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BARLEY SILAGE EFFECTS ON POULTRY BEHAVIOUR

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By

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ABSTRACT

A series of trials were conducted to determine the effect of feeding barley silage to laying hens and broiler breeder pullets on performance, stress and behaviour. In the first study, two trials were conducted each with 20 hens and 2 roosters (n=176) randomly assigned to one of 8 community cages. The birds in 4 cages were provided with a nutritionally balanced soy/wheat–based laying hen diet *ad libitum*, whereas the birds in another 4 cages were given free access to barley silage in addition to the regular laying hen diet. In both trials, the control birds consumed more feed ($P < 0.05$) than the birds given barley silage. Birds fed barley silage had significantly decreased ($P < 0.05$) aggressive and feather pecking behaviours as well as time spent in their nest boxes at different ages. Time spent drinking, resting, preening and eating a large particle calcium source was similar between the two treatments. No treatment effects ($P > 0.05$) were found in regards to egg quality, egg production and bird weights at various ages; however yolk colour was darker by silage treatment in each trial. At the end of each trial, the feather score was improved in silage-fed birds compared to the control birds. It was concluded that feeding barley silage as a supplement to laying hens can improve their welfare without negatively affecting the egg production and egg quality.

A second study was conducted to determine the effect of feeding barley silage on body weight, stereotypic behaviour, stress and fear on broiler breeder pullets during the brooding and rearing periods. The 3 week old broiler breeder pullets (n=180) were randomly allocated into 12 straw litter floor pens having 15 birds per pen. The birds in 6 pens were provided with a nutritionally balanced corn/oat-soybean/canola meal-based broiler breeder diet at recommended restricted levels, whereas the birds in another 6 pens were given free access to barley silage in addition to a regular broiler breeder diet. Total DM intake was significantly higher ($P < 0.05$) for silage-fed birds compared to their control counterparts without affecting mean body weights. Dietary treatment had no significant effect on bird behaviour with the exception of object pecking behaviour which was reduced with silage feeding. Aggressive and gentle feather pecking behaviour was consistently numerically higher in the control birds than the silage-fed birds, although not significantly. Age affected many of the behaviours recorded in this study. Silage feeding had no significant effect on heterophil to lymphocyte ratios and tonic immobility values indicating that birds in both treatments were not very stressed or fearful. It was concluded that feeding barley silage to broiler breeder pullets has potential to aid in improving their welfare.

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LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
AME	Apparent metabolizeable energy
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
CARC	Canadian Agri-Food Research Council
CIE	International Commission of Light
CV	Coefficient of variation
DM	Dry matter
EDTA	Ethylene diamine tetraacetic acid (anti-coagulant)
EU	European Union
FACS	Farm Animal Council of Saskatchewan Inc.
FAWC	Farm Animal Welfare Council
PROC GLM	General linear model procedure in statistical analysis
H:L	Heterophil to lymphocyte ratio
NDF	Neutral detergent fibre
NRC	National Research Council
SEM	Standard error of the mean
TI	Tonic immobility
THDP	Total hen-day production
THHP	Total hen-housed production

1.0 GENERAL INTRODUCTION

Animal management practices and production systems are commonly a topic of debate and controversy within today's society. Although some consumers may be very well informed, generally speaking, the public has an interest but limited knowledge on factors involved in raising animals for the purposes of human consumption, sport, entertainment, medical/scientific and breeding. Consumer purchasing decisions are usually based on the information they are given and as such consumers may be skeptical when hearing or reading material from the media. General consensus is that consumers want to know the real facts but lack the scientific capabilities or knowledge to source the information themselves.

Scare tactics by animal rights extremists are often used to play on the already teetering public perceptions towards concerns of animal treatment and welfare. Producers have recently become involved as spokespeople in their areas of specialty to educate and present the facts on animal production issues in conjunction with organizations for the fair and ethical treatment of animals such as The Farm Animal Council of Saskatchewan Inc. (FACS) whose mission statement reads:

> To represent the livestock industry in advancing responsible animal care and handling practices in agriculture by seeking consensus, sharing information, presenting research, factual information and perspectives from all commodities, respect for others in their industry, quality care for animals and making a positive contribution to our world through responsible animal agriculture.

The concerns are more elevated in European countries, such as with the banning of battery cages for laying hens, however North America is also concerned with these matters. Research is currently being conducted on enhancing animal environments to decrease abnormal behaviours normally associated with boredom and frustration due to the inability to perform their natural behaviours (Whittaker *et al.,* 1998; Savory and Mann, 1999a). Examples include feeding sugar beet pulp to sows to decrease stereotypical oral behaviours such as tail biting (Olsen, 2001) and manipulating straw/chains (Whittaker *et al*., 1998) and feeding low-nutrient, fibre dense feeds to laying hens to decrease feather pecking (Zaczek *et al.,* 2003; Hocking *et al.,* 2004; De Jong *et al.,* 2005). Also included are preference tests to examine an animal's desire to work for an outcome of their choice, whether it be for food, housing conditions or pen mates (Blokhuis, 1986; De Jong *et al.,* 2003).

Laying hens have been associated with having high incidences of feather pecking and cannibalism within their flocks especially when closely housed in community cages with an absence of litter and environmental enhancements. Broiler breeder birds are thought to spend a portion of their life in a state of hunger, primarily in brooding and rearing periods, as they are severely feed restricted in order to control body weight and bird uniformity. Both types of birds exhibit stereotypic behaviours according to their environmental and feeding conditions; usually a mash in both cases which is easily and relatively quickly consumed.

It is because of these associated abnormal behaviours that research was initiated at the University of Saskatchewan. By providing birds with a less nutrient dense feedstuff, barley silage, a fermented product having various particle sizes, it was thought an increase in bird satiety levels and increased time spent eating would occur. By spending more time foraging, pecking, eating and digesting the silage, the birds would spend less time focusing on stereotypic behaviours. Barley silage was utilized due to its availability on the University of Saskatchewan farm and that it also contained various sized particles within it differing from their mash feed.

The objectives of this research were to:

- determine whether hens of either laying or broiler breeding type would eat barley silage when given free access to it in addition to their regular feeding program;
- determine whether bird behaviour would be altered from abnormal stereotypical behaviours normally associated with their specific management programs as a result of eating barley silage;
- investigate the effects barley silage had on production parameters, body and feather condition in laying hens, stress and fear responses in broiler breeder pullets.

2.0 LITERATURE REVIEW

2.1 Concerns involving commercially raised poultry

An interesting relationship exists between what an animal eats and the behaviours it exhibits. Many researchers have delved into the idea by conducting studies either inhibiting or activating stereotypic behaviours. Such behaviours have included feather pecking and feather eating in poultry, sham chewing and bar biting in swine as examples. The welfare and economic effects within the poultry industry continue to be of major concern to ethologists, researchers and producers. There are several factors which influence behaviour and in turn lead to welfare and economic areas of concern. It is important to define the language used most often; the study of animal behaviour is known as ethology which has become closely associated with the term animal welfare and in many cases, the terms are used interchangeably (Gonyou, 1994). Initially, concerns within the animal industry were focused on animal production rather than their welfare but currently there are more researchers examining all aspects of animal welfare and well-being (Gonyou, 1994). Many studies have been conducted to enable researchers to understand why an animal acts the way it does. Through the process of evolution, bird behaviours have in some ways changed, such as with broodiness, or have remained the same, such as with fearfulness and feeding (Appleby *et al.*, 2004). Internal and external factors affect how an animal reacts or behaves and each factor can be attributed to the overall health and well-being of animals. Nutrition is a key area studied including how it directly relates to the behaviour of animals involved.

There are several parameters to consider when studying behaviour and welfare concerns in today's animal production systems. Intensive production systems have spawned many concerns within the general public evolving from battery housed hens, confined sows, crating of veal calves and feed restriction of broiler breeders to only name a few. It is perhaps appropriate to start with some definitions and meaning of abnormal behaviour, stereotypies and welfare before discussing this issue further as the presence of stereotyped behaviours is usually associated with welfare impairment.

Abnormal behaviour, as explained by Fraser and Broom (1990) and Broom (1991), is indicated as a behaviour that differs in pattern, frequency or context from that which is shown by a majority of those species in conditions where a full range of behaviour is allowed. This abnormal behaviour may only be short term to enable the individual to cope in a particular situation but it will still be an indicator of poor welfare compared to another animal that does not have as much difficulty in coping. An example of this may be seen when treating cattle or moving them into different pens, younger animals not yet having much life experience may show panicked or "frozen" behaviour which elicit higher heart rates or higher injury rates compared to the older animals having experience through repetition.

Stereotyped behaviour may be characterized by a repeated, relatively invariate sequence of movements having no obvious purpose (Ödberg, 1978) and are usually shown in situations where the individual lacks control of its environment. Although stereotypies occur in healthy individuals they usually appear in situations that prove to be difficult and so are generally associated with an impaired level of welfare (Broom, 1986). A good example of this is shown by Duncan and Wood-Gush (1972) where they trained hens to feed in a particular space and then proceeded to cover the feed with a transparent cover to prevent the hens from attaining any feed. Out of frustration (from lack of control over this situation), the hens developed stereotypic pacing and also aggressively pecked other nearby birds.

Many welfare definitions exist and can be quite complicated. For the most part, welfare to most people involves general enjoyment and quality of life free of any suffering. But how would one determine whether an animal has positive or reduced welfare? An understanding of the animals' general behaviour, interaction and physiology would need to be known and understood in order for a comparison to be made. This is where complications arise as one person's view will differ from another's based on experience, scientific knowledge and/or training; all evidence and opinions must be examined before strong statements can be made. Biological parameters must also be in place in order to properly define whether an animal has reduced welfare or not.

Public awareness was probably most apparent after the arrival of a book titled *Animal Machines*, by Ruth Harrison (1964) which described her thoughts on the intensive animal production systems at that time. Her book encompassed the more extreme issues with farming focusing mostly on poultry production (both egg and table meat birds) including debeaking, disease, housing systems and loss of individual life values. Other species were discussed as well as her views on problems in the food industry and welfare concerns. Harrison stated she was not an expert in the areas of livestock production and so was not trying to advocate either way but her goal was to collect information and portray the animal industry to the public as it was. This concept had not really been done before and as it turned out, her book was very controversial. At one point in her book, Harrison stated:

> … the extent to which farming has now carried its 'interference with nature' has reached far beyond depriving the animal of its birthright of freedom, sunlight and

> green fields. It has now reached the point of frustrating practically every natural instinct in animals – except the instinct of survival.

She then stated in response to farmers' arguments that if animals were not healthy and content, they would not be productive that:

> … all their energy must go into converting food into flesh and it is hardly surprising that they put on weight. But this has nothing to do with contentment or health.

A report followed shortly after the release of Ruth Harrison's book directed by a Government appointed committee for improvements in the animal production legislation, known as the *Brambell Committee Report*, chaired by Professor F. W. Rogers Brambell (Command Paper 2836, 1965), where investigations were conducted by professionals in the industry and public individuals:

> To examine the conditions in which livestock are kept under systems of intensive husbandry and to advise whether standards ought to be set in the interests of their welfare, and if so what they should be.

From this report arose awareness of the currently well known five freedoms; where animals should have sufficient freedom 1) from thirst and hunger, 2) from discomfort, 3) from pain, injury or disease, 4) to express normal behaviour and 5) from fear and distress (FAWC, 1998). Although there have been some variations from the originals, these freedoms posses a solid basis to follow in any livestock production.

Following are some welfare definition examples taken from existing and past research to show the range of opinions:

> The welfare of an individual is its state as regards to its attempts to cope with its environment (Broom, 1986);
>
> … welfare is that it must refer to a characteristic of the individual animal rather than something given to the animal by man…it (welfare) has to be defined in such a way that it can be readily related to other concepts such

> as: needs, freedoms, happiness, coping, control, predictability, feelings, suffering, pain, anxiety, fear, boredom, stress and health (Broom, 1996);
>
> … animal welfare science operates on a playing field where social values (including ethical ones) explicitly or implicitly determine the boundaries and rules of the science (Rollin, 1996);
>
> …measurements of welfare should be based, not on the presence or absence of indicators of reduced biological function which, in evolutionary terms, could be considered primary, but on the animal's feelings or emotions (Duncan, 1996).

The author agrees with those ideas of Broom (1996) where all factors must be taken into account before determining the level of impaired welfare resulting from the situation an animal is in. As Broom also states, welfare assessment must include a direct measurement of the indicators of poor welfare as well as a sophisticated study of animal preferences. Housing and management test systems for livestock production, in order to be good, must be carried out to gain an understanding of animal preferences. As scientists, we need to understand what animals prefer (i.e. feed, environment, lighting, foraging compounds, housing space, etc…) in order to treat them in a humane way. In order to gain these results, observations from animals in their most enriched environment allowing them to spend their time as they wish would perhaps be a useful preliminary guide in developing such accommodations.

As mentioned by Mendl (1990), animal choices will depend on their existing environment at the time. An example would be where sows housed in group situations may show a preference to seek straw bedding or pen mates to sleep with in winter seasons in order to stay warm whereas in summer seasons, they may seek out wet, cooler areas. Their choice may also depend on their environment during growth and development and so may in fact not improve their welfare.

2.2 Behaviour and welfare issues related to laying hens

Feather pecking is a common behaviour vice in laying hens which can lead to cannibalism and death. It is affected by both internal (i.e. genetic) and external (i.e. environmental) factors. Research has indicated that nutrition has an important influence on feather pecking. A deficiency in nutrients, particularly for protein and amino acids, can increase the incidence but other factors such as the nature and level of fibre may also be important (Savory *et al.*, 1999; McKeegan *et al.*, 2001).

It is suggested that as laying hens are exposed to other forms of interest (i.e. wood shavings, straw, etc…) the event of feather pecking decreases (Sanotra *et al.*, 1995; Huber-Eicher *et al.*, 2001). Other research suggests that feather pecking is a redirected behaviour associated with ground pecking and so in the event ground pecking is not an option for birds, such as with caged birds, their focus turns to pen mates' feathers (Blokhuis and Arkes, 1984; Blokhuis, 1986; Rodenburg *et al.*, 2004). This behaviour may start from social exploration as suggested by McAdie and Keeling (2000) and then turn into damaging feather pecking leading to economic and welfare concerns. It is interesting to note however, that litter based facilities have noticed cannibalism as well. As Appleby *et al.* (2004) suggest, birds are social creatures and are affected by their surroundings. The actions and behaviour of one bird in a flock can easily spread and affect other bird behaviour. Even though these birds are able to forage, if they are housed within large groups or with high light intensities, this can cause frustration leading to feather pecking and aggression but also can increase the chances of curious pecking which may lead to damaging pecking. Hughes (1973) suggests that initiation of feather pecking and cannibalism relates to hormone concentrations and is most often seen in female birds around the time of sexual maturity. He found that the incidence of feather pecking increased in immature females with injections of progesterone, especially in combination with oestradiol, while the behaviour was reduced with the injection of testosterone.

It has been suggested when birds spend more time focusing on eating and foraging it decreases the time spent focusing on litter mates feathers. This is difficult to accomplish in cages because of the cage floor and nature of the feeding system. However some feed ingredients may provide at least a partial alternative. For example, silage is novel and contains many sizes and types of food particles which might stimulate foraging behaviour. In addition, chickens appear to favour silage so may spend more time eating thereby reducing the incidence of other behaviours such as feather pecking (Steenfeldt *et al*., 2007).

2.3 Behaviour and welfare concerns related to broiler breeders

Feeding programs of broiler breeders have been researched extensively. National and international animal care programs exist to ensure that a standard practice exists for producers to promote sound animal care. Government funding has been offered to promote research on improving the welfare of feed restricted broiler breeders (FAWC, 1998). The main concern regarding broiler breeder feeding programs is that birds are feed restricted during their brooding, rearing and laying periods suggesting that they spend most of their lives in a state of hunger. The idea of feed restriction came into play when it was realized that body weight and uniformity play a key role in fertility, hatchability and settable eggs. It is known to be very important to restrict feed intake in order to have control over bird feeding and prevent overeating, thereby reducing excess fat deposits. Feed restriction plays a large role in improving overall health and reproduction later in life (De Jong *et al.,* 2003).

Broiler breeders that are feed restricted elicit stereotypic pecking and pacing behaviours. Some studies suggest that with feed restriction, the incidence of boredom and frustration out of hunger enhances these behaviours leading to birds pecking obsessively at feeders in the hopes of retrieving more feed (De Jong *et al.*, 2002 and 2003). In males, aggression towards other males and also towards females during mating becomes a key factor as this behaviour leads to severe injuries or even death (Millman and Duncan, 2000b, Millman *et al.*, 2000). Lighting programs have been used to reduce aggression in birds generally as well as nutrient diluted diets (Appleby *et al.,* 2004).

2.4 Tests of welfare in poultry

Levels of fear and stress are often measured in studies involving feed restricted birds to give an indication of bird welfare. Two common tests involve looking at the heterophil to lymphocyte ratios (H:L) and a physical test of tonic immobility (TI) however these two common tests should not be the overriding factors when discussing animal welfare; they only identify one part of the welfare equation.

2.4.1 Stress (Heterophil to Lymphocyte ratios)

Changes in heterophil to lymphocyte (H:L) ratios have been shown to indicate levels of chronic stress in an animal (Gross and Siegel, 1983) as heterophils are the first line of defense in immune response and as such, the more stressed an animal is, the higher number of heterophils there will be in the blood (Gartner and Hiatt, 2000). A variation of this procedure is often conducted on various species such as with horses (neutrophil to lymphocyte ratio) during transportation (Stull, 2000), and turkeys, ducks, broilers and laying hens as a means to determine their reactions to environment and management factors (McFarlane and Curtis, 1989; Gross, 1990; Maxwell *et al.,* 1992). In birds, blood is collected from the wing vein (brachial) directly into a vacutainer tube, blood smeared slides are made and slides are examined under oil immersion microscopy to count the numbers of blood cells. More often than not, heterophils and lymphocytes are the primary cells measured in determining the H:L ratio (Robertson *et al.,* 1990). Use of the H:L ratio has suggested that high levels of fibre within a diet improve the welfare of birds (Hocking *et al.,* 2004), perhaps due to the calming effect of eating and increased time digesting the fibre.

