon on arrival. Warriss (2010a) reported that a longer journey time increased the incidence of death-on-arrival rates. Therefore, longer transportation could be detrimental for ostriches and farm economic sustainability.

4.5 Conclusion and animal welfare implications

In conclusion, longer transportation duration significantly increased body weight loss of ostriches, their plasma CPK and AST enzyme concentrations, and their plasma glucose and total
protein concentrations in this study. Nutrient supplementation decreased the weight loss of male ostriches.

We conclude that, under the present shipping conditions, long distance transportation is detrimental to ostrich welfare with significant loss to producers due to mortalities and shrinkage. Further, the use of pre-transport nutrient supplementation can partially alleviate the effect of the transportation stress.
Table 4-1 Pre-transport (baseline) values measured in this study and blood biochemistry reference ranges reported in other studies.

<table>
<thead>
<tr>
<th>Plasma concentration</th>
<th>Pre-transport value (Mean ± SE)</th>
<th>Reference value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>11.6 ± 0.4</td>
<td>10.3–13.7</td>
<td>Verstappen et al. (2002)</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>143.9 ± 0.5</td>
<td>113-181</td>
<td>Krautwald-Junghanns (2007)</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>40.9 ± 0.9</td>
<td>39-56</td>
<td>Verstappen et al. (2002)</td>
</tr>
<tr>
<td>Creatine phosphokinase (CPK) (IU/L)</td>
<td>3450 ± 328</td>
<td>1,648-4,894</td>
<td>Verstappen et al. (2002)</td>
</tr>
<tr>
<td>Aspartate aminotransferase (AST) (IU/L)</td>
<td>294 ± 10</td>
<td>243–418</td>
<td>Verstappen et al. (2002)</td>
</tr>
<tr>
<td>Uric acid (μmol/L)</td>
<td>362 ± 12</td>
<td>351 – 649</td>
<td>Verstappen et al. (2002)</td>
</tr>
<tr>
<td>Corticosterone (ng/mL)</td>
<td>5.2 ± 0.6</td>
<td>4.9 ± 2.9</td>
<td>Mitchell (1999)</td>
</tr>
</tbody>
</table>
Figure 4-1 Effect of transport duration on the weight loss of ostriches in three trials
Figure 4-2 Effects of the sex and nutrient supplementation interaction on the weight loss of ostriches.
Figure 4-3 Effect of transport duration on plasma glucose concentrations of ostriches
Figure 4-4 Pre- and post-transport plasma creatine phosphokinase (CPK) concentrations in the three transport trials

F_{2,33} = 12
P < 0.001
Figure 4-5 Pre- and post-transport plasma aspartate aminotransferase (AST) concentrations in the three transport trials
5 General discussion and recommendations

5.1 Introduction

Ostrich production is a relatively young industry and little research has been done to examine ostrich welfare during pre-transport handling and during transportation. In Canada and USA, producers must transport and slaughter their birds in a registered processing plant to be able to sell the meat to the retail sector. The main goal of this research was to gather information on how to alter pre-slaughter and transport practices to improve ostrich welfare and to minimize deterioration of product quality. I proposed and developed the holistic research model (Figure 1-1) to fulfilling the objectives of the research.

5.2 Limitations and strengths of the dissertation research

In this dissertation, I provide a substantial amount of information about current ostrich handling and transport practices in Canada and USA. This is the first study reporting the effects of the long transportation on ostrich welfare. Potential transport welfare issues in the current production system were identified, transport welfare guidelines from other countries were reviewed to find applicable guidelines for the North American ostrich industry to remedy these issues, research studies were carried out to find solutions for remaining issues, and the information gathered will be provided to policy making bodies (e.g., National Farm Animal Care Council of Canada) to develop the Codes of Practice for ostrich transportation in Canada and USA.

The limitations of the study were:

- Very little research is conducted on ostrich welfare during handling and transportation, and novelty of this study was one of the main challenges of the project. However, in addition to the ratite welfare studies, results of the related studies on other livestock handling and transport were also considered in discussing my findings.
• Even though there was no replication for the transport duration trials, the use of mixed models and advanced statistical analyses allowed me to obtain valid and reliable results (Schank and Koehnle, 2009).
• The other limitation of the study was the availability of crossbred ostriches for transportation trials. However, this was beneficial in conducting research according to Canadian and USA ostrich farming conditions because all farms in Canada and USA raise crossbred ostriches.
• I conducted all three trials based on real ostrich handling and transportation practices in ostrich farms in Canada. However, I tried to provide similar condition in all three trials by controlling most of the manageable stressors.

5.2.1 Producer survey and transport guidelines

Prior to this study there was no published information about the current ostrich handling and transport practices in Canada or USA, and the potential welfare issues related to the current practices were unknown. Furthermore, Canada and USA have no specific ratite transport guidelines. The objectives of my research in Chapter 2 of this dissertation were: 1) to identify current ostrich pre-slaughter handling and transport practices in Canada and USA through a survey of ostrich producers; 2) to identify potential welfare issues in current ostrich handling and transport practices; and 3) to review ostrich transport standards and guidelines of Australia, European Union, New Zealand and South Africa to investigate if those guidelines are applicable to Canadian and USA ostrich farming systems.

The number of respondents was small because of the small size of the industry, but the survey was well received with a very high response rate from members of the ostrich associations in USA and Canada.

