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Current status and future directions: Equine pituitary pars intermedia dysfunction and equine metabolic syndrome

Introduction

The extent to which endocrinological diseases of horses has impacted both veterinary clinical practice and equine scientific research is appreciated when one considers that a PubMed search on the terms 'equine' and 'endocrine' yields almost 800 'hits' for the antecedent 10 year period. As we reflect on all the new information that has been published during the previous decade, several especially noteworthy advances in the field have considerably changed the way that we understand the pathophysiological basis of the common endocrine diseases and our approach to their diagnosis and treatment. Central to the importance of both of the 2 common equine endocrinopathies, equine metabolic syndrome (EMS) and pituitary pars intermedia dysfunction (PPID), is the fact that laminitis is a potentially severe and career-ending and life-threatening complication of both conditions.

Advances in our understanding of endocrine disease have been paralleled by similar and remarkable advances in the understanding of endocrinopathic laminitis. Improved understanding of the pathophysiological links between both EMS and PPID and laminitis should lead to earlier clinical recognition of risk, more accurate corroborative diagnostic approaches and improved therapeutic and preventive strategies. The fact that insulin was recently shown to be an independent risk factor for endocrinopathic laminitis has especially provoked extensive scientific investigation regarding the mechanisms of insulin regulation in horses as well as elucidating the mechanisms behind insulin-induced injury to the laminae [1–3].

The recognition of EMS as a distinct entity and the characterisation of EMS as primarily a problem with glucose and insulin metabolism allowed practitioners and researchers to place an understandable framework around the cluster of clinical signs that were, up to that point, poorly understood. Current and future research efforts are now directed towards characterising the various manifestations of insulin dysregulation and addressing how best to manage horses with the condition [4,5]. Although previous definitions of EMS were centred on the demonstration of insulin resistance [6], in this issue, Frank and Tadros (2014) have proposed that it is an inappropriately excessive insulin response (hyperinsulinaemia) to dietary factors that should be regarded as the *sine qua non* of EMS and that insulin resistance, when present, may play a secondary role [4]. In this regard, it has been suggested that the term 'insulin dysregulation' might be a better clinical descriptor for those horses at risk of endocrinopathic laminitis associated with diet (especially pasture)-associated hyperinsulinaemia [4]. It is interesting to reflect on the fact that, as with the human medical and endocrine counterparts, there has always existed a healthy degree of debate and discussion pertaining to the 'ideal' name for these conditions.

To a greater and greater extent, horse owners are seeking veterinary guidance to promote the health of increasingly older horses and ponies. Coincident with the increasing number of aged horses within the equine population has been the recognition that PPID, once believed to be a rare condition of extremely aged (geriatric) horses, is present in a significant percentage of horses and ponies over 15 years of age [7]. Hormonal alterations consistent with PPID are often identified in horses that present for laminitis of unknown aetiology, and it has been recognised recently that laminitis (and other PPID-associated morbidities) may arise in a younger subset of PPID-affected horses prior to the development of the significant haircoat abnormalities (hirsutism or, more correctly, hypertrichosis) that are so implicative for the condition [5]. Identification of the 'early PPID' horse is one of the primary challenges currently facing equine practitioners.

The most obvious need for further elucidating the mechanisms at play in both PPID and EMS is to continue to study the horse in both health and disease. In both conditions, understanding the pathophysiology of the diseases can only be realised within the context of normal equine physiology. The pathological processes observed in PPID, EMS and laminitis are significantly attributable to perturbations in the physiological mechanisms that have allowed horses to succeed evolutionarily as temperate climate herbivores.

Horses evolved the physiological mechanisms that allow them to thrive in climates that impose extreme changes in temperatures throughout the year. For much of the year, their primary nutritional needs are met by consuming high-cellulose forages, in which the majority of nutrients are made available to the horse only by the presence of colonic bacteria. Both the horse and the colonic bacterial populations must adapt to radical changes in available dietary nutrients as pasture composition changes rapidly with the seasons. Horses living in temperate climates have the need to grow a protective winter hair coat in the autumn and then shed it in the spring. This requires a physiological mechanism for sensing the shortening day length and then elaborating an endocrinological message needed to stimulate the growth of a longer hair coat. Finally, they must have the ability to stand for months on frozen ground in subfreezing temperatures without compromising the function of the hooves or distal extremities. A critical feature of the distal limbs of animals that spend time in cold weather is the ability to regulate tightly the blood flow into, within, and out of the digit.

