AN ABSTRACT OF THE THESIS OF

Stephanie L. Greenway for the degree of Master of Science in Animal Science presented on July 16, 1999.

Title: Evaluation of Bamboo as Livestock Forage and Applications of *Yucca schidigera* and *Quillaja saponaria* Products in Agriculture.

Approved:_____

Peter R. Cheeke

Byproducts of bamboo processing, such as leaves and branches, may have potential as a livestock feedstuff. The objectives of this study were to evaluate seasonal changes in proximate composition of several bamboo species and reed canarygrass, and subsequently determine the digestibility of bamboo in ponies. Monthly samples of *Phyllostachys bissetii*, *Phyllostachys henon*, *Sasa pumila*, and reed canarygrass were evaluated for dry matter (DM), ash, crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), and acid detergent fiber (ADF) content over one year. Dry matter, ash, and CP for the bamboos and reed canarygrass were significantly influenced by an interaction between time of sampling and forage type ($P < .0001$; Extra-SS F-test). There were no interaction, quadratic, or linear effects of time on EE ($P > .10$; Extra-SS F-test). Neutral detergent fiber and ADF for all forages also were not affected by interactions or quadratic terms; however, a linear explanation of trends was significant. Two adult pony mares were used in a crossover design experiment to evaluate DM, CP, ADF, and NDF

digestibility (DMD, CPD, ADFD, and NDFD, respectively) of the temperate bamboo, *P. bissetii*. The diets consisted of either 1-inch chopped bamboo or grass hay. Feces were collected over 5-d periods after adaptation to diets. Dry matter digestibility, CPD, ADFD, and NDFD of the forages were generally below 30% in both ponies, with the CPD of bamboo being the only exception (75.4% for Pony A and 59.6% for Pony B). Acid detergent ash and acid detergent lignin values obtained for bamboo fed during both fecal collection periods were 2.9% and 3.6%, and 10.0% and 11.1%, respectively. The digestibility results indicate that bamboo foliage is similar in feed value to low-quality grass hay, with a DMD of approximately 30%. Feces from the two pony mares used in the previous *in vivo* experiment were collected to provide a source of inoculum for the *in vitro* dry matter disappearance (IVDMD) determination of four forages. Feedstuffs analyzed included bamboo fed during the two fecal collection periods of the previous *in vivo* experiment, as well as orchard grass hay and alfalfa hay. The effect of different levels (0, 250, 500, 750, 1500, and 3000 ppm) of *Yucca schidigera* extract (YE) on IVDMD of the bamboos and hays was determined. Addition of either 250 or 500 ppm YE did not affect bamboo IVDMD, whereas 3000 ppm decreased the digestibility of Bamboo B and increased that of orchard grass hay. Variable responses were seen when Bamboo B was treated with 750 ppm YE. No effect on alfalfa hay IVDMD was seen at any treatment level. The effects of YE treatment on feedstuffs *in vitro* are variable depending upon treatment level and type of forage evaluated.

Yucca schidigera and *Quillaja saponaria* products were evaluated for their capacity to reduce ammonia emissions from poultry excreta. Yucca extract (YE), quillaja extract (QE), yucca ultra (YU), quillaja ultra (QU), yucca powder (DK-30), and quillaja

powder (QCP) were evaluated at 0, 20, and 200 μL (mg for powders) per 5 g of excreta (wet wt.). Saponin, non-saponin, tannin, and non-tannin fractions of YE and QE (200 μL/5 g excreta) were also evaluated for ammonia reduction. Treatment with 200 μL QE/5 g excreta significantly reduced ammonia emissions when compared to all other products at either treatment level ($P < .0001$). All other treatments within the same level, but between different products were not significantly different from each other or the control ($P > .05$), except for DK-30. The higher treatment level (200 μL/5 g excreta) for all products combined was more effective ($P < .0001$) in reducing ammonia than 20 μL, which is to be expected. Treatment with the extracted fractions at 200 μL/5 g excreta were significantly different ($P < .05$) from each other when product type was not taken into account, except when comparing the percent ammonia reduction from carbohydrate treatment to that of the tannin fraction. Comparison of product means with all tannin, saponin, non-tannin, and non-saponin treatments combined were significantly different ($P < .05$). Pairwise comparisons of treatment fraction and product could not be obtained in the Mixed Linear Model. Of all standard products, QE reduced ammonia the most. The tannin-free component from both YE and QE appeared to be particularly effective in reducing emissions, with that of QE having the greater percent reduction. The reduced ammonia emissions observed when the non-saponin, and particularly the non-tannin fraction of YE and QE were applied to poultry excreta indicate the need for further investigation into determining the active compound in the non-saponin liquid.

Evaluation of Bamboo as Livestock Forage and Applications of *Yucca schidigera* and *Quillaja saponaria* Products in Agriculture

by

Stephanie L. Greenway

A Thesis Submitted

to

Oregon State University

In Partial Fulfillment of
the requirements for the
degree of

Master of Science

Presented July 16, 1999
Commencement June, 2000

Master of Science thesis of Stephanie L. Greenway presented on July 16, 1999

Approved:

Redacted for Privacy

Major Professor, representing Animal Science

Redacted for Privacy

Head of Department of Animal Sciences

Redacted for Privacy

Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Redacted for Privacy

Stephanie L. Greenway, Author

ACKNOWLEDGEMENTS

I would like to thank Dr. P. R. Cheeke, my major professor, for providing me with the opportunity to continue my graduate studies under his supervision. I would also like to thank Dr. D. J. Carroll for her guidance during my undergraduate years and subsequent first year of graduate school. Appreciation is also extended to Dr. J. Males and Dr. R. Van Saun for participating as members of my Graduate Committee. I would like to thank Dr. A. M. VanDerZanden for serving as my Graduate Council Representative.

I am extremely grateful to Mark Keller for his extensive assistance in the Department of Animal Sciences Nutrition Laboratory and to Sunny Morris for her technical support in the lab. Certainly without Mark Keller's help much of my thesis could not have been completed. Thanks also to the Oregon State University Horse Center, Scott Paxton, and Oregon State University Farm Services, in particular Russ Henning, for their cooperation in various aspects of my research. I also appreciate Dr. D. Holton and Chad Jacobs for supplying me with access to research animals. I would like to thank Dr. D. Thomas for providing statistical counseling. I am extremely grateful to Garold Nelson, Dr. D. Emenhiser, Allan Rack, Ned Jaquith, and Rick Valley for their roles in the bamboo research. My gratitude also extends to Paul Hiley at Desert King International and George Baker at OR-CAL, Inc. for their financial contributions.

Lastly, I would like to acknowledge the incredible love and support I have received from my parents, Sammy and Christiane, and my brother, Michael. Without them all of this would not have been possible.

CONTRIBUTION OF AUTHORS

Dr. P. R. Cheeke was involved in experimental design and interpretation of results. Mark Keller provided assistance in experimental design, interpretation of results, and laboratory analyses. Garold Nelson was involved in providing information regarding the evaluation of seasonal changes in bamboo composition and collecting seasonal bamboo samples. Dr. D. J. Carroll was involved in the experimental design and protocol for the *in vivo* bamboo digestibility study.

TABLE OF CONTENTS

TABLE OF CONTENTS, CONTINUED

LIST OF FIGURES

LIST OF TABLES

This thesis is dedicated to Dane, mein kleiner Flitzbombe.

EVALUATION OF BAMBOO AS LIVESTOCK FORAGE AND APPLICATIONS OF *YUCCA SCHIDIGERA* AND *QUILLAJA SAPONARIA* PRODUCTS IN AGRICULTURE

CHAPTER 1
LITERATURE REVIEW

BAMBOOS AS AN ANIMAL FEEDSTUFF

Bamboos belong to the grass family, Poaceae, subfamily Bambusoideae. Most species are perennial and spread rapidly via underground rhizomes. Pandas are the primary animal species associated with bamboo consumption, but other animal species may be better adapted to digesting its nutritive constituents. The gastrointestinal tract of giant pandas is similar to that of carnivores, allowing for limited microbial digestion (Dierenfeld et al., 1982); the panda is in fact an herbivorous carnivore. Dry matter digestibility (DMD) of bamboo in pandas is less than 20% and gut transit ranges from 5-16 h. These factors require that pandas have a daily consumption of 6-15% of their body weight in bamboo dry matter in order to meet energy needs. Structural carbohydrates, like those found in bamboo, may be more efficiently broken down by the longer rate of passage in equids (65-75 h; Evans et al., 1990), as well as the microbial fermentation that takes place in the cecum and colon. These intestinal compartments are very well developed in the horse, representing 60% of the total volume of the digestive tract while the stomach comprises less than 10% (Tisserand, 1988).

Byproducts of bamboo culm (pole) processing, as well as foliage obtained from the thinning of bamboo stands, can be fed to livestock fresh or as silage. Livestock can

additionally graze new growth and shorter varieties of bamboo. *Arundinaria racemosa* in the Himalayas (Lawson, 1968) and *Arundinaria tecta* (cane breaks) in North Carolina (Farrelly, 1984) have been evaluated for use as cattle fodder. Shepherd (1952) stated that cane breaks (*A. tecta*) provides an outstanding source of forage for cattle during winter months, requiring only supplementation with minerals. Numerous bamboos are fed to horses, cattle, and sheep worldwide (Farrelly, 1984). Trimmings of *Phyllostachys* spp. have been fed to livestock in Georgia and horses and sheep pastured on bamboo in Japan. Bamboo foliage has long been used as either a supplementary or a major source of forage (McClure, 1958). Nelson et al. (1997) evaluated the use of temperate bamboo as forage for livestock. The authors determined the *in vitro* dry matter digestibility (IVDMD) of several bamboo spp. to range from 40% – 55%. Since horses adapt to low-quality roughage via high feed intake, Nelson et al. (1997) felt bamboo could be a more reasonable forage-source for equids than ruminants. Indeed, horses and ponies may have an advantage over ruminants in that they will consume greater amounts of low quality forage, thereby compensating for low nutrient digestibility (Illius and Gordon, 1990; Cuddeford et al., 1995). *Phyllostachys bissetii* (*P. bissetii*) is a very hardy, evergreen bamboo, and thus may have potential as a winter forage source for livestock in the Pacific Northwest, when pastures are typically dormant.

SILICA AND ACID INSOLUBLE ASH IN BAMBOO

Bamboo contains a high amount of crude ash, most of which is silicate (Ueda, 1961; Dierenfeld, 1997). The acid insoluble fraction of ash (AIA) is predominately silica (Goering and Van Soest, 1970). In general, members of the grass family are found to

contain significant concentrations of silica (Jones and Handreck, 1967). Distribution of silica within plant tissues adds structural strength (Takahashi, 1974; Kaufman et al., 1985) and like lignin, it is an integral part of the matrix of plant cell walls (Jones and Handreck, 1967). Silica can be found as either monosilicic acid or solid silica. Rice straw is also high in silica (10-20% of dry matter), with the leaves having comparatively more silica than stems (Van Soest, 1993). Dierenfeld (1997) found similar differences in the silica content of stems (0.3%) versus leaves (2.5%). The author also noted that silica levels appear to increase in colder months. The overall mineral concentration of bamboo leaves may be 2- to 10-fold higher than that of culms (Dierenfeld, 1997).

The access to cell wall carbohydrates by digestive microorganisms may be inhibited by silica (Jones and Handreck, 1967; Bae et al., 1997). The anti-nutritional qualities of silica include decreased digestibility and palatability (Van Soest, 1982). There is evidence of a decrease in DMD of up to 3 units per unit increase of silica in temperate forages (Van Soest and Jones, 1968). One potential mode of action in decreasing digestibility is binding of monosilicic acid with certain elements, preventing their use by microorganisms in the gut. This could potentially decrease microbial growth, which would in turn inhibit digestion (Shewmaker et al., 1989). Minson (1971) evaluated the influence of silicon and lignin on the digestibility of cell wall constituents in sheep. He reported a strong correlation between lignification and the digestibility of hemicellulose and cellulose, but found that silica did not influence organic matter, cellulose, or hemicellulose digestibility. This is in contrast to the findings of Van Soest and Jones (1968) who stated that both silica and lignin affect digestibility, and in fact appear to have an additive effect. The digestibility of lignin is generally considered to be

minimal. Bonds between lignin and structural carbohydrates may affect accessibility of cell contents by either preventing digestion by microbial enzyme or physical attachment of microbes to cell walls (Jones and Wilson, 1987). Lignin in bamboo (*Phyllostachys aureosulcata*) was determined to be higher in branches and stems (10%) than in leaves (6%; Dierenfeld et al., 1982).

Silica could have negative impacts on animal health due to its low solubility. The majority of solid silica ingested should emerge in the feces; however, some microscopic particles of solid silica may be retained in animal tissue such as lymph nodes and kidneys (Jones and Handreck, 1967). Siliceous phytoliths (opal phytoliths) are responsible for cases of silica urolithiasis in grazing cattle and sheep of western Canada, northwestern parts of the USA, and western Australia (McDonald et al., 1995). It is not known whether horses consuming highly siliceous forages experience similar health risks.

The low digestibility of poor quality forages can prevent animals from meeting their energy needs. Equids may be able to compensate for low nutrient digestibility via *ad libitum* consumption of high fiber forages (Illius and Gordon, 1990), and thereby meet their energy requirements (Cymbaluk et al., 1989). The digestibility of bamboo could be enhanced by treatment with anhydrous ammonia or boiling. For example, use of anhydrous ammonia on low quality forages such as straw can lead to improved energy and crude protein values (Cuddeford, 1986). In Japan, a variety of bamboo processing techniques enhance the feed value of bamboo forage (Manda et al., 1990). Boiling bamboo decreases its NDF, ADF, and lignin content, and increases its palatability in goats and cattle. Similar processing may not be practical in the U. S., however, considering the availability of better quality forages.

Few nutritional analyses exist for bamboo that depict changes over time or differences among bamboo spp., especially after taking into account stage of growth, and soil and climate conditions. Dierenfeld (1997) indicated that the nutrient content of bamboo does not vary significantly throughout the year, although seasonal variation in mineral levels has been identified. Proximate analyses reported by other researchers (McClure, 1958; Nelson et al., 1997) indicate noticeable differences in nutrient constituents relative to time of sampling. Farrelly (1984) stated that the nitrogen content of bamboo leaves decreases by more than half during the summer months.

FECES AS AN INOCULUM SOURCE FOR *IN VITRO* FERMENTATION

The Tilley and Terry method (1963) has been an established procedure for *in vitro* determination of forage digestibility. This method requires maintaining fistulated animals in order to obtain rumen or cecal fluid. Recent studies have indicated the feasibility of using feces as a source of inoculum for *in vitro* digestibility experiments in both ruminants (El Shaer et al., 1987; Omed et al., 1989; Akhter et al., 1994) and non-ruminants (Sunvold et al., 1995a, 1995b, 1995c). Hintz et al. (1971) determined that only a small amount of digestion takes place in the colon and rectum of horses, indicating that microbes may escape fermentation and be excreted in the feces (Lowman et al., 1999, in press). Other researchers have found equine feces to contain viable quantities of microorganisms (Uden and Van Soest, 1982), including a large number of living protozoa (Adam, 1951). *In vitro* fermentation using horse feces has been found to be comparable to analyses using cecal fluid as a source of inoculum (Lowman et al., 1996; Kirkhope and Lowman, 1996). Digestibility determination using fecal inoculum appears to be better

suited to analysis of roughages rather than concentrates (Omed et al., 1989), although there is some evidence that even among forages there is variability in how well *in vivo* and *in vitro* values agree (El Shaer et al., 1987). El-Meadaway et al. (1998) noted that the IVDMD of several feedstuffs decreased as the ruminant fecal concentration used in the inoculum was increased from 3% to 9%. The IVDMD values obtained when a 3% fecal suspension was used were comparable to those obtained with rumen fluid, except when low-quality feeds were tested. The authors also indicated that bacterial populations varied with inoculum source, resulting in different fermentation products.

URIC ACID, UREA, AND AMMONIA

Dietary, endogenous, and urea nitrogen provide the ammonia from which bacterial-N is synthesized (Mackie, 1987). Ammonia is the primary nitrogen source for bacterial protein synthesis in the hindgut. It is postulated that ammonia is absorbed in the ruminant forestomach as NH_3 rather than NH_4 (Stevens and Hume, 1995). Microbial protein can be digested in the abomasum and small intestine, providing an additional protein source for the ruminant (McDonald et al., 1995). If the rate of protein degradation exceeds that of bacterial protein synthesis, the excess is absorbed into the portal blood and converted to urea in the liver. Under such conditions, plasma urea concentrations tend to increase (Ørskov and Kay, 1987). Urea is subsequently recycled via saliva or ruminal secretions, or excreted in the urine (McDonald et al., 1995).

Excess nitrogen in birds is voided from the body as uric acid; however, if removal from the blood is impaired, the precipitated uric acid may result in gout (Scott et al., 1982). Poultry excreta is a combination of urine, which contains excreted nitrogen as

uric acid, and feces, which has true protein as the nitrogenous waste (McDonald et al., 1995). Uric acid undergoes several steps of decomposition before yielding urea, and is subsequently degraded by microbial urease to produce ammonia and carbon dioxide (Carlile, 1984). The author reported that a number of bacteria, fungi, and actinomycetes in feces are capable of degrading uric acid into either ammonia or its precursors. Barnes and Impey (1974) identified 35 strains of anaerobic non-proteolytic bacteria in the avian cecum that degrade uric acid, with only one having urease activity.

High levels of ambient ammonia have negative effects on poultry such as poor feed intake, decreased egg quality, and respiratory disease (Charles, 1992). Levels of 10 ppm or more pose health risks to poultry farm workers; however, it is not uncommon to find levels in poultry houses that exceed 50 ppm (Headon and Walsh, 1993; Crober, 1996). Ammonia levels may be reduced by either dietary modifications or treatment of excreta. Optimal treatment of poultry litter involves neutralization of free ammonia rather than inhibition of uric acid decomposition (Carlile, 1984).

YUCCA SCHIDIGERA AND *QUILLAJA SAPONARIA*

Yucca schidigera (Agavaceae) grows in the deserts of Baja California and *Quillaja saponaria* Molina (Rosaceae) trees grow in South America (mainly Chile). Yucca and quillaja extracts are commonly used as additives in the cosmetic and soft drink industries. Yucca extract is also fed to livestock and poultry to improve health and productivity. Similar applications of *Q. saponaria* have not been greatly investigated although other research has focused on its usefulness as an immunoadjuvant in vaccines

(Kensil et al., 1996). The mode of action for both yucca and quillaja is believed to be related to their saponin content.

The three major classes of saponins are triterpene, steroid, and steroid alkaloid (Hostettman and Marston, 1995). All saponins typically have an ether-linked sugar chain at C-3, classifying them as monodesmosidic. Steroidal saponins are synthesized from squalene via steroid and cholesterol intermediates and are categorized as either spirostanol or furostanol glycosides (Hostettman and Marston, 1995). Spirostanol saponins are typically considered monodesmosidic, whereas furastanol saponins are bidemosidic with the second sugar chain located at C-26 via an ether linkage. *Y. schidigera* contains spirostan-based steroidal saponins (Tanaka et al., 1996). In the case of the primary steroid saponins identified in *Y. schidigera* (sarsasapogenin, smilagenin, and markogenin), C-3 is not occupied by a sugar moiety, but is instead linked to an –OH group (Tanaka et al., 1996). Additional yucca steroid saponins include samogenin, gitogenin, and neogitogenin, which are all spirostanol in nature (Kaneda et al., 1987). Saponins found in quillaja are bidesmosidic triterpenoids (van Setten and van de Werken, 1996). The triterpenoid saponin core is synthesized from squalene via tetracyclic and pentacyclic triterpenoid precursors (Hostettman and Marston, 1995). The term bidesmosidic indicates attachment of sugar moieties at C-3 and C-28 of the aglycone core (quillaic acid) of *Q. saponaria* (Higuchi et al., 1987). The saponin nucleus is fat-soluble, whereas the carbohydrate side chains are water-soluble (Cheeke, 1998). Extraction of steroidal saponins using butanol has been conducted by Wall et al. (1952). Headon et al. (1991), Wallace et al. (1994), and Killeen et al. (1998) conducted similar saponin extractions on *Y. schidigera*. Wallace et al. (1994) additionally removed tannins from

yucca extract, and evaluated the effects of the saponin and non-tannin (non-saponin) fractions on protozoal activity in rumen fluid. Tannins can be divided into two categories: condensed tannins and hydrolyzable tannins (Barry and Blaney, 1987). The authors state that both types of tannins can bind to proteins and form tannin-protein complexes, thereby preventing loss of N across the rumen wall. Low concentrations of tannin in ruminant diets enhances the flow of undigested dietary protein to the small intestine (Elliott and McMeniman, 1987), which can increase the efficiency with which dietary nitrogen is utilized (Ahn et al., 1997). The tannin fraction of yucca and quillaja extracts could be responsible for some of the observed physiological responses, and in fact may have an additive effect in combination with saponins (Makkar and Becker, 1996). Makkar et al. (1995) observed that total protozoal numbers and ammonia concentrations were reduced when condensed tannins were added to a RUSITEC (Rumen Simulation Technique) unit.