Appropriate management of an ostrich’s exposure to stressors during pre-slaughter handling and transport practices can improve its wellbeing and its product quality. Results of the producer survey revealed that transport duration is longer in Canada and USA compared to other countries because farms are located further from the processing plants. Further, there is no specific handling and transport guidelines which can be followed by producers.
Based on the results of the producer survey and the review of the transport standards and guidelines, I conclude that the following are potential ostrich handling and transport welfare issues in Canada and USA:

- Lack of scientific information about welfare of ostriches during transport;
- Handlers’ unfamiliarity with handling and transport practices that might promote bird welfare;
- Lack of consideration of social bonds, sexes, behaviours and physical states of birds in mixing them during the handling and transport process;
- Lack of an established maximum water and feed withdrawal duration standard for ostrich transport;
- Lack of a specific vehicle designed for ratite transportation in Canada and USA considering different physical body characteristics of ostriches compared to other species; and
- Exposure of birds to natural light during transport, overcrowding, and long transportation in Canada and USA.

To improve the welfare of the birds during transport and to decrease losses, more research is required about each one of the identified potential welfare problems related to ostrich handling and transport, and the identified issues should be considered when developing the Codes of Practice for ratite transport in Canada and USA.

5.2.2 Effects of pre-transport handling process

Based on the identified potential welfare issues from the producers’ survey and literature review, I identified the pre-slaughter handling practices of ostriches as one of the major stress factors which affect ostrich welfare during the pre-slaughter transport process and alter the product quality. Therefore, I investigated the effects of pre-transport handling stress on the behavioural and physiological stress responses of ostriches.

Analysis of pre-transport blood indicated that birds which experienced higher pre-transport handling stress had higher pre-transport plasma concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and sodium, and higher packed cell volume. An immobile sitting behaviour was observed in five out of the 11 birds that experienced
the longest pre-holding time. This immobile sitting behaviour was positively correlated with higher pre-transport handling stress, higher post-transport AST, ALT, creatine phosphokinase (CPK) and glucose concentrations and transport losses. I conclude that minimizing pre-transport handling stress will improve welfare of ostriches and will lessen product losses. Further, because of the results of physiological stress indicators, the displayed immobile sitting behaviour could be considered as one of the behavioural fear indicators in ostriches. Consequently, I concluded that immobile sitting behaviour could be used to identify the fearful birds during handling practices and to alter practices to minimize losses.

This study was conducted based on the current routine industry handling and transport condition in Canada to identify potential welfare issues. Higher stress levels of the birds and higher injuries also demonstrate the need for development of a Codes of Practice for the transportation of ratites in Canada because ostrich transportation problems may be different from the transportation problems of other species because of specific features of the ostrich anatomy.

5.2.3 Effects of nutrient supplementation and transport duration

From the producer survey, I identified that pre-transport feed/water withdrawal and long transport duration as current practices that may affect ostrich welfare in Canada and USA. I carried out transport trials to investigate the effects of pre-transport nutrient supplementation and transport duration on ostrich welfare and their physiological stress responses.

Results of this study indicated that birds which were shipped for 18 h had the most body weight loss, and 7-hour transported birds had more body weight loss compared to 30-minute transported birds. Birds which were transported for a longer period of time also had higher post-transport concentrations of plasma glucose, creatine phosphokinase, aspartate aminotransferase, total protein and uric acid. Control males lost more weight compared to the nutrient-supplemented males, and they had higher increases in their post-transport sodium and glucose concentrations. I concluded that, under the present shipping conditions, long distance transportation is detrimental to ostrich welfare with significant loss to producers due to mortality and shrinkage, and the use of pre-transport nutrient supplementation can partially alleviate the effect of the transportation stress.
To provide producers with applicable results to practical farm conditions, all three trials were actual transportation by producers within Canada. The same handling protocol was applied in all three trials to minimize potential differences among trials.

5.3 Conclusion

The developed research model (Figure 1-1) was successfully implemented to gather valuable information to improve the transport welfare of ostriches. By conducting this research, I could identify the current norms of ostrich handling and transport in Canada and USA, and could identify the potential welfare issues of the current ostrich transport practices. I could also investigate the effects of pre-transport handling, nutrient supplementation and transport duration on the stress responses of ostriches. By reviewing the ratite transport welfare standards and guidelines from Australia, European Union, New Zealand and South Africa and considering the potential welfare issues related to the current ostrich practices in North America, I provided applicable information which could be used in the development of Codes of Practice for ratite transport in Canada and USA.

5.4 Possible future research directions

The producer survey conducted for the purposes of this thesis identified many research questions which could be answered by conducting scientific research to improve ostrich handling and transport welfare and product quality in North America. Some of the identified questions (effects of the handling stress, validated behavioural response, nutrient supplementation and transport duration) were addressed in this dissertation, but there is need for additional research to investigate effects of the following potential welfare issues:

- Comparison of different ostrich handling methods on ostrich stress response;
- Effects of familiarizing birds from early production cycle with proper handling practices on their pre-slaughter handling and transport stress responses;
- Effects of transport vehicle design and adequate density inside the trailer on ostrich welfare; and
- Effects of rest stops and feed and water withdrawal duration on ostrich welfare.
In addition to the presented information in this dissertation, I have investigated the effects of the pre-transport nutrient supplementation and transport duration on the quality of the ostrich meat based on the results of the conducted transport trials. The results of this further research will be presented in a separate research paper.