2.4.2 Fear (tonic immobility)

Fear is generally a response mechanism in preparation to a dangerous situation or a reaction to danger itself (i.e. predation risk or injury caused by physical event) (Broom, 1991). A common test used to determine the level of fear is by measuring an animal's "flight or fight" reaction. In birds the test is referred to as Tonic Immobility (TI). Where some species may react in a fight or flight scenario, birds demonstrate hypnosis like behaviour or a hypnotic gaze with waxy flexibility of the limbs (Maser and Gallup, 1974). It is conducted by holding a bird on its' back within a U-shaped saddle and timing how long it takes for the bird to upright itself – the longer it takes for the bird to move, the more fearful it is (Gallup, 1979).

Birds are removed from their pen, quickly and quietly placed onto their backs in a U-shaped saddle and a hand is placed gently over their head for an induction time of 15 seconds to place them into a tonic state. If they right themselves within 10 seconds after induction, the test is repeated for a maximum three times. If they right themselves within 10 seconds all three times, the test is a fail meaning they are not fearful (or have limited fear). If in fact they stay in a tonic state after induction, it is best not to allow them to stay past a maximum 10 minutes when the tester will intervene and bring the bird out of the tonic state. De Jong *et al.* (2003) suggest that feed restricted birds are less fearful than *ad libitum* fed birds based on the fact that these birds are anxious to feed and will start to associate people with being fed. When people are present, the birds expect to be fed.

2.4.3 Behaviours

Colony caged and feed restricted birds have been shown to exhibit specific stereotypic behaviours. Layers housed in colony cages tend to exhibit aggressive feather pecking behaviour leading to cannibalism while feed restricted birds, such as with broiler breeders tend to spend a lot of time pacing, object pecking, feeder pecking and in some cases feather pecking (Hocking *et al.*, 2004).

2.4.4 Mortality

Mortality is commonly monitored in poultry production systems and is a clear indicator of welfare. An indication of a problem would exist where mortalities are abnormally high.

2.4.5 Feed intake and body weight

The key to raising healthy, productive birds with uniform body weights and adequate nutrition to meet the birds' production levels is to ensure that birds are weighed frequently to determine appropriate feed allocation. It is important to sample a representative number of birds in order to receive information which best indicates bird flock status. Any deviation from the norm, such as low or high body weights and low productions numbers, would give a clear indication of an existing problem (i.e. disease, lack of feeder space for number of birds in pen/cage, etc…). It is common for farm managers to have checklists, weekly or monthly, recording animal weights, production numbers and feed intakes as appropriate animal management practices in order to keep updates on the status of their flock/herd.

2.5 Role of nutrition in improved welfare

There are several factors to consider when investigating the cause of a stereotypic behaviour resulting in impaired welfare. Internal factors involve anything that relates to the animal itself such as genetics, social experiences, hunger and age. External factors have been studied as they relate to bird behaviour and include housing, diet composition and feeding management (Krimpen *et al.*, 2005). Krimpen *et al.* (2005) also suggest that the interaction between these internal and external factors will also cause negative stereotypical behaviours (i.e. feather pecking behaviour).

2.5.1 Internal factors

Looking at internal factors involving poultry, research states that genetics plays a large role in feather pecking and aggressive behaviour of animals (Klein *et al*., 2000; Millman and Duncan, 2000a, 2000b; Rodenburg *et al.*, 2004) and is a possible way to help solve the problem of feather pecking within a flock of birds by selecting against particular genetic lines. Social exploration and experiences also alter how birds relate to their environment and pen mates in regards to feather pecking and eating (McAdie and Keeling, 2000; McKeegan and Savory, 2001). It has been found that there are definite aggressive birds within a flock, feather peckers, who will influence others within the flock to feather peck at the non-peckers or birds that do not fight back (McAdie and Keeling, 2000; McKeegan and Savory, 2001).

2.5.2 External factors

When looking at external factors involving poultry, it makes sense when researchers say that increased housing density increases the effect of negative behaviour. Birds are very social animals but extreme densities can result in more aggressive behaviour which is thought to be related to self preservation (Smith and Daniel, 2000). Other consequences of dense housing include an increased risk of mortality due to crushing or crowding, increased disease and competition for feeder space. Strong regulations presently exist both provincially and in the European Union (EU) to control these situations. Guidelines are provided for housing density depending on the type of production (i.e. caged layers, broilers, turkeys, etc…).

Some feeding programs researched to improve the hunger and welfare issues of feed restricted birds have provided nutrient diluted diets (Zuidhof *et al.*, 1995; De Jong *et al.,* 2005), diet supplementation (Steenfeldt *et al.*, 2007), different concentrations and sources of dietary fibre (Zaczek *et al.*, 2003; Hocking *et al.*, 2004; De Jong *et al.*, 2005), low protein diets and different allocations of food during rearing and restricting feeding at onset of lay (Hocking *et al.*, 2001 and 2002a) and altering meal size and food form (Savory *et al.*, 1999).

2.6 Diet composition

2.6.1 Energy

Poultry diets are usually based on the energy content of a feedstuff, bird type and stage of development. The geographics and location of producers determines sources of feed they use to give the most efficient energy source and economic value (i.e. Western Canada uses barley and wheat whereas Eastern Canada uses corn). Typically, the higher the diet is in energy, the less the birds require. The lower the energy provided in a diet, the more the birds will have to eat to reach the same energy level as if they were fed a higher energy diet. Producers tend to feed birds higher energy diets to lower the feed conversion ratios and to remain economical.

Where behaviour problems are seen, such as aggressiveness or feather pecking, birds can be fed a low value feed to decrease the incidence of these behaviours. With this type of feeding, birds will spend most of their time eating thereby reducing aggressive behaviour. Such diets could include the feeding of oat hulls, whole cereal grains (Hetland *et al.,* 2001; De Jong *et al.*, 2005) or silage (Steenfeldt *et al.*, 2007). Birds have the ability to select food to meet their nutritional requirements on the basis of their life stage, environment and production and/or maintenance (Leeson and Summers, 2001; Appleby *et al.,* 2004). This behaviour likely was displayed more readily before the major domestication of the birds. Aside from backyard flocks, commercial poultry are not given choice and have to eat what they are provided whether they like it or not.

Energy is derived from the carbohydrates, fats and proteins within the diet and is used for the metabolic reactions of the bird; such as heat production, meat and egg production or deposition of body tissue (Leeson and Summers, 2001). Excess energy is not excreted and so it is important for producers to feed a diet which meets the birds' requirements otherwise excess energy will be deposited as fat on the bird. Birds have a great ability to monitor their energy levels and feed at a constant level. Feed formulation must take into account the needs of the birds, stage of development and production or maintenance needs. Again, it is important to ensure the energy balance is met for maintenance and production and is not exceeded otherwise the result will be increased fat deposition (Leeson and Summers, 2001).

2.6.2 Protein

Protein is essential for providing the basic structures of life; it provides structural support to soft tissues, skin, feather and beak formation, blood proteins, transport albumins and glubulins to maintain homeostasis within the body and they also transport nutrients and essential amino acids through the bloodstream and across membranes and provide immunity to disease (Leeson and Summers, 2001). The requirement for protein in chickens varies according to stage of development or production and also the chicken's growth and production potential (Appleby *et al.*, 2004). As such, the impact of specific levels of protein will vary with the type and age of chicken. Since amino acid balance is of primary importance in nutrition, correspondingly the balance of amino acids in feeds as well as their biological availability may impact behaviour. Specific protein sources may also vary in impact on behaviour based on other nutritional characteristics of the ingredient. This is suggested to be the case in a comparison of the impact of plant and animal based proteins on feather pecking. McKeegan *et al.* (2001) found that there was more damaging pecking in the plant protein fed hens than those fed animal based protein. Plant based proteins do not contain the animal factor vitamin B_{12} but do contain phytoestrogens, a steroidal oestrogen, and these differences rather than amino acid nutrition may have been responsible for the impact of protein source on behaviour.

Research completed by Schaible *et al.* (1947) has suggested that protein deficient diets increase the risk of negative behaviours such as feather pecking in laying hens, which in turn leads to cannibalism. They found that protein supplements, such as casein, liver meal, gelatin, blood meal and other protein sources to diets low in crude protein (13.5%), phosphorus (0.53%) and fibre (2.6%) reduced the incidence of feather pecking and cannibalism in young birds from 0-8 weeks of age. Another study completed by Ambrosen *et al.* (1997) suggests this same theory where feeding young birds either a low-protein (11.1%) or a high-protein (19.3%) diet resulted in 17.6 and 2.5% mortality, respectively, due to feather pecking and cannibalism. Plumage condition was also improved with the higher level of dietary protein.

Some studies have used varying levels of amino acids within the diet to control aggressive behaviours. An example is the use of increased dietary tryptophan to suppress aggression in males and feather pecking in laying hens. Tryptophan is the precursor of serotonin (a neurotransmitter in the brain) and so acts as a mood stimulator – the higher level of tryptophan in the diet, the calmer the birds will be. Shea *et al.* (1990) conducted a study of tryptophan as it relates to aggression and feather pecking by feeding broiler breeder males different doses within the diet (0, 3.8, 7.5 and 15.0 g/kg). It was discovered that at 3.8 g/kg and above, aggression and feather pecking in the male birds was significantly reduced. Savory *et al.* (1999) and Hierden *et al.* (2002) similarly found reduced feather pecking damage in laying hens fed higher levels of dietary tryptophan. It appears that feather pecking behaviour is triggered by a lowered serotonergic neurotransmission. By increasing serotonin levels (by increasing tryptophan in the diet) negative feather pecking behaviour decreases.

Feather development is heavily dependent on methionine and cysteine levels, the sulphur containing amino acids, within the diet. As McAdie and Keeling (2000) have suggested, feathers consist of approximately 89-97% protein and if birds are not fed appropriate amino acid levels to meet the requirement for proper feather development, this in turn will increase the risk of these birds being feather pecked. Birds are naturally curious and if anything stands out, such as deformed or straggly feathers, they are drawn to and out of curiosity will peck at them. This pecking may progress to damaging feather pecking and possibly cannibalism as this is also a learned behaviour; other pen mates will join in and overpower the victim birds. McAdie and Keeling (2000) suggest that if birds are deficient in protein, they may also partake in feather pecking and eating to gain that additional protein source in their diet.

It would likely be fair to say that any deficient diet will cause the bird to investigate in order to obtain the nutrients it needs which in itself could lead to the feather pecking behaviour.

2.6.3 Fibre

Several studies have been conducted on the impact of fibre on gut health, satiety and feeding behaviour of birds. Birds fed diets high in insoluble fibre have been shown to appear calmer and spend more time eating and performing other behaviours, such as standing, resting, preening rather than feather pecking (Hetland *et al*., 2004). Comparisons of feeding diets high and low in insoluble fibre have shown that those fed high fibre appeared calmer, spent more time eating and had a decreased mortality rate due to cannibalism than those with the low fibre diet (Hetland *et al., 2*001; Choct and Hartini, 2003). Digesta passage rate is affected by particle size and so by feeding a diet high in fibre, the gizzard retains the feed particles for a longer period of time until they are ground into smaller sizes allowing for continuation through the digestive tract. Possibly, the increased time digesta spends in the gizzard increases the feeling of satiety and in turn increases the feeling of contentment. Another possible explanation would be that hens are normally not hungry due to being fed *ad libitum* however do eat to maintain their energy requirements. If they have been provided with a diet lower in energy due to the high fibre content, their time spent eating will be increased to ingest the same level of energy.

A study conducted by Karlsson *et al.* (1997), showed that diet had no effect on plumage condition with high levels of oats or wheat. Birds fed high levels of oats (33% oats – 0% wheat) had a lower mortality due to cannibalism than did the birds fed a high wheat diet (25% wheat – 10% oats).

2.6.4 Vitamins and minerals

Various studies have concluded that birds have the ability to self select feeds which contain the specific nutrients required by the bird. For example, hens increase consumption of calcium prior to the onset of oviposition to ensure the shell gland has sufficient calcium to develop a strong shell. Birds have a high demand for vitamins and minerals within their diet as their stresses have increased dramatically through the domestication process. These stresses relate to environmental conditions, behaviour, disease stress and production levels (Leeson and Summers, 2001). When a diet is formulated, vitamins and minerals are usually not the focus but it is important to remember to feed at least the minimum levels of dietary NRC (1994) requirements and increase the levels as they relate to the production and stage of development of the birds. Birds with a deficiency in niacin, choline, folate, biotin and pantothenic acid may develop rough feathers which may in turn stimulate feather pecking from pen mates as an example (Leeson and Summers, 2001).

2.7 Feeding management

There are several factors resulting from feed management that relate to behaviour. Scratch feeding can be used as a management factor when males and females are housed together on litter or straw floors. Males will scratch at the floor, source out an interesting piece of feed, cluck to call the hens over to the feed so that the females will be attracted and allow mating to occur. Litter feeding also increases other behaviour such as foraging and dust-bathing, which in turn may reduce the focus of birds on the feather of pen mates. Litter floor systems provided with whole grains spread out allow other forage related behaviour to come out such as ground pecking and ground scratching whereas birds housed in cages are not able to forage and out of frustration, redirected forage behaviours are seen such as feather pecking, pacing and other abnormal behaviours (Blokhuis and Arkes, 1984; Appleby *et al.*, 2004). As mentioned earlier however, feather pecking and cannibalism is still seen in non-caged birds which may be related to light intensity, stocking density, hormone concentrations or curiosity (Appleby *et al.*, 2004).

2.7.1 Feed form

Feed form plays a huge role in the behaviours of poultry. Studies have shown that when birds are fed a mash and pellet diet, birds spend more time eating the mash. Pelleted diets are much easier to eat thereby decreasing time spent eating and more time performing other behaviours such as feather pecking (Appleby *et al*., 2001). This is in agreement with research conducted by Savory *et al*. (1999) where newly hatched chicks were divided up into three groups: group 1 was fed a standard diet in mash form, group 2 was fed the same standard diet but in pelleted form (3mm) and group 3 was fed the same mash diluted with 100g per kg of cellulose powder. They concluded that chicks fed the mash alone and cellulose supplemented mash had mean total feather pecking scores significantly less than those fed the pelleted diet.

So, as it has been briefly explained, nutrition plays an important role in the management and behaviour of poultry. The numerous studies mentioned in this thesis as well as those not mentioned, have supplied researchers with much information. By continuing to search out ways to customize or specialize diets to enrich the lives of commercial birds or better understand how birds “work/think”, will help in reducing such abnormal or stereotypic behaviour as feather pecking, feeder pecking or enhancing others such as dust-bathing, preening and feed intake control. Based on this premise, barley silage was fed to laying hens and broiler breeder pullets in order to determine whether feeding a supplemental high fibre diet would positively affect bird behaviour by reducing the stereotypic negative behaviours.

3.0 EFFECTS OF FEEDING BARLEY SILAGE ON FEED INTAKE, EGG PRODUCTION, BEHAVIOUR, STRESS AND FEATHER COVER IN LAYING HENS

3.1 ABSTRACT

Two trials were conducted to study the effect of feeding barley silage on behaviour, feather cover, stress, and egg production in laying hens. In each trial, 20 hens and 2 roosters (initiated at 20 and 19 weeks of age in trials 1 and 2, respectively) were randomly assigned to each of 8 locally designed community cages. Birds in 4 cages were provided with a nutritionally balanced soy/wheat–based laying hen diet *ad libitum*, whereas birds in another 4 cages were given free assess to barley silage in addition to the regular laying hen diet until the end of the trial (week 30 and 28 in trials 1 and 2, respectively). In both trials, control birds consumed less feed (92.1 and 87.3 g/b/d DM in trials 1 and 2, respectively) than the birds given barley silage (95.9 and 90.6 g/b/d DM in trials 1 and 2, respectively) in addition to regular laying hen diet, although results were significant in trial 2 only. Behaviour observation data from the two trials were combined and analyzed. Birds fed barley silage had significantly decreased ($P < 0.05$) aggressive and feather pecking behaviours as well as time spent in their nest boxes at different ages. Time spent drinking, resting, preening and eating a large particle calcium source was similar between the two treatments. No treatment effects ($P > 0.05$) were found in regards to egg quality, egg production and bird weights; however yolk colour was darker for the silage treatment. At the end of each trial, feather scores on different parts of the body as

well as overall feather score were improved in silage-fed birds compared to the control birds. In trial 2, treatment did not affect H:L ratio in laying hens, but feeding silage reduced this ratio in males. It was concluded that feeding barley silage to laying hens can improve their welfare without negatively affecting the egg production and egg quality.

3.2 INTRODUCTION

Feather pecking is a behavioural vice in laying hens that can lead to cannibalism and death, and is affected by both internal (i.e. genetic, innate beahviours) and external (i.e. environmental, social, management) factors (Choct and Hartini, 2003). Previous research has indicated that nutrition plays an important role in feather pecking behaviour in laying hens. A deficiency in nutrients, particularly of protein and amino acids, can increase the incidence but other factors such as the nature and level of dietary fibre may also be important (Savory *et al.*, 1999; McKeegan *et al.*, 2001).

It has been suggested that when laying hens are exposed to other forms of interest i.e. wood shavings, straw etc, the event of feather pecking decreases (Sanotra *et al.*, 1995; Huber-Eicher *et al.*, 2001). Other research suggests that feather pecking is a redirected behaviour associated with ground pecking and so in the event ground pecking is not an option for birds, such as in caged birds; their focus turns to pen mates' feathers (Blokhuis and Arkes, 1984; Blokhuis, 1986; Rodenburg *et al.*, 2004). This behaviour may start from social exploration as suggested by McAdie and Keeling (2000) which then turns into damaging feather pecking leading to economic and welfare concerns.