EFFECTS OF *YUCCA SCHIDIGERA* ON FEEDSTUFF DIGESTIBILITY

A number of equine supplements, either being comprised partially or entirely of *Y. schidigera*, have been marketed to potentially improve metabolism, and relieve arthritis, stiffness, and joint pain. Few studies have been conducted on equids to evaluate the effects of yucca extract on digestibility. *Y. schidigera* contains saponins that have species-dependent bacterial and antiprotozoal activities (Wallace et al., 1994; Wang et al., 1998), with the mechanism of antiprotozoal activity being cell membrane lysis (Cheeke, 1998). Most studies regarding the impact of *Y. schidigera* on animal nutrition have been conducted on ruminants. Although Kern et al. (1973) did not identify protozoa

common to both ponies and steers, it is possible that yucca would have similar effects on gut microbes regardless of host type. Wang et al. (1998) found that the number of protozoa decreased upon addition of 0.5 mg mL^{-1} yucca extract to diets fermented in a RUSITEC unit, while the DMD of both concentrates and hay and the total bacterial count remained unaffected. Goetsch and Owens (1985) found that the addition of 44 ppm sarsaponin (steroid saponin of *Y. schidigera*) increased total tract organic matter digestibility in dairy cows, yet had no effect on dry matter disappearance of six substrates *in situ*. Glade (1992) concluded that feeding yucca extract to equids tended to increase the digestibilities of DM, NDF, ADF, cellulose, and hemicellulose, although the treatment effect was not necessarily significant ($P > .05$). In a defaunation study conducted by Moore and Dehority (1993) on ponies, it was determined that overall DMD decreased only slightly ($P < .10$) when protozoa were removed from the hindgut. The authors concluded that the absence of protozoa had little impact on how well forages were digested, and that it was possible that bacteria and fungi compensated for the loss of protozoa and their actions in the gut. Mackie (1987) classified ciliate protozoa into different functional groups according to their apparent nutritional strategy in the rumen. The author stated that feeding patterns of ciliates overlap, particularly when the host animal is under nutritional stress. This would indicate that even if yucca extract were antiprotozoal in nature, digestibility in the equid might not be greatly affected by loss of protozoal numbers. Veira's (1986) review of literature regarding faunated and defaunated ruminants agreed with the findings of Moore and Dehority (1993) that protozoa are not essential; however, Veira (1986) also stated that when protozoa were present, ruminal ammonia concentrations were higher and bacterial numbers lower.

It is believed that changes in ammonia concentration in the gut are in response to bacterial populations remaining unchecked by protozoa, since bacteria utilize ammonia for protein synthesis (Leng and Nolan, 1984; McAllister et al., 1998). The role of bacteria and nitrogen in the large intestine of the horse is presently not well understood (Burke, 1987; Evans et al., 1990), although it appears that the predominant form of nitrogen absorbed from the large intestine is ammonia (NRC, 1989). Ammonia is then converted to urea in the liver, and excreted or returned to the gut where bacterial urease degrades the urea to ammonia (Frape, 1986; Evans et al., 1990). The ammonia can then be used for protein synthesis by intestinal bacteria (Frape, 1986) or be excreted in urine. The extent of nitrogen use from bacterial sources by equids is unknown (Evans et al., 1990), but likely to be minimal unless the animal practices coprophagy (Ørskov and Kay, 1987). Prior et al. (1974) evaluated urea recycling and metabolism in ponies. The authors stated that bacterial protein degradation in the gut affect the ability of ponies to utilize recycled urea nitrogen. Dietary nitrogen utilization was decreased when *Y. schidigera* extract (YE; 1 mL/ kg feed) was added to the diet of weanling foals, and subsequently resulted in decreased growth rate (Glade, 1992). *Y. schidigera* extract added to the diet of dairy cows did not affect ruminal ammonia or plasma urea nitrogen (Wilson et al., 1998). Ruminal organic matter and acid detergent fiber digestibilities, as well as microbial protein and ruminal ammonia were unaffected by the incorporation of 125 ppm YE in the diet of dairy cows (Wu et al., 1994). Use of YE at 100 mg/L of rumen fluid resulted in a significant decrease ($P < .05$) in mmol NH_4^+/L (Ryan et al., 1997), whereas Hussain and Cheeke (1995) found no significant decrease in ruminal ammonia-N in steers fed high roughage diets containing extract at 250 mg/kg. Bacteria

that digest forages are more greatly inhibited by yucca than those that digest concentrates (Wang et al., 1998). Killeen et al. (1998) stated that the antimicrobial activity of yucca extract would have little effect on bacterial numbers in the animal at supplementation levels presently recommended. If, however, bacteria and protozoa were affected by inclusion of yucca extract in the diet, the resulting changes in availability of energy and nitrogen to microbes may affect the efficiency with which particularly fibrous forages are digested (Alexander, 1952; Cuddeford et al., 1995).

Saponins in *Y. schidigera* are not easily degraded (George, 1965), although Wang et al. (1998) indicated that yucca saponins can be deglycosylated by rumen microbes, resulting in structural changes to the sapogenin (aglycone core). In the monogastric mammal, the majority of dietary nutrients are absorbed in the small intestine via facilitative or active transport (McDonald et al., 1995). Saponins may cause increased intestinal wall permeability and decreased active nutrient transport, resulting in impaired nutrient absorption (Johnson et al., 1986; Wang et al., 1997). Intestinal walls could also become more permeable to other, potentially toxic, substances (Johnson et al., 1986). There is evidence that other types of saponins, such as quillaja saponins (Makkar and Becker, 1997) and alfalfa saponins (Gutierrez et al., 1959; Gutierrez and Davis, 1962), are capable of being degraded by rumen microbes. It has also been determined that soybean saponins can be hydrolyzed by cecal microflora (Gestetner et al., 1968) and that alfalfa saponins possibly limit nutrient digestion by rumen microorganisms (Lu et al., 1987).

AMMONIA-REDUCING PROPERTIES OF *YUCCA SCHIDIGERA* AND *QUILLAJA SAPONARIA*

Ammonia-reducing properties of *Y. schidigera* have been studied quite extensively in a variety of non-ruminants. Dvorak and Watts (1996) found ammonia emissions from cat litter were reduced when 200 ppm YE was added to the diet. Environmental ammonia was reduced by the addition of 250 mg YE to the diet of rabbits, as well as by spraying 125 mg YE/L on rabbit feces (Al Bar et al., 1993). The authors also found that ambient NH_3 levels in a poultry facility were decreased by the dietary inclusion of YE (125 ppm). Cecal ammonia-N and cecal urease in rats were not affected by dietary supplementation with sarsaponin (steroid saponin of yucca) as determined by Preston et al. (1987). The effect of YE on synthetic urease was evaluated by Kemme et al. (1993), who found that only with 600 ppm YE did ammonia production decrease by 50%. The authors also added YE directly to pig feces (2400 ppm), or to pig diets (720 ppm), and observed that ammonia production was reduced by only 14% and 22%, respectively. These concentrations of yucca in the diet are much greater than what is typically found to reduce ammonia levels in swine units (Headon and Walsh, 1993). Ammonia emissions in swine confinement buildings were reduced by 26% when YE was simultaneously incorporated into the diet and applied to slurry (Amon et al., 1995). Ammonia levels in layer houses were reduced when YE was added to the diet of hens (Goodall et al., 1988; Crober, 1996). Goodall et al. (1988) also found that yucca incorporated into the diet of broilers reduced ammonia emissions from excreta *in vitro*, whereas Johnston et al. (1981) found no response and Anthony et al. (1994) witnessed an increase in environmental ammonia. Rowland et al. (1976) fed pullets a diet containing

up to 465 ppm YE, and observed that ammonia levels remained below 20 ppm regardless of treatment level.

Stutz and Metrokotsas (1972) indicated that urease activity is predominately (99%) located in the ceca of chicks, with excretion and metabolism of urea being rapid. When a high protein diet was supplemented with YE, plasma ammonia nitrogen in rabbits was significantly decreased (Hussain et al., 1996). The authors found a similar decrease in cecal ammonia nitrogen and plasma urea nitrogen when rabbits on a low protein diet were supplemented with both urea and YE. No significant changes in urease activity or net ammonia production in the intestines of broiler chicks were evident between those chicks fed a control diet and ones supplemented with YE (Yeo and Kim, 1997). Results obtained by Balog et al. (1994) indicated blood uric acid concentrations increased when broilers were supplemented with 125 ppm YE, whereas concentrations of uric acid in birds provided 250 ppm YE did not change. The authors also found that YE supplemented birds had lower large intestine ammonia and blood urea nitrogen, although blood ammonia levels were not affected. Killeen et al. (1998) extracted saponins from *Y. schidigera*, and evaluated the effects of both the saponin and non-saponin fractions on intestinal fermentation processes in the rat. The non-saponin fraction significantly increased hindgut urease activity and fecal ammonia concentrations, whereas the saponin fraction decreased urease activity in the gut. Serum urea and ammonia were reduced by the non-saponin fraction. Sarsasapogenin and smilagenin, two of the steroid saponins within *Y. schidigera*, were identified as being the predominant source of yucca's antimicrobial activity; however, this activity was determined on only a single strain of bacteria (Killeen et al., 1994). One must consider that, at least in the ruminant, there are

a variety of bacteria which have urease activity (Hungate, 1966), and that yucca could have an indirect effect on bacterial numbers and urease activity simply through its antiprotozoal characteristics. The antimicrobial activity of a yucca saponin fraction obtained by column chromatography was found to have little or no effect on both Gram-positive and Gram-negative bacteria (Tanaka et al., 1996), which contradicts the findings of Wallace et al. (1994). The butanol-soluble extract of *Y. schidigera* was found to have both antiprotozoal and antibacterial activity, although in some instances bacterial growth was stimulated or unaffected (Wallace et al., 1994). Removal of tannins from the saponin fraction did not alter its efficacy. Headon et al. (1991), using a standard NH_4Cl solution, determined the ammonia-binding activity of yucca to reside in the water-soluble (non-saponin) fraction. *Q. saponaria* saponins were evaluated *in vitro* using rumen liquor and found to increase the efficiency of microbial protein synthesis (Makkar and Becker, 1996).

CHAPTER 2
EVALUATION OF TEMPERATE BAMBOO FOLIAGE AS AN ANIMAL FEEDSTUFF: SEASONAL CHANGES IN COMPOSITION AND DIGESTIBILITY IN PONIES

S. L. Greenway[1], M. Keller[1], G. Nelson[2], P. R. Cheeke[1], and D. J. Carroll[3]
[1]Department of Animal Sciences, Oregon State University, Corvallis, OR 97331
[2]RKR Bamboo Plantation, Coquille, OR 97423
[3]Purina Mills, St. Louis, MO 63144

ABSTRACT

Bamboo is an evergreen grass, with potential as an animal feedstuff. The objectives of this study were to (1) determine seasonal changes in composition of several bamboo spp. compared to reed canarygrass, and to (2) determine the digestibility of *Phyllostachys bissetii* winter foliage in ponies. Monthly samples of *P. bissetii*, *P. henon*, *Sasa pumila*, and reed canarygrass were dried, ground, and evaluated for DM, ash, CP, EE, NDF, and ADF content over one year. Bamboo DM values ranged from 31.9% in *S. pumila* to 53.6% in *P. bissetii*, with reed canarygrass being as low as 12.9%. The lowest and highest ash content for the three temperate bamboos was present in *P. bissetii* (6.1% and 16.1%). Reed canarygrass ash ranged from 9.3% to 13.2% over the twelve-month sampling period. *P. henon* contained the highest CP (18.2%) of the bamboos for any given month, whereas *P. bissetii* contained the lowest (11.1%). Reed canarygrass had the highest monthly CP (32.7%) of all forages tested. Ether extract ranged from 2.1% to 4.9% for *S. pumila* and *P. bissetii*, respectively, with percentage EE for reed canarygrass falling within this range. Of the three bamboos, *P. henon* contained the lowest monthly NDF (67.5%), as well as the lowest ADF (32.8%). *P. bissetii* was highest in ADF (44.2%) for any given month, whereas *P. henon* was highest in NDF (81.4%). Reed canarygrass NDF and ADF values were within the ranges presented for the bamboo spp. Mean values for DM, ash, and CP for all forages were significantly influenced by an interaction between time of sampling and forage sampled ($P < .0001$; Extra-SS *F*-test). Ether extract showed neither an effect of time of sampling nor an interaction thereof ($P > .10$; Extra-SS *F*-test); therefore, any trend or change in EE was inherent to the forage

analyzed. Interactions between forage and month, as well as the quadratic term, were not significant ($P > .10$; Extra-SS F-test) in explaining trends of either NDF or ADF for all forages; however, the explanatory variables were independently significant with evidence of a linear correlation over time. Two adult pony mares were used in a crossover design experiment to evaluate the digestibility of the temperate bamboo, *P. bissetii*. The diets consisted of either 1-inch chopped bamboo or grass hay. Feces were collected over 5-d periods after adaptation to diets. Dry matter digestibility (DMD) for grass hay was 33.6% for Pony A and 28.3% for Pony B. *P. bissetii* DMD was 33.7% and 19.4% for Pony A and Pony B, respectively. Crude protein digestibility (CPD) was 22.0% (Pony A) and 34.0% (Pony B) for grass hay and 75.4% (Pony A) and 59.6% (Pony B) for *P. bissetii*. Pony A had a grass hay acid detergent fiber digestibility (ADFD) of 24.4% and neutral detergent fiber digestibility (NDFD) of 25.5%. Grass hay ADFD for Pony B was 16.4% and NDFD was 20.2%. ADFD for the bamboo was lowest (13.8%) for Pony B and highest (16.0%) for Pony A. NDFD for *P. bissetii* was 31.5% and 14.8% for Pony A and Pony B, respectively. Acid detergent ash (ADA) and acid detergent lignin (ADL) values were obtained for the bamboo fed during the fecal collection periods. ADL in the bamboo fed during Period 1 was 11.1% and 10.0% for Period 2. Bamboo A had 2.9% and Bamboo B had 3.6% ADA. The digestibility results indicate that bamboo foliage is similar in feed value to low-quality grass hay, with a DMD of approximately 30%.

INTRODUCTION

Bamboos belong to the grass family, Poaceae, subfamily Bambusoideae. Most species are perennial and spread rapidly via underground rhizomes. Many bamboos reach their mature height in just 60 days (McDonald, 1998).

Bamboo has been used as livestock fodder on a global basis (Shepherd, 1952; Lawson, 1968; Farrelly, 1984). Livestock may graze shorter bamboo varieties, as well as new growth. Bamboo foliage (byproducts of culm processing) can be fed fresh or as silage. Nelson et al. (1997) evaluated the use of temperate bamboo as forage for livestock. *In vitro* dry matter digestibility for the same bamboo spp. tested in the present study ranged from 44.6% to 54.5%. The authors felt that since horses adapt to low quality roughage via high feed intake bamboo could prove to be a reasonable forage source for equids. Horses and ponies may have an advantage over ruminants in that they will consume greater amounts of low quality forage, thereby compensating for low nutrient digestibility (Illius and Gordon, 1990; Cuddeford et al., 1995).

Phyllostachys bissetii (*P. bissetii*; use of *P.* henceforth refers to the genus *Phyllostachys*) is a very hardy, evergreen bamboo, and thus may have potential as a winter forage source for livestock in the Pacific Northwest, when pastures are dormant.

MATERIALS AND METHODS:

Over the course of one year, monthly samples of approximately 400 leaves were randomly harvested from each bamboo stand and reed canarygrass patch at the RKR Bamboo Plantation in Coquille, Oregon. Leaves from *P. bissetii*, *P. nigra 'Henon'*

(henceforth referred to as *P. henon*), *Sasa pumila* (*S. pumila*), and reed canarygrass clippings were placed in individually labeled plastic bags and mailed to Oregon State University where they were frozen until further analysis. Samples were dried in a forced air oven at 55°C and ground with a Wiley mill using a 1-mm screen. Dry matter, ash, EE, and CP were analyzed according to the AOAC Official Methods of Analysis (1995). ADF and NDF values were obtained using the Goering and Van Soest method (1970).

Data for the monthly changes in bamboo composition were analyzed using Multiple Linear Regression in SAS v. 6.12 (SAS, 1988). The model $Y_{ij} = F_i + M_j + M_j^2 + (M_j \times F_i) + (M_j^2 \times F_j)$ was used to determine the presence of a non-linear trend through time, where Y is the nutritive constituent, F is the forage type, and M is the month. Quadratic and linear effects of time, as well as interactions of time and forage type on proximate composition were evaluated for significance using the Extra-Sum-of-Squares *F*-test. When all terms except forage lacked significance ($P > .10$), the data was analyzed using One-way ANOVA. Pairwise comparisons were made for all applicable models. Means and standard errors for the sampling year for each proximate component of a given forage were obtained using distribution analysis in SAS v. 6.12 (SAS, 1988).

Bamboo for the digestibility trial was harvested by sawing the culms (poles) at the base. Culms were subsequently debranched and whole branches (stems and leaves) were fed. Entire branches were fed during the first week of the trial, but selective consumption by Pony B and difficulties in retrieving orts dictated a need to process the feed; therefore, a hammer mill with a 1-inch screen was used to process the bamboo for the remainder of the trial. The source of the bamboo fed during the fecal collection of Period 1 differed from that of Period 2, which was reflected most prominently in the CP content. Bamboo

for Period 1 was obtained from both Alsea, Oregon and Corvallis, Oregon, and contained an overall CP content of 7.9%. The bamboo for Period 2 was harvested from four separate locations in the Willamette Valley (Oregon) and contained 10.9% CP. After each harvest, bamboo branches were stored indoors and kept fresh by misting.

Two adult pony mares were used in a crossover design experiment to evaluate the digestibility of the temperate bamboo, *P. bissetii.* Pony A (178 kg BW) and Pony B (260 kg BW) were fed a diet of either grass hay or *P. bissetii* at 1.8% BW (NRC, 1989). The study had initially been designed as a 3 x 3 Latin square with a third pony being fed the bamboo *P. aureosulcata.* The pony refused up to 50% of the feed offered. Other bamboo spp. were also offered (*P. nigra* and *Pseudosasa japonica*) but intake was not adequate, and the pony was ultimately removed from the study.

Ponies were adapted to diets for at least 12 days prior to fecal collection and were fed twice a day at 700 and 1700. Ponies were confined to their stalls during the fecal collection period, but were otherwise provided daily turnout in outdoor pens devoid of feed. Water intake and body weight of ponies was monitored for assessment of pony health.

Samples of *P. bissetii* were taken after each bamboo harvest. Grass hay fed throughout the trial was cored at trial onset and samples composited. Orts were collected before the morning feeding and composited for each week. All feed and orts samples were frozen for later analysis. Samples were dried in a forced air oven at 55°C and ground in a Wiley mill through a 1-mm mesh screen.

During Period 1, feces were collected for five days, three times a day, at 8-h intervals. The collection time for each subsequent day was advanced by one hour to

eliminate any effect of diurnal variation. Lack of an adequate amount of *P. bissetii* resulted in a 4-d fecal collection for Period 2. When possible, manure samples were taken from recently defecated, undisturbed feces rather than being obtained intra-rectally. Samples were frozen immediately after collection. Individual samples were dried in a forced air oven at 55°C, ground in a Wiley mill using a 1-mm mesh screen, and composited according to day of collection.

All feed, orts, and fecal samples were analyzed for DM, CP, ADF, and NDF using the standard procedures defined previously (AOAC, 1995). Only feed and orts samples were additionally analyzed for EE and ash content. Bamboo fed during the fecal collection periods was also analyzed for acid detergent ash (ADA) and acid detergent lignin (ADL; Goering and Van Soest, 1970). Acid insoluble ash (AIA) values were obtained for feed, orts, and fecal samples. Acid insoluble ash was used as an internal marker to determine dry matter digestibility (DMD) as described by the 2N HCl method of Van Keulen and Young (1977). In determination of DMD, orts AIA content was taken into account as suggested by Block et al. (1981) and Thonney (1981) and the mean AIA for each fecal collection period used to obtain the digestibility values for forages consumed by each pony. Crude protein digestibility (CPD), acid detergent fiber digestibility (ADFD), and neutral detergent fiber digestibility (NDFD) were also calculated.

Data for the digestibility trial could not be statistically analyzed because differences in the three explanatory variables (pony, feed, period) could not be disregarded.

RESULTS AND DISCUSSION

Figures 2.1 – 2.6 depict seasonal changes in proximate nutrients. The effect of month on mean DM, ash, and CP was influenced by forage type. All month and forage interactions for DM were different ($P < .01$), with mean values for *P. bissetii* and *P. henon* being the only exception ($P > .10$). Percent ash of reed canarygrass and *P. bissetii* were different from *S. pumila* ($P < .02$), as was *P. henon* ($P < .10$). The seasonal change of CP in reed canarygrass was different ($P < .001$) from the three bamboos; however, the bamboos did not differ from each other ($P > .10$). Neutral detergent fiber and ADF were influenced by both month and forage, but no interactions were found to be significant ($P > .10$; Extra-SS *F*-test). The linear trend of both NDF and ADF differed for reed canarygrass when compared to all other forages ($P < .0001$), but did not differ among the other forages ($P > .10$). The trends observed in this study cannot be conclusively attributed with seasonal patterns, and may have instead been influenced by serial correlation or a missing term in the model. Dierenfeld (1997) indicated lack of significant changes in nutrient content of bamboo throughout the year. In contrast, proximate analyses reported by other researchers (McClure, 1958; Farrelly, 1984; Nelson et al., 1997) indicate similar nutrient fluctuations as found here (Table 2.1) which could be attributed to a seasonal effect.