The other important issue that should be considered in future research projects is consumer perception. I included this in my research model (Figure 1-1) because of consumers’ major role in demanding changes in animal farming practices to improve welfare. To complete the cycle expressed in the research model, market research is required to identify consumers’ perceptions regarding current ostrich farming, handling and transport practices and their willingness to purchase ostrich products by improving the welfare of the birds, and developing and applying Codes of Practice for ratite handling and transport in Canada and USA.
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Appendix

Producer survey

Introduction letter and consent

Dear Ostrich Producer/Processor,

You are cordially invited to participate in a survey about ostrich production and processing. In this survey, you, as ostrich experts, are being asked to complete a survey which contains questions about farming, handling, shipment and processing of ostriches to explore efficient ways of ostrich pre-slaughter handling/transport process. It will take approximately 20 – 40 minutes to complete the questionnaire depending on the sections you answer. Please return your survey by December 15, 2011.

The data from this survey will be used as part of a Doctoral dissertation to be completed at The University of British Columbia. An electronic copy of the dissertation will be available in 'cIRcle: UBC's Institutional Repository' upon completion (https://circle.ubc.ca/).

Please note the following:

- You have been identified as an ostrich farming/processing expert, and your contact information located during a web-search, from yellow pages or professional website (e.g., association website).
- Your participation in this study is on a voluntary basis. There are no foreseeable risks associated with this project. However, if you feel uncomfortable answering any of the questions, you can skip that question or withdraw from the survey at any point.
- Your survey responses will be confidential and data from this research will be reported only in the aggregate and for academic research purposes only. Your information will be coded for documentation purposes only and will remain confidential.
- We most welcome participation of ostrich industry experts (farmers, handlers and processors) in our survey. Please feel free to provide your colleagues with this invitation letter, the online address of the survey
(http://app.fluids surveys.com/surveys/ostrich/expert/) or our contact information, so that we can send the invitation email or the paper version of the survey upon their request.

- If the questionnaire is submitted, it will be assumed that consent has been given.

Please keep a copy of this consent for your records.

Your participation in this survey is greatly appreciated. If you have questions at any time about the survey, you may contact Dr. Kimberly Cheng at +1-604-822-2480 (kmtc@mail.ubc.ca) or Masoumeh Bejaei at +1-604-822-3959 (mbejaei@interchange.ubc.ca) in the Avian Research Centre at the University of British Columbia (2357, Main Mall, Vancouver, British Columbia, Canada, V6T1Z4).

Please start the survey on the next page, or go to the following link to start the survey online:

http://app.fluids surveys.com/surveys/ostrich/expert/
Section A: General Questions

A.1. In which country and in which state/province is your facility located?
(Please write in the provided spaces.)

<table>
<thead>
<tr>
<th>Country</th>
<th>State/Province</th>
</tr>
</thead>
</table>

A.2. For how many years have you been involved in ostrich production, shipping or processing?
(Please write the number in the provided space.)

A.3. In your opinion what are the potential strengths of the ostrich industry?
(Please choose top four factors.)

- Generates higher income compared to other livestock products
- Product demand exceeds supply
- Production is more environmentally friendly than other livestock production
- Ostrich leather has high quality and unique characteristics/pattern
- Higher protein and lower fat/cholesterol levels of ostrich meat
- Good feed conversion ratio of ostriches
- Other, please specify:________________________________________

A.4. In your opinion, what are the major weaknesses of the ostrich industry?
(Please choose top four factors.)

- Lack of strong co-operation among producers
- Lack of government support
- Safety problems related to working with ostriches
- Export limitations
- Competition with a strong beef/red meat market
- Lack of research support
- Variation in product quality in different farms
- Exotic nature of ostrich products
- Other, please specify:________________________________________

A.5. You are: □ Male □ Female

A.6. Which of the following age group do you fall into?
(Please select one of the following).

- 20 yrs. or younger
- 21 – 30 yrs.
- 31 – 40 yrs.
- 41 – 50 yrs.
- 51 – 60 yrs.
- 61 – 70 yrs.
- 71 yrs. or older
A.7. The highest level of your formal education is:
(Please select one of the following.)
- □ No formal education
- □ Completed junior college/trade school/technical school
- □ Some high school
- □ Completed Bachelors degree
- □ Completed high school
- □ Graduate degree (Masters, PhD or equivalent)
- □ Some college/technical school
- □ Other, please specify: _______________________

A.8. What is the minimum and maximum outside temperature that ostriches may experience at your facility in a year?
(Please write in the provided spaces.)

<table>
<thead>
<tr>
<th>Minimum temperature</th>
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<td></td>
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<table>
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<tr>
<th>Maximum temperature</th>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A.9. Please select statements that best describe your activities in the ostrich industry.
(Please select all that apply.)
- □ I am an ostrich farmer.
- □ I ship ostriches (or I am involved in ostrich shipment).
- □ I process ostriches (or I am involved in ostrich slaughtering).

Please continue the survey by selecting the starting section for the rest of the survey based on your involvement in the ostrich industry.

<table>
<thead>
<tr>
<th>I am active in ostrich ...</th>
<th>Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production</td>
<td>B</td>
</tr>
<tr>
<td>Shipping</td>
<td>C</td>
</tr>
<tr>
<td>Processing</td>
<td>D</td>
</tr>
</tbody>
</table>

If you are involved in only one of the above activities, please answer the questions in the section associated with that activity.

If you are involved in two activities, please answer the questions associated with those two activities (for example, if you are involved in shipping and processing please answer questions in both sections C and D).