Comparisons of equine physiological processes with those of tropical omnivores, such as man and rodents, have obvious limitations. While there are doubtless many similarities in physiological pathways, as there would be with all mammalian species, equine researchers and practitioners must be cognisant of the possibility that similar end-stage manifestations of disease may occur through completely different pathophysiological processes.

Results of significant research throughout the last 10 years have demonstrated that there exist striking differences between horses and man regarding what were originally thought to be similar pathophysiological/endocrinopathic conditions. For example, the term 'Cushing's disease', used to describe the clinical manifestations of hyperadrenocorticism resulting from an adrenocorticotrophic hormone (ACTH)-producing corticotrophinoma of the human or canine pituitary pars distalis, should probably not be used as a descriptor for the equine PPID syndrome, which is a consequence of neurodegeneration affecting dopaminergic nerves that arise in the hypothalamus. Likewise, extensive research has demonstrated that there are as many (if not more) differences between the human and equine metabolic syndromes. In the future, we should strive to avoid inappropriately and misleadingly adopting attractive human medical terminologies for purposes of naming equine diseases.

Pituitary pars intermedia dysfunction

The current hypothesis that is most widely accepted is that PPID is primarily a disease of the dopaminergic neurons that originate in the hypothalamus and extend to the pars intermedia, where they exert tonic inhibition of the melanotrophs that constitute that portion of the pituitary gland. These neurons are particularly sensitive to oxidative damage, and when as-yet-unidentified causes of oxidative damage affect these neurons, they decrease in number. This dopaminergic neurodegeneration results in a decrease in the amount of dopamine at the level of the melanotroph. Relatively uninhibited, the melanotrophs respond by synthesising the preprohormone polypeptide, pro-opiomelanocortin, at higher levels than in a normal horse. The cleavage products (melanocortins) of pro-opiomelanocortin include β-endorphin, α-melanocyte-stimulating hormone, corticotrophin-like intermediate peptide and ACTH. Increased circulating plasma concentrations of these substances exert endocrine effects, either directly or indirectly, that result in the clinical signs associated with PPID. With prolonged loss of hypothalamic inhibition, the melanotrophs hypertrophy and eventually undergo adenomatous change. In normal horses, these same events occur to some degree during times of shortening day length. Increased melanocortin

influence is responsible for the development of a winter hair coat and possibly other seasonal adaptations to the onset of cold temperatures and decreased nutrient availability. This normal seasonal elevation in ACTH and other melanocortins and pars intermedia hyperplasia must be recognised when evaluating a horse suspected of PPID.

Although horses with PPID represent a significant subset of all horses with insulin dysregulation, not all PPID-affected horses develop insulin resistance. Why some horses with PPID develop insulin resistance whereas others do not is currently not known. Likewise, not all PPID-affected horses develop clinical laminitis. Although PPID is a well-recognised risk factor for endocrinopathic laminitis, current evidence suggests that laminitis is principally significant for those PPID-affected horses in which insulin dysregulation is present as a comorbidity. Although the relationship between EMS (insulin dysregulation/hyperinsulinaemia) and PPID is incompletely understood, there exist (at least) 2 schools of thought, i.e. either that EMS and PPID are absolutely independent conditions and either one or both endocrinopathies may be identified in any given individual or that one or other condition tends to predispose to the other. Further elucidation of the relationship between these 2 important conditions will surely be the focus of significant further research in this discipline in the future.

One of the major current research focuses in the study of PPID is how best to identify the animal with 'early' PPID, i.e. the horse with morbidity due to pituitary dysfunction that does not yet have the characteristic/pathognomonic hypertrichosis associated with advanced stages of the condition. Horses developing early stage PPID may have exaggerated elevations in the pro-opiomelanocortin-derived peptides in the autumn or may be more readily identified using evocative testing, such as the thyrotrophin-releasing hormone (TRH) stimulation or the domperidone response test. Often, however, diagnostic testing results in conflicting results. *It must be emphasised that there is no 'gold standard' diagnostic test that can reliably identify every horse with PPID*. As is the case with many other diseases that are insidious in onset, an animal with advanced PPID can be identified positively using any of the published diagnostic tests including physical examination.