The DMD (Table 2.2) of *P. bissetii* was 33.7% for Pony A and 19.4% for Pony B. Pony A had a grass hay DMD of 33.6% versus 28.3% for Pony B. This is markedly lower than the DMD (50.4%) of untreated oat straw fed to ponies (Slagsvold et al., 1979). The bamboo CPD for Pony A was 75.4% and 59.6% for Pony B. Grass hay had a CPD

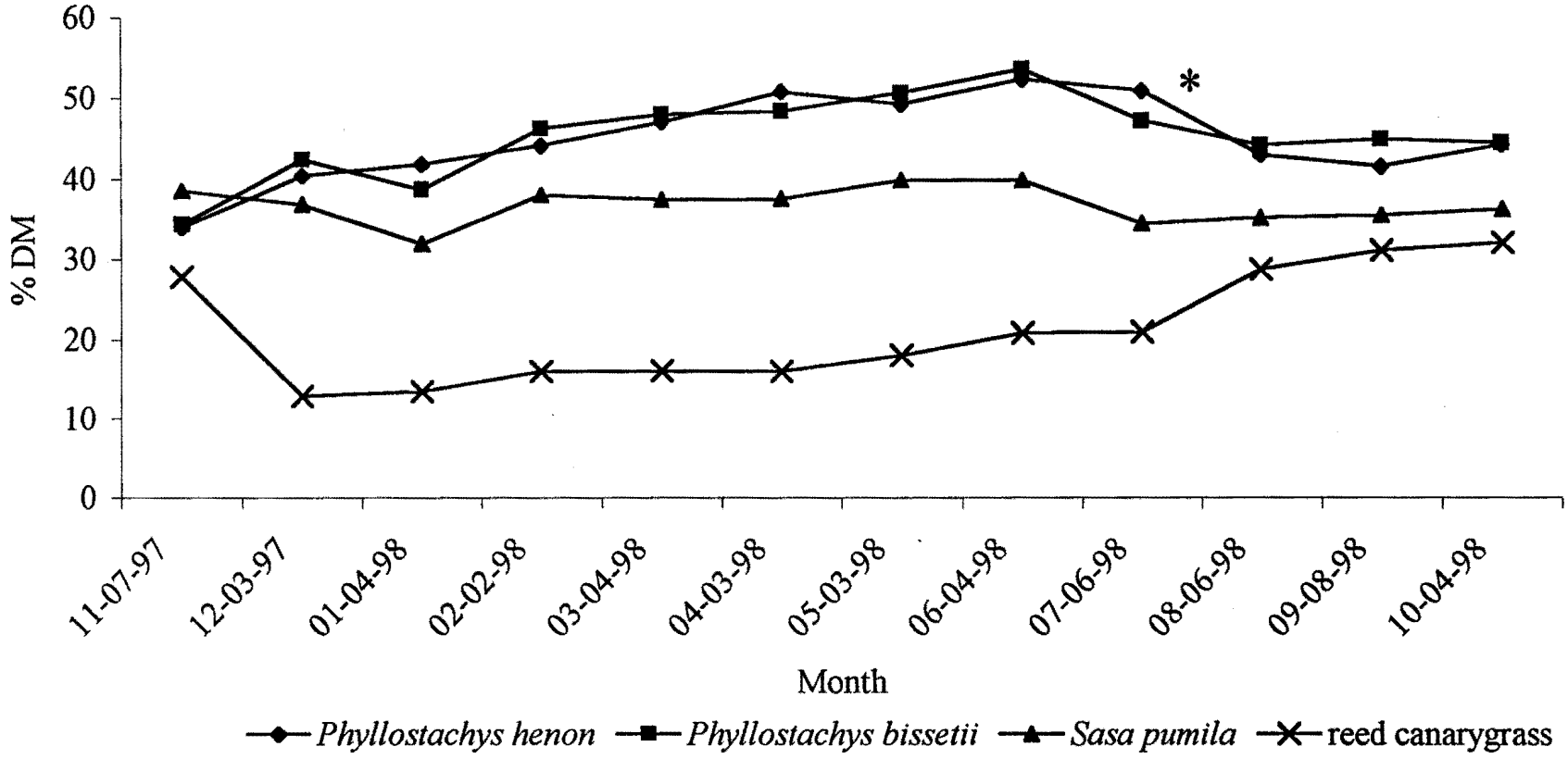

Figure 2.1. Seasonal changes in percent dry matter (% DM) of *P. henon*, *P. bissetii*, *S. pumila*, and reed canarygrass. Month^2 x Forage interaction was significant ($P < .001$; Extra-SS *F*-test). *Trend for *P. henon* and *P. bissetii* were not significantly different ($P > .10$) from each other; all other forage comparisons were significant ($P < .01$). Each data point represents the single monthly sample taken.

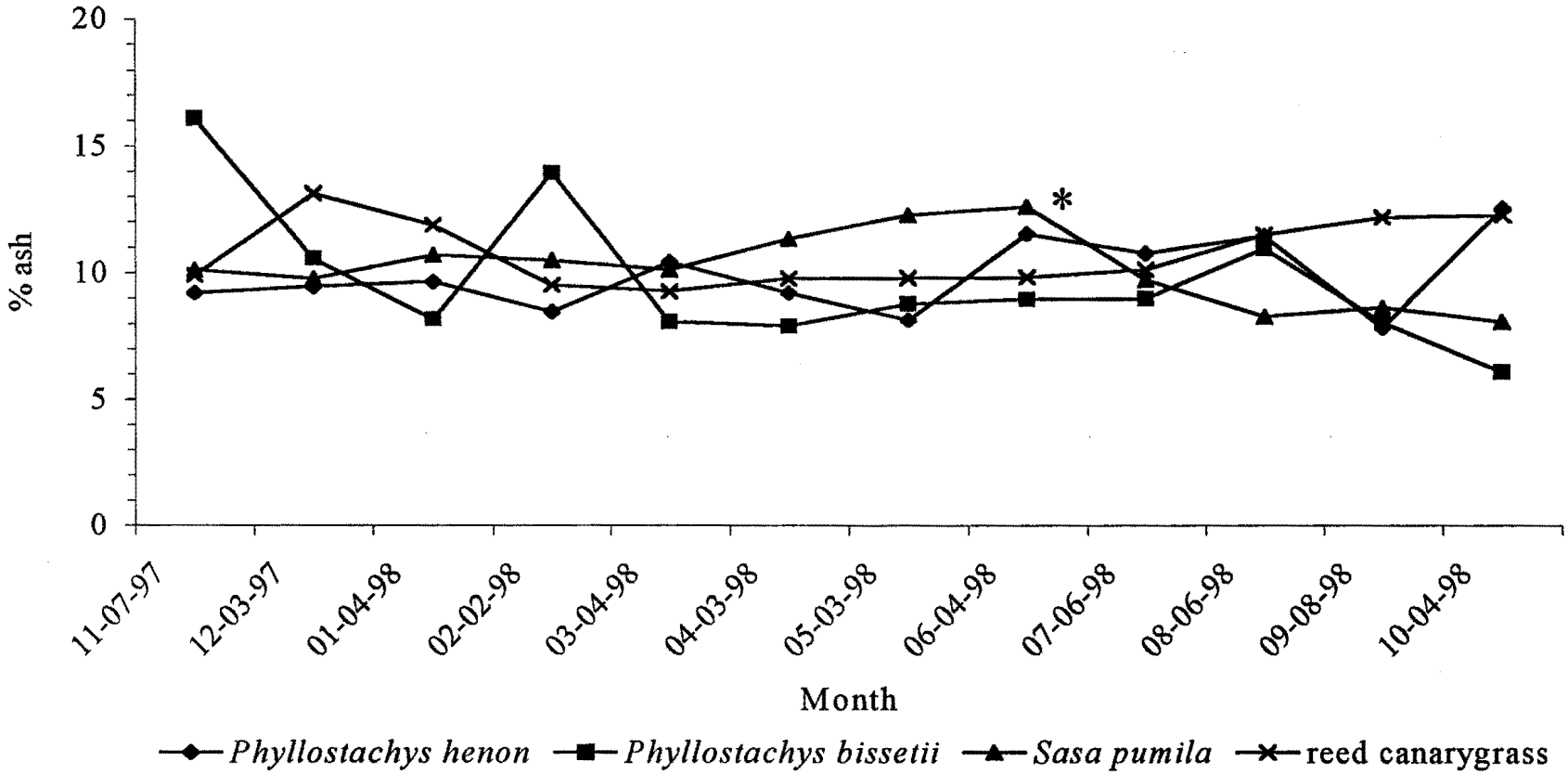

Figure 2.2. Seasonal changes in percent ash (% ash) of *P. henon*, *P. bissetii*, *S. pumila*, and reed canarygrass. Month[2] x Forage interaction was significant ($P < .01$; Extra-SS *F*-test). *Trend for *S. pumila* was significantly different from reed canarygrass and *P. bissetii* ($P < .05$), and from *P. henon* ($P < .10$). Each data point represents the single monthly sample taken.

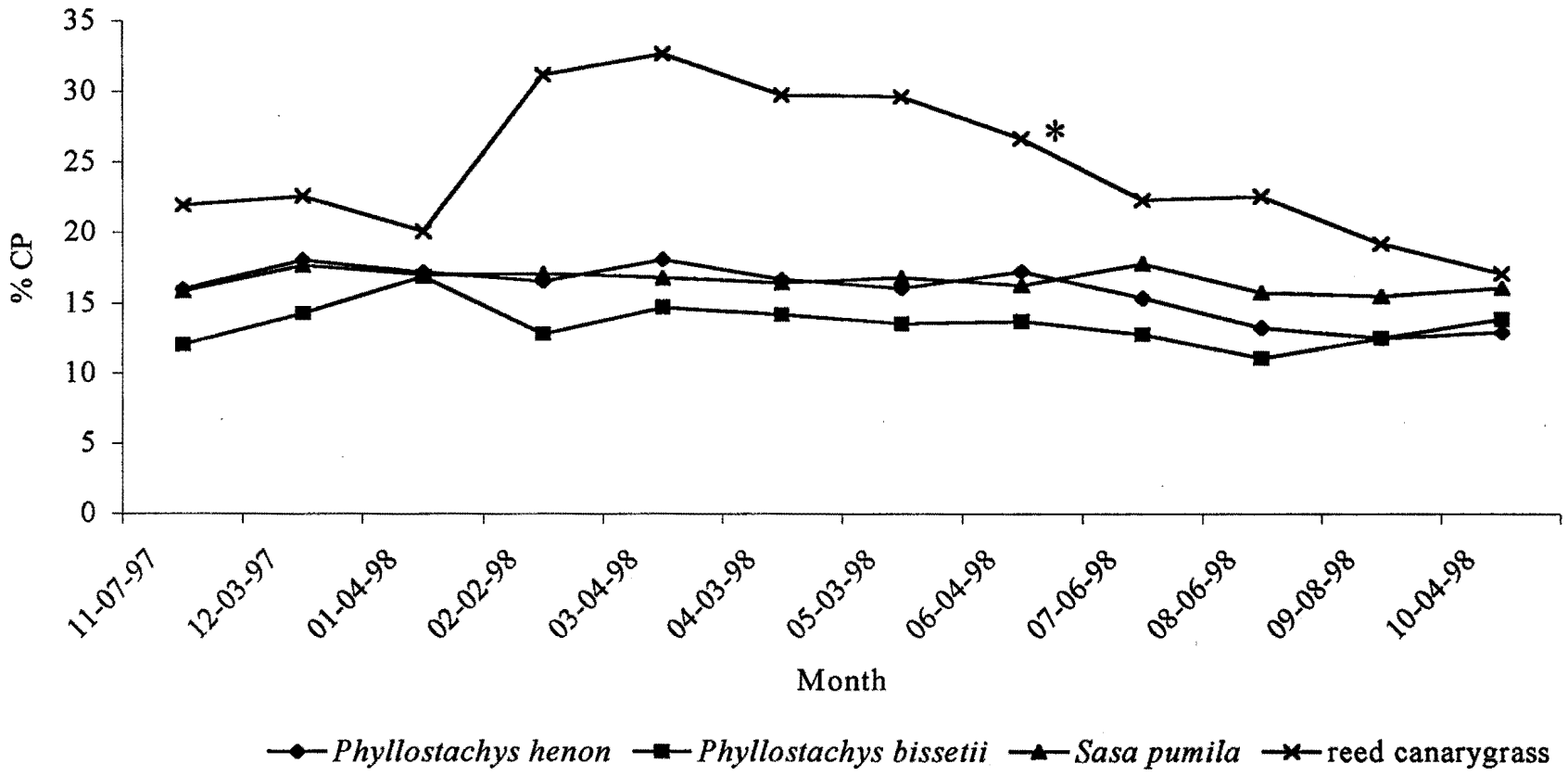

Figure 2.3. Seasonal changes in percent crude protein (% CP) of *P. henon*, *P. bissetii*, *S. pumila*, and reed canarygrass. Month^2 x Forage interaction was significant ($P < .001$; Extra-SS *F*-test). *Trend for reed canarygrass was significantly different ($P < .001$) from the three bamboos. Each data point represents the single monthly sample taken.

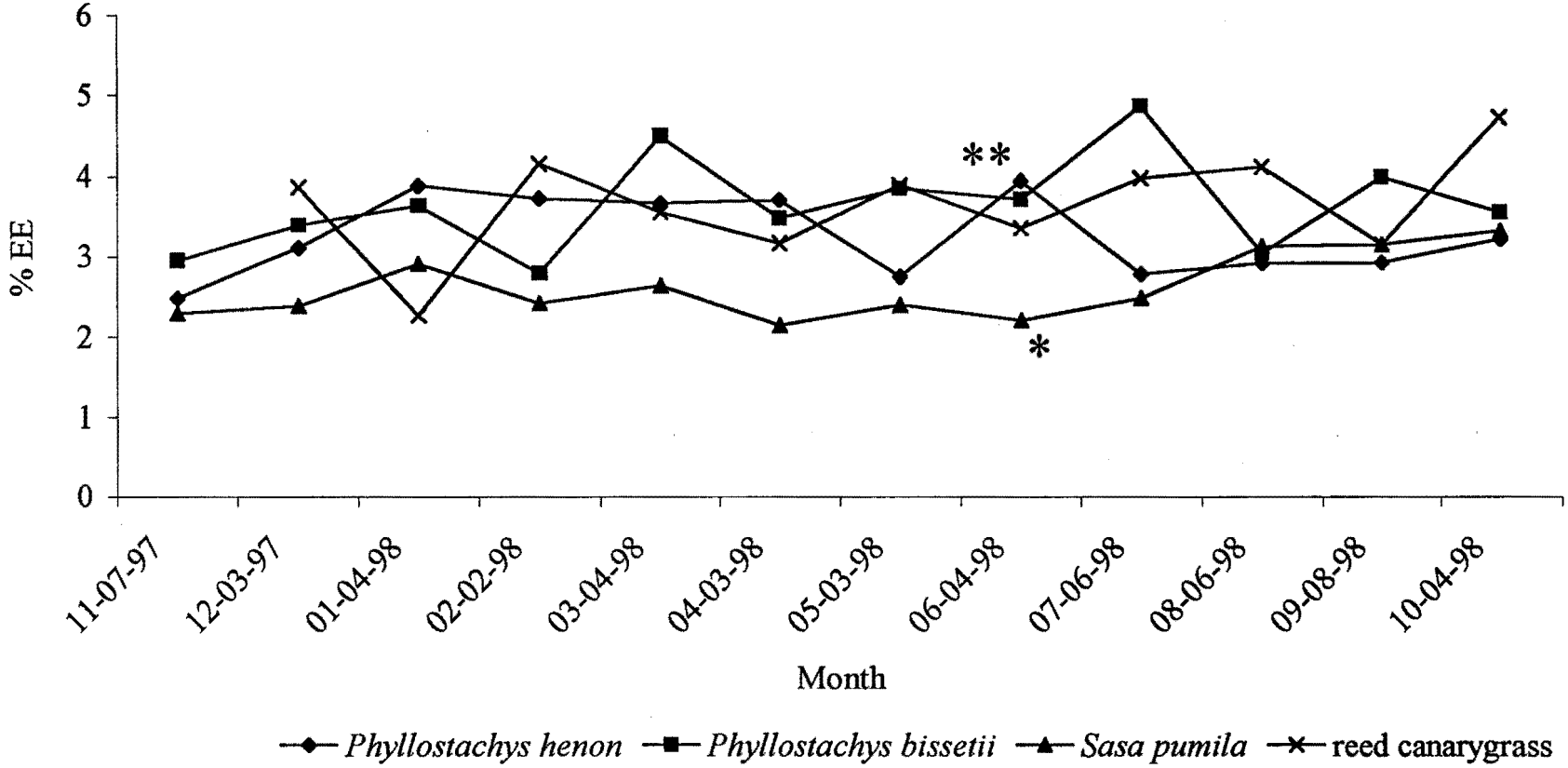

Figure 2.4. Seasonal changes in percent ether extract (% EE) of *P. henon*, *P. bissetii*, *S. pumila*, and reed canarygrass. Month^2 and Month^2 x Forage lacked significance ($P > .10$; Extra-SS *F*-test). *Mean EE for reed canarygrass differed significantly ($P < .01$) from the three bamboos; **mean EE for *P. henon* differed significantly ($P < .01$) from *S. pumila*, and from *P. bissetii* ($P < .10$). Each data point represents the single monthly sample taken.

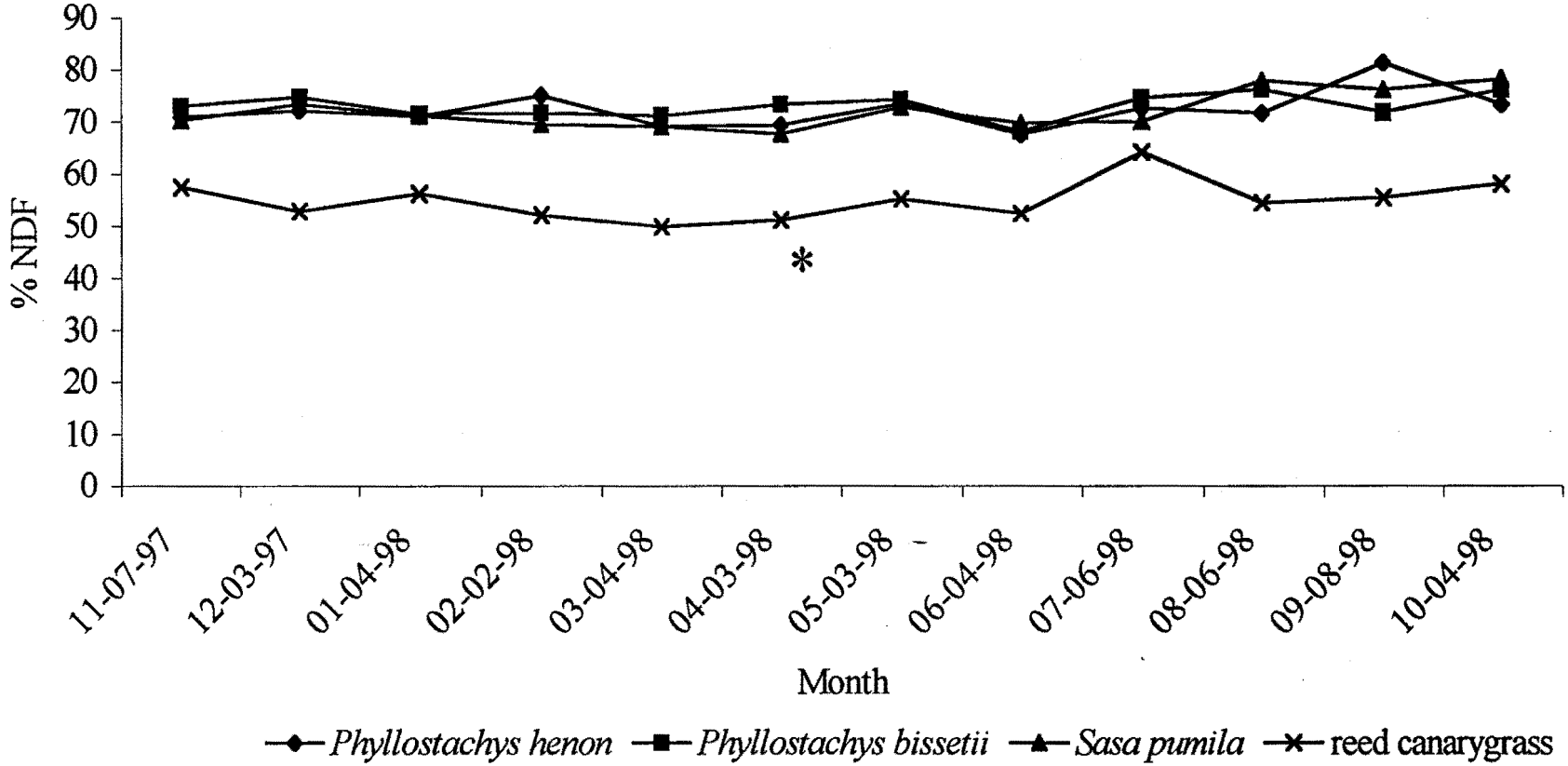

Figure 2.5. Seasonal changes in percent neutral detergent fiber (% NDF) of *P. henon*, *P. bissetii*, *S. pumila*, and reed canarygrass. Month^2 x Forage lacked significance ($P > .10$; Extra-SS *F*-test). *Trend for reed canarygrass was significantly different ($P < .0001$) from the three bamboos. Each data point represents the single monthly sample taken.