If you are involved in all three activities, please answer the questions associated with all three activities (sections B, C and D).
Section B: Ostrich Production

B.1. Which production system do you primarily use for market ostrich farming? (Market ostriches are the birds that you raise for their meat, skin or fat products.)
(Please select one of the following.)
- □ Extensive system (birds are totally dependent on natural or cultivated pasture)
- □ Semi-intensive system (birds graze on pasture and receive a feed concentrate as supplement)
- □ Intensive system (birds receive a full balanced feed)
- □ Other, please specify: ____________________________

B.2. On your farm, do you usually keep the newly hatched chicks with breeders?
(Please select one of the following.)
- □ Never          □ Rarely          □ About half the time            □ Often          □ Always

B.3. On average, what is the mortality rate of the ostrich chicks (up to 3 months of age) at your farm?
(Please write the percentage in the provided space.)

B.4. What is the neck color of the adult ostriches on your farm?
(Please select all that apply.)
- □ Black               □ Blue-neck               □ Red-neck
- □ Other, please specify: ____________________________

B.5. How many ostriches from each age category did you have on your farm as of September 30, 2011?
(Please write the number in the provided space.)

<table>
<thead>
<tr>
<th>Age Category</th>
<th>Number of birds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicks (up to 3 months of age)</td>
<td></td>
</tr>
<tr>
<td>Juveniles (3 to 6 months of age)</td>
<td></td>
</tr>
<tr>
<td>Yearlings (6 to 18 months of age)</td>
<td></td>
</tr>
<tr>
<td>Female breeders (above 18 months of age)</td>
<td></td>
</tr>
<tr>
<td>Male breeders (above 18 months of age)</td>
<td></td>
</tr>
</tbody>
</table>
### B.6. What method do you use to identify non-breeder ostriches at your farm? (Please select all that apply.)
- □ Color on the back
- □ Color on the neck
- □ Color on the leg
- □ Color/number band on the leg
- □ Other, please specify: ________________________________
- □ Tags on the neck
- □ Tags on the wing
- □ DNA marker
- □ Microchip

### B.7a. Do you have the ostriches on your farm declawed?  □ Yes □ No

### B.7b. If yes, which method was used to declaw the ostriches at your farm? (Please select all that apply.)
- □ Declawing using a heated blade
- □ The microwave claw treatment process
- □ Other, please specify: ________________________________

### B.8. How often do you weigh the ostriches that are 18 months old or less at your farm? (Please select one of the following.)
- □ Never □ Annually □ biannually □ Seasonally □ Monthly or more

### B.9. Do you do the following activities in your ostrich farming? (Please select one of the provided options for each activity.)

<table>
<thead>
<tr>
<th>Activity</th>
<th>Never</th>
<th>Rarely</th>
<th>Sometimes</th>
<th>Often</th>
<th>Always</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observe your ostriches</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Speak to your ostriches</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Touch your ostriches</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Name your ostriches</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
</tbody>
</table>

### B.10. On average, how many market ostriches do you rear per one pen on your farm? (Please write the number in the provided space).

_________Number of market ostriches per pen
### B.11. On average, what is the covered area of one pen used for market ostriches on your farm?
(Please write the number in one of the provided spaces).

<table>
<thead>
<tr>
<th>_________ Square feet</th>
<th>or</th>
<th>_________ Square meter</th>
</tr>
</thead>
</table>

### B.12. On average, what is the paddock area of one pen used for market ostriches on your farm?
(Please write the number in one of the provided spaces).

<table>
<thead>
<tr>
<th>_________ Square feet</th>
<th>or</th>
<th>_________ Square meter</th>
</tr>
</thead>
</table>

### B.13. On average, what percentage of your market ostriches can access feed troughs in a pen at the same time?
(Please select **one** of the following.)

- □ 1 – 10 %
- □ 11 – 20 %
- □ 21 – 30 %
- □ 31 – 40 %
- □ 41 – 50 %
- □ 51 – 60 %
- □ 61 – 70 %
- □ 71 – 80 %
- □ 81 – 90 %
- □ 91 – 100 %

### B.14. On average, what percentage of your market ostriches can access water troughs in a pen at the same time?
(Please select **one** of the following.)

- □ 1 – 10 %
- □ 11 – 20 %
- □ 21 – 30 %
- □ 31 – 40 %
- □ 41 – 50 %
- □ 51 – 60 %
- □ 61 – 70 %
- □ 71 – 80 %
- □ 81 – 90 %
- □ 91 – 100 %

### B.15. Which form of the feed do you provide for the ostriches at your farm?
(Please select all that apply.)

- □ Chopped
- □ Crumbles
- □ Mash
- □ Pellets
- □ Other, please specify: ____________________________________________

### B.16. In the case you provide concentrate feed (crumble, mash or pellet forms) to ostriches at your farm, what are the main **ingredients** of the **concentrate feed**?
(Please select all that apply.)

- □ Alfalfa/Lucerne
- □ Barley
- □ Clover
- □ Corn
- □ Fishmeal
- □ Oils
- □ Soybean meals
- □ Vitamins and minerals premix
- □ Wheat
- □ Other, please specify: ____________________________________________
- □ I do not know.
- □ I do not use concentrate feed.
B.17. On average, how much concentrate do market ostriches consume per day on your farm? (Please write the number in one of the provided spaces.)