The diagnosis of PPID is difficult to confirm in animals during the early stages of the disease. The dexamethasone suppression test had been labelled a gold standard test, but that label was given in an era when extremely advanced cases of PPID had been compared with much younger control animals. Early studies that reported 100% sensitivities and specificities for various endocrine test protocols for PPID have not held up when examined for repeatability and seasonal effects [8]. Even histopathological examination of the pituitary gland may not be definitive, because season can also alter the microscopic appearance of the pars intermedia (Dianne McFarlane, personal communication).

A current and future challenge to equine researchers and practitioners pertains to how to identify reliably the horses with pars intermedia changes that are not consistently symptomatic. In addition, lacking a reliable set of clinical or laboratory criteria for case definition provides a significant challenge to investigators needing to identify and separate age-matched horses into subject and control populations for the purpose of conducting research trials regarding PPID. Currently, researchers find that they must make a trade-off between studying horses with confirmed, symptomatic PPID and those with suspected PPID that more closely reflect the horse population of interest. Use of the TRH stimulation test to classify horses with resting ACTH levels close to laboratory cut-off values is currently the best way to provide a case definition for research and clinical purposes, but there is certainly room for improvement in this area. In this manner, Rendle *et al*. (2013) employed results of TRH stimulation testing for the purpose of identifying both symptomatic and nonsymptomatic horses with PPID [9].

Another important clinical and research-oriented goal to improve understanding of PPID is the development and characterisation of acceptable methods to evaluate the clinical response following initiation of treatment with pergolide. Necessarily, follow-up diagnostic tests intended to characterise the clinical response to pergolide treatment must account for the inherent variability in measured plasma ACTH concentrations, especially from the perspective of the seasonal effect. Presently, veterinarians assess treatment response on the basis of improvement in the horse's physical examination and repeated plasma ACTH concentrations. For those PPID-affected horses with laminitis, follow-up assessment of measures of insulin dysregulation (such as fasting insulin concentration or results of an oral sugar test) should also be considered following institution of pergolide treatment. More information is needed regarding the effect of pergolide treatment on measurements of insulin sensitivity and dietary hyperinsulinaemia. As noted above, the TRH stimulation test is being employed to a greater extent for purposes of identifying PPID in younger horses (before the development of hypertrichosis); it will likewise be important to obtain and present data that address the anticipated outcome of TRH stimulation test results following administration of pergolide (including seasonal influence).

The overall clinical picture for PPID varies significantly between affected individuals. Differences in clinical expression may be partly explained by heterogeneity in the pars intermedia-derived melanocortin repertoire being produced in a given patient, the extent to which adrenocortical stimulation is occurring and the presence or absence of insulin dysregulation as a comorbidity. It is likely that the mixture of secreted melanocortins (α-melanocyte-stimulating hormone, β-endorphin, ACTH, etc) differs significantly between horses and over time (perhaps with a seasonal effect). Moreover, the extent to which each melanocortin is processed by post translational modification (glycosylation, *N*-acetylation, etc) before it is secreted into the circulation is also highly variable between individuals [10,11]. These potentially highly variable post translational modifications add an additional layer of heterogeneity to the composition of the (final) secreted product. Future work might expand our understanding of the extent of post translational modification of secreted melanocortins and its effect on clinical expression and prognosis.

Cachexia (wasting) is a very serious component of several severe chronic disease states, including cancer, heart failure and renal disease. Cachexia, characterised by anorexia, loss of lean body mass, increased metabolism and decreased quality of life, causes severe clinical illness in patients already suffering with other severe chronic diseases. There is increasing interest and recognition of the role and importance of the hypothalamic melanocortin system (which is stimulated by elevated inflammatory cytokine production) in the development of cachexia [12,13]. Recent work suggests that novel drugs that act through melanocortin antagonism are useful for the reversal of some of the clinical features of cachexia. Future studies pertaining to the role of melanocortin effects in horses affected with PPID will probably be informed by some of the novel work seeking to reverse the effects of melanocortin-mediated cachexia in human patients.