It has been suggested that when birds spend more time focusing on eating and foraging it limits the time spent focusing on litter mates' feathers. This is difficult to accomplish in cages because of the cage floor and nature of the feeding system. However some feed ingredients may provide at least a partial alternative. For example, silage contains many sizes and types of food particles which might stimulate foraging behaviour. In addition, chickens appear to favour silage so may spend more time eating/investigating thereby reducing the incidence of feather pecking (Steenfeldt *et al.*, 2007). Based on this premise, this study was designed to determine the consumption of barley silage by laying hens when given free access to both regular laying hen feed and silage, and to determine if feeding silage alters bird behaviour and/or reduces feather pecking when hens are housed in community cages where feather pecking is very common.

3.3 MATERIALS AND METHODS

Experimental protocol was approved by the Animal Care Committee of the University of Saskatchewan and was performed in accordance with recommendations of the Canadian Council on Animal Care as specified in the Guide to the Care and Use of Experimental Animals (1993).

3.3.1 Birds and Housing

Two trials were conducted each with 160 White Leghorn hens (Shaver White) and 16 roosters (Brown Leghorn roosters in trial 1 and White Leghorn roosters in trial 2). The chicks were beak trimmed at 1 day of age at the hatchery (Steinbach Hatchery and Feed Ltd., Steinbach, MB) using a hot blade trimmer. They were floor raised on litter until housing in experimental cages. Pullets were brooded and reared together with roosters. In trial 1, 20 hens and 2 roosters were randomly assigned to one of 8 community cages at 18 weeks of age with a trial duration of 11 weeks (20 to 30 weeks of age inclusive). Trial 2 was initiated at 19 weeks and took place until 28 weeks of age.

The cages used in this research have been previously shown to elicit moderate to severe feather pecking (Schwean, 1995). The locally manufactured cages were 1.2 m in width x 1.8 m in length with a height of 1.2 m, and were suspended over a shallow manure scraping track (Figures 3.1 and 3.2). CARC (2003) recommends at least 432 cm^2 for adult battery caged white-egg laying hens but the community cages used in the current study allowed for 982 cm^2 cage floor space per bird (not including perch space) which far exceeds that recommendation. The cage floor consisted of a 75% open plastic grid allowing waste and excreta to pass through. The front of the cage had two separate panels of horizontal bars extending the full length of the cage. Two feeders (1.80 m feeder space each) were attached to each side of the cage exterior just above floor level. Three perches were mounted at a height of 0.60 m which ran along the length of the cage. The perches were spaced 0.38 m from each other and allowed a roosting space of 16.2 cm per bird. The water was delivered through nipple drinker lines which hung underneath the two outside perches (4 per line totaling 8 nipple drinkers per cage). In order to meet the laying needs of the hens, a 1.20 m long x 0.54 m wide colony nest was mounted on the rear section of the cage (0.60 m above cage floor) with two nest openings and one metal divider to form two interior sections. The nest had a floor slope of 7° and was made of 2.54 cm x 5.08 cm wire mesh, covered with plastic, non-backed pliable broiler breeder Astroturf. Solid wall dividers were placed into each cage so birds could not watch birds in adjacent cages and potentially learn abnormal or feather pecking behaviours (McAdie and Keeling, 2002). In both trials, lighting increased from 8L:16D to 14L:10D at 19 weeks of age with a light intensity level of 10 lux. The room temperature was kept relatively constant at 20°C for the duration of each trial.

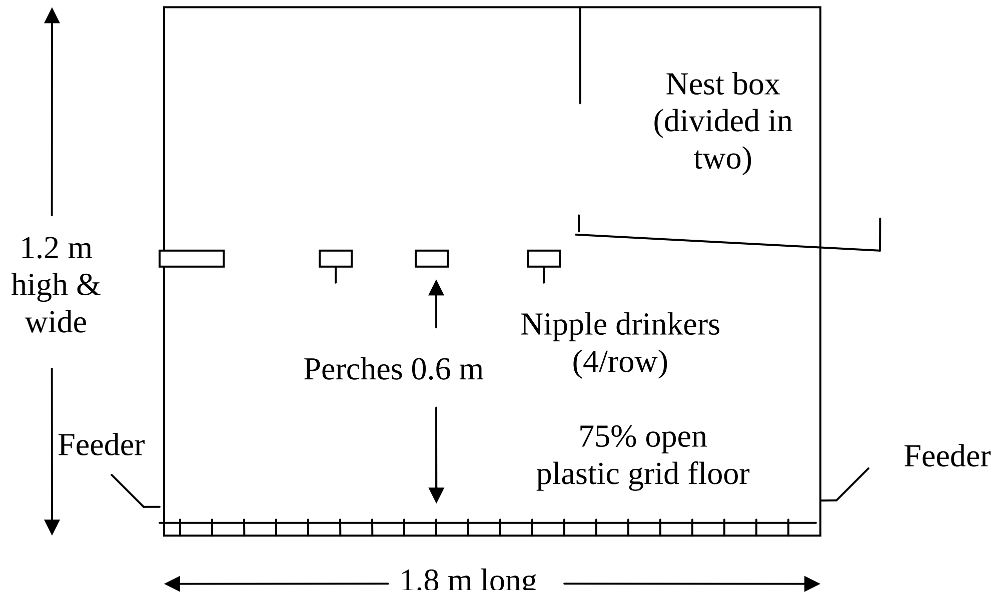

FIGURE 3.1. Design of a community cage used in the current study

(982 cm^2 floor space per bird).

FIGURE 3.2. Side view of community cage used in laying hen trials.

3.3.2 Experimental Diets and Design

Each dietary treatment was replicated 4 times and treatments were arranged in a completely randomized block design. From 20 (trial 1) and 19 (trial 2) weeks of age onwards, birds in 4 cages were provided with a nutritionally balanced wheat/soy–based laying hen diet (mash form) *ad libitum* in both feeders, whereas the birds in another 4 cages were given free access to barley silage in one feeder in addition to regular laying hen diet in another feeder (Table 3.1) A large particle calcium source, Sure Shel Calcium (IMASCO Minerals Inc., Surrey, B.C.), was also provided *ad libitum* within each cage to supplement bird diets in the event a high proportion of silage was consumed and therefore prevented hens from meeting their calcium requirement.

Small amounts of barley silage were given to birds in each of the four silage-fed community cages 3 weeks before initiation of data collection, in each trial, to allow the birds to acclimate to the feedstuff. The birds exhibited a strong desire to consume the silage in addition to the regular laying hen diet. The amount of silage given to the birds daily was based on the experience prior to the experiment. The intention was to allow the birds to feed *ad libitum* with minimum or no wastage.

Trial One - Barley silage was collected from the University of Saskatchewan dairy silo approximately every two weeks into tin pails with lids, and kept in the freezer to ensure freshness. Due to the high moisture content of the barley silage (~ 66%), it was sub-sampled weekly (550 g) into plastic bags, and frozen to prevent spoilage. The bags of silage were taken out of the freezer daily approximately five minutes before feeding for thawing. Loose silage was given to the birds twice a day in the provided feeder; in the morning at 1030 h and in the afternoon at 1530 h to ensure freshness.

TABLE 3.1. Ingredient and nutrient composition (%) of layer diet used in trials 1 and 2.

Ingredient composition		Analyzed nutrient composition			
Wheat	74.62	AME (Kcal/kg)	2850	Met	0.34
Soybean meal – 48%	10.96	DM	89.45	Met + Cys	0.69
Canola oil	2.34	Moisture	10.55	Thr	0.59
Dicalcium phosphate	1.00	Crude protein	18.61	Val	0.73
Limestone	9.95	Calcium	4.00		
Vitamin-mineral premix[1]	0.50	Non-phytate Phosphorus	0.40		
Common salt	0.26	Total P	0.55		
Choline chloride	0.10	Sodium	0.18		
DL-Methionine	0.11	Lys	0.78		
L-Threonine	0.01	Arg	0.98		
L-Lysine HCl	0.15	Iso	1.25		

[1]Layer Vitamin-mineral premix composition (Appendix A – Table A1).

ND = not determined.

***Trial Two* –** Dietary treatments were the same as in trial 1 except a different barley silage source was used which was greener, had more of a sour smell and also had a higher moisture content (~73%). It was collected from the University of Saskatchewan dairy barn silos at the beginning of the trial into several large tin garbage pails, sealed and frozen immediately. Before each daily feeding of silage, a pail was removed from the freezer and scraped to collect the appropriate weight of silage for each cage. Initial silage feeding was provided in 200 g increments per day for each cage increasing up to 2200 g per day in some of the 4 cages. Silage was provided to the birds at 0930 h and 1300 h to ensure freshness throughout the day.

Nutrient analysis of fresh and left over silage samples was conducted in both trials in order to determine the average moisture, dry matter (AOAC, 1990), neutral detergent fibre (Van Soest *et al.*, 1991), acid detergent fibre (AOAC, 1990), starch (Salmonsson *et al.*, 1984)**,** crude protein (AOAC, 1990), calcium (Zasoski and Burau, 1977) and lysine (AOAC, 1994) levels.

3.3.3 Data Collection

3.3.3.1. Feed Intake and Body Weight

Birds were weighed at the beginning and end of each trial. Fresh silage samples were taken weekly for chemical analysis. Pre-weighed amounts of fresh silage were added to feeders after remaining silage from previous feeding had been removed. Left-over samples were then weighed and sampled for chemical analysis. The laying hen diet and Sure Shel Calcium intake were recorded weekly (trial 1) or bi-weekly (trial 2).

3.3.3.2. Behavioural Observations

In trial 1, the behaviour of birds was observed by one observer in each cage at 20, 22, 24, 26, 28 and 30 weeks of age via scan sampling. Observations were made over a 10-min period after 5-min for the birds to adjust to the presence of the observer. Behaviours of birds were scored for each cage, by a single observer, by counting the number of birds performing different activities at a specific time according to the ethogram (Table 3.2). Scan sampling occurred every 30 seconds. Bird behaviour was observed mid-day from 1300 h to 1500 h approximately three hours after fresh silage was provided to test bird cages (silage was provided at 1030 h and 1530 h daily). All observations were made at a lighting intensity of 40 lux.

In trial 2, the behavioural observations were recorded at 20, 22, 24, 26 and 28 weeks of age, and were taken at 1400 h to 1600 h, one hour after the second feeding of fresh silage.

3.3.3.3. Feather Scores

All birds were feather scored by two observers at the end of each trial as per the method described by Davami *et al.* (1987). Visual scores on five areas of the body were made (neck, breast, back, wings and tail) by trained observers based on a score varying from 1 (no feathers, skin injuries) to 4 (full feather cover, no injuries).

TABLE 3.2. Measured behaviours.

Behaviours	**Description**
Aggression	Striking purposely at head and/or pulling out feathers with response from receiver
Feather pecking	Gentle pecks towards feathers of other birds - no response from receiver
Standing	Standing and not performing any other behaviour
Preening	Self manipulation of feathers (self grooming)
Nest box	Any time spent in the nest box
Feed front feeder	Time spent feeding at front feeder (contained layer diet in all cages)
Feed back feeder	Time spent feeding at back feeder (contained silage in experimental cages)
Calcium	Time spent eating additional calcium source
Drinking	Time spent pecking at water nipple
Resting	Time spent resting and not performing any other behaviour
Object pecking	Gentle pecks towards inanimate objects in cage or cage itself

Modified from De Jong *et al*. (2003).

3.3.3.4. Egg Production and Egg Quality

In both trials, egg production data were collected for 5 days per week and corrected to 7 days. Total hen day production (THDP; based on the mortality in each trial) and total hens housed production (THHP; based on total hens housed in the beginning of each trial) were calculated for each week. Total egg weight per cage, egg specific gravity (Solomon, 1991) and yolk colour using a Roche Yolk Colour Fan (colours characterized by tristimulus values of the CIE (International Commission of Light, 1931) standard colourimetric system from 1 to 14) were determined at 21, 23, 25, 27 and 29 weeks of age in trial 1, while at 22, 26 and 29 weeks of age in trial 2.

3.3.3.5. Heterophil to Lymphocyte Ratios

At 29 weeks of age, blood samples were collected into 2.0 mL vacutainer tubes (Becton, Dickinson and Company, New Jersey, USA) containing EDTA from all the birds in trial 2 by superficial venipuncture of the brachial vein. Tubes containing blood samples were placed on a mixer immediately after collection to mix the blood and EDTA to prevent clot formation. The blood samples were kept at 4°C for approximately 24 hours before smears were prepared (in duplicate). Smears were dried for another 24 hours before staining with Wright-Giesma Stain (EMD Chemicals purchased from VWR, Lot number 4215). Proportions of heterophils and lymphocytes in 60 white blood cells were determined using oil immersion microscopy (Figure 3.3) as described by Robertson *et al*. (1990) and Gross and Siegel (1983).

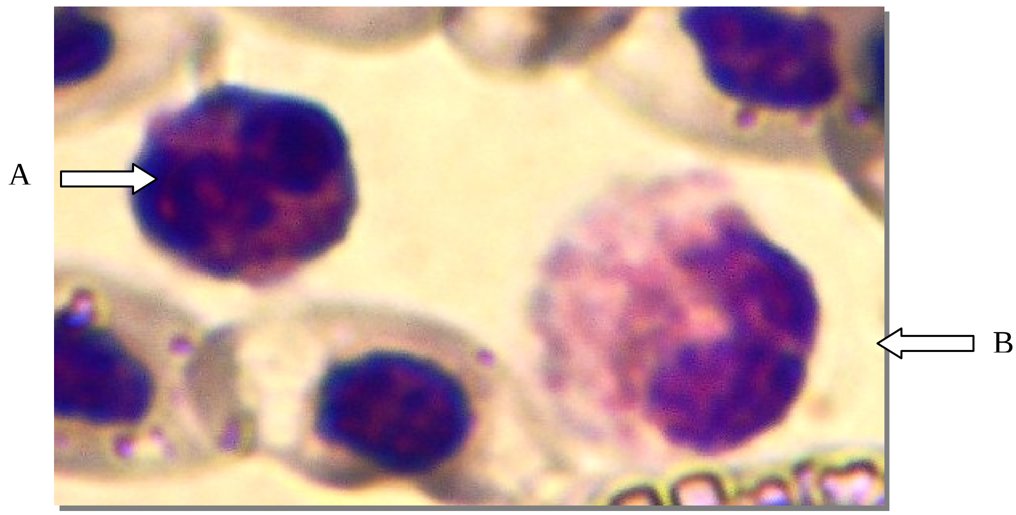

FIGURE 3.3. Photomicrograph of a blood smear showing a lymphocyte (A – bluish-purple cytoplasm with dark purple nucleus) and a heterophil (B – pink cytoplasm with purple nucleus). Magnified 100x.

3.3.4 Statistical Analysis

In order to determine the effect of two dietary treatments (control and silage-fed) on egg production, feed intake, egg quality and H:L ratios in laying hens, a randomized complete block design was used. Data were analyzed as a one way analysis of variance (ANOVA) using Proc GLM of SAS (SAS Institute, Cary NC).

Behavioural data was expressed as a percentage of birds performing a specific behaviour. All data was log transformed prior to statistical analysis. A mixed model (Proc Mixed) repeated measured design was applied using sample days over the experiment as the repeated component. Trials were used in a randomized complete block design with treatment and age as the main effects measured. Differences were considered significant when $P < 0.05$.

3.4 RESULTS

3.4.1 Feed Intake and Body Weight

Intakes of laying hen diet and silage were calculated on a dry matter basis while intake of Sure Shel Calcium (calcium source) is shown on an as is basis. In each trial, control birds ate more regular laying hen diet than their counterpart silage-fed birds (Table 3.3). Silage intake was relatively consistent for both trials with 13.9 and 13.5 grams DM/hen/day for trials 1 and 2, respectively. The latter values mean that hens ate approximately 41 and 49 grams/hen/day of silage on an as is basis. In trial 1, the total dry matter intake (layer diet and silage) was not affected by treatment although the silage-fed hens ate numerically more. Overall consumption of Sure Shel Calcium was not affected by dietary treatment in trial 1, but silage-fed ate more than the control birds.

TABLE 3.3. Overall average daily feed and silage intake (grams per hen per day; DM basis), total DM intake and calcium intake (grams per hen per day; as is-basis) of laying hens fed regular laying hen diet with and without *ad libitum* barley silage.

	Age (weeks)	
	Trial 1	**Trial 2**
Layer diet intake	20-30	19-27
Control	92.1	87.3
Silage-fed	82.0	77.2
SEM	2.11	2.06
P value	0.00	0.00
Silage intake		
Silage intake	13.9	13.5
SEM	2.64	2.63
Total DM intake		
Control	92.1	87.3
Silage-fed	95.9	90.6
SEM	1.20	0.85
P value	0.11	0.04
Calcium intake		
Control	6.7	8.4
Silage-fed	8.0	8.0
SEM	0.44	0.32
P value	0.15	0.58

SEM = standard error of mean.

$P < 0.05$ indicates a significant effect.

When examining the total DM intake of layer diet and silage in trial 2, silage-fed birds had a higher intake than the control hens with overall means of 90.6 and 87.3 grams/hen/day, respectively. There were no effects of treatment on Sure Shel Calcium intake.

Examination of nutrient analysis of the fresh and left-over silage (Table 3.4) revealed that the silage lost moisture between feedings but overall, hens consumed all portions of the silage. There was no effect of dietary treatment (i.e. silage feeding) on body weight gain in either of the trials (Table 3.5).

TABLE 3.4. Nutrient composition (%) of fresh and left-over barley silage fed in trials 1 and 2 on dry matter basis (where values are expressed as sample means±SEM).

	Trial 1[1]		**Trial 2[2]**	
	Fresh silage	Leftover silage	Fresh silage	Leftover silage
DM	32.9±0.26	57.5±2.60	31.4±0.52	62.5±2.32
Crude protein	17.5±0.19	19. 6±0.24	14.3±0.15	17.8±0.21
NDF	61.7±0.33	58.8±0.74	49.6±0.70	53.1±0.32
ADF	36.2±0.17	33.9±0.70	31.6±0.50	33.3±0.34
Starch	11.5±0.00	ND	8.2±0.35	7.1±0.15
Calcium	0.4±0.00	0.5±0.00	0.4±0.01	0.4±0.01
Total Phosphorus	0.3±0.00	0.3±0.00	0.3±0.00	0.3±0.01
Lysine	0. 5±0.00	ND	0.3±0.01	ND

ND = not determined.