<table>
<thead>
<tr>
<th>Amount</th>
<th>Pounds</th>
<th>Kilograms</th>
</tr>
</thead>
<tbody>
<tr>
<td>__________________________</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

☐ I do not feed concentrate.

B.18. Do you provide **bedding material** for ostriches at your farm? (Please select one of the following.)

- ☐ Never
- ☐ Rarely
- ☐ About half the time
- ☐ Often
- ☐ Always

☐ I do not feed concentrate.

B.19. Does the change of feed type put your ostriches off-feed? (Please select one of the following.)

- ☐ Never
- ☐ Rarely
- ☐ About half the time
- ☐ Often
- ☐ Always

☐ I do not feed concentrate.

B.20. **How often** are the market ostriches fed on your farm? (Please select one of the following.)

- ☐ Once a day
- ☐ Four times per day or more (not continuous access)
- ☐ Two times per day
- ☐ *Ad libitum* (continued access)
- ☐ Three times per day

B.21. **How often** do the market ostriches at your farm have access to water? (Please select one of the following.)

- ☐ Once a day
- ☐ Four times per day or more (not continuous access)
- ☐ Two times per day
- ☐ *Ad libitum* (continued access)
- ☐ Three times per day

B.22. On average, what is the age of your ostriches that are sent to market for their meat, fat or skin products? (Please write the number in the provided space.)

<table>
<thead>
<tr>
<th>Age in Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>____________</td>
</tr>
</tbody>
</table>

B.23. On average, what is the weight of your ostriches that are sent to market for their meat, fat or skin products? (Please write the number in one of the provided spaces.)

<table>
<thead>
<tr>
<th>Weight in Pounds</th>
<th>Weight in Kilograms</th>
</tr>
</thead>
<tbody>
<tr>
<td>_______________</td>
<td>_______________</td>
</tr>
</tbody>
</table>
B.24a. If you do on-farm slaughtering, how many hours before slaughtering are the ostriches kept off-feed?
(Please write the number in the provided space.) __________ Hours

□ No on-farm slaughtering

B.24b. If you ship your ostriches, how many hours before shipping are the ostriches kept off-feed?
(Please write the number in the provided space.) __________ Hours

□ No shipment

B.25a. If you do on-farm slaughtering, how many hours before slaughtering are ostriches kept off-water?
(Please write the number in the provided space.) __________ Hours

□ No on-farm slaughtering

B.25b. If you ship your ostriches, how many hours before shipping are ostriches kept off-water?
(Please write the number in the provided space.) __________ Hours

□ No shipment

B.26. Do you consider the following factors when you allot ostriches to the holding pens before slaughtering/shipment?
(Please select one of the provided options for each factor.)

<table>
<thead>
<tr>
<th>Age of the birds</th>
<th>□ Never</th>
<th>□ Rarely</th>
<th>□ Sometimes</th>
<th>□ Often</th>
<th>□ Always</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of the birds</td>
<td>□ Never</td>
<td>□ Rarely</td>
<td>□ Sometimes</td>
<td>□ Often</td>
<td>□ Always</td>
</tr>
<tr>
<td>Sex of the birds</td>
<td>□ Never</td>
<td>□ Rarely</td>
<td>□ Sometimes</td>
<td>□ Often</td>
<td>□ Always</td>
</tr>
<tr>
<td>Familiarity of the birds with each other</td>
<td>□ Never</td>
<td>□ Rarely</td>
<td>□ Sometimes</td>
<td>□ Often</td>
<td>□ Always</td>
</tr>
<tr>
<td>Size of the pen</td>
<td>□ Never</td>
<td>□ Rarely</td>
<td>□ Sometimes</td>
<td>□ Often</td>
<td>□ Always</td>
</tr>
</tbody>
</table>

□ I do not put ostriches in holding pens before slaughtering/shipment.
### B.27. What **behavioural changes** have you noticed in ostriches on your farm during the pre-slaughter/transport handling process?

(Please select all that apply.)

- □ Attacking
- □ Climbing on top of each other
- □ Compulsive repetitive movements
- □ Fearfulness
- □ Fighting
- □ Feather pecking
- □ Kicking
- □ Running
- □ Sitting
- □ Stop feeding
- □ Stop drinking
- □ Trampling
- □ Vocalization
- □ Other, please specify: ___________________________________________
- □ No change in their behaviour

### B.28. What method do you use to **restrain** ostriches to start pre-shipping/pre-slaughter process?

(Please select all that apply.)

- □ Hooding
- □ Using a hook/crook
- □ Using no device
- □ Prod
- □ Tranquillizer
- □ Other, please specify: ___________________________________________

### B.29. On average, what **percentage** of ostriches on your farm **slide or fall** while being rounded up for loading on to a truck/trailer (before they leave the farm)?

(Please write the percentage in the provided space.)

___________Percent

### B.30. On average, what **percentage** of ostriches are **injured** (or show signs of bruises, bleeding or cuts) while being loaded on to a truck/trailer (before they leave the farm)?

(Please write the percentage in the provided space.)

___________Percent

### B.31. What type of pre-shipping/pre-slaughter **feed supplement** do you provide to your ostriches?

(Please select all that apply.)

- □ Minerals and vitamins premix
- □ Sugar solution
- □ Amino acids mixed in water
- □ Special pre-slaughter mixed concentrate
- □ Other, please specify: ___________________________________________
- □ I do not use any pre-slaughter supplement.
B.32. In your opinion, what are the **major stress factors** for ostriches during the pre-slaughter process?  
(Please choose **top four** factors.)