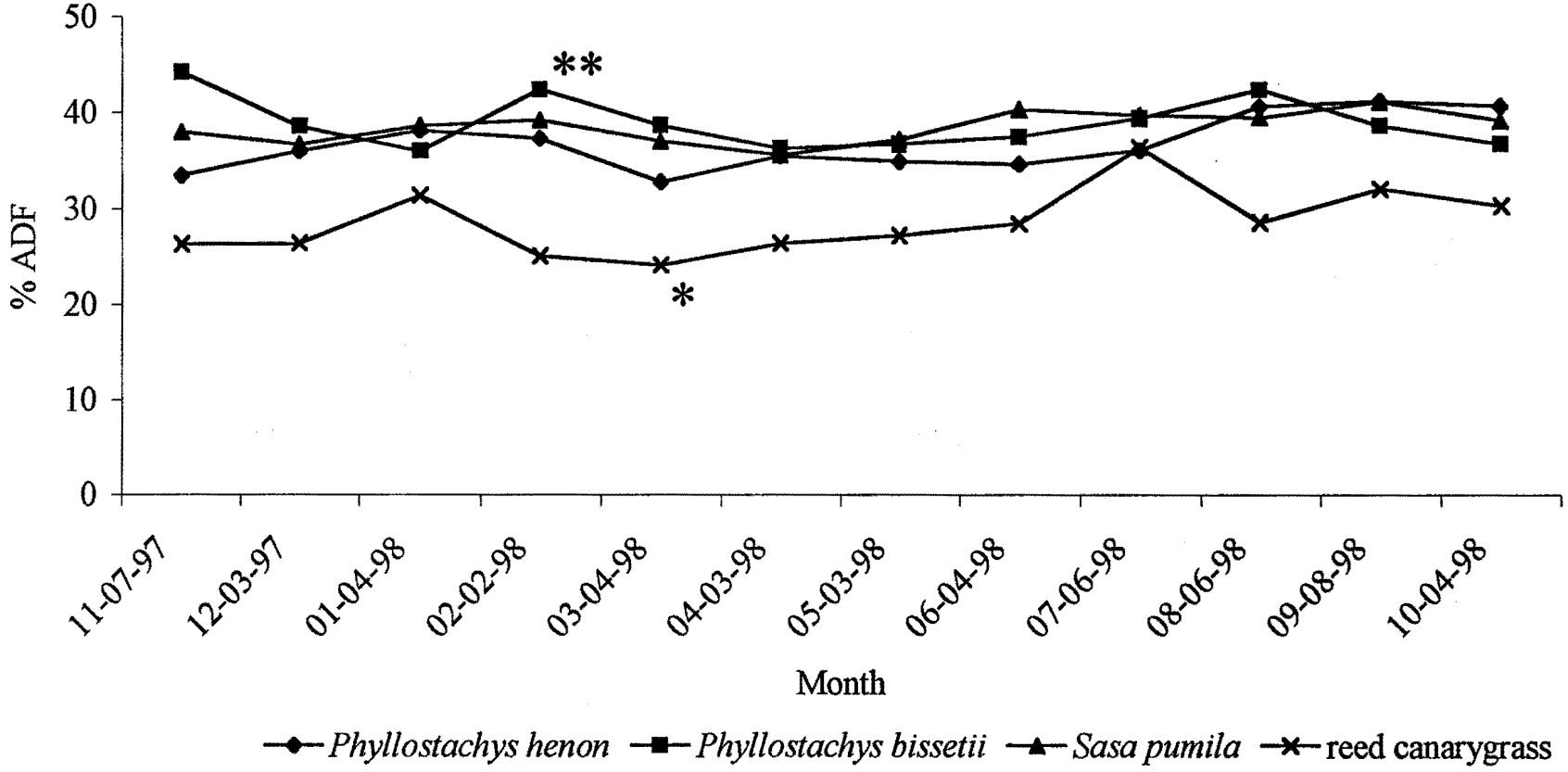

Figure 2.6. Seasonal changes in percent acid detergent fiber (% ADF) of *P. henon*, *P. bissetii*, *S. pumila*, and reed canarygrass. Month^2 x Forage lacked significance ($P > .10$; Extra-SS *F*-test). *Trend for reed canarygrass was significantly different ($P < .0001$) from the three bamboos; **trend for *P. henon* differed significantly from *P. bissetii* ($P < .05$). Each data point represents the single monthly sample taken.

Table 2.1. Proximate analysis for *P. henon*, *P. bissetii*, *S. pumila*, and reed canarygrass

Item	*P. henon*[b]	*P. bissetii*	*S. pumila*	Reed canarygrass
DM[a] Range	34.0-52.4	34.4-53.6	31.9-39.9	12.9-32.1
Mean ± SE	45.0 ± 1.5	45.3 ± 1.5	36.8 ± .7	21.2 ± 2.0
ASH Range	7.9-12.6	6.1-16.1	8.1-12.6	9.3-13.2
Mean ± SE	9.9 ± .4	9.7 ± .8	10.2 ± .4	10.8 ± .04
CP Range	12.6-18.2	11.1-16.9	15.5-17.8	17.1-32.7
Mean ± SE	15.9 ± .6	13.5 ± .4	16.6 ± .2	24.7 ± 1.5
EE Range	2.5-3.9	2.8-4.9	2.1-3.3	2.3-4.7
Mean ± SE	3.3 ± .1	3.6 ± .2	2.6 ± .1	3.7 ± .2
ADF Range	32.8-41.3	36.0-44.2	35.6-41.2	24.1-36.4
Mean ± SE	36.8 ± .8	39.0 ± .8	38.5 ± .5	28.6 ± 1.0
NDF Range	67.5-81.4	68.1-76.3	67.8-78.4	49.8-64.1
Mean ± SE	72.3 ± 1.0	73.1 ± .7	72.2 ± 1.0	55.0 ± 1.1

[a] DM, ash, CP, EE, ADF, and NDF values on a percent DM basis.
[b] n = 12, except for reed canarygrass EE where n = 11.

of 22.0% and 34.0% for Pony A and B, respectively. The digestibility values of CP for bamboo were comparable to the CPD (> 60%) of a natural grassland hay as was determined by Vermorel et al. (1997) in ponies. ADFD and NDFD for *P. bissetii* in Pony B (13.8%; 14.8%) were lower than that of Pony A (16.0%; 31.5%). Grass hay ADFD for Pony B was also lower than that of Pony A (16.4% versus 24.4%). NDFD for the grass hay diet was 25.5% for Pony A and 20.2% for Pony B. Vermorel et al. (1997) reported ADFD and NDFD values of over 45% for natural grassland hay. Differences in the nutrient content of the bamboo fed during Period 1 compared to Period 2 can be

attributed to a number of factors such as time of harvest, stage of growth, as well as soil and climate conditions.

Table 2.2. Digestibility of dry matter (DMD, crude protein (CPD), acid detergent fiber (ADFD), and neutral detergent fiber (NDFD) of grass hay and bamboo (*P. bissetii*)

Diet	Pony	DMD %	CPD %	ADFD %	NDFD %
Grass hay	A	33.6	22.0	24.4	25.5
	B	28.3	34.0	16.4	20.2
Bamboo (*P. bissetii*)	A	33.7	75.4	16.0	31.5
	B	19.4	59.6	13.8	14.8

Low digestibility of bamboo may be related to its high insoluble ash content. Most of the crude ash in bamboo leaves is silicate (Ueda, 1961). Dierenfeld (1997) stated that bamboo is quite high in silica, not unlike other members of the grass family (Jones and Handreck, 1967). Levels of silica vary throughout the year with the highest amounts evident in the coldest months. Insoluble ash can be used as an estimate of forage silica content (Goering and Van Soest, 1970).

The access to cell wall carbohydrates by digestive microorganisms may be inhibited by silica (Jones and Handreck, 1967; Bae et al., 1997), with the effects of silica including decreased digestibility and palatability (Van Soest, 1982). There is evidence of a decrease in DMD of up to 3 units per unit of silica in temperate forages (Van Soest and Jones, 1968). Minson (1971) evaluated the influence of silicon and lignin on the digestibility of cell wall constituents in sheep. He reported a strong correlation between the digestibility of hemicellulose and cellulose as related to lignification, but found that silica did not play a role in organic matter, cellulose, or hemicellulose digestibility. This

is in contrast to the findings of Van Soest and Jones (1968) who found that both silica and lignin affect digestibility, and more so when considered in combination with each other. Lignin, like silica, is a structural component of cell walls, and its digestibility is minimal. ADL in the bamboo fed during Period 1 of this trial was 11.1% and during Period 2 was 10.0%, which is similar to the lignin content of oat straw (11%; Slagsvold et al., 1979). Bamboo A had 2.9% ADA and Bamboo B had 3.6%.

Differences in the digestibility of bamboo between the two ponies may in part be due to the nutrient content of the forage offered compared to that which was refused (Table 2.3). Additionally, differences in dry matter intake (DMI) of both grass hay and bamboo by the two ponies may have influenced digestibility (Table 2.4). The lower DMI by Pony A could explain the higher digestibility values obtained for that animal.

Table 2.3. Proximate composition of bamboo (*P. bissetii*) offered to and refused by two pony mares

Pony	Bamboo diet	AIA %	CP %	ADF %	NDF %
A	Offered	4.1	10.9	43.0	73.5
	Refused	.7	3.1	53.9	80.9
B	Offered	3.3	7.9	46.6	74.5
	Refused	.6	3.5	52.3	81.3

Few nutritional analyses exist for bamboo, especially ones that depict changes over time or differences among bamboo spp. In the present study, the trends in proximate composition of bamboo were explained only in terms of bamboo spp. and time

of sampling; however, other factors such as stage of growth, as well as soil and climate conditions may influence bamboo composition.

Table 2.4. Dry matter intake (DMI) of grass hay and bamboo diets by two pony mares

	DMI	
Diet	Pony A	Pony B
Grass hay (kg)	3.9	4.3
Bamboo (kg)	3.0	3.6

Use of bamboo as a sole source of fodder for ponies can not be recommended at this time. Dry matter digestibility, CPD, ADFD, and NDFD of the forages were generally below 30% in both ponies, with the CPD of bamboo being the only exception. The low nutrient digestibility may prevent the animal from consuming enough to meet energy needs. Equids may be able to compensate for low digestibility via *ad libitum* consumption of high fiber forages (Illius and Gordon, 1990), and thereby meet their energy requirements (Cymbaluk et al., 1989). The digestibility of bamboo may be enhanced by treatment with anhydrous ammonia or boiling. For example, use of anhydrous ammonia on low quality forages such as straw can lead to improved energy and crude protein values (Cuddeford, 1986). In Japan, processing of bamboo enhances its feed value (Manda et al., 1990). Boiling bamboo decreases its NDF, ADF, and lignin content, and increases bamboo palatability in goats and cattle compared to untreated bamboo. Such processing may, however, not be practical in the U. S., since better quality forages are available.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Don Emenhiser, Alan Rack, Ned Jaquith, and Rick Valley for graciously donating bamboo for this study, Russ Henning from Oregon State University Farm Services for his assistance in harvesting bamboo, and Scott Paxton from the Oregon State University Beef Center for providing the facilities for bamboo storage.

REFERENCES

AOAC. 1995. Official Methods of Analysis (16th Ed.). Association of Official Analytical Chemists, Washington, DC.

Bae, H. D., McAllister, T. A., Kokko, E. G., Leggett, F. L., Yanke, L. J., Jakober, K. D., Ha, J. K., Shin, H. T., and K.-J. Cheng. 1997. Effect of silica on the colonization of rice straw by ruminal bacteria. Anim. Feed Sci. Technol. 65:165-181.

Block, E., Kilmer, L. H., and L. D. Muller. 1981. Acid insoluble ash as a marker of digestibility for sheep fed corn plants or hay and for lactating dairy cattle fed hay *ad libitum*. J. Anim. Sci. 52:1164-1169.

Cuddeford, D. 1986. Equine practice: Alternative feedstuffs for horses. In Practice, March, pp 68-70.

Cuddeford, D., Pearson, R. A., Archibald, R. F., and R. H. Muirhead. 1995. Digestibility and gastro-intestinal transit times of diets containing different proportions of alfalfa and oat straw given to Thoroughbreds, Shetland ponies, Highland ponies and donkeys. Anim. Sci. 61:407-417.

Cymbaluk, N. F., Christison, G. I., and D. H. Leach. 1989. Nutrient utilization by limit- and ad libitum-fed growing horses. J. Anim. Sci. 67:414-425.

Dierenfeld, E. S. 1997. Chemical composition of bamboo in relation to giant panda nutrition. In: The Bamboos. pp 205-211. The Linnean Society, London.

Farrelly, D. 1984. The Book of Bamboo. p 280. Sierra Club Books, San Francisco, CA.

Goering, H. K, and P. J. Van Soest. 1970. Forage fiber analysis. Agric. Handbook No. 379. ARS, USDA, Washington, DC.

Illius, A. W., and I. Gordon. 1990. Constraints on diet selection and foraging beviour in mammalian herbivores. In: R. N. Hughes (Ed.) Behavioural Mechanisms of Food Selection. Springer-Verlag, Berlin.

Jones, L. H. P., and K. A. Handreck, K. A. 1967. Silica in soils, plants, and animals. Adv. Agron. 19:107-149.

Lawson, A. H. 1968. Commercial uses of the bamboo. In: Bamboos: A Gardener's Guide to their Cultivation in Temperate Climates. pp 68-69. Taplinger Publishing Company, NY.

Manda, M., Osa, E., Tokuda, H., and I. Kurohiji. 1990. Feeding value of bamboo. In: Bulletin of the Faculty of Agriculture. pp 173-179. Kagoshima University, Japan.

McClure, F. A. 1958. Bamboo as a source of forage. In: Pacific Sci. Assoc., 8th Pacific Sci. Congr. Proc. 4:609.

McDonald, E. 1998. Gardening with bamboo. Pacific Northwest Bamboo 14 (2):6-8.

Minson, D. J. 1971. Influence of lignin and silicon on a summative system for assessing the organic matter digestibility of *Panicum*. Aust. J. Agric. 22:589-598.

Nelson, G., Keller, M., and P. R. Cheeke. 1997. Evaluation of temperate bamboo species as forages for livestock. Paper presented at the Pacific Northwest Bamboo Agro-Forestry Workshop, June 1997, Port Townsend, WA.

NRC. 1989. Nutrient Requirements of Horses (5th Ed.). National Academy Press, Washington, DC.

SAS. 1988. SAS/STAT® User's Guide (Release 6.03). SAS Inst. Inc., Cary, NC.

Shepherd, W. O. 1952. Highlights of forest grazing research. J. For. 50:280-283.

Slagsvold, P., Hintz, H. F., and H. F. Schryver. 1979. Digestibility by ponies of oat straw treated with anhydrous ammonia. Anim. Prod. 28:347-352.

Thonney, M. L. 1981. Acid insoluble ash as digestion marker. In: Proc. Cornell Nutrition Conference for Feed Manufacturers. pp 118-122. Cornell University, Ithaca, NY.

Ueda, K. 1961. Report on a study on improvement in bamboo plantation in India. Agricultural Department of the Kyoto University on behalf of the Japan Consulting Institute.

Van Keulen, J., and B. A. Young. 1977. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. J. Anim. Sci. 44:282-287.

Van Soest, P. J. 1982. Silica. In: Nutritional Ecology of the Ruminant. pp 72-74. Cornell University Press, USA.

Van Soest, P. J., and L. H. P. Jones. 1968. Effect of silica in forages upon digestibility. J. Dairy Sci. 51:1644-1648.

Vermorel, M., Vernet, J., and W. Martin-Rosset. 1997. Digestive and energy utilisation of two diets by ponies and horses. Livest. Prod. Sci. 51:13-19.

CHAPTER 3
EVALUATION OF *YUCCA SCHIDIGERA* ON *IN VITRO* DRY MATTER DISAPPEARANCE IN PONIES USING FECES AS A SOURCE OF INOCULUM

S. L. Greenway, M. Keller, and P. R. Cheeke
Department of Animal Sciences
Oregon State University

ABSTRACT

Feces from two mature pony mares were collected to provide a source of inoculum for the *in vitro* dry matter disappearance (IVDMD) determination of four forages. Feedstuffs analyzed included bamboo (Bamboo A, Bamboo B) used in a previous *in vivo* experiment, as well as orchard grass hay and alfalfa hay. The effect of *Yucca schidigera* extract (YE) on IVDMD for all forages was determined. Treatment levels were 0, 750, 1500, and 3000 ppm YE for bamboo and hay. Bamboo IVDMD was additionally analyzed at 250 and 500 ppm YE. IVDMD of Bamboo A increased ($P < .10$) with 750 ppm YE treatment during the first experiment, but not the second. *In vitro* analysis of Bamboo B indicated that addition of 750 ppm YE lowered IVDMD when compared to the control and 500 ppm YE ($P < .01$), as well as 250 ppm ($P < .05$). Comparisons among the control, 250, and 500 ppm showed no significant difference ($P > .10$) in mean IVDMD for Bamboo B. A second analysis of 750 ppm extract added to Bamboo B increased IVDMD ($P < .10$) when compared to the control, contrary to the decrease seen in the first experiment. IVDMD of Bamboo B was not different between the control and 1500 ppm ($P > .10$); however, mean IVDMD with the addition of 1500 ppm was lower than 750 ppm and 3000 ppm ($P < .05$). No treatment effect was seen on the mean IVDMD of alfalfa hay ($P > .10$). Application of 3000 ppm YE to orchard grass hay *in vitro* resulted in a significant increase ($P < .01$) in the mean IVDMD when compared to the control, 750 ppm, and 1500 ppm. IVDMD for the control, 750 ppm, and 1500 ppm did not differ from each other ($P > .10$). Addition of YE had no effect on IVDMD of either Bamboo A or alfalfa hay. The effects of YE treatment on feedstuffs *in vitro* were variable depending upon treatment level and type of forage evaluated.

INTRODUCTION

A number of equine supplements comprised either partially or entirely of *Yucca schidigera* (*Y. schidigera*) have been marketed to potentially improve metabolism, and relieve arthritis, stiffness, and joint pain. Few studies have been conducted on equids to evaluate the effects of yucca extract on digestibility.

The Tilley and Terry method (1963) has been an established method for *in vitro* determination of forage digestibility. This method requires maintaining fistulated animals in order to obtain rumen or cecal fluid. Recent studies have indicated the feasibility of using feces as a source of inoculum for *in vitro* digestibility experiments in both ruminants (El Shaer et al., 1987; Omed et al., 1989; Akhter et al., 1994) and non-ruminants (Sunvold et al., 1995a, 1995b, 1995c). *In vitro* fermentation using horse feces is comparable to analyses using cecal fluid as a source of inoculum (Lowman et al., 1996; Kirkhope and Lowman, 1996). The digestibility analysis of roughages appears to be better suited to this procedure than concentrates (Omed et al., 1989), although there is some evidence that even among forages there is variability in how well the *in vivo* and *in vitro* values agree (El Shaer et al., 1987).

Y. schidigera contains saponins which have species-dependent bacterial and antiprotozoal activities (Wallace et al., 1994; Wang et al., 1998), with the mechanism of antiprotozoal activity being cell membrane lysis (Cheeke, 1998). Most studies regarding the impact of *Y. schidigera* on animal nutrition have been conducted on ruminants (Goetsch and Owens, 1985; Wang et al., 1998). Although Kern et al. (1973) did not identify protozoa common to both ponies and steers, it is possible that yucca would have similar effects on gut microbes regardless of host type. Glade (1992) concluded that

feeding yucca extract to equines tended to increase the digestibilities of DM, NDF, ADF, cellulose, and hemicellulose, but the treatment effect was not necessarily significant ($P > .05$). In a defaunation study done by Moore and Dehority (1993) on ponies it was determined that overall DMD decreased only slightly ($P < .10$) by defaunation. The authors concluded that the absence of protozoa had little impact on how well forages were digested, and that it was possible that bacteria and fungi compensated for the loss of protozoa and their actions in the gut. This would indicate that even if yucca extract were antiprotozoal in nature, digestibility in the equid might not be greatly affected by loss of protozoal numbers.

Bacterial populations that remain unchecked by protozoa could potentially affect the concentration of ammonia in the gut, since bacteria utilize ammonia for protein synthesis (Leng and Nolan, 1984; McAllister et al., 1998). Dietary nitrogen utilization was decreased when yucca extract (1 mL/ kg feed) was added to the diet of weanling foals (Glade, 1992), and subsequently resulted in decreased growth rate. Bacteria that digest forages are more greatly inhibited by yucca than those that digest concentrates (Wang et al., 1998). Killeen et al. (1998) stated that the antimicrobial activity of yucca extract would have little effect on bacterial numbers in the animal at supplementation levels presently recommended, but those levels are not reflected in what is fed to equids (see Materials and Methods). Saponins may cause increased intestinal wall permeability and decreased active nutrient transport, resulting in impaired nutrient absorption (Johnson et al., 1986; Wang et al., 1997). If, however, bacteria and protozoa were affected by inclusion of yucca extract in the diet, the resulting changes in availability of energy and

nitrogen to microbes may affect the efficiency with which particularly fibrous forages are digested (Alexander, 1952; Cuddeford et al., 1995).

The present study was conducted to determine the effects of YE on the *in vitro* dry matter disappearance of bamboo, alfalfa hay, and orchard grass hay, thereby predicting possible effects of *Y. schidigera* supplementation in horses.

MATERIALS AND METHODS

Two pony mares previously used for *in vivo* analysis of the bamboo, *Phyllostachys bissetii* (*P. bissetii*; Greenway et al., 1999), were provided a diet of timothy hay, oat hay, and grain, morning and evening. Ponies were on the diet for a week prior to the first fecal collection. The ponies had free access to water and were housed together in an outdoor corral.

Fecal samples were collected immediately after defecation, placed in separate plastic bags, and immediately transported in a pre-warmed (37°C) thermos to the laboratory for processing. After each collection equal amounts of feces from the two ponies were composited for *in vitro* analysis. Feces for the first determination of IVDMD were processed immediately upon return to the laboratory. In contrast, feces for the second IVDMD analysis were frozen directly after collection, and thawed prior to analysis as described by Akhter et al. (1994).