SEM = standard error of the mean.

[1]Trial 1: 88 fresh and leftover samples were analyzed for nutrient analysis except for starch and lysine (n=44 samples).

[2]Trial 2: 80 fresh and leftover samples were analyzed for nutrient analysis except for leftover starch (n=48 samples) and lysine (n=40 samples).

TABLE 3.5. Effects of feeding barley silage on overall weight gain (kg) in hens and roosters in combined trials (CRBD model).

	Treatment			
	Control	**Silage-fed**	**SEM**	***P* value**
Female	0.370	0.383	0.01	0.65
Male	0.110	0.113	0.01	0.92

SEM = standard error of the mean.

$P < 0.05$ indicates significant effect.

3.4.2 Behaviour

Effects of dietary treatment and age on recorded behaviours (trials were combined) are shown in Table 3.6 and Figures 3.4 to 3.8. Behaviour data were combined and each observation collection day was analyzed as a week; so there were 6 collection weeks in total for behaviour data. Dietary treatment only affected aggression, gentle feather pecking, nest box activity and feeding behaviour while age affected all of the behaviours recorded except object pecking, feeding from back feeder and resting behaviours. Age effects for some behaviour did not demonstrate a consistent trend but the incidence of aggression, preening and drinking tended to increase with age and the proportion of hens consuming Sure Shel Calcium and standing tended to decrease. The interactions between treatment and age were not significant for any of the behaviour categories recorded in this study.

There were no interaction effects although numerically, the interaction of aggressive and gentle feather pecking behaviour, and age approached significance (*P*-value = 0.08 and 0.09, respectively, Figures 3.4 and 3.5).

Control birds spent significantly more time in the nest box than their silage-fed counterparts. Again the effect was consistent and interactions were not significant (Figure 3.6).

Time spent feeding from the front feeders (contained layer diet in all cages) and back feeders (control cages - layer diet, experimental cages - silage) was higher in the silage-fed birds compared to control birds (Figures 3.7 and 3.8). Overall (i.e. front and back feeders combined), silage-fed birds spent more time feeding than their control counterparts.

TABLE 3.6. Combined results of various behavioural measurements in both trials.

Item	Treatment		Age (weeks)						SEM	*P* value		
	Control	Silage-fed	20	22	24	26	28	30[3]		Trt	Age	Trt x Age
n	8	8	16	16	16	16	16	8	-	-	-	-
Aggression	0.7[a]	0.2[b]	0.1[b]	0.5[a]	0.4[a]	0.4[a]	0.5[a]	0.6[a]	0.06	0.00	0.01	0.08
Gentle	9.5[a]	4.6[b]	5.1[c]	9.3[a]	7.4[b]	6.9[bc]	8.7[ab]	4.9[c]	0.44	0.00	<.0001	0.09
Nest Box	7.0[a]	3.8[b]	6.3[a]	4.4[b]	3.8[b]	5.5[ab]	5.2[ab]	7.1[a]	0.36	0.01	0.04	0.73
Object Peck	0.8	0.6	1.0	0.7	0.8	0.9	0.7	0.0	0.11	0.32	0.32	0.26
Feed Front	14.4[b]	17.7[a]	11.9[c]	15.7[abc]	17.1[ab]	17.8[ab]	14.5[bc]	19.4[a]	0.78	0.01	0.02	0.39
Feed Back	10.0[b]	16.0[a]	11.3	14.4	11.5	14.2	16.6	10.0	0.70	0.00	0.08	0.71
Feed Total[2]	24.39[b]	33.70[a]	23.1	30.1	28.7	31.9	31.1	29.4	1.13	0.00	0.12	0.79
Preen	17.2	18.7	12.5[b]	9.7[b]	20.9[a]	19.5[a]	22.2[a]	22.9[a]	0.83	0.75	<.0001	0.42
Drink	6.5	6.3	4.9[c]	5.5[bc]	5.4[abc]	7.2[ab]	6.6[abc]	8.9[a]	0.33	0.69	0.03	0.87
Rest	5.3	6.3	4.0	4.1	6.9	6.9	5.3	7.4	0.42	0.49	0.19	0.52
Sure Shel Calcium	1.4	1.2	1.5[b]	2.2[a]	1.1[b]	0.8[b]	1.0[b]	1.0[b]	0.12	0.37	0.00	0.26
Stand	27.3	24.7	41.5[a]	33.7[b]	24.9[c]	20.0[d]	18.8[d]	17.9[d]	1.28	0.09	<.0001	0.28

[1] Means represent a percentage of birds engaged in a particular as measured by an instantaneous scan sample every 10 minutes.
[2] Feed front and back added together to see overall feeding behaviour (not included in sum of all other behaviours).
[3] Data results from trial 1 only.
[abcd] Means within the same row within treatment and age with different superscripts differ significantly ($P < 0.05$).
SEM = standard error of the mean. Variability of real numbers (means of main effects).

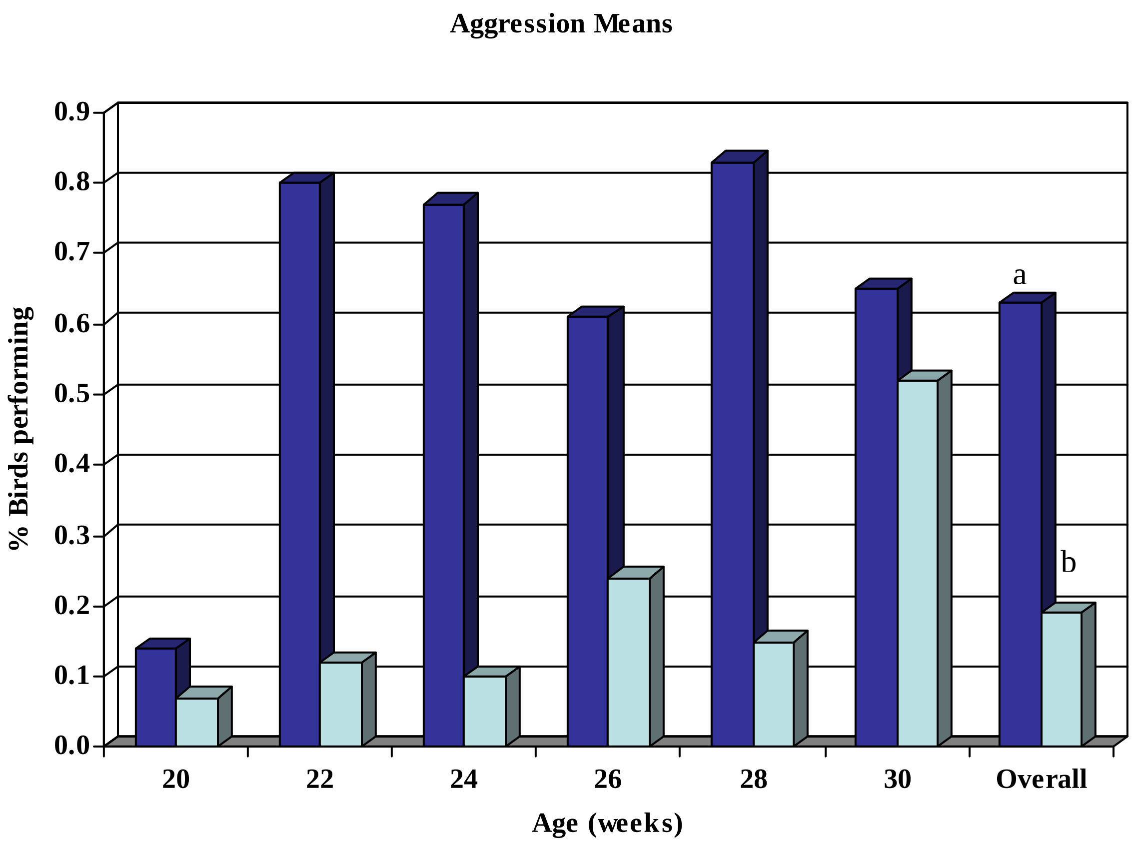

FIGURE 3.4. Percentage of birds (interaction means) displaying aggressive behaviour (where ■ = control and □ = silage-fed) on bi-weekly (repeated measure) basis (overall SEM ± 0.06) and overall basis (combined data, main effects) where different letters indicate significant differences ($P < 0.05$). Main effects where significant for treatment and age, whereas interaction means were not.

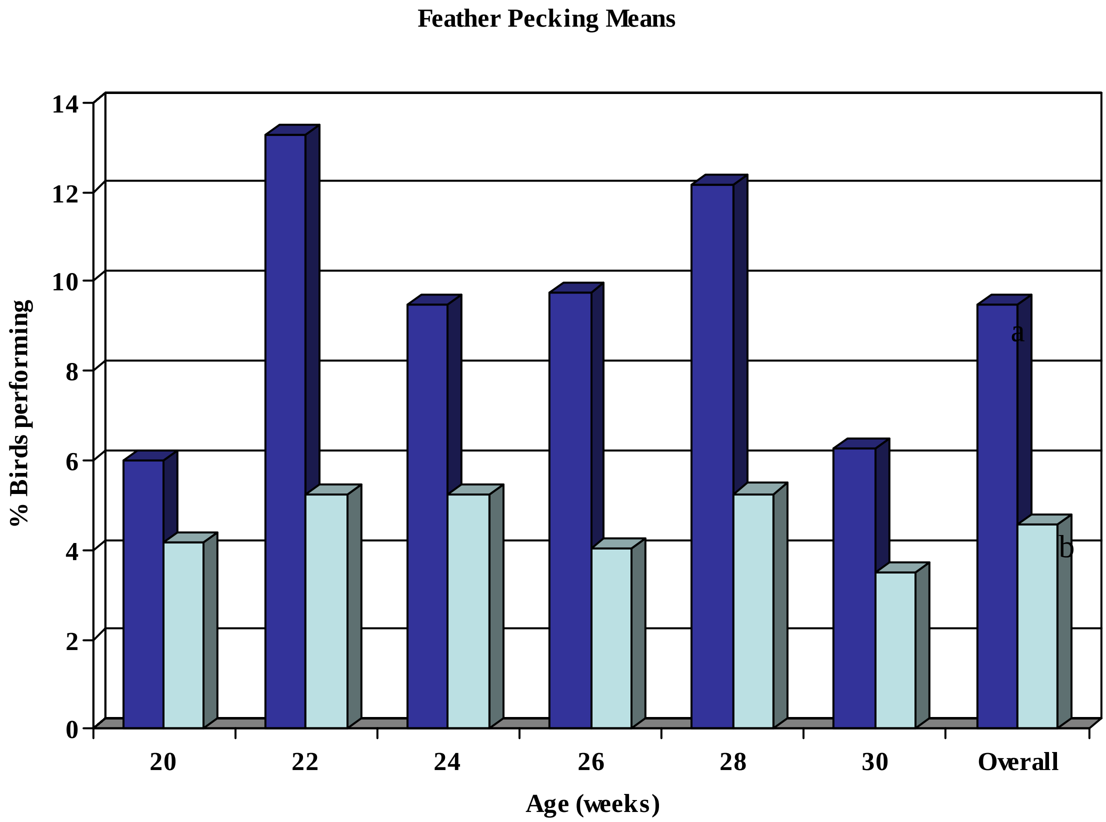

FIGURE 3.5. Percentage of birds (interaction means) displaying gentle feather pecking behaviour (where ■ = control and □ = silage-fed) on bi-weekly (repeated measure) basis (overall SEM±0.44) and overall basis (combined data, main effects) where different letters indicate significant differences (*P*<0.05). Main effects where significant for treatment and age, whereas interaction means were not.

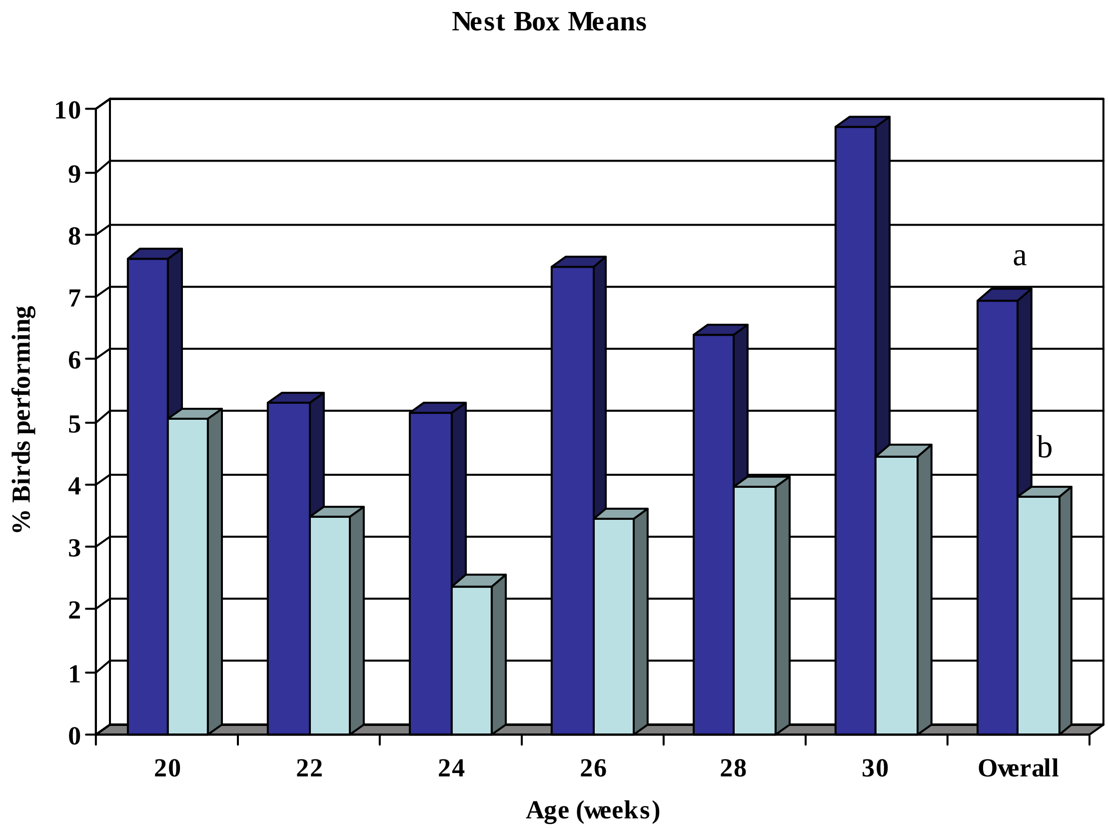

FIGURE 3.6. Percentage of birds (interaction means) using the provided nest boxes (where ■ = control and □ = silage-fed) on bi-weekly (repeated measure) basis (overall SEM±0.36) and overall basis (combined data, main effects) where different letters indicate significant differences (*P*<0.05). Main effects where significant for treatment and age, whereas interaction means were not.

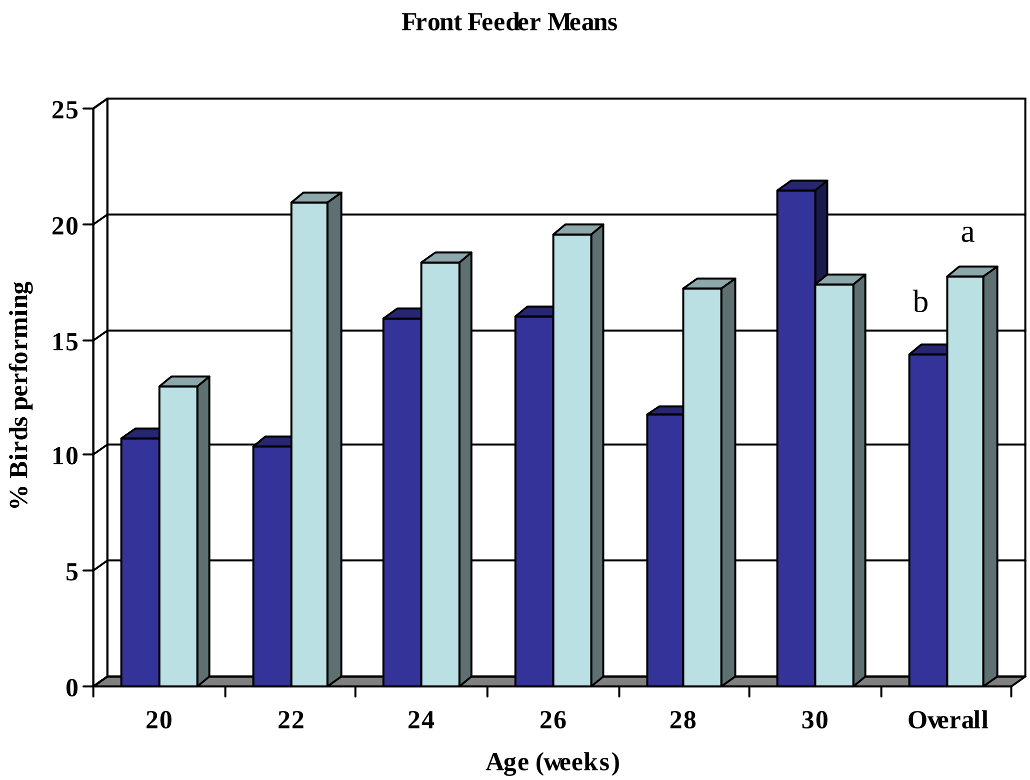

FIGURE 3.7. Percentage of birds (interaction means) feeding from the front feeder (mash in all cages where = control and = silage-fed) provided on bi-weekly (repeated measure) basis (overall SEM±0.78) and overall basis (combined data, main effects) where different letters indicate significant differences (*P*<0.05). Main effects where significant for treatment and age, whereas interaction means were not.