- □ Contact with humans  
- □ Capturing the bird  
- □ Feed withdrawal  
- □ Water withdrawal  
- □ Hooding  
- □ Mixing unfamiliar ostriches  
- □ New environment  
- □ Loading in a truck  
- □ Restraining  
- □ Any changes in their feed  
- □ Separation from familiar ostriches  
- □ Unfamiliar handlers  
- □ Feed withdrawal  
- □ Water withdrawal  
- □ Restraining  
- □ Any changes in their feed  
- □ Separation from familiar ostriches  
- □ Unfamiliar handlers  
- □ New environment  
- □ Loading in a truck  

☐ Other, please specify: ____________________________________________

B.33. **Where** do you **slaughter** your ostriches?  
(Please select all that apply.)

- □ My own farm  
- □ Another farm  
- □ Ostrich abattoir  
- □ Red-meat abattoir  
- □ Multi-species abattoir  
- □ Mobile abattoir  
- □ Other, please specify: ____________________________________________

B.34. **How far is** your farm from the closest **abattoir** where you could slaughter your ostriches?  
(Please write the number in one of the provided spaces.)  
__________ Miles or _______ Kilometres

B.35. What percentage of your **farm net income** is from ostrich farming?  
(Please select one of the following.)

- □ 1 – 20 %  
- □ 21 – 40 %  
- □ 41 – 60 %  
- □ 61 – 80 %  
- □ 81 – 100 %

B.36. What percentage of your farm net income comes from each one of the following ostrich products?  
(Please write the percentages in the provided spaces.)

<table>
<thead>
<tr>
<th>Product</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat</td>
<td>%</td>
</tr>
<tr>
<td>Skin</td>
<td>%</td>
</tr>
<tr>
<td>Fat</td>
<td>%</td>
</tr>
<tr>
<td>Feather</td>
<td>%</td>
</tr>
<tr>
<td>Fertile eggs</td>
<td>%</td>
</tr>
<tr>
<td>Infertile eggs</td>
<td>%</td>
</tr>
<tr>
<td>Breeders</td>
<td>%</td>
</tr>
<tr>
<td>Live chicks</td>
<td>%</td>
</tr>
</tbody>
</table>

☐ Other, please specify: ____________________________________________
B.37. How do you market your ostrich products?

(Please select all that apply.)

☐ Direct sale to wholesalers  ☐ Online Sale
☐ Direct sale to retailers  ☐ Sale to pet food companies
☐ Farm gate sale  ☐ Selling meat to hotels/restaurants
☐ Farm market sale
☐ Other, please specify: ________________________________________________

Please let us know if you have any comments or suggestions about the ostrich farming questions.

If you ship your ostriches or are involved in the shipping of birds, please go to Section C (shipping).

If you slaughter ostriches but you are not involved in the shipping of birds, please go to Section D (Processing).
**Section C: Shipping Ostriches**

**C.1.** Which **type of vehicle** do you use to ship ostriches?
(Please select all that apply.)

- □ Ostrich trailer
- □ Horse trailer
- □ Cattle transport truck
- □ Other trailer
- □ Other, please specify: __________________________________________________________

**C.2.** Is the vehicle being used for ostrich shipping an **open top vehicle**?

- □ Yes
- □ No

**C.3.** What is the **height of the compartment** of the vehicle used for shipping ostriches?
(Please write the number in one of the provided spaces.)

_________ Feet  or  _______ Meters

**C.4.** What is the **total area of the shipping compartment** in the vehicle?
(Please write the number in one of the provided spaces.)

___________ Square feet  or  _________ Square meters

**C.5.** How many **partitions** does the **vehicle** have in the compartment?
(Please write the number in the provided space.)

_______ Number of

**C.6.** What is the source of **lighting** inside the compartment?
(Please select one of the following.)

- □ No light (dark)  □ Artificial light
- □ Natural light  □ Both natural and artificial light

**C.7.** Is there a **ventilation** system installed in the vehicle compartment?

- □ Yes
- □ No

**C.8.** Do you provide **bedding material** for **ostriches inside the compartment**?
(Please select **one** of the following.)

- □ Never  □ Rarely  □ About half the time  □ Often  □ Always
C.9. On average, **how many** market ostriches do you ship **per vehicle**?
(Please write the number in the provided space.)

__________ Number of ostriches

C.10. On **average, how long** does it take to **load** one bird onto the vehicle (including the time to round up the bird)?
(Please write the number in the provided space.)

__________ Minutes

C.11. Do you consider the following factors when you allot ostriches to the same compartment/partition?
(Please select one of the provided options for each factor.)

<table>
<thead>
<tr>
<th>Age of the birds</th>
<th>□ Never</th>
<th>□ Rarely</th>
<th>□ Sometimes</th>
<th>□ Often</th>
<th>□ Always</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of the birds</td>
<td>□ Never</td>
<td>□ Rarely</td>
<td>□ Sometimes</td>
<td>□ Often</td>
<td>□ Always</td>
</tr>
<tr>
<td>Sex of the birds</td>
<td>□ Never</td>
<td>□ Rarely</td>
<td>□ Sometimes</td>
<td>□ Often</td>
<td>□ Always</td>
</tr>
<tr>
<td>Familiarity of the birds with each other</td>
<td>□ Never</td>
<td>□ Rarely</td>
<td>□ Sometimes</td>
<td>□ Often</td>
<td>□ Always</td>
</tr>
<tr>
<td>Size of the compartment</td>
<td>□ Never</td>
<td>□ Rarely</td>
<td>□ Sometimes</td>
<td>□ Often</td>
<td>□ Always</td>
</tr>
</tbody>
</table>

□

C.12. At what time of the day do you usually **start transporting** ostriches?
(Please select one of the following.)