Insulin dysregulation

Frank and Tadros (2013) have reviewed the recent literature regarding equine insulin dysregulation, including EMS [4]. They make the important distinction between the various manifestations of insulin dysregulation in the horse. The equid with EMS as defined by the recent American College of Veterinary Internal Medicine consensus statement typically has a characteristic phenotype with a high body condition score, including abnormal adipose distribution along the topline and a 'cresty' neck, abnormal response to insulin injection or an i.v. glucose load and a predisposition to laminitis [6]. Often, there is evidence that chronic laminitis has occurred by the time the animal first receives veterinary attention.

After reading this review, one might consider that it is time to re-examine the basis of that EMS definition. Newer thinking suggests that the principal underlying endocrinopathic problem that causes increased susceptibility to laminitis in affected individuals is an excessive hyperinsulinaemic response to dietary factors in ingested food. A satisfactory explanation for this response in some horses has not yet been identified, but Frank and Tadros (2013) suggest that an excessive intestinal incretin response could be involved and certainly warrants further investigation [4]. Incretin hormones include glucagon-like peptide and glucose-dependent insulinotrophic peptide, produced in the L and K cells of the intestinal tract, respectively. Incretins act on the pancreas to increase insulin secretion and also inhibit gastric emptying, actions that minimise postprandial hyperglycaemia. It is possible that chronic pancreatic stimulation by high incretin concentrations may lead to hyperinsulinaemia.

The gut-level endocrinological response to various dietary constituents is an important topic for future research. For example, one might also consider that substances produced by colonic bacteria in response to a sudden dietary carbohydrate load could affect pancreatic insulin secretion and glucose kinetics. Much like the rumen in cattle, the bacteria of the equine large intestine produce volatile fatty acids that enter the circulation and are then used as an energy source by skeletal muscle and other tissues. Very little attention has been paid to the effect of volatile fatty acid concentrations on circulating insulin levels in horses, although Argenzio and Hintz (1971) demonstrated that butyrate produced an increased insulin response in fasted ponies [14].

A curious observation one can make in general when reading the current literature is that there is a complete absence of comparison between the equine conditions of insulin dysregulation, caecal and colonic response to high carbohydrate loads, and laminitis with the corresponding conditions in cattle and other ruminants. Unlike man and rodents, ruminants suffer from clinical laminitis, a fact that suggests comparisons between these species might be fruitful. Subacute rumen acidosis is a syndrome of cattle that has many similarities to colonic acidosis and pasture-associated laminitis in horses. The review by Kleen and others (2003) is a good introduction to subacute rumen acidosis for equine researchers [15]. It may be the case that considering horses to be purely monogastric when performing research investigating insulin dysfunction will result in researchers missing a large piece of the puzzle. How horses with insulin dysregulation respond to the abnormal or altered volatile fatty acid concentrations that accompany sudden dietary changes is an area ripe for further study.

Although a diet-induced, excessive hyperinsulinaemic response might be the principal problem leading to endocrinopathic laminitis sensitivity in EMS-affected horses and ponies, other clinical factors that act to reduce insulin sensitivity should also be regarded as potential contributors to hyperinsulinaemia and risk of laminitis. Therefore, diagnostic tests for EMS should include both an oral sugar test (looking for evidence of an excessive insulin response to ingested food) and a test of peripheral insulin action (either an insulin challenge test or a combined insulin and glucose test). It is likely that laminitis risk is greatest in those horses with both abnormalities.

Insulin sensitivity varies a great deal in an individual animal when body weight, level of exercise and diet are altered. The complex interplay of genetics, current physiological state, effect of disease and diet has begun to be investigated. Future research is also needed to improve understanding of pancreatic and hepatic physiology as it relates to carbohydrate metabolism in normal horses and those with insulin dysregulation and EMS [16]. In spite of considerable interest in the role of insulin resistance in EMS-affected horses and the development of methods to assess insulin sensitivity either directly or indirectly in the clinical setting, there has been a dearth of information that addresses the molecular basis for insulin resistance at the cellular and tissue level in affected equids.

The hyperinsulinaemic–euglycaemic clamp technique is widely held to be the most definitive method to determine an animal's insulin sensitivity, and it is used exclusively in rigorous studies of insulin resistance in human subjects. Its use in the horse has been extremely limited to date. Pratt *et al*. (2005) demonstrated that it is superior to the minimal model analysis with regard to repeatability over time [17], but a study demonstrating its clear superiority over other tests of insulin sensitivity in horses has yet to be published. Conducting a study using the hyperinsulinaemic–euglycaemic clamp is difficult, and totally impractical in a clinical setting. In order to understand thoroughly the effects of various interventions on insulin dynamics in a research setting, however, it may be time for equine researchers to adopt its use unless it can be proved that employing easier tests yields similar information.