In vitro artificial digestion was conducted using a modified Tilley and Terry (1963) method. Bamboo (*P. bissetii*) fed during the two fecal collection periods of the previous *in vivo* digestibility trial was used for the present *in vitro* analysis. In addition to

the bamboos, an orchard grass hay and an alfalfa hay were evaluated for IVDMD. Dry matter, CP, and ADF for all forages is presented in Table 3.1. Forage samples (350 mg)

Table 3.1. Nutrient composition of forages used for *in vitro* analysis

Forage	% DM	% CP	% ADF
Bamboo A[a]	61.9	7.9	46.6
Bamboo B	47.4	10.9	43.0
Alfalfa hay	94.0	22.4	24.0
Orchard grass hay	92.1	11.8	35.3

[a] Bamboo A refers to the bamboo fed during the fecal collection of Period 1 of the *in vivo* study, Bamboo B refers to the bamboo fed during the fecal collection of Period 2 of the same study.

were added to each *in vitro* tube (40 mL) followed by treatment with *Yucca schidigera* extract (YE; Foamation *R*, Desert King International, Chula Vista, CA). Label directions on a number of yucca supplements for horses simply indicate feeding on a scoop-per-day basis, with no reference to feed intake of the animal. If the contents of a scoop weighed approx. 6 g, then the ppm YE fed to a 200-kg pony would differ from that fed to a 500-kg horse. A scoop for a 200-kg pony consuming feed at 1.8% (NRC, 1989) of its body weight (3.6 kg) would be equivalent to 1667 ppm, whereas the 600-kg horse eating 10.8 kg of feed per day would only be receiving 556 ppm of yucca in its diet. To simulate the ranges of possible YE supplementation, incremental levels of YE were used. Treatment levels for the first *in vitro* analysis (Exp. 1) were 0, 250, 500, and 750 ppm and the second analysis (Exp. 2) evaluated 0, 750, 1500, and 3000 ppm of YE. The DM of the

YE used in this experiment was 47.5%; however, all treatment levels were quantified on a liquid basis.

A Waring blender was used to macerate and combine feces with McDougall's buffer (McDougall, 1948), which was then strained through four layers of cheesecloth. The fecal liquor (35 mL) was then added to each *in vitro* tube and contents artificially digested at 39° C for 48 h, as recommended by Nsahlai and Umunna (1996) and Sunvold et al. (1995c). After incubation, contents were centrifuged at 348·*g* and the supernatant removed. HCl-pepsin (25 mL) was added to each tube, and contents digested for another 48 h (39° C). Centrifugation and removal of supernatant was repeated as before. Tubes were placed in a 100° C oven for 24 h and subsequently weighed.

Data were analyzed using One-way ANOVA in SAS v. 6.12 (SAS, 1988). Multiple Linear Regression indicated significant differences of some, but not all treatment-forage interactions; therefore, the effects of varying treatment levels were evaluated for each forage via pairwise comparisons. Means and standard errors were obtained using distribution analysis in SAS v. 6.12 (SAS, 1988). No statistical comparisons were made between forage types.

RESULTS AND DISCUSSION

Effects of YE on forage IVDMD are shown in Table 3.2 and 3.3. The mean IVDMD of Bamboo A for both Exp. 1 and Exp. 2 showed no evidence of a treatment effect, except with the addition of 750 ppm in Exp. 1 ($P < .10$). The IVDMD analysis (Exp. 1) of Bamboo B indicated that 750 ppm reduced dry matter disappearance ($P < .01$) compared to the control and 500 ppm and was moderately lower than that obtained for

250 ppm ($P < .05$). Comparisons among the control, 250, and 500 ppm showed no significant difference ($P > .10$) in mean IVDMD for Bamboo B. The second IVDMD analysis of Bamboo B showed no evidence ($P > .10$) of a difference in mean IVDMD between 1500 ppm extract and the control. Mean IVDMD differed between 750 ppm and 1500 ppm ($P < .05$), and the control ($P = 05$). Use of 3000 ppm YE decreased IVDMD when compared to the control and 1500 ppm ($P < .05$), as well as 750 ppm ($P < .01$). No treatment effect was seen on the mean IVDMD of alfalfa hay ($P > .10$). The use of 3000 ppm of YE resulted in a significant increase ($P < .01$) in the mean IVDMD of orchard grass hay as compared to the control, 750 ppm, and 1500 ppm. IVDMD for the control, 750 ppm, and 1500 ppm did not differ from each other ($P > .10$). IVDMD for Bamboo A and Bamboo B with no YE treatment were similar to the DMD determined in the previous *in vivo* experiment (Greenway et al., 1999). The DMD *in vivo* of Bamboo A was lower (19.4%) than Bamboo B (33.7%), with each value representing the

Table 3.2. Experiment 1: Effects on percent *in vitro* dry matter disappearance (% IVDMD) of bamboo with addition of varying levels of *Yucca schidigera* extract (YE)

		ppm YE			
Forage	Item	0	250	500	750
Bamboo A	Mean[ab]	28.8[ef]	29.0[ef]	28.6[e]	29.9[f]
	SE	.4	.5	.5	.5
Bamboo B	Mean	33.4[c]	33.2[ce]	33.9[c]	31.7[d]
	SE	.2	.2	.7	.4

[a] Values on a percent basis.
[b] n = 10
[cd] Means in a row with different superscripts differ ($P < .01$).
[de] Means in a row with different superscripts differ ($P < .05$).
[ef] Means in a row with different superscripts differ ($P < .10$).

Table 3.3. Experiment 2: Effects on percent *in vitro* dry matter disappearance (% IVDMD) of bamboo, alfalfa hay, and orchard grass hay with addition of varying levels of *Yucca schidigera* extract (YE)

		ppm YE			
Forage	Item	0	750	1500	3000
Bamboo A	Mean[ab]	26.1	26.7	26.9	26.6
	SE	.7	.3	.3	.2
Bamboo B	Mean	29.4[ef]	30.7[c]	29.3[e]	27.9[d]
	SE	.4	.6	.2	.5
Alfalfa hay	Mean	76.0	75.6	76.5	74.7
	SE	.7	1.0	1.1	1.4
Orchard grass hay	Mean	59.5[c]	59.5[c]	60.7[c]	63.4[d]
	SE	.6	.5	.6	.6

[a] Values on a percent basis.
[b] n = 5, except 0 and 3000 ppm for both Bamboo B and orchard grass hay, where n = 4.
[cd] Means in a row with different superscripts differ (P < .01).
[de, ce] Means in a row with different superscripts differ (P < .05).
[cf] Means in a row with different superscripts differ (P < .10).

digestibility within a particular pony. In the present experiment, however, feces from the two ponies were composited and used to determine the IVDMD of each bamboo. Although the differences in digestibility of the two bamboos were not as apparent as in the *in vivo* study, Bamboo A was still lower in digestibility than Bamboo B. *In vitro* values obtained for alfalfa and orchard grass hay were in agreement with those obtained by other researchers for equids *in vivo* (Table 3.4). The similar results *in vitro* versus *in vivo* confirm the validity of using feces as a source of inoculum for *in vitro* fermentation. In addition, the use of frozen and then thawed feces for inoculum appeared to be as effective in predicting digestibility as the use of fresh feces. Most other research concerning YE has been conducted on ruminants. YE had no effect on DMD in the RUSITEC unit (Wang et al., 1997), whereas supplementation increased organic matter

Table 3.4. Percent dry matter digestibility (% DMD) for alfalfa hay and orchard grass hay

Forage	% DMD	Reference
Alfalfa hay	64.4 (h)[a]	Cymbaluk (1990)
	62.0 (p)	Cymbaluk and Christensen (1986)
	60.4 (h)[b] and 62.5 (p)	Slade and Hintz (1969)
	53.4 (p)[c] and 55.2 (p)	Carle et al. (1975)
	60.81 (h)	Vander Noot and Gilbreath (1970)
	52.1 (h)[d] and 58.5 (h)	Fonnesbeck et al. (1967)
Orchard grass hay	49.99 (h)	Vander Noot and Gilbreath (1970)
	47.2 (h)	Fonnesbeck et al. (1967)

[a] (h) refers to horse and (p) refers to pony.
[b] Values are for organic matter digestibility.
[c] Values are for alfalfa hay from two different sources.
[d] Values are from two separate experiments.

digestibility *in vivo*, but not *in situ* (Goetsch and Owens, 1985). Glade (1992) evaluated the effects of *Y. schidigera* on weanling foals and found that DMD tended to be enhanced by supplementation with YE; however, the data was not statistically significant. Although microbial numbers were not assessed in the present study, it is possible that defaunation played a role in the effects seen on dry matter disappearance. The effects of YE on digestibility varied depending upon forage type. The microorganisms effective in degrading bamboo, which is high in silica, may have been negatively affected by yucca, whereas the bacteria involved in the digestion of orchard grass hay were stimulated or at least indirectly affected by yucca's antiprotozoal activity. *In vivo* responses may differ from those obtained here due to the effects of saponins on membrane permeability, which was not recreated *in vitro*.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to Desert King International for financial support, as well as Sunny Morris for her assistance in the Oregon State University Animal Nutrition Laboratory.

REFERENCES

Akhter, S., Owen, E., Fall, A., O'Donovan, F., and M. K. Theodorou. 1994. Use of fresh or frozen cow faeces instead of sheep rumen liquor to provide micro-organisms for *in vitro* digestibility assays for forages. Anim. Prod. 58: 452 (Abstr.).

Alexander, F. 1952. Some functions of the large intestine of the horse. Q. J. Exp. Physiol. 37:205-214.

Carle, C. J., Fontenot, J. P., and K. E. Webb, Jr. 1975. Digestibility of nutrients from alfalfa hay, corn and oats by ponies. In: Livestock Research Report. pp 151-154. Virginia Polytechnic Institute and State University, Blacksburg, VA.

Cheeke, P. R. 1998. Saponins: Surprising benefits of desert plants. In: The Linus Pauling Institute Newsletter. pp 4-5. Oregon State University, Corvallis, OR.

Cuddeford, D., Pearson, R. A., Archibald, R. F., and R. H. Muirhead. 1995. Digestibility and gastro-intestinal transit time of diets containing different proportions of alfalfa and oat straw given to Thoroughbreds, Shetland ponies, Highland ponies and donkeys. Anim. Sci. 61:407-417.

Cymbaluk, N. F. 1990. Comparison of forage digestion by cattle and horses. Can. J. Anim. Sci. 70:601-610.

Cymbaluk, N. F., and D. A. Christensen. 1986. Nutrient utilization of pelleted and unpelleted forages by ponies. Can. J. Anim. Sci. 66:237-244.

El Shaer, H. M., Omed, H. M., Chamberlain, A. G., and R. F. E. Axford. 1987. Use of faecal organisms from sheep for the *in vitro* determination of digestibility. J. Agric. Sci., Camb. 109:257-259.

Fonnesbeck, P. V., Lydman, R. K., Vander Noot, G. W., and L. D. Symons. 1967. Digestibility of the proximate nutrients of forage by horses. J. Anim. Sci. 26:1039-1045.

Glade, M. J. 1992. Effects of *Yucca shidigera* extract on feed utilization by equine weanlings. Equine Veterinary Science 12:93-98.

Goetsch, A. L., and F. N. Owens. 1985. Effects of sarsaponin on digestion and passage rates in cattle fed medium to low concentrate. J. Dairy Sci. 68:2377-2384.

Greenway, S. L., Keller, M., Nelson, G., Cheeke, P. R., and D. J. Carroll. 1999. Evaluation of temperate bamboo foliage as an animal feedstuff: Seasonal changes in composition and digestibility in ponies. In: Proc., Western Section, Amer. Soc. Anim. Sci. 50:193-196.

Johnson, I. T., Gee, J. M., Price, K., Curl, C., and G. R. Fenwick. 1986. Influence of saponins on gut permeability and active nutrient transport in vitro. J. Nutr. 116:2270-2277.

Kern, D. L., Slyter, L. L., Weaver, J. M., Leffel, E. C., and G. Samuelson. 1973. Pony cecum *vs.* steer rumen: The effect of oats and hay on the microbial ecosystem. J. Anim. Sci. 37:463-469.

Killeen, G. F., Connolly, C. R., Walsh, G. A., Duffy, C. F., Headon, D. R., and R. F. Power. 1998. The effects of dietary supplementation with *Yucca schidigera* extract or fractions thereof on nitrogen metabolism and gastrointestinal fermentation processes in the rat. J. Sci. Food Agric. 76:91-99.

Kirkhope, R. T. S., and R. S. Lowman. 1996. Use of an *in-vitro* gas production technique with faeces as inoculant to assess tropical forage quality for equids. Anim. Sci. 62:690.

Leng, R. A., and J. V. Nolan. 1984. Nitrogen metabolism in the rumen. J. Dairy Sci. 67:1072-1089.

Lowman, R. S., Theodorou, M. K., Longland, A. C., and D. Cuddeford. 1996. A comparison of equine faeces or caecal digesta as sources of inoculum for *in vitro* fermentation studies using the pressure transducer technique. Anim. Sci. 62: 683-684 (Abstr.).

McAllister, T. A., Wang, Y., Hristov, A. N., Olson, M. E., and P. R. Cheeke. 1998. Applications of *Yucca schidigera* in livestock production. In: Proc., 33rd Annual Pacific Northwest Animal Nutrition Conference. pp 109-119. Vancouver, B. C., Canada.

McDougall, E. I. 1948. Studies on ruminant saliva. 1. The composition and output of sheep's saliva. Biochem. J. 43:99-109.

Moore, B. E., and B. A. Dehority. 1993. Effects of diet and hindgut defaunation on diet digestibility and microbial concentrations in the cecum and colon of the horse. J. Anim. Sci. 71:3350-3358.

NRC. 1989. Nutrient Requirements of Horses (5th Ed.). National Academy Press, Washington, DC.

Nsahlai, I. V., and N. N. Umunna. 1996. Comparison between reconstituted sheep faeces and rumen fluid inocula and between *in vitro* and *in sacco* digestibility methods as predictors of intake and *in vivo* digestibility. J. Agric. Sci., Camb. 126:235-248.

Omed, H. M., Axford, R. F. E., Chamberlain, A. G., and D. I. Givens. 1989. A comparison of three laboratory techniques for the estimation of the digestibility of feedstuffs for ruminants. J. Agri. Sci., Camb. 113:35-39.

SAS. 1988. SAS/STAT® User's Guide (Release 6.03). SAS Inst. Inc., Cary, NC.

Slade, L. M., and H. F. Hintz. 1969. Comparison of digestion in horses, ponies, rabbits and guinea pigs. J. Anim. Sci. 28:842-843.

Sunvold, G. D., Fahey, G. C., Jr., Merchen, N. R., Bourquin, L. D., Titgemeyer, E. C., Bauer, L. L., and G. A. Reinhart. 1995a. Dietary fiber for cats: In vitro fermentation of selected fiber sources by cat fecal inoculum and in vivo utilization of diets containing selected fiber sources and their blends. J. Anim. Sci. 73:2329-2339.

Sunvold, G. D., Fahey, G. C., Jr., Merchen, N. R., Titgemeyer, E. C., Bourquin, L. D., Bauer, L. L., and G. A. Reinhart. 1995b. Dietary fiber for dogs: IV. In vitro fermentation of selected fiber sources by dog fecal inoculum and in vivo digestion and metabolism of fiber-supplemented diets. J. Anim. Sci. 73:1099-1109.

Sunvold, G. D., Hussein, H. S., Fahey, G. C., Jr., Merchen, N. R., and G. A. Reinhart. 1995c. In vitro fermentation of cellulose, beet pulp, citrus pulp, and citrus pectin using fecal inoculum from cats, dogs, horses, humans, and pigs and ruminal fluid from cattle. J. Anim. Sci. 73:3639-3648.

Tilley, J. M. A., and R. A. Terry. 1963. A two-stage technique for the *in vitro* digestion of forage crops. J. Brit. Grassland Soc. 18:104-111.

Vander Noot, G. W., and E. B. Gilbreath. 1970. Comparative digestibility of components of forages by geldings and steers. J. Anim. Sci. 31:351-355.

Wallace, R. J., Arthaud, L., and C. J. Newbold. 1994. Influence of *Yucca shidigera* extract on ruminal ammonia concentrations and ruminal microorganisms. Appl. Environ. Microbiol. 60:1762-1767.

Wang, Y., McAllister, T. A., Newbold, C. J., Cheeke, P. R., and K.-J. Cheng. 1997. Effects of yucca extract on fermentation and degradation of saponins in the RUSITEC. In: Proc., Western Section, Amer. Soc. Anim. Sci. 48:149-152.

Wang, Y., McAllister, T. A., Newbold, C. J., Rode, L. M., Cheeke, P. R., and K.-J. Cheng. 1998. Effects of *Yucca schidigera* extract on fermentation and degradation of steroidal saponins in the rumen simulation technique (RUSITEC). Anim. Feed Sci. Technol. 74:143-153.

CHAPTER 4
USE OF *YUCCA SCHIDIGERA* AND *QUILLAJA SAPONARIA* IN REDUCING AMMONIA EMISSIONS FROM CHICKEN MANURE

S. L. Greenway, M. Keller, P. R. Cheeke
Department of Animal Sciences
Oregon State University

ABSTRACT

Yucca schidigera and *Quillaja saponaria* products were evaluated for their capacity to reduce ammonia emissions from poultry excreta. A standard and saponin-concentrated liquid of both yucca (YE and YU, respectively) and quillaja (QE and QU, respectively) were added to 5 g of excreta at 0, 20, and 200 μL. Treatment levels for yucca (DK-30) and quillaja powders (QCP) were 0, 20, and 200 mg/5g excreta. Saponins were extracted from YE and QE using butanol, and the ammonia-reducing efficacy of the saponin and non-saponin fractions compared (200 μL/5 g excreta). The non-saponin fractions of YE and QE were further separated into tannin and non-tannin constituents, which were also used to treat excreta at 200 μL/5 g. Dry weights were obtained for all products and fractions to provide a reference level of dry solids. QE reduced ammonia emissions when compared to all other products ($P < .0001$). YE and YU were not significantly different from each other within the same treatment level. Effects on ammonia emissions of the two powder products (DK-30 and QCP), as well as YU and QU, were not significantly different when compared within the same treatment level. Comparisons between the powder products (DK-30 and QCP), the 'Ultra' products (YU and QU), and yucca products (YE and YU) were not significantly different when the means of both treatment levels were combined ($P > .05$).

INTRODUCTION

High levels of ambient ammonia (NH_3) have negative effects on poultry, resulting in poor feed intake, decreased egg quality, and respiratory disease (Charles, 1992).

Although levels of 10 ppm NH_3 or more pose health risks to farm workers, it is not uncommon to find levels exceeding 50 ppm in poultry houses (Headon and Walsh, 1993; Crober, 1996). Ammonia levels may be reduced by either dietary modifications or treatment of excreta. Optimal treatment of poultry litter would result in neutralization of free ammonia rather than inhibition of uric acid decomposition (Carlile, 1984).

Ammonia reducing properties of *Y. schidigera* have been studied quite extensively in a variety of non-ruminants. Ammonia binding, urease inhibition, and antiprotozoal activity are among the proposed, but as yet unconfirmed modes of action. Birds excrete excess nitrogen as uric acid (Scott et al., 1982). It is possible that sufficient numbers of microorganisms in poultry excreta are affected by either yucca extract so as to decrease the conversion of uric acid to NH_3. If, however, the process were one of ammonia binding, this would conform to Carlile's (1984) classification of optimal excreta treatment.

The majority of research has focused on reducing ammonia by including yucca in the diet of monogastrics (Al Bar et al., 1993; Dvorak and Watts, 1996). Ammonia levels in layer houses were reduced when YE was added to the diet of hens (Goodall et al., 1988; Crober, 1996). Goodall et al. (1988) also found that yucca incorporated into the diet of broilers reduced ammonia emissions from excreta *in vitro*, whereas Johnston et al. (1981) found no response and Anthony et al. (1994) witnessed an increase in environmental ammonia. Rowland et al. (1976) fed pullets a diet containing up to 465 ppm YE, and observed that ammonia levels remained below 20 ppm regardless of treatment level.

Killeen et al. (1998) extracted saponins from *Y. schidigera*, and evaluated the effects of both the saponin and non-saponin fractions on intestinal fermentation processes in the rat. The non-saponin fraction significantly increased hindgut urease activity and fecal ammonia concentrations, whereas the saponin fraction decreased urease activity in the gut. Sarsasapogenin and smilagenin, two of the saponins within *Y. schidigera*, were identified by the authors as the predominant source of yucca's antimicrobial activity.

Y. schidigera contains spirostan-based steroidal saponins, whereas *Q. saponaria* contains bidesmosidic triterpenoid saponins. The potential ammonia-ameliorating effects of *Q. saponaria* have not been adequately determined. If indeed saponins are responsible for ammonia reduction via binding, different effects may be seen depending upon the predominant saponin type present in the extract (George, 1965).