□ 12 am – 3 am □ 6 am - 9 am □ 12 pm – 3 pm □ 6 pm - 9 pm
□ 3 am - 6 am □ 9 am - 12 pm □ 3 pm - 6 pm □ 9 pm - 12 am

C.13. On average, what are the **minimum and maximum hours** you spend per one shipment of ostriches?
(Please write the numbers in the provided spaces.)

<table>
<thead>
<tr>
<th>Average minimum hours</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Average maximum hours</td>
<td></td>
</tr>
</tbody>
</table>
### C.14. On average, how many times do you stop the vehicle when shipping ostriches?

(Please write the number in the provided space.)

_______ Number of stops

### C.15. Which type of roads do you have to use when shipping ostriches?

(Please select all that apply.)

- □ Mostly asphalt highways
- □ Mostly paved roads
- □ Mostly gravel road
- □ Various types of roads
- □ Other, please specify: _____________________________

### C.16. How frequently do you (or a handler) monitor the status of the ostriches during shipping?

(Please select one of the following.)

- □ Once during every hour of drive
- □ Once during every 2 hours of drive
- □ Once during every 3 hours of drive
- □ Once during every 4 hours of drive
- □ Once during every 5 hours of drive
- □ Once during every 6 hours of drive
- □ Once during every 7 hours of drive
- □ Once during every 8 hours of drive
- □ Other, please specify: _____________________________
- □ I do not monitor the status of the ostriches during shipping.

### C.17. Do the ostriches you ship have access to feed and water in the vehicle compartment during shipping?

(Please select one of the following.)

- □ None
- □ Only feed
- □ Only water
- □ Both

### C.18. On average, what percentage of ostriches that you ship do not survive the drive?

(Please write the percentage in the provided space.)

_______ Percent

### C.19. On average, what percentage of ostriches that you ship are injured (or show signs of bruises, bleeding or cuts) during the drive?

(Please write the percentage in the provided space.)

_______ Percent

### C.20. On average, what percentage of shrinkage do ostriches (that you ship) have when being shipped?

(Please write the percentage in the provided space.)

_______ Percent
C.21. Have you noticed any **behavioural changes** in the ostriches **during** your **shipping** of ostriches? (Please select all that apply.)

- □ Attacking
- □ Climbing on top of each other
- □ Compulsive repetitive movements
- □ Fearfulness
- □ Fighting
- □ Feather pecking
- □ Kicking
- □ Sitting
- □ Trampling
- □ Vocalization
- □ Other, please specify: ________________________________
- □ No change in their behaviour

C.22. On average, **how long** do the ostriches that you ship **stay in the vehicle after arrival** at the destination? (Please write the **time** in the provided space.)

- _______Hours and _______Minutes

C.23. **On average, how long** does it take to **off-load one ostrich** at the destination from the vehicle that you used to ship the ostriches? (Please write the time in minutes in the provided space.)

- _______Minutes

C.24. **On average, what percentage** of ostriches **slip or fall during unloading**? (Please write the percentage in the provided space.)

- _______%

C.25. How often have you noticed any **thick white concentrated urine** inside the shipping compartment? (Please select **one** of the following.)

- □ Never
- □ Rarely
- □ Sometimes
- □ Often
- □ Always

Please let us know if you have any comments or suggestions about the ostrich shipment questions.

If you slaughter ostriches, please go to **Section D (Processing)**.
### Section D: Ostrich Processing

**D.1. Do you consider the following **factors** when you **allot ostriches to the same pre-slaughter holding pen**?**

( Please select one of the provided options for each factor.)

<table>
<thead>
<tr>
<th>Age of the birds</th>
<th>□ Never</th>
<th>□ Rarely</th>
<th>□ Sometimes</th>
<th>□ Often</th>
<th>□ Always</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of the birds</td>
<td>□ Never</td>
<td>□ Rarely</td>
<td>□ Sometimes</td>
<td>□ Often</td>
<td>□ Always</td>
</tr>
<tr>
<td>Sex of the birds</td>
<td>□ Never</td>
<td>□ Rarely</td>
<td>□ Sometimes</td>
<td>□ Often</td>
<td>□ Always</td>
</tr>
<tr>
<td>Familiarity of the birds with each other</td>
<td>□ Never</td>
<td>□ Rarely</td>
<td>□ Sometimes</td>
<td>□ Often</td>
<td>□ Always</td>
</tr>
<tr>
<td>Size of the holding pen</td>
<td>□ Never</td>
<td>□ Rarely</td>
<td>□ Sometimes</td>
<td>□ Often</td>
<td>□ Always</td>
</tr>
</tbody>
</table>

**D.2. Do you provide **bedding material** for **ostriches in the pre-slaughter holding pens**?**

( Please select one of the following.)

□ Never □ Rarely □ About half the time □ Often □ Always

**D.3. What is the total area of each holding pen at your facility?**

( Please write the area in one of the provided spaces.)

___________ Square feet or _________ Square meter

**D.4. How many ostriches** do you usually keep in **one holding pen** at your facility?

( Please write the number in the provided space.)