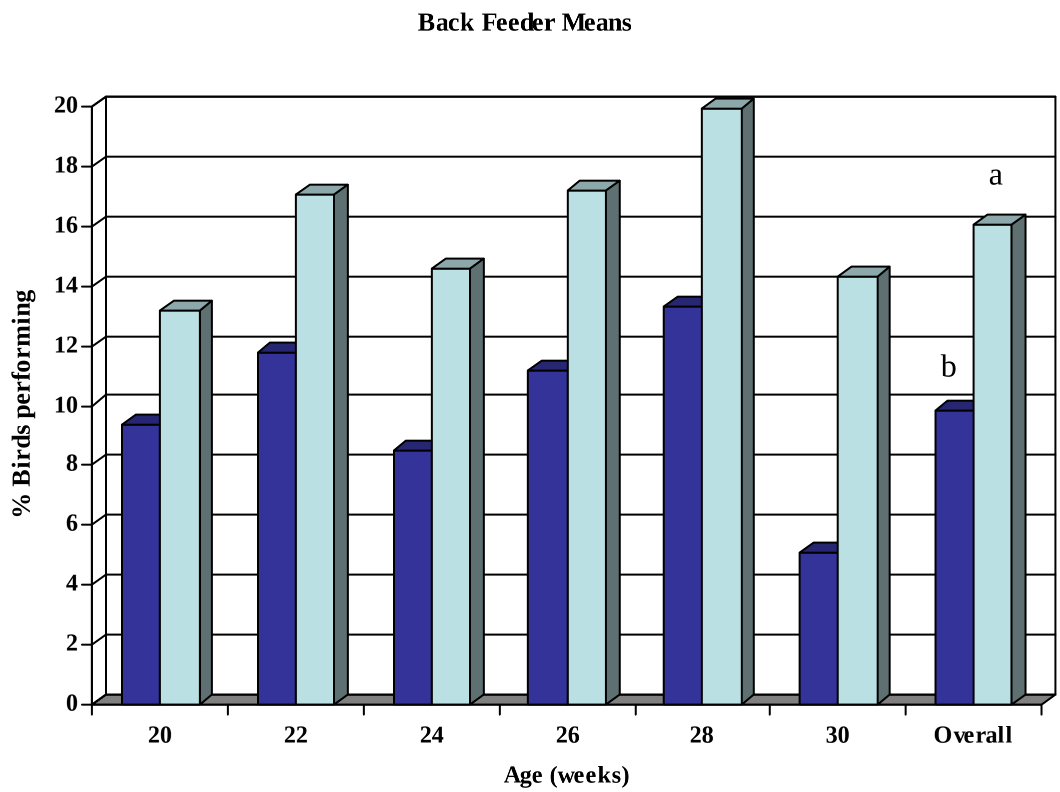

FIGURE 3.8. Percentage of birds (interaction means) feeding from the back feeder (silage in half of the cages where ■ = control and □ = silage-fed) provided on bi-weekly (repeated measure) basis (overall SEM±0.70) and overall basis (combined data, main effects) where different letters indicate significant differences (*P*<0.05). Main effects where significant for treatment and age, whereas interaction means were not.

Time spent resting, preening, drinking, object pecking, standing and eating Sure Shel Calcium were not affected by dietary treatment.

3.4.3 Feather Scores

At the end of each trial, feather scores on different parts of the body as well as overall feather score were improved in silage-fed birds compared to the control birds (Table 3.7 and Figure 3.9). Sex differences were also noted with silage-fed males having better feathering than females, particularly on the neck, breast, back and vent areas.

3.4.4 Egg Production and Egg Quality

Hen-day egg production (THDP) and hen-housed egg production (THHP) values for trial 1 and 2 are presented in Table 3.8. The THDP and THHP values were very similar in both trials because of the fact that only 3 and 5 birds died during the course of trials 1 and 2, respectively. Silage feeding had no effect on either THDP or THHP in both trials. In trial 2, the effect of silage feeding on overall THDP during 19-28 weeks of age was close to significant ($P = 0.08$) with silage-fed birds having higher production than the control birds.

The specific gravity and weight of eggs were not affected by dietary treatment in either trial. However, a significant increase in yolk colour due to silage feeding was observed in both trials (Table 3.8).

Table 3.7. Average feather score[1] means of birds at the end of trials.

	Treatment		Sex			*P* value		
	Control	**Silage-fed**	**Male**	**Female**	**SEM**	**Trt**	**Sex**	**Trt x Sex**
Neck	3.6[b]	3.9[a]	3.9[a]	3.6[b]	0.08	<.001	0.01	0.06
Breast	2.8[b]	3.4[a]	3.6[a]	2.6[b]	0.16	<.001	<.001	0.34
Back	3.0[b]	3.4[a]	3.1[b]	3.4[a]	0.09	<.001	0.00	0.26
Wings	3.7[b]	3.9[a]	3.7	3.8	0.05	0.00	0.24	0.51
Vent	2.1[b]	3.2[a]	2.8[a]	2.4[b]	0.17	<.001	0.04	0.53
Total	15.1[b]	17.8[a]	17.1[a]	15.6[b]	0.43	<.001	0.02	0.51

[1] Feather scores are based on a subjective scoring system of 1 to 4 with a score of 4 being full feather cover. Total is the sum of scores from the 5 areas of the body (neck, breast, wings, back and vent). Means are the combined average of both trials.

SEM = standard error of the mean.

$P < 0.05$ indicates significant effect.

A

B

FIGURE 3.9. Comparison of relatively full feather cover in trial 2 from silage-fed birds (A) to those of feather pecked birds from control pens (B) at 28 weeks of age.

TABLE 3.8. Effect of silage feeding on egg production and quality in trials 1 and 2.

	Trial 1				Trial 2			
	Control	**Silage-fed**	**SEM**	***P* value**	**Control**	**Silage-fed**	**SEM**	***P* value**
Hen-day egg production (THDP)	90.8	90.8	0.57	1	83.5	87.4	1.16	0.08
Hen-housed egg production (THHP)	90.3	89.8	0.64	0.73	83.5	85.1	1.06	0.49
Specific Gravity	1.096	1.091	0	0.41	1.094	1.093	0	0.59
Egg Weight (g)	51.6	51.5	0.57	0.91	51.8	51.0	0.52	0.48
Yolk Colour[1]	3.7[b]	8.3[a]	0.47	<.0001	3.1[b]	8.4[a]	1.09	<.0001

SEM = standard error of the mean.

$P < 0.05$ indicates a significant effect.

[1]Determined using a Roche Yolk Colour Fan standard colourimetric system from 1 to 14 (colours characterized by tristimulus values of the CIE (International Commission of Light, 1931)).

3.4.5 Heterophil to Lymphocyte Ratios (H:L)

Blood lymphocytes and heterophils were counted only in trial 2 and the data were analyzed separately for hens (n = 73) and roosters (n = 7). Feeding silage did not affect the H:L ratio in hens with mean values of 0.17 and 0.16 for control and silage-fed hens, respectively (SEM = 0.01). In contrast, the values for control and silage-fed males were 0.15 and 0.09, respectively (SEM = 0.01) with the differences statistically significant.

3.5 DISCUSSION

3.5.1 Feed Intake and Nutrient Analysis

Birds given silage also consumed a significant amount of regular laying hen diet. As suggested by Hetland and Svihus (2001), birds fed diets of incremental oat hull inclusions and various oat hull particle sizes, spent more time eating in order to meet their energy requirements. The increased time spent eating led to increased gut-fill capacity and less time digesting these feedstuffs as the passage rate of the digesta also increased as particle size increased. This may also have led to less time for microbial fermentation within the small intestine. The increased gizzard activity likely aided in the birds feeling full, or satiated. Hetland and Svihus (2001) also found that despite the reduced nutrient concentration of their test diets, bird weight gain was not adversely affected which is also the case in the current study. It should be remembered that the barley silage provided to the birds in the current study had whole grains scattered throughout it and was provided separately (as a choice) from the nutritionally balanced diet.

Hetland *et al.* (2002) fed diets containing whole grain inclusions of wheat, barley and oats. They found that as the level of whole grains increased within the diets, consumption decreased likely due to the limited gizzard capacity for grinding the whole grains followed by the slower passage rate through the digestive tract. The increased gizzard activity could also cause the feeling of satiety leading to the reduced feed intake. Bird weight gain was reduced with moderate and high inclusion levels of all cereal grains. Spillage was also increased with the addition of whole grains to the diets. The spillage in the current trial was minimal with only the long pieces of plant stalks being left in or around the feeder. There were no barley kernels visible however the left over silage analysis indicates kernels were still present.

Steenfeldt *et al.* (2007) discovered that by supplementing laying hens with different types of ensiled products (maize and barley-pea) or carrots caused gizzard size and weights to increase likely due to the increased mechanical requirement of the organ to digest the dietary fibre and coarser feed. They determined that a full gizzard was more likely to lead to a feeling of satiation resulting in calmer birds which may contribute to decreased bouts of feather pecking. They concluded that silages are fermented feeds and will stimulate gizzard function due to their high fibre content. Slower digesta passage rates also occurred after the intake of silages. Higher concentrations of acetic, propionic and butyric acid (also known as volatile fatty acids (VFA's) derived from fermentation) were also found in the supplemented hens caecal fermentation analysis. The number of lactic acid bacteria were lower in the small intestine of birds fed supplemental barley silage compared to those fed maize silage and carrots, however, the concentration of lactic acid was highest in gizzard contents of both silage fed groups compared to the hens fed carrots.

In the current trials, hens consumed relatively large amounts of silage (~ 41 and 50 grams/bird/day on an as-is basis in trials 1 and 2, respectively) when given *ad libitum* access despite the fact that they also had free access to a nutritionally balanced laying hen diet. Steenfeldt *et al.* (2007) also found that hens supplemented with barley silage consumed approximately 35% of their daily feed intake (as-is basis; 11% on DM basis). It was also noted during both trials that hens were notably excited about silage feeding and were anxious to eat it once it had been placed into the feeder which is also consistent with findings of Steenfeldt *et al.* (2007). These findings support our premise that hens find silage to be an attractive feed ingredient. It is of interest to consider the reasons that hens consume silage. The fact that the silage used in these trials is very low in digestible nutrients for chickens and that hens had access to a nutritionally balanced diet suggest that consumption is not to fulfill a nutritional requirement for maintenance and production. Silage is high in moisture (67 to 73%) and it is possible that this increases its palatability and attractiveness. The amount of water intake was not monitored; however, one can assume that birds would not drink as much water because of the high moisture content of the barley silage.

Silage is a fermentation product and it is possible that products of fermentation are attractive to laying hens. It is interesting to speculate that chickens have evolved to selectively consume fermented material to meet nutritional requirements that are associated with fermentation, such as vitamin B_{12}. Finally, silage has a large amount of fibre with a wide range in particle size and shape. The presence of fibre may be attractive to hens fed a relatively uniform and low fibre laying hen mash. Understanding the reasons that hens eat large amounts of silage is of interest because it may help direct management/nutrition strategies to reduce behavioural vices such as feather pecking and cannibalism.

In the current trial, one might expect preferential selection of the small amount of barley grain in the silage but the starch content of fresh and left-over silage was not markedly different indicating the birds were eating all components of the silate. Despite the fact that nearly 15% of the dry matter intake of silage-fed hens was silage, egg production, egg size and body weight gain were comparable to the control hens. In both trials, silage-fed hens ate 3 to 4 grams more dry matter but this would not compensate for the low nutrient content of the silage. This suggests that silage consumption aids nutrient utilization.

Choct and Hartini (2003) found that high fibre diets were more preventative than commercial diets when it came to cannibalism and mortality in pre and early-lay flocks. Although the mechanisms of the insoluble fibre diets on the cannibalistic behaviour are unknown, they developed a couple of hypotheses: 1) Birds may require structural components in their diet to regulate passage rate for digestion which current commercial diets lack. Low fibre diets reduce the time feed spends in the crop and gizzard, which in turn may lead to an increased volume of nutrients entering the small intestine in a short span of time. Increased nutrient load may cause discomfort for the birds, which in turn leads them alter their behaviour. 2) Cannibalism may be related to increased transit time of digesta. As also suggested by Hetland *et al*. (2004), with increased levels of insoluble fibre in the diet, the digesta is more bulky and passage rate increases leading to side effects of cannibalism due to the fact birds are left feeling hungry. The silage used in the current trials was selected for ready access rather than particular nutrient characteristics and research is warranted on other silage products with a better nutritional profile.

3.5.2 Behaviour and Feather Condition

Aggression and feather pecking were the two key behaviours of interest in the current study. Feeding barley silage altered the behaviour of the birds housed in the community cages as both aggressive and gentle feather pecking behaviours were reduced. This trend was consistent throughout the duration of both trials. As a general observation, the control birds appeared to be more assertive and chased each other around the cages. Direct pecking at the heads of other birds, as well as purposely pulling out feathers (by hens jumping up from the bottom of the cages to pull feathers out of hens perching above them) was also observed. When feathers were pulled out, birds would fight over and eat them very quickly which is consistent with the findings of McKeegan and Savory (2001) where feathers gathered from pen mates were selected and eaten when offered to them. During the course of the trial, the feather pecking behaviour increased which is consistent with the social learning or imitation as described by McAdie and Keeling (2002) and Nicol (1995) whose research suggests that hens are very adept at social learning through two mechanisms – stimulus enhancement and imitation.

As would be expected, based on reduced feather pecking, feeding silage also improved feather condition. Our results are in close agreement with other research. Steenfeldt *et al.* (2001 and 2007) found that providing laying hens with carrots and maize-silage reduced feather pecking behaviour (both gentle and severe) and improved feather condition when compared to the control group. Steenfeldt *et al.* (2007) similarly reported that hens fed supplemental barley-pea silage, in addition to their pelleted layer diet, spent more time eating and had improved plumage condition in comparison to hens without access to these feedstuffs. Other characteristics of the silage, such as slower passage rate, moisture content, VFA production as a result of caecal fermentation and particle size, are also likely to be involved in the positive behaviour effects of decreasing feather pecking in laying hens. By feeding high fibre, low energy based diets, they determined that reduced feather pecking and improved plumage occurred.

A review conducted by Choct and Hartini (2003) reported that feeding laying hens diets high in insoluble fibre reduced aggression and cannibalistic behaviour. Effective fibre sources used in reviewed experiments were millrun oat hulls, rice hulls and lucerne meal. Conclusions made were that the “calming” effect was due to the physical properties of the fibre which regulated the function of the gizzard and also due to the increased passage rate of the digesta, the appearance of hunger increased which in turn caused them to spend more time eating and less time performing other behaviours, such as feather pecking. Hens were provided one diet throughout the trials and so did not have a choice of what to eat.

An increase in feeding time and feed consumption is to be expected when fibre is included in the diet to reduce diet energy. Krimpen *et al.* (2007) reduced diet energy by 10% using oat hulls and found that hens compensated by increasing their intake by 9.3%. Krimpen *et al.* (2005) demonstrated similar effects with high inclusions of other fibre rich materials were provided. Although fibre addition has received considerable attention as a method of altering behaviour in laying hens and broiler breeders, considerable research is required to fully understand which fibre characteristics are of most importance.

Silage-fed hens in this study spent approximately 9.6% more time eating (mash and silage) than the control hens, which is similar to the reduced time (8.5%) these hens spent demonstrating aggressive and feather pecking behaviour or being in the community nests. This suggests that the reason for the reduced negative behaviours is that the silage-fed hens spent more time eating instead of expressing these behaviours, which is supportive of earlier research (Choct and Hartini, 2003; Steenfeldt *et al,*. 2001 and 2007). Although this is a logical interpretation, alternative hypotheses may be possible. Silage contains volatile fatty acids and these products may have a physiological effect on the birds. Another possibility could be the mechanism of action where post-absorptive effects of volatile fatty acids produced in the gastrointestinal tract affect the birds. The impact of additional fibre on the gastrointestinal tract is another potential mechanism (Choct and Hartini, 2003).

The focus of feather pecking on damaged feathers has been shown previously (Savory and Mann, 1999a; McAdie and Keeling, 2002). In the latter research, birds appeared to peck at objects (i.e. litter, objects in environment) and feathers of others which were of different colours (feather colour or stained from faeces/feed) and manipulated (cut or damaged). Authors also suggested that feather pecking is usually initiated on the tail feathers and then the rump of birds, likely due to the chance of these areas being damaged first from rubbing on cage walls. In both of the current trials, hens initiated feather pecking on the males, however, the effect of feather pecking was more severe in trial one where roosters were of a different feather colour than the hens. Hens continued to peck until the males' tail feathers were mostly gone. In trial two, the roosters were the same colour as the hens and maintained their tail feathers suggesting that having the same feather colour is advantageous with regard to feather pecking. Whenever birds had food or excreta particles on their feathers, others would be attracted to it and peck at them as well.

There were no significant treatment effects on the comfort behaviours of preening and resting which may have been expected based on less aggressive and feather pecking behaviour in silage-fed hens.

The control birds spent more time in the nest boxes than the birds fed silage along with the regular laying hen diet. The birds were likely not laying eggs since observations were made ~8-10 hours after the lights came on and therefore most eggs had already been laid. These data could suggest a few things. With the increased aggressive behaviour in the control treatment, hens were hiding in the nest boxes to flee pecking attacks. It could be argued that silage-fed birds were spending more time feeding thereby less time focusing on other behaviours, such as feather pecking as suggested by Zuidhof *et al.* (1995) and De Jong *et al.* (2005). It could also be argued that the control birds spent their time eating the mash and so without the additional feed source, were spending their free time in the nest boxes. We observed that sometimes there were up to four birds at a time in each next box in the control treatment. The increased use of nest boxes by control hens continued to rise as the experiment progressed, possibly utilizing nest boxes as a safe haven when being chased or attacked as suggested by Millman and Duncan (2000a, 2000b) where female broiler breeders hid in nest boxes from overly aggressive males.