MATERIALS AND METHODS

Excreta were collected from beneath layer battery cages of no less than five birds within the same feeding group. Layers were fed a standard diet containing a minimum of 15.5% CP and 2800 cal/kg ME. Excreta were thoroughly mixed with a hand mixer, frozen in plastic tubs, and thawed as needed. Fisherbrand Weigh Dishes (1½" x 1"; Cat. No. 2-202A) were individually filled with 5 g (wet wt.) chicken excreta. Initial data indicated that these dimensions would emit ammonia at approximately those levels found to cause irritation of mucous membranes, as mentioned previously. Each excreta-filled weigh dish was placed in a randomly assigned plastic tub and immobilized with a piece of tape (Figure 4.1). A 2-holed stopper was inserted through the center of each tub lid, with a tube inserted for airflow *in* and a tube for airflow *out*. Tub rims and the area

directly around the stoppers, were sealed using Dow Corning vacuum grease to prevent air leaks. Air from a hood outlet was forced through each tub containing excreta and then through boric acid traps comprised of 12 randomly assigned 125-mL Erlenmeyer flasks each containing 59 mL 2% boric acid (Figure 4.2). Flasks were sealed with 2-holed stoppers, with one hole connected to the airflow *out* tube from the tubs, and the second hole remaining open for escape of air. Airflow rate from each open hole was evaluated using a Hewlett-Packard Soap Film Flowmeter (0101-0113; 1-10-100 mL), and valve adjustments made to ensure a similar flow rate through each boric acid trap. Initially each flask was attached to an additional trap to evaluate how much, if any, free ammonia escaped binding in boric acid. Less than 1% NH_3 escaped the first trap and was bound in the secondary traps, which agrees with data obtained by Canh et al. (1998); therefore, only a single flask was attached to each tub. After 3 h, the boric acid solution was titrated with 0.15 N HCl and the percent ammonia in solution determined. Values were calculated as percent of the control, and presented in terms of the amount of ammonia emitted in one hour.

Y. schidigera and *Q. saponaria* products (Desert King International, Chula Vista, CA) were analyzed for their ammonia-reducing properties. Current industry recommendations suggest that yucca or quillaja extract be incorporated into the diet at 100 to 125 ppm; however, data obtained in preliminary studies indicated that these levels were not effective in reducing ammonia emissions from yucca or quillaja treated excreta (data not shown). Products evaluated in the present study included Foamation *R* (YE), Quillaja Extract (QE), Yucca Ultra (YU), Quillaja Ultra (QU), DK Sarsaponin 30 (DK-30), and Quillaja Crude Powder (QCP). The two 'Ultra' liquids are commercially

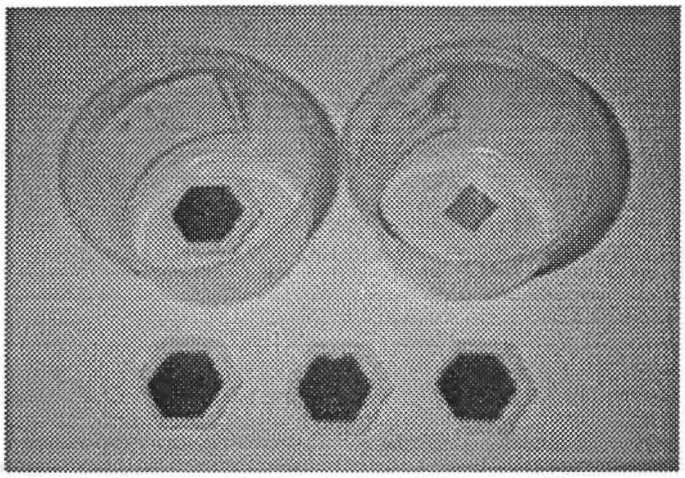

Figure 4.1. Fisherbrand weigh dishes filled with poultry excreta and immobilized in tubs with tape.

Figure 4.2. Boric acid trap system used to capture ammonia emitted from poultry excreta.

prepared via dialysis filtration to remove the majority of non-saponin compounds, and are therefore considered saponin-enriched extracts. Treatment levels were 0, 20, and 200 μL/5 g excreta (wet wt.) for liquid products and 0, 20, and 200 mg/5 g excreta for powder products (DK-30 and QCP). All treatments were applied on a uniform volume or weight basis; however, solids (100% DM) varied among the products and product fractions. Saponin-extraction was performed on YE and QE using a modified method of Wall et al. (1952). The butanol-soluble fraction was resuspended in a small amount of water and both the saponin and non-saponin fractions for YE and QE were evaluated for their ammonia lowering capacity at 200 μL/5 g excreta. Tannins were extracted from YE and QE using 0.1 M trivalent ytterbium according to the procedure of Giner-Chavez et al. (1997). Ytterbium (III) acetate hydrate 99.9% (316480100; Acros Organics, NJ) was added in sufficient quantities until little or no tannin was precipitated. The tannin-free fraction was obtained by a procedure modified from that of Anderson and Sowers (1968). Polyvinylpolypyrrolidine (PVPP; P-6755, SIGMA, St. Louis, MO) was used to bind tannins within the non-saponin fractions of YE and QE. The tannin and tannin-free fractions (200 μL/5 g excreta) of YE and QE were evaluated for their ability to reduce ammonia emissions from poultry excreta. It should be noted that tannin-free fractions may still contain small amounts of tannin even after using PVPP (Churms and Stephen, 1991). Carbohydrates were extracted from DK-30, and .5 g thereof dissolved in 5 mL dH_2O. Excreta was treated with the carbohydrate solution (200 μL/5 g) to determine its effects on ammonia emissions. Products and product fractions were thoroughly mixed with excreta prior to being placed in the appropriate weigh dish.

To ensure that responses seen with treatments were not related to dilution of manure or an effect of water used to resuspend some of the fractions, the effect of 200 μL dH_2O/5 g excreta was compared to controls with no water added (data not shown). Since no effect was seen with the addition of water, it was concluded that the observed treatment responses were not directly affected by water or via dilution. A significant number of small bubbles, resembling carbonation, appeared when the tannin-free extracts of both YE and QE were added to excreta. Similar responses were seen to a lesser degree with the non-saponin and whole yucca and quillaja extracts. High levels of dietary calcium carbonate may be excreted in the feces of layers. Calcium carbonate may have been affected by the acidic nature of the tannin-free extract (pH 3.4), potentially resulting in CO_2 bubbles. To test this possibility dH_2O was acidified (pH 3.4) using acetic acid and 1 mL used to treat a small amount of excreta. A response similar to that of the tannin-free extract was not seen. Several chemicals were used to obtain the tannin-free portion of YE and QE. The possibility that the 10% methanol and water-saturated butanol used during the extraction process resulted in the reactions observed between the excreta and tannin-free fraction could not be discounted. These chemicals were also added to a small amount of manure and no response was observed. It is therefore concluded that neither acidification nor the extraction chemicals were responsible for the carbonation, and subsequent ammonia reduction.

Data was analyzed using a Mixed Linear Model in SAS v. 6.12 (SAS, 1988). Type III expected mean squares were obtained for the terms product, runs within products, treatments, product and treatment interactions, and run and treatment interactions within products. The F-value for the run and treatment interaction within

products indicated it would be appropriate to pool it and the error term ($P > .10$). Differences between means, least squares means, and pairwise comparisons were determined for all products and treatment levels, but not treatment fractions.

RESULTS AND DISCUSSION

Percent ammonia reduction for all products on a 100% DM basis is presented in Figure 4.3. Responses between treatment levels were significant ($P < .05$) when all products were combined. When treatment level was disregarded and comparisons were made between the six products, it was evident that QE resulted in a significantly greater reduction of ammonia emissions ($P < .05$). There was no significant difference between treatment levels of DK-30; however, all other products did differ between levels. Treatments of 20 μL/5 g excreta for YE, YU, QE, QU, and QCP did not reduce ammonia emissions ($P > .10$). YU and QU have a greater concentration of saponins than their YE and QE counterparts, yet contain only half the amount of solids (100 mg versus 45 mg) for 200 μL. YU appeared to reduce ammonia emissions to a greater extent than YE at 200 μL/5 g excreta. Treatment with 200 μL QE/5 g excreta significantly reduced ammonia emissions when compared to all other products at either treatment level ($P < .0001$). The higher treatment level (200 μL) for all products combined was more effective ($P < .0001$) in reducing ammonia than 20 μL, which is to be expected.

Ammonia reduction for the saponin and non-saponin fractions is presented in Figure 4.4. Treatment with the extracted fractions (200 μL/5 g excreta) were significantly different ($P < .05$) from each other when product type was not taken into account, except when comparing the percent ammonia reduction from carbohydrate

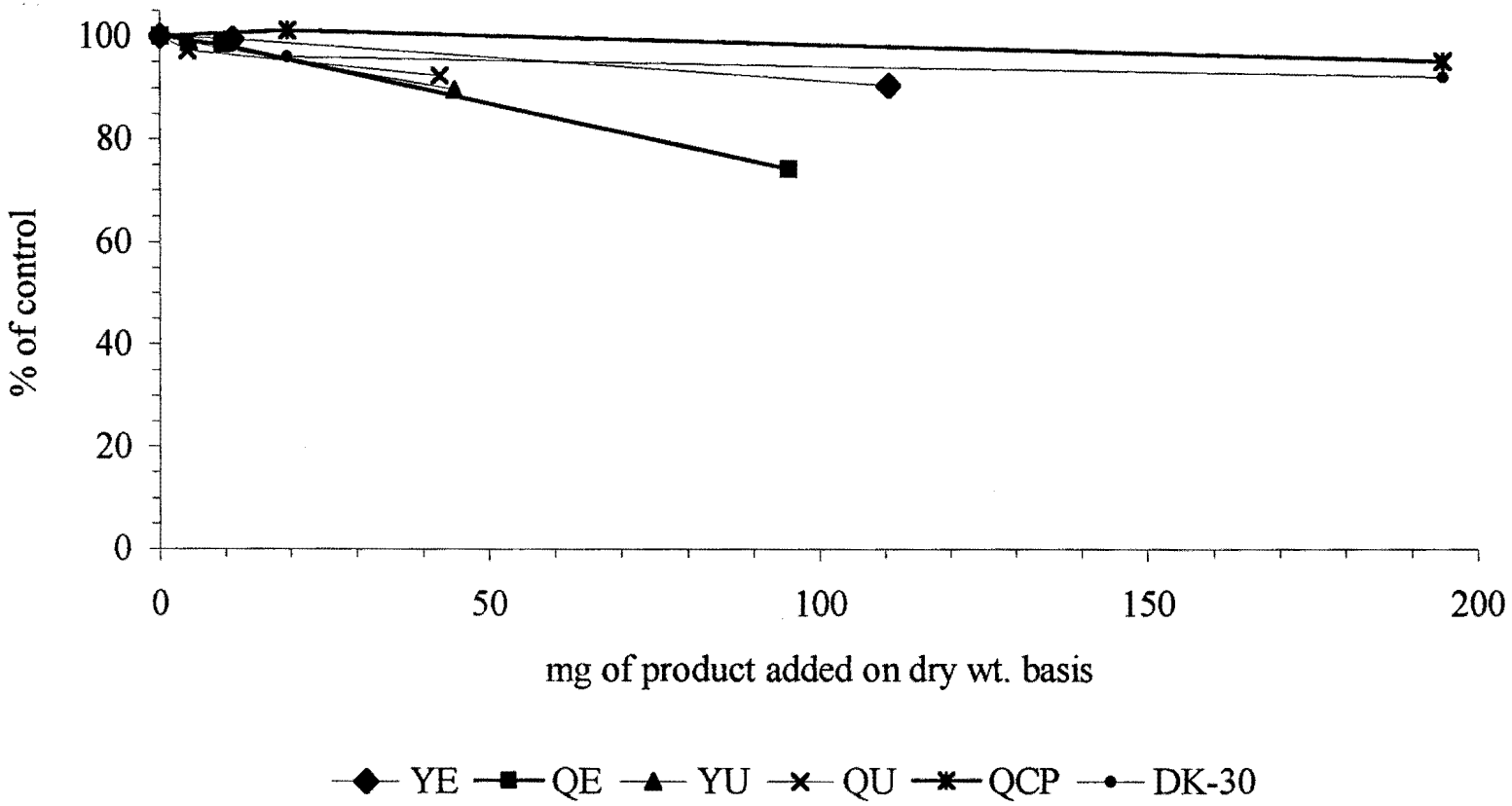

Figure 4.3. Ammonia concentration in 1 h as % of control for *Yucca schidigera* extract (YE), *Quillaja saponaria* extract (QE), yucca ultra (YU), quillaja ultra (QU), quillaja powder (QCP), and yucca powder (DK-30). Wt. of 200 µL of liquid products (100% DM): YE = 110.7 mg, QE = 95.4 mg, YU = 44.8 mg, QU = 42.5 mg; wt. of 200 mg of powder products (100% DM): QCP = 195 mg, DK-30 = 195 mg.

treatment with that of the tannin fraction. Comparisons of product means with all tannin, saponin, non-tannin, and non-saponin treatments combined were significantly different ($P < .05$). Pairwise comparisons of treatment fraction and product could not be obtained in the Mixed Linear Model. The tannin-free component from both YE and QE appeared to be particularly effective in reducing emissions (Figure 4.5), with that of QE having the greater percent reduction.

Preliminary work using a standard NH_4Cl solution (1000 ppm NH_3) similar to that used by Headon et al. (1991) with treatment of yucca and quillaja products indicated ammonia-reducing effects resembling those found with excreta. Dose response curves comparing YE and YU, as well as QE and QU indicated that those liquids with the higher saponin content (YU and QU) were less effective in inhibiting the release of NH_3 (data not shown). An ammonia electrode was used to evaluate the effects of .5 mL increments of the saponin, non-saponin, and tannin-free fractions of YE and QE on their ability to reduce free ammonia emissions from the NH_4Cl solution (data not shown). Trends were similar to those observed when excreta was treated with the fractions, especially for the tannin-free extract. These results support the idea that the mode of action lies in ammonia binding.

Previous research has focused primarily on saponins as being the active ammonia-reducing component. The effects of both saponin and non-saponin fractions of *Y. schidigera* were evaluated by Killeen et al. (1998). The non-saponin fraction significantly increased hindgut urease activity and fecal ammonia concentrations in the rat, whereas the saponin fraction decreased urease activity in the gut. Serum urea and

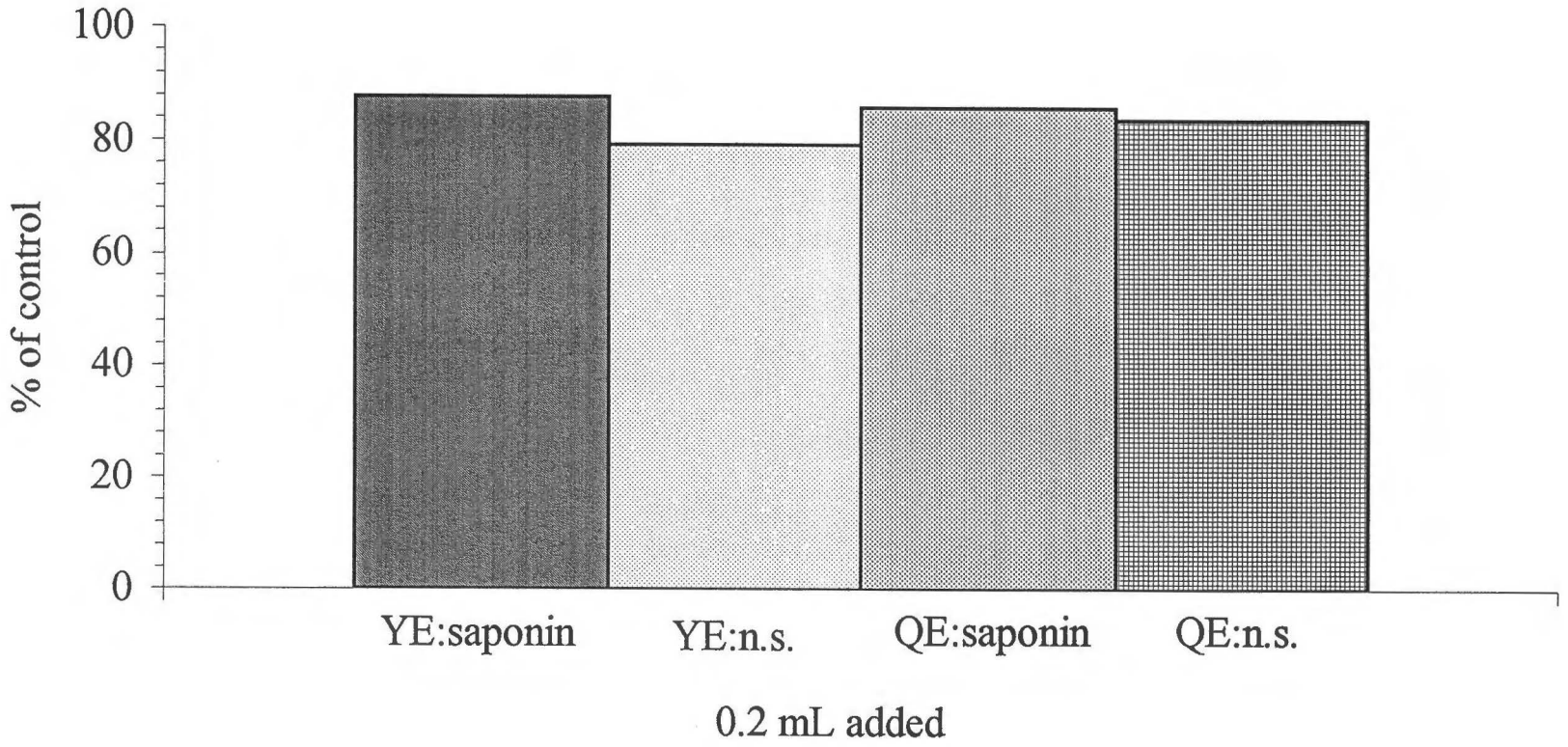

Figure 4.4. Ammonia concentration in 1 h as % of control for the saponin and non-saponin (n.s.) fractions of *Yucca schidigera* extract (YE) and *Quillaja saponaria* extract (QE). Wt. of 200 µL (100% DM): YE: saponin = 72.8 mg, n.s. = 53.6 mg; QE: saponin = 30.1 mg, n.s. = 55.6 mg.

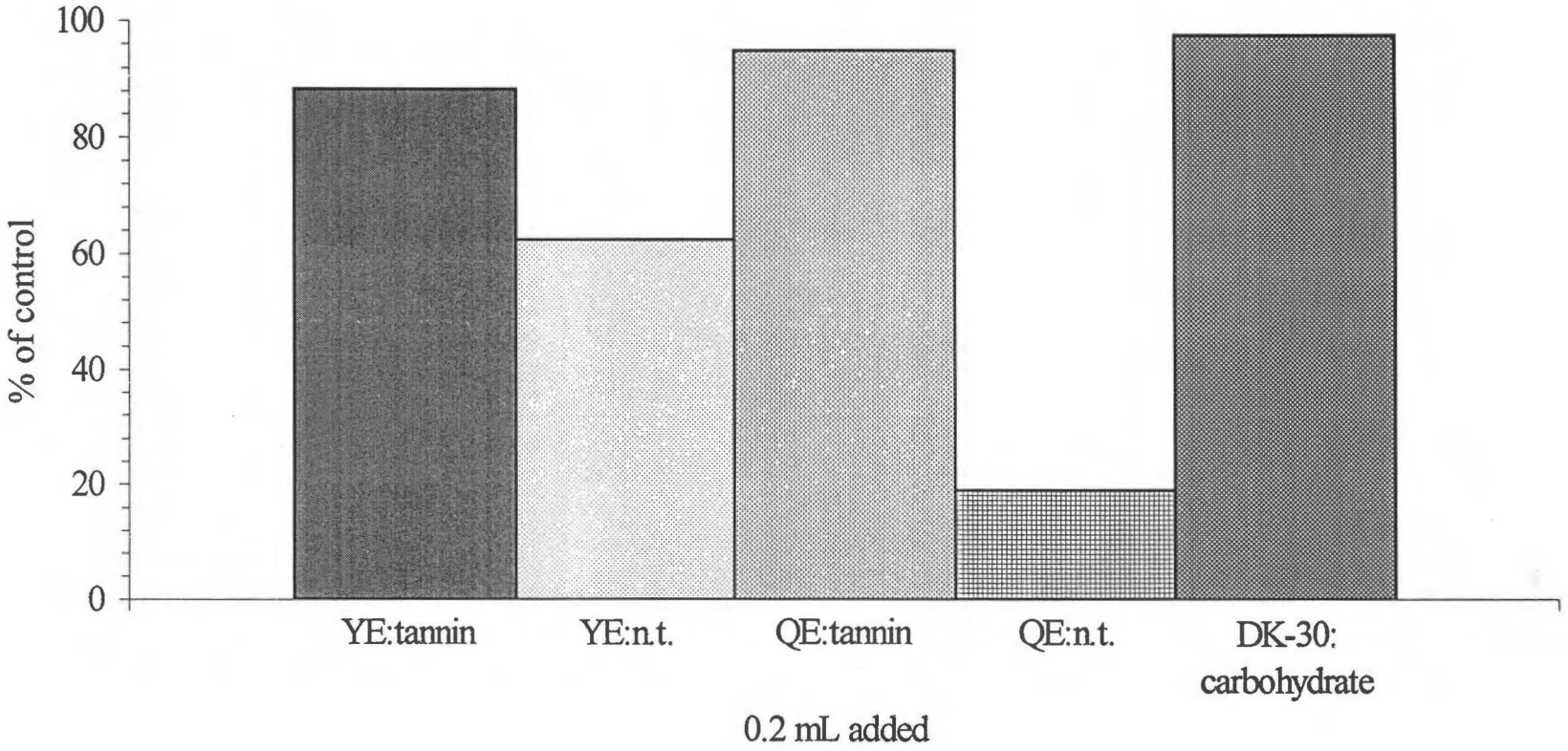

Figure 4.5. Ammonia concentration in 1 h as % of control for the tannin and non-tannin (n.t.) fraction of *Yucca schidigera* extract (YE) and *Quillaja saponaria* extract (QE). Wt. of 200 µL (100% DM): YE: tannin = 14.4 mg, n.t. = 23.1 mg; QE: tannin = 9.9 mg, n.t. = 25.9 mg; DK-30: carbohydrate = 15.2 mg.

ammonia were reduced by the non-saponin fraction. Sarsasapogenin and smilagenin, two of the steroid saponins within *Y. schidigera*, were identified as being the predominant source of yucca's antimicrobial activity. The antimicrobial activity of a yucca saponin fraction obtained by column chromatography was found to have little or no effect on both Gram-positive and Gram-negative bacteria (Tanaka et al., 1996), which contradicts the findings of Wallace et al. (1994). The butanol-soluble extract of *Y. schidigera* was found to have both antiprotozoal and antibacterial activity, although in some instances bacterial growth was stimulated or unaffected (Wallace et al., 1994). Removal of tannins from the saponin fraction did not alter its efficacy. Headon et al. (1991), using a standard NH_4Cl solution, determined the ammonia-binding activity of yucca to reside in the water-soluble (non-saponin) fraction. *Q. saponaria* saponins were evaluated *in vitro* using rumen liquor and found to increase the efficiency of microbial protein synthesis (Makkar and Becker, 1996). Observed responses are often attributed to saponin-effects when in fact saponins only comprise a portion of the entire extract. Results obtained in the present study contradict the findings of those researchers who have identified saponins as the active ammonia-reducing component. The non-saponin constituents, particularly when lacking tannins, were shown to reduce ammonia emissions from poultry excreta. The bubbling action witnessed with the tannin-free liquid has not been previously reported. It does not appear that yucca carbohydrates are responsible for the effects seen; however, it would be appropriate to identify the remaining compounds which are present in the YE and QE tannin-free extracts to determine their activity. Future investigation into the possible *in vivo* effects of YE and QE fractions may prove beneficial. Results could vary if fractions were included in the diet rather than applied to excreta.