___________ Number of ostriches in one

**D.5a. Are the ostrich holding pens rectangle (four sides) at your facility? □ Yes □ No**

**D.5b. If not, please describe:**

________________________________________________________

**D.6. Do the ostriches have access to feed and water** in the pre-slaughter holding pens at your facility?

( Please select one of the following.)

□ None □ Only feed □ Only water □ Both
**D.7.** For how many **hours** do you keep the ostriches in the **pre-slaughter holding pens** at your facility?  
(Please write the time in hours in the provided space.)  

<table>
<thead>
<tr>
<th>Hours</th>
</tr>
</thead>
</table>

**D.8.** On average, what **percentage** of ostriches do not survive the **pre-slaughter holding time before stunning** at your facility?  
(Please write the percentage in the provided space.)  

<table>
<thead>
<tr>
<th>Percent</th>
</tr>
</thead>
</table>

**D.9.** On average, what **percentage of ostriches show any signs of new injuries** (e.g., cuts, trauma, bruises or bleeding) **while in the holding pen before stunning** at your facility?  
(Please write the percentage in the provided space.)  

<table>
<thead>
<tr>
<th>Percent</th>
</tr>
</thead>
</table>

**D.10.** On which **part(s) of the body** of the slaughtered ostrich can you see most of the **bruises, cuts or injuries**?  
(Please select all that apply.)  

- □ Neck  
- □ Back  
- □ Thigh front  
- □ Thigh back  
- □ Other, please specify: ________________________________

**D.11.** How **often** do ostriches excrete thick **white concentrated urine** inside the **pre-slaughter holding pen** at your facility?  
(Please select **one** of the following.)  

- □ Never  
- □ Rarely  
- □ Sometimes  
- □ Often  
- □ Always  

**D.12.** How many **ostriches** do you usually **slaughter** per day at your facility?  
(Please write the number in the provided space.)  

<table>
<thead>
<tr>
<th>Number of</th>
</tr>
</thead>
</table>

**D.13.** Which **stunning method** do you use at your facility?  
(Please select **all that apply**.)  

- □ Captive bolt  
- □ Gases  
- □ Electrical stunning  
- □ Other, please specify: ________________________________  
- □ Free bullets  
- □ No stunning
D.14. Which **slaughter method** do you use in your operation?

(Please select **all that apply**.)

- [ ] Complete ventral cut to the neck
- [ ] Thoracic sticking
- [ ] Religious slaughter (e.g., Halal or Shechita)
- [ ] Other, please specify: ________________

D.15. **On average**, what is the **carcass weight** of the ostrich in your operation?

(Please write **weight** in one of the provided spaces.)

- [ ] Did not measure

<table>
<thead>
<tr>
<th></th>
<th>Pounds</th>
<th>Kilograms</th>
</tr>
</thead>
<tbody>
<tr>
<td>__________</td>
<td>________</td>
<td>________</td>
</tr>
</tbody>
</table>

D.16a. **Do you** assess the ostrich meat **quality** at your facility?  

- [ ] Yes
- [ ] No

D.16b. If yes, please select the **method** that you use to **assess the meat quality**.

(Please select all that apply.)

- [ ] Color
- [ ] Initial pH (15 minutes)
- [ ] Ultimate pH (after 24 hours)
- [ ] Shear-force value
- [ ] Shelf-life
- [ ] Water-holding capacity
- [ ] Other, please specify: ________________________________

D.17. On average, what is the **weight of the fat** obtained from one ostrich in your operation?

(Please write weight in one of the provided spaces.)

- [ ] Did not measure

<table>
<thead>
<tr>
<th></th>
<th>Pounds</th>
<th>Kilograms</th>
</tr>
</thead>
<tbody>
<tr>
<td>__________</td>
<td>________</td>
<td>________</td>
</tr>
</tbody>
</table>

D.18. On average, what is the **skin size of a slaughtered ostrich** in your operation?

(Please write the **skin size** in one of the provided spaces.)

- [ ] Did not measure

<table>
<thead>
<tr>
<th></th>
<th>Square feet</th>
<th>Square meter</th>
</tr>
</thead>
<tbody>
<tr>
<td>__________</td>
<td>________</td>
<td>________</td>
</tr>
</tbody>
</table>

D.19a. Do you assess the **quality of the ostrich skin** at your facility?  

- [ ] Yes
- [ ] No

D.19b. If yes, please select the **factors** that you consider when you are assessing the **skin quality** at your facility. (Please select all that apply.)

- [ ] Any sign of a bacterial contamination
- [ ] Color
- [ ] Healed wound
- [ ] Number of cuts on skin
- [ ] Loose scab
- [ ] Scratch
- [ ] Shape
- [ ] Signs of bruises or injuries
- [ ] Size
- [ ] Other, please specify: ________________________________
D.20. What percentage of the slaughtered ostrich skins fall in each grading category in your operation?

(Please write the percentages in the provided spaces.)

<table>
<thead>
<tr>
<th>Grading Category</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Grade (the best)</td>
<td></td>
</tr>
<tr>
<td>Second Grade</td>
<td></td>
</tr>
<tr>
<td>Third Grade</td>
<td></td>
</tr>
<tr>
<td>Fourth Grade (the poorest)</td>
<td></td>
</tr>
</tbody>
</table>

Please let us know if you have any comments or suggestions about the ostrich processing questions.

Thank you very much for your time and support.