In recent years, the extent to which the microbial population (microbiome) of the intestinal tract influences metabolic and endocrinological processes in the mammalian host has received substantial attention [18]. In one study, individuals with marked overall adiposity, insulin resistance, dyslipidaemia and a more pronounced inflammatory phenotype (all components of metabolic syndrome) had a distinctly different gut microbiome compared with lean control animals [19]. Of particular interest to veterinarians working to improve our understanding of the relationship between diet, obesity, insulin dysregulation/insulin resistance and laminitis is the fact that this microbial community acts within the body as a discrete (microbial) 'organ' and contributes significantly to homeostasis, directly affecting energy metabolism and insulin sensitivity. As a first step, Steelman and her coworkers (2012) demonstrated that there is a distinct difference between the microbiome of healthy horses vs. those affected with chronic laminitis [20]. Further investigation of the equine microbiome regarding its influence on metabolism and all aspects of equine endocrinology are needed.

Finally, the welfare considerations of managing horses with PPID and EMS, particularly those with laminitis, are an important area for future research. The veterinary profession is asked to provide leadership when making animal welfare-related decisions, but is often poorly equipped to do so. In order to do this responsibly, veterinarians must become familiar with animal welfare science, which has evolved over the last 30 years as a scientific discipline in its own right. Equine welfare assessments lag behind those for other species, although application of the concepts of welfare science to horses is currently taking place worldwide. Prevention of disease and lameness through good husbandry and foot care is seen as the owner's primary responsibility [21]. The ethical and welfare issues surrounding the long-term care of a horse with chronic disease suffering from laminitis have yet to be addressed fully.

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References

1. Asplin, K.E., Sillence, M.N., Pollitt, C.C. and McGowan, C.M. (2007) Induction of laminitis by prolonged hyperinsulinaemia in clinically normal ponies. *Vet. J.* **174**, 530-535.
2. Burns, T.A., Watts, M.R., Weber, P.S., McCutcheon, L.J., Geor, R.J. and Belknap, J.K. (2013) Distribution of insulin receptor and insulin-like growth factor-1 receptor in the digital laminae of mixed-breed ponies: an immunohistochemical study. *Equine Vet. J.* **45**, 326-332.
3. Venugopal, C.S., Eades, S., Holmes, E.P. and Beadle, R.E. (2013) Insulin resistance in equine digital vessel rings: an *in vitro* model to study vascular dysfunction in equine laminitis. *Equine Vet. J.* **43**, 744-749.
4. Frank, N. and Tadros, E.M. (2014) Insulin dysregulation. *Equine Vet. J.* **46**, 103-112.
5. Tadros, E.M. and Frank, N. (2013) Endocrine disorders and laminitis. *Equine Vet. Educ.* **23**, 152-162.
6. Frank, N., Geor, R.J., Bailey, S.R., Durham, A.E. and Johnson, P.J. (2010) Equine metabolic syndrome. *J. Vet. Intern. Med.* **24**, 467-475.
7. McGowan, T.W., Pinchbeck, G.P. and McGowan, C.M. (2012) Evaluation of basal plasma α-melanocyte-stimulating hormone and adrenocorticotrophic hormone concentrations for the diagnosis of pituitary pars intermedia dysfunction from a population of aged horses. *Equine Vet. J.* **45**, 66-73.
8. McFarlane, D. (2011) Equine pituitary pars intermedia dysfunction. *Vet. Clin. Equine* **27**, 93-113.
9. Rendle, D., Litchfield, E., Heller, J. and Hughes, K.J. (2014) Investigation of rhythms of secretion and repeatability of plasma adrenocorticotropic hormone concentrations in healthy horses and horses with pituitary *pars intermedia* dysfunction. *Equine Vet. J.* **46**, 113-117.
10. Millington, W.R., Dybdal, N.O., Mueller, G.P. and Chronwall, B.M. (1992) *N*-acetylation and C-terminal proteolysis of β-endorphin in the anterior lobe of the horse pituitary. *Gen. Comp. Endocrinol.* **85**, 297-307.
11. Millington, W.R., Dybdal, N.O., Dawson, R. Jr, Manzini, C. and Mueller, G.P. (1988) Equine Cushing's disease: differential regulation of β-endorphin processing in tumors of the intermediate pituitary. *Endocrinology* **123**, 1598-1604.
12. Krasnow, S.M. and Marks, D.L. (2010) Neuropeptides in the pathophysiology and treatment of cachexia. *Curr. Opin. Support Palliat. Care* **4**, 266-271.
13. Grossberg, A.J., Scarlett, J.M. and Marks, D.L. (2010) Hypothalamic mechanisms in cachexia. *Physiol. Behav.* **100**, 478-489.
14. Argenzio, R.A. and Hintz, H.F. (1971) Volatile fatty acid tolerance and effect of glucose and VFA on plasma insulin levels in ponies. *J. Nutr.* **101**, 723-730.
15. Kleen, J.L., Hooijer, G.A., Rehage, J. and Noordhuizen, J.P.T.M. (2003) Subacute ruminal acidosis (SARA): a review. *J. Vet. Med.* **50**, 406-414.