3.5.3 Egg Production and Egg Quality

Feeding barley silage had no adverse effect on egg production and egg quality in either of the trials which was different than what was observed by Steenfeldt *et al*. (2007) where average egg weight was not affected by feeding barley silage (similar to the current trial), however, rate of lay and number of eggs produced were lower in the barley silage fed hens. Feeding silage changed yolk colour in both trials with colour initially similar to the control eggs but within two weeks of feeding silage, the yolks turned bright orange as compared to pale yellow in the control diet. This finding is consistent with the level of pigment in silage and will vary according to the pigment level in the diet. The layer diet fed to the birds was a soy/wheat mash and contained very little pigment. The yolk colour reflects a consumer preference issue and therefore would vary worldwide.

3.5.4 Heterophil to Lymphocyte Ratios

Heterophil to Lymphocyte ratios have been shown to assess levels of stress (Gross and Siegel, 1983; De Jong *et al.,* 2005). H:L ratios have indicated that high levels of dietary fibre improve the welfare of broiler breeders (Zuidhof *et al.,* 1995; Hocking *et al.,* 2004) and that providing forage substrates reduces stress and results in an improved immune response (El-Lethey *et al.*, 2000). It was therefore of interest to see if feeding barley silage which might provide both fibre and increased foraging potential would affect H:L ratio. The evidence from our work is not definitive as H:L ratios in females were not affected but in males they were. A possible reason for the higher H:L ratios in control males could be related to the fact that aggression and gentle feather pecking in the control birds was higher and for the most part, the males were primarily the first birds to be feather pecked.

Normal heterophil and lymphocyte counts for an adult White Leghorn hen are approximately 13.1 and 76.1 respectively out of 100 cell counts and as such, the normal H:L ratio should read about 0.17 (Sturkie, 1986). The values in this experiment were comparable as well as in the controls mentioned in other trials (Gross and Siegel, 1983).

3.6 CONCLUSIONS

This study showed that feeding barley silage to laying hens reduced aggressive and feather pecking behaviours and as a consequence, improved plumage condition. The control birds appeared to be more agitated and restless, although these were not scientifically measured during these trials. There were no effects of barley silage feeding on egg production and egg quality.

Using silage in a commercial situation is logistically difficult but should not be dismissed. Engineering technology undoubtedly could be developed to make feeding more user friendly and thereby allow the benefits of feeding silage to be achieved.

4.0 EFFECTS OF FEEDING BARLEY SILAGE ON FEED INTAKE, BODY WEIGHT, BEHAVIOUR, STRESS AND FEAR IN BROILER BREEDER PULLETS

4.1 ABSTRACT

A trial was conducted to determine the effect of feeding a low-energy, high-fibre feedstuff (barley silage) on body weight, stereotypic behaviour, stress and fear levels in broiler breeder pullets. Three week-old broiler breeder pullets (n=180) were randomly allocated into 12 straw litter floor pens having 15 birds per pen. Birds in 6 pens were provided with a nutritionally balanced corn/oat-soybean/canola meal-based broiler breeder pullet diet at recommended restricted levels, whereas birds in another 6 pens were given free access to barley silage in addition to a regular broiler breeder pullet diet until the end of trial at week 18. The body weight, feed intake, heterophil to lymphocyte (H:L) ratio and tonic immobility scores were recorded at regular intervals. Various behaviour measurements were made in each pen at 5, 9, 13 and 17 weeks of age via scan sampling.

The mean body weights and uniformity coefficients of variation of the control and silage-fed hens were not significantly different throughout the trial. There was almost a linear increase in silage intake with age with total dietary DM intake significantly higher ($P < 0.05$) for silage-fed birds compared to their control counterparts.

Dietary treatment had no significant effect on bird behaviour with the exception of object pecking behaviour which was reduced with silage feeding. Aggressive and gentle feather pecking behaviour was consistently higher in the control birds than the silage-fed birds for the duration of the trial, but the differences were not significant at the end of the trial. Age affected many of the behaviours recorded in this study. In general, gentle feather pecking, watching observers, perching, pecking feeders and drinking increased significantly ($P < 0.05$) with age while resting, silage pecking, foraging and preening decreased with age. Silage feeding had no significant effect on H:L ratios or tonic immobility values indicating that birds in both treatments were neither very stressed nor fearful. It was concluded that feeding barley silage to broiler breeder pullets has potential to aid in improving their welfare, however further research is required.

4.2 INTRODUCTION

Demand for high growth rate and breast muscle yield has been a fundamental aspect of genetic selection in broiler strains. Associated with dramatic changes in these characteristics has been a reduction in productivity in *ad libitum* fed broiler breeders (Renema and Robinson, 2004). As a result, feed restriction programs were developed to increase reproductive capacity and at the same time improve bird health (De Jong *et al*., 2003; Hocking *et al*., 1996 and 2004; Renema and Robinson, 2004). Broiler breeder growth continues to increase with each generation of selection and as a consequence the degree of restriction required to maintain breeder health and productivity has also increased. This has raised concerns about the welfare of broiler breeders because of hunger associated with feed restriction (Renema and Robinson, 2004).

The Farm Animal Welfare Council (FAWC) (1998) encouraged further research into alternative feeding strategies and management practices because of the increasing concern about the degree of hunger experienced by broiler breeders particularly during the brooding phase due to commercial feed restriction practices. A number of strategies have been examined to reduce hunger and improve the welfare of feed restricted birds. These have included providing nutrient diluted diets (Zuidhof *et al*., 1995), feeding different concentrations and sources of dietary fibre (Hocking *et al*., 2004; De Jong *et al*., 2005), feeding low protein diets, using different allocations of feed (Hocking *et al*., 2001, 2002a and 2002b) and using mash instead of pelleted diets (Savory *et al.*, 1999b).

Feed restricted broiler breeders have been shown to elicit stereotypic feeder pecking, aggressive and pacing behaviours (Hocking *et al.*, 2002; Mench, 2002; Zaczek *et al.*, 2003). It has been suggested that the incidence of boredom and frustration due to hunger enhances these behaviours leading to birds pecking obsessively at the feeders in the hopes of retrieving more feed (Hocking *et al*., 2004). In males, aggression between males and also towards females has also been associated with hunger (Millman and Duncan, 2000a and 2000b; Millman *et al*., 2000). Diluting diets has been one method of nutritional manipulation with the intent to reduce feeding motivated behaviour due to frustration and hunger, such as stereotypic pecking behaviour and aggression (Zuidhof *et al.*, 1995; Savory *et al.*, 1999; De Jong *et al.*, 2005).

Levels of fear and stress are often measured in studies involving feed restricted birds in order to determine the level of impaired welfare. Numerous tests are used in determining physiological stress on an animal indicating its state of well-being (Archer, 1979; Broom, 1981, 1996; Campo and Dávila, 2002). Heterophil to lymphocyte ratio (H:L) as well as a physical test of tonic immobility (TI) are two common tests used to measure the degree of stress and fear, respectively in feed restricted birds. The H:L ratio gives an indication of the level of physiological stress resulting in higher numbers of heterophils in the blood (Gross and Siegel, 1983; Gross, 1990). High levels of fibre within a diet have been shown to improve the welfare of birds when examining the H:L ratio (Hocking *et al*., 2004; De Jong *et al*., 2005).

Gallup (1979) suggested that TI is a good way to measure fear in birds as it assesses the bird's state of fight or flight reaction by monitoring how long it takes them to react to the situation they are placed in. Hocking *et al*. (1996) suggest that feed restricted birds are less fearful than *ad libitum* fed birds based on the premise that feed restricted birds are essentially looking for feed and associate humans with receiving it; therefore are less likely to be fearful when humans are present.

We hypothesized that the level of welfare in broiler breeder hens can be improved by providing them a low-energy, high-fibre feedstuff (barley silage) *ad libitum.* We suggest that improved bird welfare, as it relates to hunger, will result from a modification of stereotypic abnormal behaviours (i.e. pacing, pecking at feeders, aggression) normally associated with feed restriction. The objective of this study was to assess the impact of providing feed restricted broiler breeder pullets *ad libitum* access to barley silage in addition to their regular mash diet on their behaviour, fear and stress levels, and body weight uniformity.

4.3 MATERIALS AND METHODS

This experimental protocol was approved by the Animal Care Committee of the University of Saskatchewan and was performed in accordance with recommendations of the Canadian Council on Animal Care as specified in the Guide to the Care and Use of Experimental Animals (1993).

4.3.1 Birds and Housing

Beak trimmed broiler breeder female chicks (Ross 308) obtained from a commercial hatchery (Lilydale Hatchery, Calgary, AB) were housed in litter floor pens until 3 weeks of age and brooded according to industry practices. Birds in all 12 pens were initially provided a starter diet (corn/oat/soy-based) on an *ad libitum* basis until 3 weeks of age. At 3 weeks of age, the birds were weighed, wing banded and randomly allocated to each of 12 straw litter floor pens having 15 birds per pen (2.36 m long x 1.98 m wide) and received a grower ration in mash form provided at restricted levels following the recommended guidelines of Aviagen Broiler Breeder Management practices for growing and rearing phases (Ross Breeders, 2000). Restricted amounts of feed were fed on a daily basis throughout the trial. Solid wall dividers were placed between each pen to prevent birds from seeing birds in adjacent pens. Each pen contained a hanging tube feeder (0.40 m diameter with 0.10 m lip) and 6 nipple drinkers. At 10 weeks of age, an additional tube feeder was added to each pen to accommodate simultaneous feeding of all birds. Silage was provided in a separate trough feeder (0.84 m long x 0.08 m wide with 0.10 m lip) along the side of the experimental pens. The birds were given 8 hours of light per day at 10 lux intensity. Room temperature was lowered 0.3°C daily starting from 33.8°C on day 1 until it reached 21°C on day 42.

4.3.2 Treatments

Barley silage was provided *ad libitum* to the birds in 6 of the 12 pens along with the regular broiler breeder diet initiating at 3 weeks of age when birds could start to ingest the silage. Therefore, the dietary treatments consisted of a control, where birds were feed restricted with a standard broiler breeder grower mash diet for the entire experiment and a silage-fed treatment, where birds were provided barley silage *ad libitum* in addition to the usual feed restricted diet (Table 4.1).

Barley silage was collected from the University of Saskatchewan dairy silo approximately every two weeks into sealed containers and was subdivided into plastic bags according to individual pen requirements on a weekly basis. Both pails and plastic bags were kept frozen (-20°C) due to the high moisture content of silage (~73%) to ensure freshness and prevent spoilage. The bags of silage were taken out of the freezer approximately 5 minutes before feeding for thawing. Silage was given to the birds twice a day, in the morning at 0900 h (approximately 45 minutes after receiving their restricted

TABLE 4.1. Composition of broiler breeder diets

Ingredients: (%)	Starter[1]	Grower[2]
Corn	35.68	41.52
Oats	34.47	35.00
Canola meal	0.00	10.00
Soybean meal – 48%	24.43	8.69
Canola oil	1.00	1.00
Dicalcium phosphate	1.41	1.27
Limestone	1.54	1.29
Common salt	0.33	0.27
Vitamin-mineral premix[3]	0.50	0.50
Choline chloride	0.10	0.10
DL-Methionine	0.21	0.11
L-Lysine HCL	0.13	0.05
Coccistac[4]	0.10	0.10
Feed enzyme[5]	0.10	0.10
Analyzed nutrients: (%, as-is basis)		
AME (Kcal/kg)	2865	2865
DM	88.56	88.55
Crude protein	18.69	15.36
Calcium	1.00	0.90
Phosphorus - total	0.61	0.62
Lysine	1.00	0.73

[1]Starter diet was fed from 1-3 weeks of age.

[2]Grower diet was fed from 3-18 weeks of age.

[3]Broiler Breeder Vitamin-mineral premix composition (Appendix A – Table A2).

[4]Coccistac (Phibro Animal Health).

[5]Avizyme 1100 (1 kg/tonne feed to allow higher inclusion of oat in diet), Danisco (manufactured by FinnFeed Oy, Vaasa, Finland for Danisco Animal Nutrition, Wiltshire, England).

ND = not determined.

fed mash diet) and in the afternoon at 1300 h. Silage was weighed in and out on a weekly basis to determine dry matter consumption.

Fresh silage was sub-sampled at time of collection and left-over silage sampled weekly for determination of moisture, dry matter (AOAC, 1990), neutral detergent fibre (Van Soest *et al.*, 1991), acid detergent fibre (AOAC, 1990), starch (Salmonsson *et al.*, 1984) and crude protein (AOAC, 1990).

4.3.3 Data Collection

4.3.3.1. Feed Intake and Body Weight

Feed and management practices followed Ross Broiler Breeder Management guidelines (Ross Breeders, 2000). Birds received a specific amount of feed based on their weekly body weights in order to maintain specific Ross broiler breeder body weight goals during the growing phase. Grower mash was provided to all birds daily at 0815 h.

Birds were weighed weekly on a pen basis except on weeks 2, 6, 10, 14 and 18 of age when birds were weighed individually to assess body weight uniformity. Uniformity was assessed by mathematically analyzing the variability of the individual bird weights per treatment every 4 weeks and calculating the coefficient of variation (CV%) (Ross Breeders, 2000). From these values, it was then determined whether feed provided needed to be reduced, maintained or increased in order to maintain the suggested growth rates. The goal was to maintain body weight uniformity as per standard broiler breeder management practices.

4.3.3.2. Behavioural Observations

Behaviour of birds was observed in each pen at 5, 9, 13 and 17 weeks of age via scan sampling by 3 observers. Each observer rotated between and viewed 4 pens (balanced by treatment) for each of 3 sessions so all 12 pens received the same attention throughout the observation periods. Observers were trained before-hand to ensure that behaviours were classified consistently. Observations were made for an 8-minute period per pen after the birds were allowed to adjust to the presence of the observer for 2 minutes. During each period, the number of birds engaged in a particular behaviour was recorded every 30 seconds according to the list shown in Table 4.2. The behavioural observations were recorded three times daily: in the morning before mash feeding, late morning after silage feeding and in the afternoon one hour after silage feeding.

4.3.3.3 Tonic Immobility

Tonic immobility (TI) was used to estimate fear level in all birds at 9 and 17 weeks of age using two trained observers. As described by Zulkifli (2005), birds were randomly removed as quickly and quietly as possible from pens on an individual basis, taken into a quiet room away from the rest of the birds and immediately tested for TI. Tonic immobility was conducted by manually securing the birds legs with one hand and wings with the other hand to prevent flapping and then quickly placing them on their backs in a wooden U-shaped saddle. TI was induced for 15 seconds by gently covering the bird's head with one hand while securing its body with the other hand. This usually induced a state of TI but if birds started flapping or immediately jumped up, the test was conducted again. If this occurred 3 times, the TI test was considered a fail (scored 0) due to the birds having no fear. If birds remained immobile for a period of 10 minutes, the

TABLE 4.2. Measured behaviours

Behaviour	**Description**
Pacing	Pacing back and forth
Aggression	Seeing response from receivee
Gentle Feather Pecking	No response from receivee
Perching	Jumping up and sitting on water lines
Resting	On floor resting (breast on floor)
Standing	Standing without doing anything else
Peck feeder (empty)	Pecking at the feeder (both empty and containing mash)
Peck silage	Pecking at the silage in the feeder
Peck water nipple	Pecking at the water nipple
Peck Object	Pecking at parts of the cage
Foraging	Pecking, scratching at the litter
Preening	Self grooming/manipulating feathers
Dust bathing	Time spent dust bathing in the litter
Watching	Standing at front of cage watching observer

Modified from De Jong *et al*. (2003).

observer stopped the test and assigned a score of 600 seconds (indicating high level of fear). All other birds received a score of time in seconds (from 0 - 600) to indicate their specific level of fear and each received a spray colour (different for each observer) on their backs indicating completion of the test. At 17 weeks of age, observers tested the opposite coloured birds to ensure consistent sampling occurred.

4.3.3.4 Heterophil to Lymphocyte Ratios

At 18 days, and 6, 10, 14 and 18 weeks of age, blood samples were collected into 2.0 mL vacutainer tubes (Becton, Dickinson and Company, New Jersey, USA) containing EDTA from all the birds by superficial venipuncture of the brachial vein. Blood was sampled very quickly after catching a bird. Additionally, utmost care was taken while catching the birds and collecting the blood to minimize stress on the rest of the birds. The blood samples were kept at 4°C for approximately 24 hours before smears were prepared (in duplicate). Smears were dried for another 24 hours before staining with Wright-Giesma Stain (EMD Biosciences, San Diego, USA). Proportions of heterophils and lymphocytes in 60 white blood cells were determined using oil immersion (100x) as described by Robertson and Maxwell (1990) and Zulkifli (2005).

4.3.4. Statistical Analysis

In order to determine the effect of two dietary treatments (control and silage-fed) on body weight, uniformity, silage feed intake, TI and H:L ratios in broiler breeders, a completely randomized experimental design was used. The body weight, uniformity, silage feed intake, TI and H:L ratio data was analyzed as one way analysis of variance (ANOVA) using Proc GLM of SAS (SAS Institute, Cary NC).

Behavioural data was expressed as a percentage of birds performing a specific behaviour. All data was log transformed prior to statistical analysis. A mixed model (Proc Mixed) repeated measured design was applied using sample days over the experiment as the repeated component with treatment and age as the main effects measured. Differences were considered significant when $P < 0.05$.

4.4 RESULTS

4.4.1 Body Weight and Feed Intake

The mean body weights of the control and silage-fed pullets were not significantly different throughout the trial and were in close agreement with the breeder recommendations (Figure 4.1) with the exception of week 10 where pullet body weights were significantly lower ($P < 0.05$) than their control counterparts (Table 4.3). The average uniformity coefficient of variation (CV%) was 12.9% with no treatment differences. This value is higher than the recommended <11% by Ross Breeder guidelines (Ross Breeders, 2000) falling into the “fair” category which was maintained for the duration of the experiment.