ACKNOWLEDGEMENTS

The authors would like to thank Desert King International for financial support, Dr. W. Oleszek at the Department of Biochemistry, Institution of Soil Science and Plant Cultivation, Pulawy, Poland for yucca carbohydrate extraction, and Sunny Morris for her extensive assistance in the Oregon State University Animal Nutrition Laboratory.

REFERENCES

Al-Bar, A., Ismail, A., Cheeke, P. R., and H. S. Nakaue. 1993. Effect of dietary *Yucca shidegria* extract (Deoderase) on environmental ammonia and growth performance of chickens and rabbits. In: Proc., Western Section, Amer. Soc. Anim. Sci. 44:106-108.

Anderson, R. A., and J. A. Sowers. 1968. Optimum conditions for bonding of plant phenols to insoluble polyvinylpyrrolidine. Phytochemistry 7:293-301.

Anthony, N. B., Balog, J. M., Staudinger, F. B., Wall, C. W., Walker, R. D., and W. E. Huff. 1994. Effect of a urease inhibitor and ceiling fans on ascites in broilers. 1. Environmental variability and incidence of ascites. Poult. Sci. 73:801-809.

Canh, T. T., Aarnink, A. J. A., Verstegen, M. W. A., and J. W. Schrama. 1998. Influence of dietary factors on the pH and ammonia emission of slurry from growing-finishing pigs. J. Anim. Sci. 76:1123-1130.

Carlile, F. S. 1984. Ammonia in poultry houses: A literature review. World's Poultry Science Journal 40:99-113.

Charles, D. R. 1992. Pollution problems associated with intensive poultry production: Indirect effects of ammonia on health, performance and yield. In: T. P. Lyons (Ed.) Biotechnology in the Feed Industry, Proc., 8th Annual Alltech Symposium. pp 33-42. Alltech Technical Publications, Nicholasville, KY.

Churms, S. C., and A. M. Stephen. 1991. Chromatographic separation and examination of carbohydrate and phenolic components of the non-tannin fraction of black wattle (*Acacia mearnsii*) bark extract. J. Chromatogr. 550:519-537.

Crober, D. 1996. Reducing ammonia and flies in layer operations with yucca. Feeding Times, Dec-Jan, pp 7-9.

Dvorak, R. A., and D. Watts. 1996. Effect of De-Oderase® added to canned cat food on ammonia arising from cat litter. J. Anim. Sci. 74 (Suppl. 1):35 (Abstr.).

George, A. J. 1965. Guest reviews: Legal status and toxicity of saponins. Fd. Cosmet. Toxicol. 3:85-91.

Giner-Chavez, B. I., Van Soest, P. J., Robertson, J. B., Lascano, C., Reed, J. D., and A. N. Pell. 1997. A method for isolating condensed tannins from crude plant extracts with trivalent ytterbium. J. Sci. Food Agric. 74:359-368.

Goodall, S. R., Curtis, S. E., and J. M. McFarlane. 1988. Reducing aerial ammonia by adding Micro Aid to poultry diets: Laboratory and on-farm evaluations. In: Livestock Environment III. pp 286-290. American Society of Agricultural Engineers, St. Joseph, MI.

Headon, D. R., Buggle, K., Nelson, A., and G. Killeen. 1991. Glycofractions of the yucca plant and their role in ammonia control. In: T. P. Lyons (Ed.) Biotechnology in the Feed Industry, Proc., 7th Annual Alltech Symposium. pp 95-108. Alltech Technical Publications, Nicholasville, KY.

Headon, D. R., and G. Walsh. 1993. *Yucca schidigera* extracts and ammonia control. In: Livestock Environment III. pp 286-290. American Society of Agricultural Engineers, St. Joseph, MI.

Johnston, N. L., Quarles, C. L., Fagerberg, D. J., and D. D. Caveny. 1981. Evaluation of yucca saponin on broiler performance and ammonia suppression. Poult. Sci. 60:2289-2292.

Killeen, G. F., Connolly, C. R., Walsh, G. A., Duffy, C. F., Headon, D. R., and R. F. Power. 1998. The effects of dietary supplementation with Yucca schidigera extract or fractions thereof on nitrogen metabolism and gastrointestinal fermentation processes in the rat. J. Sci. Food Agric. 76:91-99.

Makkar, H. P. S., and K. Becker. 1996. Effect of quillaja saponins on *in vitro* rumen fermentation. In: G. R. Waller and K. Yamasaki (Eds.) Saponins Used in Food and Agriculture. pp 387-394. Plenum Press, NY.

Rowland, L. O., Jr., Plyler, J. E., and J. W. Bradley. 1976. *Yucca schidigera* extract effect on egg production and house ammonia levels. Poult. Sci. 55:2086.

SAS. 1988. SAS/STAT® User's Guide (Release 6.03). SAS Inst. Inc., Cary, NC.

Scott, M. L., Nesheim, M. C., and R. J. Young. 1982. Nutrition of the Chicken (3rd Ed.). pp 78-82. M. L. Scott & Associates, Ithaca, NY.

Stutz, M. W., and M. J. Metrokotsas. 1972. Urease activity in the digestive tract of the chick and metabolism of urea. Poult. Sci. 51:1876 (Abstr.).

Tanaka, O., Tamura, Y., Masuda, H., and K. Mizutani. 1996. Applications of saponins in foods and cosmetics: Saponins in Mohave yucca and *Sapindus mukurossi*. In: G. R. Waller and K. Yamasaki (Eds.) Saponins Used in Food and Agriculture. pp 1-11. Plenum Press, NY.

Wall, M. E., Krider, M. M., Rothman, E. S., and C. R. Eddy. 1952. Steroidal sapogenins: 1. Extraction, isolation and identification. J. Biol. Chem. 198:533-543.

Wallace, R. J., Arthaud, L., and C. J. Newbold. 1994. Influence of *Yucca shidigera* extract on ruminal ammonia concentrations and ruminal microorganisms. Appl. Environ. Microbiol. 60:1762-1767.

CHAPTER 5
SUMMARY

Seasonal effects were evident for some, but not all of the proximate nutrients evaluated in *P. henon*, *P. bissetii*, *S. pumila*, and reed canarygrass. The resulting seasonal data did, however, indicate that bamboo contained adequate nutrients to justify its consideration as a livestock feedstuff. A subsequent *in vivo* digestibility study was conducted using ponies. Although crude protein digestibility of bamboo (*P. bissetii*) was fairly high (60-75%) the comparatively low dry matter, acid detergent fiber, and neutral detergent fiber digestibilities indicated that animals fed a diet consisting entirely of bamboo may experience negative energy balance. Treatment of bamboo could improve digestibility, but given the multitude of better quality forages in the U. S. such processing would be neither time- nor cost-effective. The *in vivo* experiment provided reference data by which an *in vitro* digestibility trial using horse feces as inoculum could be conducted. Bamboo that was fed during the fecal collection periods, as well as orchard grass hay and alfalfa hay, were used as feedstuff samples. Samples were treated with varying levels of *Y. schidigera* extract (YE), and the effects on in vitro dry matter disappearance (IVDMD) determined. Horse supplements containing yucca products have been touted to improve metabolism and alleviate joint problems; however, the effect these products have on digestibility in equids has not been adequately investigated. Digestibility values obtained were variable, depending upon the type of forage being analyzed. IVDMD for orchard grass hay increased at high levels of YE (3000 ppm), but decreased for bamboo. The microorganisms effective in degrading bamboo, which is high in silica, may have been negatively affected by yucca, whereas the bacteria involved

in the digestion of orchard grass hay were stimulated or at least indirectly affected by yucca's antiprotozoal activity. Potential *in vivo* responses may vary from those found *in vitro*, since other proposed actions of saponins, such as effects on gut permeability were not simulated in the artificial digestion process. The control values obtained *in vitro* for bamboo digestibility agreed with those obtained *in vivo*. IVDMD of alfalfa hay and orchard grass hay were comparable to the *in vivo* digestibilities obtained by other researchers, indicating the validity of using feces as a source of inoculum. *Y. schidigera* extract (YE) has been determined to have ammonia-reducing effects, with the active component believed to be saponins. Little research regarding the effects of *Q. saponaria* extract (QE) on ammonia emissions has been conducted. In the present study the saponin, non-saponin, tannin, non-tannin, and carbohydrate fractions of yucca and quillaja were used to treat poultry excreta and a boric acid trap system used to capture ammonia emitted from the excreta. Data indicated that of all YE and QE fractions, the tannin-free fraction reduced ammonia the most (37.5% for YE and 81.0% for QE). The non-saponin fraction, which still contains both tannins and non-tannins, was also quite effective. Potential impurities from the extraction process may have been responsible for the decreases in ammonia levels observed with the other fractions. The mode of action by which ammonia emissions were reduced could not be elucidated in this study. Binding of ammonia, urease inhibition, and antiprotozoal activity may not individually characterize the mode of action, and instead may combine in a synergistic effect. Further research on the effects of YE and QE fractions when incorporated into poultry diets should be considered, including the remaining unidentified components in the tannin-free liquid.

BIBLIOGRAPHY

Adam, K. M. G. 1951. The quantity and distribution of the ciliate protozoa in the large intestine of the horse. Parasit. 41:301-311.

Ahn, J.-H., Elliott, R., and B. W. Norton. 1997. Oven drying improves the nutritional value of *Calliandra calothyrsus* and *Gliricidia sepium* as supplements for sheep given low-quality straw. J. Sci. Food Agric. 75:503-510.

Akhter, S., Owen, E., Fall, A., O'Donovan, F., and M. K. Theodorou. 1994. Use of fresh or frozen cow faeces instead of sheep rumen liquor to provide micro-organisms for *in vitro* digestibility assays for forages. Anim. Prod. 58: 452 (Abstr.).

Al-Bar, A., Ismail, A., Cheeke, P. R., and H. S. Nakaue. 1993. Effect of dietary *Yucca shidegria* extract (Deoderase) on environmental ammonia and growth performance of chickens and rabbits. In: Proc., Western Section, Amer. Soc. Anim. Sci. 44:106-108.

Alexander, F. 1952. Some functions of the large intestine of the horse. Q. J. Exp. Physiol. 37:205-214.

Amon, M., Dobeic, M., Misselbrook, T. H., Pain, B. F., Phillips, V. R., and R. W. Sneath. 1995. A farm scale study on the use of De-Oderase® for reducing odour and ammonia emissions from intensive fattening piggeries. Bioresource Technology 51:163-169.

Anderson, R. A., and J. A. Sowers. 1968. Optimum conditions for bonding of plant phenols to insoluble polyvinylpyrrolidine. Phytochemistry 7:293-301.

Anthony, N. B., Balog, J. M., Staudinger, F. B., Wall, C. W., Walker, R. D., and W. E. Huff. 1994. Effect of a urease inhibitor and ceiling fans on ascites in broilers. 1. Environmental variability and incidence of ascites. Poult. Sci. 73:801-809.

AOAC. 1995. Official Methods of Analysis (16th Ed.). Association of Official Analytical Chemists, Washington, DC.

Bae, H. D., McAllister, T. A., Kokko, E. G., Leggett, F. L., Yanke, L. J., Jakober, K. D., Ha, J. K., Shin, H. T., and K.-J. Cheng. 1997. Effect of silica on the colonization of rice straw by ruminal bacteria. Anim. Feed Sci. Technol. 65:165-181.

Balog, J. M., Anthony, N. B., Wall, C. W., Walker, R. D., Rath, N. C., and W. E. Huff. 1994. Effect of a urease inhibitor and ceiling fans on ascites in broilers. 2. Blood variables, ascites scores, and body and organ weights. Poult. Sci. 73:810-816.

Barnes, E. M., and C. S. Impey. 1974. The occurence and properties of uric acid decomposing anaerobic bacteria in the avian caecum. J. Appl. Bact. 37:393-409.

Barry, T. N., and B. J. Blaney. 1987. Secondary compounds of forages. In: J. B. Hacker and J. H. Ternouth (Eds.) The Nutrition of Herbivores. pp 91-119. Academic Press Australia, Marrickville, NSW, Australia.

Block, E., Kilmer, L. H., and L. D. Muller. 1981. Acid insoluble ash as a marker of digestibility for sheep fed corn plants or hay and for lactating dairy cattle fed hay *ad libitum*. J. Anim. Sci. 52:1164-1169.

Burke, D. J. 1987. The role of forages in the horse program. Paper presented at the Forage and Grassland Conference, March 1987, Springfield, IL.

Canh, T. T., Aarnink, A. J. A., Verstegen, M. W. A., and J. W. Schrama. 1998. Influence of dietary factors on the pH and ammonia emission of slurry from growing-finishing pigs. J. Anim. Sci. 76:1123-1130.

Carle, C. J., Fontenot, J. P., and K. E. Webb, Jr. 1975. Digestibility of nutrients from alfalfa hay, corn and oats by ponies. In: Livestock Research Report. pp 151-154. Virginia Polytechnic Institute and State University, Blacksburg, VA.

Carlile, F. S. 1984. Ammonia in poultry houses: A literature review. World's Poultry Science Journal 40:99-113.

Charles, D. R. 1992. Pollution problems associated with intensive poultry production: Indirect effects of ammonia on health, performance and yield. In: T. P. Lyons (Ed.) Biotechnology in the Feed Industry, Proc., 8th Annual Alltech Symposium. pp 33-42. Alltech Technical Publications, Nicholasville, KY.

Cheeke, P. R. 1998. Saponins: Surprising benefits of desert plants. In: The Linus Pauling Institute Newsletter. pp 4-5. Oregon State University, Corvallis, OR.

Churms, S. C., and A. M. Stephen. 1991. Chromatographic separation and examination of carbohydrate and phenolic components of the non-tannin fraction of black wattle (*Acacia mearnsii*) bark extract. J. of Chromatogr. 550:519-537.

Crober, D. 1996. Reducing ammonia and flies in layer operations with yucca. Feeding Times, Dec-Jan, pp 7-9.

Cuddeford, D. 1986. Equine practice: Alternative feedstuffs for horses. In Practice, March, pp 68-70.

Cuddeford, D., Pearson, R. A., Archibald, R. F., and R. H. Muirhead. 1995. Digestibility and gastro-intestinal transit times of diets containing different proportions of alfalfa and oat straw given to Thoroughbreds, Shetland ponies, Highland ponies and donkeys. Anim. Sci. 61:407-417.

Cymbaluk, N. F. 1990. Comparison of forage digestion by cattle and horses. Can. J. Anim. Sci. 70:601-610.

Cymbaluk, N. F., and D. A. Christensen. 1986. Nutrient utilization of pelleted and unpelleted forages by ponies. Can. J. Anim. Sci. 66:237-244.

Cymbaluk, N. F., Christison, G. I., and D. H. Leach. 1989. Nutrient utilization by limit- and ad libitum-fed growing horses. J. Anim. Sci. 67:414-425.

Dierenfeld, E. S. 1997. Chemical composition of bamboo in relation to giant panda nutrition. In: The Bamboos. pp 205-211. The Linnean Society, London.

Dierenfeld, E. S., Hintz, H. F., Robertson, J. B., Van Soest, P. J., and O. T. Oftedal. 1982. Utilization of bamboo by the giant panda. J. Nutr. 112:636-641.

Dvorak, R. A., and D. Watts. 1996. Effect of De-Oderase® added to canned cat food on ammonia arising from cat litter. J. Anim. Sci. 74 (Suppl. 1):35 (Abstr.).

Elliott, R., and N. P. McMeniman. 1987. Supplementation of ruminant diets with forages. In: J. B. Hacker and J. H. Ternouth (Eds.) The Nutrition of Herbivores. pp 409-428. Academic Press Australia, Marrickville, NSW, Australia.

El-Meadaway, A., Mir, Z., Zaman, M. S., and L. J. Yanke. 1998. Relative efficacy of inocula from rumen fluid or faecal solution for determining in vitro digestibility and gas production. Can. J. Anim. Sci. 78:673-679.

El Shaer, H. M., Omed, H. M., Chamberlain, A. G., and R. F. E. Axford. 1987. Use of faecal organisms from sheep for the *in vitro* determination of digestibility. J. Agric. Sci., Camb. 109:257-259.

Evans, J. W., Borton, A., Hintz, H. F., and L. D. Van Vleck. 1990. Digestive Physiology. In: The Horse (2nd Ed.). p 194. W. H. Freeman and Company, NY.

Farrelly, D. 1984. The Book of Bamboo. p 280. Sierra Club Books, San Francisco, CA.

Fonnesbeck, P. V., Lydman, R. K., Vander Noot, G. W., and L. D. Symons. 1967. Digestibility of the proximate nutrients of forage by horses. J. Anim. Sci. 26:1039-1045.

Frape, D. 1986. Equine Nutrition and Feeding. Churchill Livingstone, Inc., NY.

George, A. J. 1965. Guest reviews: Legal status and toxicity of saponins. Fd. Cosmet. Toxicol. 3:85-91.

Gestetner, B., Birk, Y., and Y. Tencer. 1968. Soybean saponins: Fate of ingested soybean saponins and the physiological aspect of their haemolytic activity. J. Agric. Food Chem. 16:1031-1035.

Giner-Chavez, B. I., Van Soest, P. J., Robertson, J. B., Lascano, C., Reed, J. D., and A. N. Pell. 1997. A method for isolating condensed tannins from crude plant extracts with trivalent ytterbium. J. Sci. Food Agric. 74:359-368.

Glade, M. J. 1992. Effects of *Yucca shidigera* extract on feed utilization by equine weanlings. Equine Veterinary Science 12:93-98.

Goering, H. K, and P. J. Van Soest. 1970. Forage fiber analysis. Agric. Handbook No. 379. ARS, USDA, Washington, DC.

Goetsch, A. L., and F. N. Owens. 1985. Effects of sarsaponin on digestion and passage rates in cattle fed medium to low concentrate. J. Dairy Sci. 68:2377-2384.

Goodall, S. R., Curtis, S. E., and J. M. McFarlane. 1988. Reducing aerial ammonia by adding Micro Aid to poultry diets: Laboratory and on-farm evaluations. In: Livestock Environment III. pp 286-290. American Society of Agricultural Engineers, St. Joseph, MI.

Greenway, S. L., Keller, M., Nelson, G., Cheeke, P. R., and D. J. Carroll. 1999. Evaluation of temperate bamboo foliage as an animal feedstuff: Seasonal changes in composition and digestibility in ponies. In: Proc., Western Section, Amer. Soc. Anim. Sci. 50:193-196.

Gutierrez, J., and R. E. Davis. 1962. Isolation of saponin-digesting bacteria from the rumen of bloating cattle on Ladino clover pasture. J. Anim. Sci. 21:819-823.

Gutierrez, J., Davis, R. E., and I. L. Lindahl. 1959. Characteristics of saponin-utilizing bacteria from the rumen of cattle. Appl. Microbiol. 7:304-308.

Headon, D. R., Buggle, K., Nelson, A., and G. Killeen. 1991. Glycofractions of the yucca plant and their role in ammonia control. In: T. P. Lyons (Ed.) Biotechnology in the Feed Industry, Proc., 7th Annual Alltech Symposium. pp 95-108. Alltech Technical Publications, Nicholasville, KY.

Headon, D. R., and G. Walsh. 1993. *Yucca schidigera* extracts on ammonia control. In: E. Collins and C. Boon (Eds.) Livestock Environment IV. pp. 686-693. American Society of Agricultural Engineers, St. Joseph, MI.

Higuchi, R., Tokimitsu, Y., Fujioka, T., Komori, T., Kawasaki, T., and D. G. Oakenful. 1987. Structure of desacylsaponins obtained from the bark of Quillaja saponaria. Phytochemistry 26:229-235.

Hintz, H. F., Hogue, D. E., Walker, E. F., Jr., Lowe, J. E., and H. F. Schryver. 1971. Apparent digestion in various segments of the digestive tract of ponies fed diets with varying roughage-grain ratios. J. Anim. Sci. 32:245-248.

Hostettman, K., and A. Marston. 1995. Saponins. Cambridge University Press, Great Britain.

Hungate, R. E. 1966. Conversions of nitrogenous materials. In: The Rumen and Its Microbes. pp 281-330. Academic Press, NY.