16. Tóth, F., Frank, N., Martin-Jiménez, T., Elliott, S.B., Geor, R.J. and Boston, R.C. (2010) Measurement of C-peptide concentrations and responses to somatostatin, glucose infusion, and insulin resistance in horses. *Equine Vet. J.* **42**, 149-155.
17. Pratt, S.E., Geor, R.J. and McCutcheon, L.J. (2005) Repeatability of 2 methods for assessment of insulin sensitivity and glucose dynamics in horses. *J. Vet. Intern. Med.* **19**, 883-888.
18. Cani, P.D. and Delzenne, N.M. (2009) The role of the gut microbiota in energy metabolism and metabolic disease. *Curr. Pharm. Des.* **15**, 1546-1558.
19. Le Chatelier, E., Nielsen, T., Qin, J., Prifti, E., Hildebrand, F., Falony, G., Almeida, M., Arumugam, M., Batto, J.M., Kennedy, S., Leonard, P., Li, J., Burgdorf, K., Grarup, N., Jørgensen, T., Brandslund, I., Nielsen, H.B., Juncker, A.S., Bertalan, M., Levenez, F., Pons, N., Rasmussen, S., Sunagawa, S., Tap, J., Tims, S., Zoetendal, E.G., Brunak, S., Clément, K., Doré, J., Kleerebezem, M., Kristiansen, K., Renault, P., Sicheritz-Ponten, T., de Vos, W.M., Zucker, J.D., Raes, J., Hansen, T., MetaHIT Consortium, Bork, P., Wang, J., Ehrlich, S.D., Pedersen, O., Guedon, E., Delorme, C., Layec, S., Khaci, G., van de Guchte, M., Vandemeulebrouck, G., Jamet, A., Dervyn, R., Sanchez, N., Maguin, E., Haimet, F., Winogradski, Y., Cultrone, A., Leclerc, M., Juste, C., Blottière, H., Pelletier, E., LePaslier, D., Artiguenave, F., Bruls, T., Weissenbach, J., Turner, K., Parkhill, J., Antolin, M., Manichanh, C., Casellas, F., Boruel, N., Varela, E., Torrejon, A., Guarner, F., Denariaz, G., Derrien, M., van Hylckama Vlieg, J.E., Veiga, P., Oozeer, R., Knol, J., Rescigno, M., Brechot, C., M'Rini, C., Mérieux, A. and Yamada, T. (2013) Richness of human gut microbiome correlates with metabolic markers. *Nature* **500**, 541-546.
20. Steelman, S.M., Chowdhary, B.P., Dowd, S., Suchodolski, J. and Janečka, J.E. (2012) Pyrosequencing of 16S rRNA genes in fecal samples reveals high diversity of hindgut microflora in horses and potential links to chronic laminitis. *BMC Vet. Res.* **8**, 231.
21. Lunn, D.P. and McIlwraith, C.W. (2011) Equine health and disease – General welfare aspects. In: *Equine Welfare*, Eds: C.W. McIlwraith and B.E. Rollins, Blackwell Publishing Ltd, Ames, Iowa. pp 59-70.