FIGURE 4.1. Target body weight curve for feed restricted broiler breeder pullets from 3 to 18 weeks of age (Ross Breeders, 2000) and the growth curves for the actual body weights of birds fed either restricted standard broiler breeder grower diet (control) or barley silage *ad libitum* along with restricted grower diet (silage-fed).

TABLE 4.3. Treatment effects on pullet body weights.

	Bird Weights (g)				
Age	**Control**	**Silage-Fed**	**SEM**	***P*-value**	**CV**
2	410.3	403.6	2.99	0.26	10.8
6	841.2	822.7	6.79	0.17	13.3
10	1134.3[a]	1074.9[b]	12.13	0.01	13.3
14	1500.1	1462.6	18.96	0.32	13.7
18	1911.3	1888.0	26.20	0.66	13.5

[ab] Means within the same row with different superscripts differ significantly ($P<0.05$).

SEM = standard error of the mean.

CV = coefficient of variation (%) on bird uniformity.

Pullets were fed the same amount of grower feed per day regardless of treatment until 17 weeks of age when the intake of silage-fed birds was reduced (compared to control birds) for weeks 17 and 18 in order to maintain the desired growth rate (Table 4.4). There was almost a linear increase in silage intake with age. Silage intake on a dry matter basis increased from less than one gram per bird per day in week 4 to nearly 14 grams at week 18 (Table 4.5, Figure 4.2). This was equivalent to approximately 3 and 51 grams per pullet per day, respectively on an as-is basis. The total DM intake was significantly higher for silage-fed birds compared to their control counterparts throughout the trial. The difference in the total DM intake increased with age. At 18 weeks of age, silage-fed birds had almost 22.4% higher (58.1 verses 71.1 g) total DM intake than the control birds.

Fresh silage contained 75.1% moisture, 12.8% crude protein, 64.8% NDF and 41.8 % ADF. The left-over feed prior to the next feeding averaged 49.7% moisture and 13.6% protein (Table 4.6).

TABLE 4.4. Summary of weekly feed intake (g) and body weight (g) of broiler breeder pullets fed standard broiler breeder grower diets with and without *ad libitum* barley silage.

Age (wk)	# of birds	Body weight (g)			Target[1] Feed	Feed Given
		Target	Silage-fed birds	Control birds		
0	180	36	0.0	40.4	*ad lib*	*ad lib*
1	180	113	0.0	100.0	*ad lib*	*ad lib*
2	180	204	0.0	406.9	*ad lib*	*ad lib*
3	180	340	425.6	442.2	40.0	40.0
4	180	454	533.2	519.8	42.0	40.0
5	180	544	605.9	623.2	46.3	42.0
6	180	635	705.0	717.8	49.9	42.0
7	180	726	797.2	811.7	53.5	46.3
8	179	817	905.0	916.8	56.7	46.3
9	179	907	1002.2	1010.6	60.3	46.3
10	179	998	1105.6	1112.3	63.5	46.3
11	179	1089	1194.0	1198.0	66.7	48.0
12	179	1179	1284.4	1284.3	69.4	50.0
13	179	1270	1367.2	1368.6	72.6	53.5
14	178	1360	1499.1	1463.6	75.3	53.5
15	178	1451	1550.0	1548.2	80.7	55.0
16	178	1565	1632.5	1623.3	87.1	60.0
17	178	1701	1745.2	1718.1	91.2	66/65[2]
18	178	1837	1953.3	1910.2	94.8	66/65[2]

[1] Target feed = expected feed to provide based on Ross breeder guidelines.
[2] Control pullets were fed 66 grams per day and silage-fed pullets 65 grams per day.

TABLE 4.5. Average daily feed intake (g per day, DM basis) of broiler breeders at different weeks of age fed restricted standard broiler breeder grower. diets with and without *ad libitum* barley silage.

	Grower diet intake				Silage intake			Total DM intake			
Age (weeks)	**Control**	**Silage-fed**	**SEM**	***P* value**	**Silage intake**	**SEM**	***P* value**	**Control**	**Silage-fed**	**SEM**	***P* value**
4	35.2	35.2	0.00	NS	0.8	0.1	0.1	35.2	36.0	0.14	<.0001
5	37.0	37.0	0.00	NS	1.9	0.3	0.3	37.0	38.9	0.30	<.0001
6	37.0	37.0	0.00	NS	3.1	0.5	0.5	37.0	40.1	0.48	<.0001
7	40.7	40.7	0.00	NS	4.1	0.6	0.6	40.7	44.8	0.61	<.0001
8	40.7	40.7	0.00	NS	4.2	0.6	0.6	40.7	45.0	0.64	<.0001
9	40.7	40.7	0.00	NS	4.3	0.6	0.6	40.7	45.0	0.64	<.0001
10	40.7	40.7	0.00	NS	4.3	0.7	0.7	40.7	45.1	0.65	<.0001
11	42.2	42.2	0.00	NS	5.0	0.8	0.8	42.2	47.2	0.76	<.0001
12	44.0	44.0	0.00	NS	6.1	0.9	0.9	44.0	50.8	0.93	<.0001
13	47.1	47.1	0.00	NS	6.3	1.0	1.0	47.1	53.3	0.95	<.0001
14	47.1	47.1	0.00	NS	7.0	1.1	1.1	47.1	54.1	1.05	<.0001
15	48.4	48.4	0.00	NS	6.5	1.0	1.0	48.4	54.9	0.99	<.0001
16	52.8	52.8	0.00	NS	10.2	1.6	1.6	52.8	63.0	1.58	<.0001
17	58.1	57.2	0.13	<.0001	11.6	1.8	1.8	58.1	68.8	1.62	<.0001
18	58.1	57.2	0.13	<.0001	13.9	2.1	2.1	58.1	71.1	1.96	<.0001

P < 0.05 indicates significant effects.

SEM = standard error of the mean.

NS = not significant.

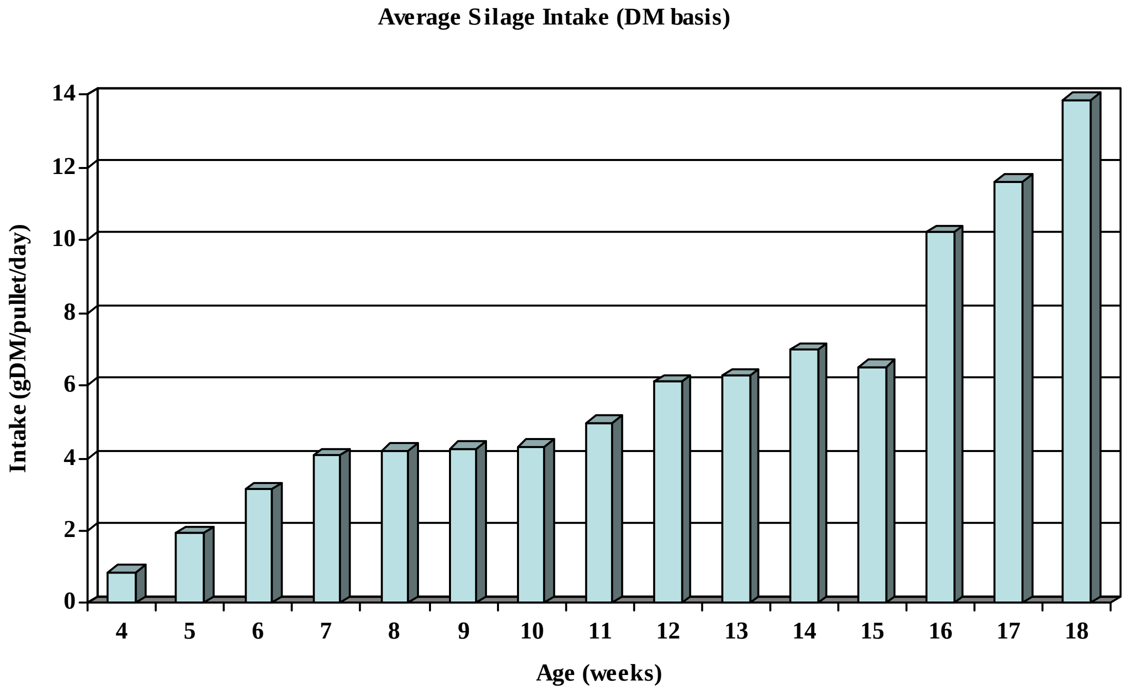

FIGURE 4.2. The average silage intake (g/pullet/day; DM basis) of broiler breeder pullets given barley silage in addition to restricted regular broiler breeder diet from 4 to 18 weeks of age.

TABLE 4.6. Nutrient composition (%) of fresh and left-over barley silage fed during broiler breeder pullet trial on dry matter basis (where values expressed are sample means±SEM).

	Barley Silage[1]	
	Fresh	**Left over**
DM	24.91±0.18	50.26±0.90
Crude Protein	12.84±0.09	13.55±0.09
Neutral Detergent Fibre	64.83±0.73	ND
Acid Detergent Fibre	41.80±0.48	ND
Starch	3.72±0.30	ND
Calcium	0.32±0.00	ND
Lysine	0.19±0.00	ND
Phosphorus	0.39±0.00	ND

[1]180 fresh and left-over samples were analyzed for nutrient analysis except for ADF, NDF, starch, calcium, lysine and phosphorus (where n=90).

ND = not determined.

SEM = standard error of the mean.

4.4.2 Behaviour

Effects of treatment and age on recorded behaviours are shown in Table 4.7 and Figure 4.3. Dietary treatment had minimal effects on bird behaviour with the exception of silage pecking and object pecking behaviour. Only the silage-fed birds had access to peck silage and the control birds object pecked more than the silage-fed pullets. In contrast, age affected many of the behaviours recorded in this study. In general, gentle feather pecking, watching observers, perching, pecking feeders and drinking increased with age while resting, silage pecking, foraging and preening tended to decrease with age. Pacing, aggression, dust bathing and standing were not affected by dietary treatment or age. The interactions between age and dietary treatment were not significant for any of the behaviours recorded in this study.

Time spent pacing back and forth at different weeks of age was variable between treatments and was not affected overall by treatment. However, if data are examined on a weekly basis, pacing behaviours at 13 weeks of age were increased in the control compared to silage-fed birds. Time spent pecking at the feeders, whether they contained feed or not, occupied the majority of birds' time; however treatment effects were not seen.

Table 4.7. Effect of feeding silage and broiler breeder pullet age on behaviour measurements.

	Treatment		**Age (weeks)**					***P* value**		
Item	**Control**	**Silage-fed**	**5**	**9**	**13**	**17**	**SEM**	**Trt**	**Age**	**Trt x Age**
n	6	6	12	12	12	12	-	-	-	-
Pacing	0.7	0.6	0.6	0.8	1.2	0.0	0.18	0.62	0.12	0.86
Aggression	0.3	0.2	0.1	0.3	0.3	0.3	0.04	0.13	0.24	0.39
Feather Pecking	3.8	0.5	0.3^{b}	0.5^{b}	2.9^{ab}	4.7^{a}	0.44	0.12	0.02	0.23
Watching Observers	8.7	11.5	5.2^{b}	14.0^{a}	11.1^{a}	10.0^{ab}	0.82	0.21	0.00	0.99
Resting	0.6	1.8	3.7^{a}	0.3^{b}	0.6^{b}	0.3^{b}	0.37	0.36	<.0001	0.85
Perching	0.4	0.4	0.0^{c}	0.4^{b}	0.4^{b}	0.7^{a}	0.05	0.60	<.0001	0.86
Peck Feeder	33.4	33.6	26.0^{b}	33.8^{ab}	38.6^{a}	35.5^{a}	1.42	0.99	0.01	0.94
Drinking	6.7	7.4	5.7^{b}	6.5^{b}	7.1^{ab}	9.0^{a}	0.39	0.37	0.01	0.72
Peck Silage	0.0^{b}	5.5^{a}	5.3^{a}	1.4^{b}	2.3^{b}	2.0^{b}	0.49	0.00	0.01	0.01
Object Peck	4.6^{a}	1.4^{b}	2.5	6.0	2.2	1.3	0.50	0.04	0.11	0.41
Foraging	21.9	18.6	32.6^{a}	15.7^{b}	17.8^{b}	15.0^{b}	1.22	0.43	0.00	0.55
Preening	6.1	6.6	10.2^{a}	5.7^{b}	4.2^{b}	5.4^{b}	0.41	0.73	<.0001	0.43
Dust-bathing	0.4	0.3	0.1	1.3	0.0	0.0	0.23	0.91	0.19	1.00
Standing	12.4	11.7	7.8	13.3	11.4	15.6	1.15	0.84	0.40	0.97

[1] Means represent a percentage of time engaged in a particular behaviour as measured by an instantaneous scan sample every 8 minutes.

[abc] Means within the same row within treatment and age with different superscripts differ significantly ($P < 0.05$).

SEM = standard error of the mean. Variability of real numbers (means of main effects).

Overall, aggressive behaviour was not affected by dietary treatment although it was seen in higher incidence in the control birds. Similarly, overall feather pecking behaviour was numerically higher in the control birds throughout the trial; however, differences were not significant.

Time spent object pecking (Figure 4.3A) was highest at 9 weeks for control birds but was otherwise minimal for the duration of the trial. Time spent pecking at silage was highest at 5 weeks of age and lower for the other observation periods. Bird interest in the feedstuff was maintained throughout the trial (Figure 4.3B).

Birds spent similar amounts of time performing various other behaviours such as watching observers, resting, perching, foraging, drinking and dustbathing, and no treatment effects were noted for these behaviours. Although there were no treatment effects, birds of both treatments spent a fair amount of time drinking water with a higher percentage in the silage-fed birds even though the silage provided them with an additional water source.

It was observed that most of the birds spent a large amount of time standing at the front of the pens “watching” the observers with the assumption they were expecting to be fed or were just curious. Birds in both groups tended to use the hanging water lines as perches, and there was no significant difference in this behaviour due to barley silage feeding.

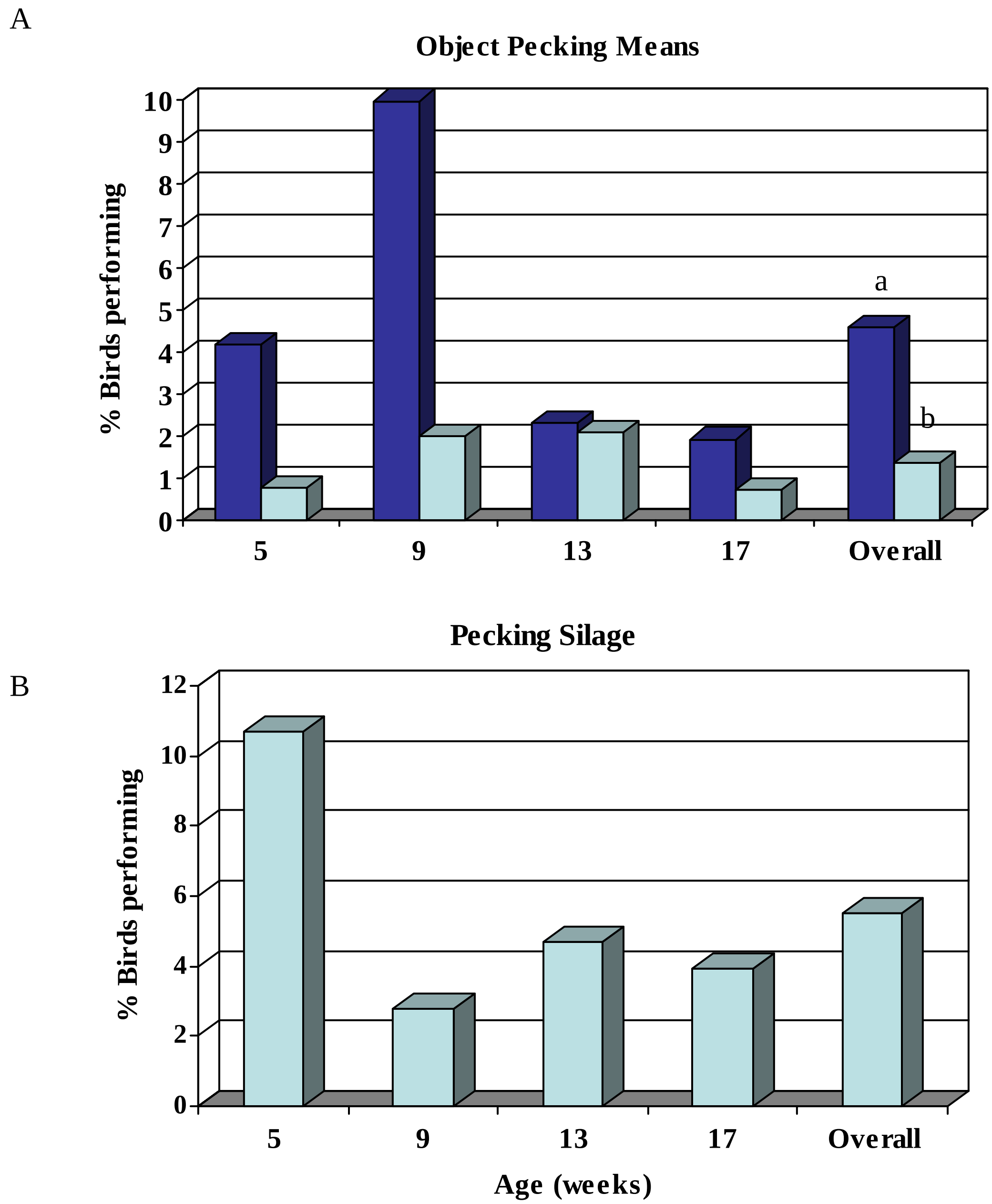

FIGURE 4.3. Effect of feeding silage on stereotypic behaviours in broiler breeder pullets (where = control and = silage-fed). Percentage of birds (interaction means) object pecking (A, overall SEM ± 0.50) and pecking at silage (B, overall SEM ± 0.49) at different weeks of age where different letters indicate significant differences (P<0.05). Main effects (overall bars) where significant for treatment and age, whereas interaction means were not.