Hussain, I., and P. R. Cheeke. 1995. Effect of dietary *Yucca schidigera* extract on rumen and blood profiles of steers fed concentrate- or roughage-based diets. Anim. Feed Sci. Technol. 51:231-242.

Hussain, I., Ismail, A. M., and P. R. Cheeke. 1996. Effects of feeding *Yucca schidigera* extract in diets varying in crude protein and urea contents on growth performance and cecum and blood urea and ammonia concentrations of rabbits. Anim. Feed Sci. Technol. 62:121-129.

Illius, A. W., and I. Gordon. 1990. Constraints on diet selection and foraging beviour in mammalian herbivores. In: R. N. Hughes (Ed.) Behavioural Mechanisms of Food Selection. Springer-Verlag, Berlin.

Johnson, I. T., Gee, J. M., Price, K., Curl, C., and G. R. Fenwick. 1986. Influence of saponins on gut permeability and active nutrient transport in vitro. J. Nutr. 116:2270-2277.

Johnston, N. L., Quarles, C. L., Fagerberg, D. J., and D. D. Caveny. 1981. Evaluation of yucca saponin on broiler performance and ammonia suppression. Poult. Sci. 60:2289-2292.

Jones, L. H. P., and K. A. Handreck. 1967. Silica in soils, plants, and animals. Adv. Agron. 19:107-149.

Jones, D. I. H., and A. D. Wilson. 1987. Nutritive quality of forage. In: J. B. Hacker and J. H. Ternouth (Eds.) The Nutrition of Herbivores. pp 65-89. Academic Press Australia, Marrickville, NSW, Australia.

Kaneda, N., Nakanishi, H., and E. J. Staba. 1987. Steroidal constituents of *Yucca shidigera* plants and tissue cultures. Phytochemistry 26:1425-1429.

Kaufman, P. B., Dayanadan, P., Franklin, C. I., and Y. Takeoka. 1985. Structure and function of silica bodies in the epidermal system of grass shoots. Ann. Bot. 55:487-507.

Kemme, P. A., Jongbloed, A. W., Dellaert, B. M., and F. Krol-Kramer. 1993. The use of a Yucca schidigera extract as 'urease inhibitor' in pig slurry. In: Nitrogen Flow in Pig Production and Environmental Consequences. EAAP Publ. No. 69. pp 330-335. Pudoc, Wageningen, The Netherlands.

Kensil, C. R., Soltysik, S., Wheeler, D. A., and J.-Y. Wu. 1996. Structure/function studies on QS-21, a unique immunological adjuvant from *Quillaja saponaria*. In: G. R. Waller and K. Yamasaki (Eds.) Saponins Used in Traditional and Modern Medicine. pp 165-172. Plenum Press, NY.

Kern, D. L., Slyter, L. L., Weaver, J. M., Leffel, E. C., and G. Samuelson. 1973. Pony cecum *vs.* steer rumen: The effect of oats and hay on the microbial ecosystem. J. Anim. Sci. 37:463-469.

Killeen, G. F., Buggle, K. A., Hynes, M. J., Walsh, G. A., Power, R. F., and D. R. Headon. 1994. Influence of *Yucca schidigera* preparations on the activity of urease from *Bacillus pasteurii*. J. Sci. Food Agric. 65:433-440.

Killeen, G. F., Connolly, C. R., Walsh, G. A., Duffy, C. F., Headon, D. R., and R. F. Power. 1998. The effects of dietary supplementation with *Yucca schidigera* extract or fractions thereof on nitrogen metabolism and gastrointestinal fermentation processes in the rat. J. Sci. Food Agric. 76:91-99.

Kirkhope, R. T. S., and R. S. Lowman. 1996. Use of an *in-vitro* gas production technique with faeces as inoculant to assess tropical forage quality for equids. Anim. Sci. 62:690.

Lawson, A. H. 1968. Commercial uses of the bamboo. In: Bamboos: A Gardener's Guide to their Cultivation in Temperate Climates. pp 68-69. Taplinger Publishing Company, NY.

Leng, R. A., and J. V. Nolan. 1984. Nitrogen metabolism in the rumen. J. Dairy Sci. 67:1072-1089.

Lowman, R. S., Theodorou, M. K., Hyslop, J. J., Dhanoa, M. S., and D. Cuddeford. 1999. Evaluation of an in vitro batch culture technique for estimating the in vivo digestibility energy content of equine feeds using equine faeces as the source of microbial inoculum. (in press).

Lowman, R. S., Theodorou, M. K., Longland, A. C., and D. Cuddeford. 1996. A comparison of equine faeces or caecal digesta as sources of inoculum for *in vitro* fermentation studies using the pressure transducer technique. Anim. Sci. 62: 683-684 (Abstr.).

Lu, C. D., Tsai, L. S., Schaeffer, D. M., and N. A. Jorgensen. 1987. Alteration of fermentation in continuous culture of mixed rumen bacteria by isolated alfalfa saponins. J. Dairy Sci. 70:799-805.

Mackie, R. I. 1987. Microbial digestion of forages in herbivores. In: J. B. Hacker and J. H. Ternouth (Eds.) The Nutrition of Herbivores. pp 233-265. Academic Press Australia, Marrickville, NSW, Australia.

Makkar, H. P. S., and K. Becker. 1996. Effect of quillaja saponins on *in vitro* rumen fermentation. In: G. R. Waller and K. Yamasaki (Eds.) Saponins Used in Food and Agriculture. pp 387-394. Plenum Press, NY.

Makkar, H. P. S., and K. Becker. 1997. Degradation of quillaja saponins by mixed culture of rumen microbes. Letters in Appl. Microb. 25:243-245.

Makkar, H. P. S., Becker, K., Abel, H., and C. Szegletti. 1995. Degradation of condensed tannins by rumen microbes exposed to quebracho tannins (QT) in rumen simulation technique (RUSITEC) and effects of QT on fermentative processes in the RUSITEC. J. Sci. Food Agric. 69:495-500.

Manda, M., Osa, E., Tokuda, H., and I. Kurohiji. 1990. Feeding value of bamboo. In: Bulletin of the Faculty of Agriculture. pp 173-179. Kagoshima University, Japan.

McAllister, T. A., Wang, Y., Hristov, A. N., Olson, M. E., and P. R. Cheeke. 1998. Applications of *Yucca schidigera* in livestock production. In: Proc., 33rd Annual Pacific Northwest Animal Nutrition Conference. pp 109-119. Vancouver, B. C., Canada.

McClure, F. A. 1958. Bamboo as a source of forage. In: Pacific Sci. Assoc., 8th Pacific Sci. Congr. Proc. 4:609.

McDonald, E. 1998. Gardening with bamboo. Pacific Northwest Bamboo 14 (2):6-8.

McDonald, P., Edwards, R. A., Greenhalgh, J. F. D., and C. A. Morgan. 1995. Minerals. In: Animal Nutrition (5th Ed.). pp 124-125. Longman Scientific & Technical, New York, NY.

McDougall, E. I. 1948. Studies on ruminant saliva. 1. The composition and output of sheep's saliva. Biochem. J. 43:99-109.

Minson, D. J. 1971. Influence of lignin and silicon on a summative system for assessing the organic matter digestibility of *Panicum*. Aust. J. Agric. 22:589-598.

Moore, B. E., and B. A. Dehority. 1993. Effects of diet and hindgut defaunation on diet digestibility and microbial concentrations in the cecum and colon of the horse. J. Anim. Sci. 71:3350-3358.

Nelson, G., Keller, M., and P. R. Cheeke. 1997. Evaluation of temperate bamboo species as forages for livestock. Paper presented at the Pacific Northwest Bamboo Agro-Forestry Workshop, June 1997, Port Townsend, WA.

NRC. 1989. Nutrient Requirements of Horses (5th Ed.). National Academy Press, Washington, DC.

Nsahlai, I. V., and N. N. Umunna. 1996. Comparison between reconstituted sheep faeces and rumen fluid inocula and between *in vitro* and *in sacco* digestibility methods as predictors of intake and *in vivo* digestibility. J. Agric. Sci., Camb. 126:235-248.

Omed, H. M., Axford, R. F. E., Chamberlain, A. G., and D. I. Givens. 1989. A comparison of three laboratory techniques for the estimation of the digestibility of feedstuffs for ruminants. J. Agri. Sci., Camb. 113:35-39.

Ørskov, E. R., and R. N. B. Kay. 1987. Non-microbial digestion of forages by herbivores. In: J. B. Hacker and J. H. Ternouth (Eds.) The Nutrition of Herbivores. pp 267-280. Academic Press Australia, Marrickville, NSW, Australia.

Preston, R. L., Bartle, S. J., May, T., and S. R. Goodall. 1987. Influence of sarsaponin on growth, feed and nitrogen utilization in growing rats fed diets with added urea or protein. J. Anim. Sci. 65:481-487.

Prior, R. L., Hintz, H. F., Lowe, J. E., and W. J. Visek. 1974. Urea recycling and metabolism of ponies. J. Anim. Sci. 38:565-571.

Rowland, L. O., Jr., Plyler, J. E., and J. W. Bradley. 1976. *Yucca schidigera* extract effect on egg production and house ammonia levels. Poult. Sci. 55:2086.

Ryan, J. P., Quinn, T., and B. F. Leek. 1997. Initial effects of *Yucca shidigera* extract and high strength yeast culture on ovine ruminal fermentation of hay *in vitro*. J. Phys. 501P.

SAS. 1988. SAS/STAT® User's Guide (Release 6.03). SAS Inst. Inc., Cary, NC.

Scott, M. L., Nesheim, M. C., and R. J. Young. 1982. Nutrition of the Chicken (3rd Ed.). pp 78-82. M. L. Scott & Associates, Ithaca, NY.

Shepherd, W. O. 1952. Highlights of forest grazing research. J. For. 50:280-283.

Shewmaker, G. E., Mayland, H. F., Rosenau, R. C., and K. H. Asay. 1989. Silicon in C-3 grasses: Effects of forage quality and sheep preference. Journal of Range Management 42:122-127.

Slade, L. M., and H. F. Hintz. 1969. Comparison of digestion in horses, ponies, rabbits and guinea pigs. J. Anim. Sci. 28:842-843.

Slagsvold, P., Hintz, H. F., and H. F. Schryver. 1979. Digestibility by ponies of oat straw treated with anhydrous ammonia. Anim. Prod. 28:347-352.

Stevens, C. E., and I. D. Hume. 1995. Secretion and absorption of electrolytes and water. In: Comparative Physiology of the Vertebrate Digestive System (2nd Ed.). pp 229-267. Cambridge University Press, Great Britain.

Stutz, M. W., and M. J. Metrokotsas. 1972. Urease activity in the digestive tract of the chick and metabolism of urea. Poult. Sci. 51:1876 (Abstr.).

Sunvold, G. D., Fahey, G. C., Jr., Merchen, N. R., Bourquin, L. D., Titgemeyer, E. C., Bauer, L. L., and G. A. Reinhart. 1995a. Dietary fiber for cats: In vitro fermentation of selected fiber sources by cat fecal inoculum and in vivo utilization of diets containing selected fiber sources and their blends. J. Anim. Sci. 73:2329-2339.

Sunvold, G. D., Fahey, G. C., Jr., Merchen, N. R., Titgemeyer, E. C., Bourquin, L. D., Bauer, L. L., and G. A. Reinhart. 1995b. Dietary fiber for dogs: IV. In vitro fermentation of selected fiber sources by dog fecal inoculum and in vivo digestion and metabolism of fiber-supplemented diets. J. Anim. Sci. 73:1099-1109.

Sunvold, G. D., Hussein, H. S., Fahey, G. C., Jr., Merchen, N. R., and G. A. Reinhart. 1995c. In vitro fermentation of cellulose, beet pulp, citrus pulp, and citrus pectin using fecal inoculum from cats, dogs, horses, humans, and pigs and ruminal fluid from cattle. J. Anim. Sci. 73:3639-3648.

Takahashi, E. 1974. Comparative Plant Nutrition. Yokendo, Tokyo.

Tanaka, O., Tamura, Y., Masuda, H., and K. Mizutani. 1996. Applications of saponins in foods and cosmetics: Saponins in Mohave yucca and *Sapindus mukurossi*. In: G. R. Waller and K. Yamasaki (Eds.) Saponins Used in Food and Agriculture. pp 1-11. Plenum Press, NY.

Thonney, M. L. 1981. Acid insoluble ash as digestion marker. In: Proc. Cornell Nutrition Conference for Feed Manufacturers. pp 118-122. Cornell University, Ithaca, NY.

Tilley, J. M. A., and R. A. Terry. 1963. A two-stage technique for the *in vitro* digestion of forage crops. J. Brit. Grassland Soc. 18:104-111.

Tisserand, J. L. 1988. Non-ruminant herbivores; horses and rabbits. Livest. Prod. Sci. 19:279-288.

Uden, P., and P. J. Van Soest. 1982. Comparative digestion of timothy (*Phleum pratense*) fibre by ruminants, equines and rabbits. Br. J. Nutr. 47:267-272.

Ueda, K. 1961. Report on a study on improvement in bamboo plantation in India. Agricultural Department of the Kyoto University on behalf of the Japan Consulting Institute.

Vander Noot, G. W., and E. B. Gilbreath. 1970. Comparative digestibility of components of forages by geldings and steers. J. Anim. Sci. 31:351-355.

Van Keulen, J., and B. A. Young. 1977. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. J. Anim. Sci. 44:282-287.

van Setten, D. C., and G. van de Werken. 1996. Molecular structures of saponins from *Quillaja saponaria* Molina. In: G. R. Waller and K. Yamasaki (Eds.) Saponins Used in Traditional and Modern Medicine. pp 185-193. Plenum Press, NY.

Van Soest, P. J. 1982. Silica. In: Nutritional Ecology of the Ruminant. pp 72-74. Cornell University Press, USA.

Van Soest, P. J. 1993. Cell wall matrix interactions. In: H. G. Jung, D. R. Buxton, R. D. Hatfield, and J. Ralph (Eds.) Forage Cell Wall Structure and Digestibility. pp 389-390. Amer. Soc. Agr, Inc., Crop Sci. Soc. of Amer., Inc., Soil Sci. Soc. of Amer., Inc., Madison, WI.

Van Soest, P. J., and L. H. P. Jones. 1968. Effect of silica in forages upon digestibility. J. Dairy Sci. 51:1644-1648.

Veira, D. M. 1986. The role of ciliate protozoa in nutrition of the ruminant. J. Anim. Sci. 63:1547-1560.

Vermorel, M., Vernet, J., and W. Martin-Rosset. 1997. Digestive and energy utilisation of two diets by ponies and horses. Livest. Prod. Sci. 51:13-19.

Wall, M. E., Krider, M. M., Rothman, E. S., and C. R. Eddy. 1952. Steroidal sapogenins: 1. Extraction, isolation and identification. J. Biol. Chem. 198:533-543.

Wallace, R. J., Arthaud, L., and C. J. Newbold. 1994. Influence of *Yucca shidigera* extract on ruminal ammonia concentrations and ruminal microorganisms. Appl. Environ. Microbiol. 60:1762-1767.

Wang, Y., McAllister, T. A., Newbold, C. J., Cheeke, P. R., and K.-J. Cheng. 1997. Effects of yucca extract on fermentation and degradation of saponins in the RUSITEC. In: Proc., Western Section, Amer. Soc. Anim. Sci. 48:149-152.

Wang, Y., McAllister, T. A., Newbold, C. J., Rode, L. M., Cheeke, P. R., and K.-J. Cheng. 1998. Effects of *Yucca schidigera* extract on fermentation and degradation of steroidal saponins in the rumen simulation technique (RUSITEC). Anim. Feed Sci. Technol. 74:143-153.

Wilson, R. C., Overton, T. R., and J. H. Clark. 1998. Effects of *Yucca schidigera* extract and soluble protein on performance of cows and concentrations of urea nitrogen in plasma and milk. J. Dairy Sci. 81:1022-1027.

Wu, Z., Sadik, M., Sleiman, F. T., Simas, J. M., Pessarakli, M., and J. T. Huber. 1994. Influence of *yucca* extract on ruminal metabolism in cows. J. Anim. Sci. 72:1038-1042.

Yeo, J., and K.-I. Kim. 1997. Effect of feeding diets containing an antibiotic, a probiotic, or yucca extract on growth and intestinal urease activity in broiler chicks. Poult. Sci. 76:381-385.

APPENDIX

POTENTIAL APPLICATIONS OF *YUCCA SCHIDIGERA* AS A MOLLUSCICIDE OR ANTIFEEDANT

The saponins of *Phytolacca dodecandra* (endod) have been extensively studied for their molluscicidal activity and their consequent role in inhibiting the life cycle of schistosomes (Thiilborg et al., 1994; Hostettman et al., 1996). Although there is evidence that slug damage is not greatly minimized when plants are treated with saponins (Ester and Nijënstein, 1996), several patents exist which incorporate the use of saponins for control of slugs and other molluscs (Patent Nos. 5,290,557; 5,639,794; 5,698,191). The effects of the active ingredients could potentially be enhanced by the increased membrane permeability caused by saponins (Price et al., 1987). Molluscicidal activity appears to be inherent to monodesmosidic triterpenoids and not those classified as bidesmosidic (Hostettman et al., 1982; Domon and Hostettman, 1983). *Yucca aloifolia* contains steroid saponins identified to have molluscicidal activities (Hostettman and Marston, 1995).

Metaldehyde, one of the common active ingredients in slug baits, has been implicated in the poisoning of birds and mammals (Patent No. 5,437,870; Brown et al., 1996). It was desired to determine if the proportion of metaldehyde in slug bait pellets could be reduced, while maintaining or enhancing the effectiveness through the addition of *Y. schidigera* powder (DK-30). Molluscicidal and antifeedant experiments were conducted according to a modified procedure (Heim, 1997). Slugs were harvested from a local nursery and five slugs randomly assigned to individually labeled plastic containers (Rubbermaid shoeboxes). Holes were drilled in lids to provide air circulation. Moistened paper towels were placed in the bottom of the containers to provide humidity. To assess the antifeedant and molluscicidal properties of DK-30, containers were

randomly assigned to two treatments. The first treatment involved a single carrot rolled in yucca powder being placed in the appropriate container, and the other treatment had a yucca powder barrier around the carrot. During the 7-d length of the trial, a piece of the carrot covered with DK-30 was eaten only once. Data was analyzed using SAS v. 6.12 (SAS, 1988). Mean mortality was greater for those containers with the carrot surrounded by the powder barrier ($P = .05$; SLR), than for the powder-covered carrot. Slugs attempted to feed on carrots, despite the surrounding powder barrier. This may have resulted in yucca saponins being absorbed through the membranes of the foot, culminating in death. A second trial was conducted to determine the synergistic effect DK-30 may have with metaldehyde. All combinations of yucca powder (0, 5, 10%) and metaldehyde (0, 2, 4%) were separately incorporated into bran-based pellets. The molluscicidal effectiveness of the pellets was evaluated using the same setup protocol as that used for the previous experiment. The mean mortality for 5% yucca - 0% metaldehyde pellets was greater ($P < .10$; MLR) than that obtained for 0% yucca - 2% metaldehyde, 0% yucca – 4% metaldehyde, and all 10% yucca – metaldehyde combinations. Slug mortality was also higher ($P < .10$) for 5% yucca – 4% metaldehyde when compared to 0% yucca – 2% or -4% metaldehyde, and 10% yucca – 0% or –2% metaldehyde. These results indicate that there may be a synergistic effect between metaldehyde and *Y. schidigera* saponins, which could be confirmed with additional studies, both in the laboratory and in the field.

I would like to thank George Baker at OR-CAL, Inc. for his financial generosity and expertise, as well as Shonnard's Nursery & Florist (Philomath, Oregon) and Garland Nursery (Corvallis, Oregon) for providing slugs.

REFERENCES

Brown, P., Charlton, A., Cuthbert, M., Barnett, L., Ross, L., Green, M., Gillies, L., Shaw, K., and M. Fletcher. 1996. Identification of pesticide poisoning in wildlife. J. Chromatogr. A 754:463-478.

Domon, B., and K. Hostettman. 1983. Saponins with molluscicidal properties from *Lonicera nigra* L. Helvetica Chimica Acta 66 (36):422-428.

Ester, A., and J. H. Nijënstein. 1996. Molluscicidal seed treatment of barley, wheat and perennial ryegrass to control the field slug (*Deroceras reticulatum*). Netherlands Journal of Agricultural Science 44:241-248.

Heim, I. 1997. Protocol: Biological efficacy test for pellets containing molluscicides used against slugs. Lonza Ltd., Switzerland.

Hostettman, K., Kizu, H., and T. Tomimori. 1982. Molluscicidal properties of various saponins. Journal of Medicinal Plant Research 44:34-35.

Hostettman, K., and A. Marston. 1995. Molluscicidal activity. In: Saponins. pp 291-295. Cambridge University Press, Great Britain.

Hostettman, K., Marston, A., Maillard, M., and J.-L. Wolfender. 1996. Search for molluscicidal and antifungal saponins from tropical plants. In: G. R. Waller and K. Yamasaki (Eds.) Saponins Used in Traditional and Modern Medicine. pp 117-128. Plenum Press, NY.

Price, K. R., Johnson, I. T., and G. R. Fenwick. 1987. The chemistry and biological significance of saponins in foods and feeding stuffs. CRC Critical Reviews in Food Science and Nutrition 26:27-135.

SAS. 1988. SAS/STAT® User's Guide (Release 6.03). SAS Inst. Inc., Cary, NC.

Thiilborg, S. T., Christensen, S. B., Cornett, C., Olsen, C. E., and E. Lemmich. 1994. Molluscicidal saponins from a Zimbabwean strain of *Phytolacca dodecandra*. Phytochemistry 36:753-759.