4.4.3 Tonic Immobility Scores

Dietary treatment had no significant effect on the duration of TI in broiler breeder pullets but interestingly, TI duration was numerically lower for silage-fed birds compared with the control birds at both 9 and 17 weeks (Table 4.8).

4.4.4 Heterophil to Lymphocyte Ratios

Feeding silage did not affect blood H:L ratio at any measurement age. At 18 weeks of age, the effect of treatment approached significance ($P = 0.06$) when silage-fed birds had a lower H:L ratio than the control birds (Table 4.8).

4.5 DISCUSSION

4.5.1 Body Weight and Feed Intake

Dietary treatment had minimal effects on weight gain with only a minor adjustment in the amount of grower mash fed to silage-fed birds in comparison to the control birds to maintain equal growth rates near the end of the trial. This is somewhat surprising considering that the silage-fed pullets were eating a considerable amount of silage, which resulted in increased dry matter intakes for these birds. The range in increase in dry matter intake was a relatively minor 2.4% at 4 weeks of age to a high of 22.3% for the pullets at 18 weeks of age. This supports the concept that silage has very little direct nutritional value for chickens. However, as found with laying hens (Chapter 3) broiler breeders readily consumed the silage. The change in consumption with age undoubtedly reflects the difficulty young birds had consuming the relatively long

TABLE 4.8. The effect of barley silage feeding on heterophil to lymphocyte ratio (H:L) and duration of Tonic Immobility (means of 90 birds per treatment) in broiler breeder pullets at different weeks of age.

	Treatment			
	Control	**Silage**	**SEM**	***P*-value**
Tonic Immobility (duration in sec)				
9 weeks	96.8	73.2	8.87	0.18
17 weeks	75.0	65.2	9.17	0.59
H:L ratio				
6 weeks	0.387	0.394	0.01	0.76
10 weeks	0.269	0.267	0.01	0.93
14 weeks	0.230	0.260	0.01	0.24
18 weeks	0.238	0.210	0.01	0.06

SEM = standard error of the mean.

P<0.05 indicates a significant effect.

particles in the silage. The amount of silage given to the silage-fed birds daily was based on their preference to eat it. Initially, they received small amounts each in the morning and afternoon which gradually increased with time. The birds showed more interest in eating their mash feed first in the morning but showed considerable interest in eating silage in the afternoon.

The author is unaware of other research on the use of silage in feeding broiler breeders but silage has been fed to turkey breeders to control body weight gain in both toms and hens (Canada Poultryman, 1994; Muirhead, 1994; B. Wentworth, *personal communication*).

Controlling body weight via feed restriction to improve reproductive performance is common in other species as well, such as with gestating sows (Lawrence *et al*., 1988; Lawrence and Terlouw, 1993). Similar to the current study where broiler breeder pullets were feed restricted, sows are provided a restricted mash diet but tend to develop and show strong motivation for feeding throughout the day, resulting in stereotypic abnormal behaviours such as chain chewing, sham chewing (chewing at the air on nothing in particular) and bar biting. This behaviour usually only occurs where feed restriction is a management practice (Lawrence and Terlouw, 1993). It has been suggested by Zonderland *et al*. (2004) that a reduction in feeding motivation could be achieved by increasing an animal's physiological satiation by altering the animal's diet.

In the current trial, broiler breeder pullets were supplemented with a fibre dense feedstuff to aid in satiation between feedings. Bird behaviour indicated a motivation to feed continuously, measured by the time spent at feeder pecking showing interest in finding more feed. Other studies suggest that longer feeding duration is a fair indicator of behaviour and stress (De Jong *et al*., 2002 and 2005). Although feeding duration was not measured in the current study, bouts of feeding or feeder pecking indicating high feeding motivation was observed in both treatments. Silage was attractive to the pullets but the very low nutrient content and bulky constituents did not seem to affect their satiety as was expected in comparison to the control birds. Other options to increase feeding duration without providing a bulky feedstuff would be to place chains within the feeders or casting feed onto the floor amongst the straw litter. Either of these options would force the birds to spend more time pecking at the feed and less time performing other behaviours. Other options to possibly increase their satiety levels without providing fibre would be to provide plastic shavings or other zero nutrient products provided their health would not be compromised.

4.5.2 Behaviour

As reported previously by Hocking *et al.* (2004) and De Jong *et al.* (2005), the most exhibited behaviour in broiler breeder pullets during rearing is time spent pecking at feeders, whether they are full or empty. In the current study, feeder pecking was not affected by silage feeding. This may indicate that the silage-fed birds were not receiving enough nutrients from the supplemental feedstuff (i.e. barley silage) or it could also imply that their motivation to eat is so strong that their focus remains in order to meet a need. Initially in the trial, the birds spent a significant amount of time eating the silage but as the trial progressed, the eating time started to decrease. Because silage intake increased markedly during the trial, the change in eating time probably reflects the ability of the pullets to eat the silage. Motivation to eat silage remained strong throughout the trial.

Even though some earlier research suggests that feed restricted birds tend to demonstrate pacing behaviours (Savory and Mann, 1999b; Kubikova *et al*., 2001), the birds in the present study did not clearly demonstrate this behaviour with the exception of an increased tendency of pacing behaviour in the control birds later in the trial. A very small portion of their time was spent performing this behaviour.

Hocking *et al.* (2004) measured severe feather pecks and aggression during their experiments and found that birds fed higher inclusions of sugar beet pulp and ground oat hulls had a decrease in these behaviours when compared to birds fed other sources of dietary fibre (sunflower meal) as well as lower levels of ground oat hulls. Although overall treatment effects were not noticed in the current study, the silage-fed birds tended to have numerically lower incidences of aggressive and feather pecking behaviours. Thus the results of this study suggest that feeding barley silage to feed restricted broiler breeder pullets has the possibility to positively influence aggressive and feather pecking behaviours. The decrease in these behaviours can likely be attributed to the general contentment of the birds and a feeling of satiety as has also been suggested by Hetland and Svihus (2001) and Choct and Hartini (2003). Hetland and Svihus (2001) and Choct and Hartini (2003) discovered that feed passage rate is delayed by larger particle size. When a diet high in fibre is fed, the gizzard retains the feed particles until they are ground into smaller sizes allowing for continuation through the digestive tract. The effects of feeding silage in this trial are very similar to the reduction in aggressiveness and feather pecking seen in silage-fed laying hens in Chapter 3.

4.5.3 Tonic Immobility Scores

The method of conducting the TI test to assess fear has been proven to work well in previous research by Jones and Faure (1981). This test has been conducted on many species of animals but the one which shows the highest response has been domestic fowl (Jones and Faure, 1981), although strain differences have also been shown to occur (Gallup *et al*., 1976). Where some species may react in a fight or flight scenario, birds demonstrate hypnosis like behaviour or hypnotic gaze with waxy flexibility of the limbs (Maser and Gallup, 1974).

Although there were no treatment effects on the TI scores, it is interesting to note that the control birds consistently scored higher than the test birds at 97 and 75 seconds for the control birds compared to 73 and 65 seconds for the silage-fed birds at 9 and 17 weeks of age, respectively. However, the lack of significance indicates no treatment differences in fear. The birds in this trial were handled frequently and were very accustomed to human presence. This may have reduced fear in all birds regardless of treatment. This is supported by a number of other studies where TI values are all higher than in the present research. Jones and Faure (1981) found TI scores that ranged from 385 to 776 seconds in laying hens. Hocking *et al*. (2001) conducted a study involving broiler breeders, in the growing and rearing phase, and reported TI scores ranged from 186 to 234 seconds in commercially restricted birds, 198 to 278 seconds in birds fed a modified restricted diet and 191 to 565 seconds in the *ad libitum* fed birds when sampled four times throughout a period of 6 to 24 weeks of age. Interestingly, these scores align with the results of our trial in that variable degrees of body weight control had no impact on fear level. In contrast, Hocking *et al.* (1996) suggested that the fearfulness decreased with increasing levels of feed restriction. De Jong *et al.* (2001) found a negative linear relationship between hunger level and time spent being inactive and hypothesized that hungry animals overcome their fearfulness due to the strong motivation to find food.

4.5.4 Heterophil to Lymphocyte Ratios

In the avian species, lymphocytes and heterophils are more responsive to stressors making them easier to detect compared to the other leukocytes (Maxwell *et al*., 1992). Overall, feeding silage did not affect H:L ratio but at trial end, the lower ratio for silage-fed than control birds approached significance (P = 0.06). Additional research is required to confirm if silage feeding reduces stress and thereby the welfare of broiler breeder pullets. Other research has shown that feeding various forms of fibre to broiler breeders decreases H:L ratios (Zuidhof *et al.*, 1995; De Jong *et al*., 2002; Zaczek *et al*., 2003). The H:L ratios found in the present trial are similar to those found by Hocking *et al*. (2001), Mckee and Harrison (1995) and Zuidhof *et al*. (1995).

An important aspect of this trial to remember is that these birds were housed and treated in an ideal environment. They were handled regularly, housed comfortably, had clean housing conditions with straw litter and were very healthy birds. As with fear, it is quite possible that for these reasons, stress levels were not reduced by feeding silage.

4.6 CONCLUSIONS

This study may not have given definitive results as far as improved welfare goes but the fact that it had trends similar to the laying hen trials is reassuring. These birds are hungry and have reduced welfare because they are restrict fed. By making efforts to improve bird welfare is still a positive step in the right direction however further research is warranted.

Further research would be beneficial in determining whether the time spent eating the silage is what reduces aggressive and feeder pecking behaviours. In terms of increasing the welfare of broiler breeder pullets during rearing, consideration of silage more specifically designed to be consumed by young birds (reduced particle size) and to provide a more continual supply of nutrients may be beneficial in increasing the sense of satiety. If there is a gut-fill factor which discourages further intake of any other feed (i.e. feather eating), a reduction in these behaviours is accomplished by feeding silage. Silage fulfills the foraging factor or another possibility is the production of volatile fatty acids may have a calming effect. Furthermore, trials conducted within the scope of industry standards may provide more pronounced results based on the more stressful housing and environmental conditions.

5.0 OVERALL DISCUSSION AND SUMMARY

The main objectives of this thesis were: 1. To determine whether laying hens or broiler breeder pullets would eat barley silage when given free access to it in addition to their regular feeding program; 2. To determine whether bird behaviour would be altered from undesirable and abnormal stereotypical behaviours normally associated with their specific management programs as a result of eating barley silage; and 3. To study the effects of barley silage on production parameters, feed intake, body and feather condition in laying hens, and stress and fear responses in broiler breeder hens.

Both laying hens and broiler breeder pullets were very much interested in eating barley silage along with the commercially recommended mash feed. These results are consistent and suggest that chickens not only find silage to be palatable but actually will consume relatively large amounts even when they have *ad libitum* access to a nutritionally balanced diet as shown with the laying hens. The fact that birds had a choice and still chose to eat large amounts of silage is different than many other experiments on the effects of fibre addition on bird behaviour. In many experiments, diets are diluted with fibre and birds must spend more time eating and eat more to ensure adequate nutrient intake. This is an important difference in experimental design. The reason chickens consume silage is not clear but likely it provides them with some novelty or reward. Establishing the reason for the consumption of silage may lead to alternate methods of behaviour modification and is worthy of research efforts.

The silage-fed birds consumed more feed on an as is basis than their control counterparts because of the silage consumed. The increased intake was associated with more time spent eating and this attribute has often been associated with changes in other behaviours like feather pecking and aggression. The concept is that birds spend more time eating and less time completing less desirable behaviours. In both laying hens and broiler breeder pullets, feeding silage decreased feather pecking and aggressive behaviour. Therefore, this supports the concept of increased feeding time reducing feather pecking and aggressive behaviour. In laying hens the increased time spent feeding in silage-fed birds matched the reduced time associated with feather pecking, aggressive behaviour and time spent in the nest fairly closely. The proportion of the hens feeding was also high at 33% of the time with 16% eating silage. In contrast, a relatively small proportion of broiler breeder pullets were eating silage (5.5%) and most silage-fed and control pullets spent their time pecking at the empty feeder. This may relate to the relatively low nutrient content of the silage which would have little impact on bird satiety. Therefore, in broiler breeder pullets, feeding silage in this study did not have much impact on the important stereotypic behaviour of feeder pecking. Although, the hypothesis that increased feeding time decreases feather pecking and aggressive behaviours is attractive, there may be alternative mechanisms whereby feeding silage affects behaviour. These have been previously discussed (Chapter 3 and 4). It is important to not lose sight of alternate explanations for the beneficial effects of feeding silage on feather pecking and aggressive behaviour. An important impact of reduced feather pecking is the improved feather condition as shown in the laying hen trials.

Production parameters were not affected by feeding barley silage to laying hens. This is somewhat surprising since total dry matter intake of silage fed hens was only marginally higher than for control hens (approximately 4%) despite the fact that low nutrient content silage represented approximately 14% of their dry matter intake. This suggests that factors in silage may enhance digestive function. In contrast, *ad libitum* silage consumption did not produce very much additional growth in broiler breeder pullets, which indicates that little direct nutritional advantage was gained. Possibly the lack of effect in broiler breeders is related to another nutritional factor in the feed (i.e. amino acid, lactic acid) limiting growth despite enhanced utilization of some nutrients. The source of silage was different in all three trials and it may be that they varied in beneficial nutritional properties.

One aspect of silage variability is the type and number of bacteria present, which can be affected by the procedure of silage making. Undi (1988) reported an extremely high number of total microbes and lactic acid producing bacteria on the standing barley crop. He also reported a significant effect of duration of ensilage on the bacterial counts. The anaerobic bacteria mainly lactic acid producing bacteria and *Clostridium spp.* in the barley silage increased with increased duration of ensilage.

Feeding silage caused darkening of yolks as expected due to the level of dietary pigments in the silage. One might expect similar changes in skin colour in chickens fed silage but that characteristic was not measured or noticed in any of the trials. Pigment changes are likely to show up more in situations where low pigments feeds (wheat-soybean meal) diets predominate. In areas with already high levels of dietary carotenoid pigments additional colouration would be less obvious.

The effect of barley silage feeding on bird behaviour and stress variables were more pronounced in the laying hens compared to broiler breeder pullets although significant stress measurement results were only shown in the males (Chapter 3). It should be remembered that in the present studies, the birds were reared under very good environmental and management conditions, so the implications of barley silage feeding on bird behaviour might be different under less perfect commercial conditions.

Feeding silage in a commercial poultry setting presents logistical problems in regards to handling and delivering the feedstuff to the birds. In broiler breeder barns, it may be possible to hand deliver silage onto the floor. This would encourage foraging and exploratory behaviour in broiler breeder birds, which would allow natural behaviours to be performed in addition to providing them with a feedstuff thereby occupying their time and aiding in satiation. Undoubtedly, automated delivery systems could also be adapted to the floor environment. The silage feeding system would be more difficult to establish in a cage laying hen operation. However, if producers have ready access to silage and utilize it regularly, modifications to poultry trough feeding systems could be developed. This could involve a separate feeder line for silage or preferably feeding of silage and regular diet interchangeably during the day. Silage is much cheaper than the regular commercial mash feed and it can be made and stored on farm. So the silage feeding might be an economical option when birds are consuming approximately 15% of their daily intake as silage without any adverse effect on their production or body condition. An important aspect of feeding silage to poultry would be to develop fermentation products more appropriate in particle size and nutrient content than the barley used in the present studies.

Currently there is limited information on the effects of supplying roughages, silage in particular, to laying hens and broiler breeder hens as an aid in reducing feather pecking and aggressive behaviours. Although many questions concerning the effect of silage feeding on poultry behaviour and performance characteristics were able to be answered in this research, it has also led to many questions and directions for further research. Some questions are as follows:

What components of silage make it attractive as a feedstuff for poultry?

How does feeding silage affect bird behaviour?

What is the impact of silage digestion, metabolism and volatile fatty content?

How does the level of gut-fill/distention due to feeding silage to poultry affect bird behaviour, satiety level and digesta passage rate compared to regular mash diets normally provided to the birds?

What types of silage (e.g. pea or corn silage) are best suited for poultry feeding and use in a commercial setting?

In conclusion, our results demonstrate that poultry are attracted to silage as a feed ingredient and that feeding silage to laying hens or broiler breeder pullets reduces the negative behaviours of feather-pecking and aggression. Our results confirm that nutrition can play an important role in behavioural modification in poultry stocks.

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APPENDIX A

Table A1. Vitamin and mineral premix composition for laying hens (0.5% inclusion)

Nutrient	Units	Nutrient amount per 1 kg premix
Vitamin A	MU	1.60
Vitamin D	MU	0.60
Vitamin E	KU	5.00
Menadione	GM	0.30
Pantothenic AC	GM	1.60
Riboflavin	GM	1.00
Folic acid	GM	0.10
Niacin	GM	6.00
Thiamine	GM	0.30
Pyridoxine	GM	0.30
Vitamin B_{12}	MG	2.40
Biotin	MG	12.00
Iodine	GM	0.16
Copper	GM	2.00
Iron	GM	16.00
Manganese	GM	16.00
Zinc	GM	16.00
Selenium	GM	0.06
Calcium carbonate	GM	100.00

Source:

DSM Nutritional Products Canada Inc., Ayr, ON, Canada.

Table A2. Vitamin and mineral premix composition for broiler breeders (0.5% inclusion)

Nutrient	Units	Nutrient amount per 1 kg premix
Vitamin A	MU	2.20
Vitamin D	MU	0.44
Vitamin E	KU	6.00
Menadione	GM	0.40
Pantothenic AC	GM	2.00
Riboflavin	GM	1.20
Folic acid	GM	0.12
Niacin	GM	12.00
Thiamine	GM	0.30
Pyridoxine	GM	0.80
Vitamin B_{12}	MG	4.00
Biotin	MG	30.00
Iodine	GM	0.16
Copper	GM	2.00
Iron	GM	16.00
Manganese	GM	16.00
Zinc	GM	16.00
Selenium	GM	0.06
Calcium carbonate	GM	100.00

Source:

DSM Nutritional Products Canada Inc., Ayr, ON, Canada.