Effect of diet composition, gut microbial status and fibre forms on Cannibalism in Layers

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Foreword

Since the introduction of the Rispen's Marek's Disease vaccine to Australia in 1998, cannibalism has become the major cause of mortality in Australian layer hens, especially for the imported strains. Mortality from cannibalism in some strains ranges from 10% to 20%, depending on the production system and management strategies (Cumming *et al*., 1998).

The Australian egg industry has a flock size of approximately 10 million layers and 10% mortality during lay means a loss of 10 million dollars annually. Currently, the majority of Australian egg farmers have adopted either 5-bird cages or 3-bird cages, which exacerbate cannibalism problems. In addition to the direct cost through loss of birds in lay there is a considerable cost associated with lost productivity, disposal of birds, and flock morbidity. With a sluggish egg market and increasing awareness of animal welfare issues in the community, cannibalism problems must be addressed in a scientific and cost-effective way.

The industry needs to consider not only management and husbandry issues, but also nutritional practices that may be used to minimise cannibalism in poultry. The current project examined the problems of cannibalism predominantly from a nutritional management-point of view, but breed and husbandry were also monitored.

The project aimed to identify both nutritional and husbandry factors that may contribute to increase cannibalism, and to develop strategies to minimise it.

This project was funded from industry revenue which is matched by funds provided by the Federal Government.

This report is an addition to AECL's range of research publications and forms part of our R&D program, which aims to support improved efficiency, sustainability, product quality, education and technology transfer in the Australian egg industry.

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List of publications

- Effect of diet composition and beak trimming on the incidence of cannibalism in laying hens. S. Hartini, M. Choct, G. N. Hinch and J.V. Nolan. 2001. Australian Poultry Science Symposium, 13: 216-219.

- Effect of light intensity during rearing and beak trimming and dietary fiber sources on mortality, egg production, and performance of ISA Brown laying hens. S. Hartini, M. Choct, G. N. Hinch, A. Kocher, and J. V. Nolan. 2002. J. Appl. Poult. Res. 11:104-110

- Effect of diet composition and feed form on the behaviour of ISA Brown laying hens. S. Hartini, M. Choct, G. N. Hinch and J.V. Nolan. 2002. Australian Poultry Science Symposium, 14: 104-107.

- Dietary prevention of cannibalism in layers. M.Choct, S. Hartini, G. N. Hinch and J.V. Nolan. 2002. Australian Poultry Science Symposium, 14: 157.

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Executive Summary

Cannibalism, the behaviour where birds start pecking or eating flesh of other birds, is a major problem for the Australian layer industry. Mortality from cannibalism in some strains ranges from 10% to 20%, depending on the production system and management strategies (Cumming *et al*., 1998) and appears highest in imported strains. The Australian egg industry has a flock size of approximately 10 million layers and 10% mortality during lay means a loss of 10 million dollars annually. Currently, the majority of Australian egg farmers have adopted either 5-bird cages or 3-bird cages, which may exacerbate cannibalism problems. In addition to the direct cost through loss of birds in lay, there is also a considerable cost associated with lost productivity, disposal of birds, and flock morbidity. With a sluggish egg market and increasing awareness of animal welfare issues in the community, cannibalism problems must be addressed in a scientific, cost-effective way.

Prevention of cannibalism through nutritional manipulation has not been widely used. Although nutritional deficiencies are often difficult to detect since feed is changed regularly in layer operations, preventing behavioural problems through dietary manipulation appears an attractive and feasible option. So, in an effort to identify the nutritional factors that influence cannibalism, a series of experiments were carried out using different diets. The effects of diet on cannibalism were observed by measuring performance (feed intake, egg production and mortality), behaviour and digestive function of birds in lay.

Each diet was formulated to contain different types of cereal grains as sources of fibre and different fibre levels. The diets were formulated according to commercial specifications and were produced at a commercial mill (Ridley AgriProducts, Tamworth, NSW), except the diets used in Auxiliary Trial 3, which were formulated and produced at the University of New England.

An initial experiment was conducted to provide information on the effect of rearing conditions, beak trimming and diet composition on the development of cannibalism in laying hens. Commercial day-old pullets obtained from Baiada hatchery (Kootingal, Tamworth, NSW) were reared at the Kirby Research Station, University of New England, where they received different light conditions for 14 weeks. At 15 weeks of age, they were transferred to the Laureldale Research Station and housed in five-bird cages and offered 4 different dietary fibres. Bird behaviour and performance were monitored until the birds were 24 weeks of age. Birds reared under bright light showed more cannibalistic behaviour than those reared under dim light. Beak trimming had a profound effect on cannibalism and feed intake, with cannibalism occurring predominantly in untrimmed birds. A diet high in insoluble fibre was effective in reducing the incidence of cannibalism.

The next experiment was designed to investigate the effect of diets used in the previous experiment on the bird's digestive function. Thirty-two ISA Brown hens kept in individual cages were used. Results from this experiment showed that soluble and insoluble NSP had a marked effect on the digestive function of birds. However, the physiological effect of both soluble and insoluble NSP in the gut may be modified by interactions with each other and with other cell-wall components. In addition, in this experiment the β-glucans present in the barley diet appeared to be of low molecular weights. The high insoluble NSP level in the intestine of barley-fed birds also indicates that the β-glucans in this particular barley sample were less soluble than usual. Therefore, the soluble NSP in the barley diet did not elicit a similar physiological effect to that of the wheat diet. The wheat diet increased the intestinal digesta viscosity and VFA concentrations, whereas the barley diet only increased the jejunal viscosity and had a lower VFA concentration. It was also found that the wheat diet decreased the rate of digesta passage, whereas barley diet increased it. However, the birds fed a wheat diet

containing high soluble NSP had a higher body weight than those given other diets. The results suggest that a small increase in digesta viscosity may not affect bird performance, but enough to modify bird behaviour. Birds fed a diet high in insoluble NSP exhibited lower mortality from cannibalism, suggesting that insoluble NSP with the properties to increase rate of digesta passage have the potential to reduce cannibalistic behaviour of birds and highlighting the question as to whether this will hold true for fibre provided by other cereal grains.

Another experiment was conducted to examine the effect of different fibre sources on cannibalism. Diets differing in fibre sources were offered to birds from the flock used in the previous experiment. There were no statistically significant effects of diet observed in this experiment. It was shown however, that birds given a diet containing a higher insoluble NSP content had a higher percentage of birds alive than those fed other diets. This result provided further evidence that insoluble NSP has a role in reducing cannibalism mortality.

Different feed forms have been reported to influence the cannibalistic behaviour of birds. An experiment was conducted to observe the effect of fibre levels and feed forms on performance and behaviour of laying hens. The results indicate that fibre level and the form in which a diet is fed (mash vs pellet) can influence the pecking behaviour of laying hens. The incidence of feeding, pecking, escape and freezing behaviour was all useful indicators of factors leading to the onset of cannibalism. Changes in diets altered the incidence of social pecking behaviour and, to a degree, this was associated with changes in the proportion of time spent feeding. A high level of social pecking behaviour in birds fed a high-fibre mash diet suggested that these birds were not fully satisfied with the amount of energy and nutrients provided.

A second phase of a laying trial was carried out to verify the effect of fibre sources and feed form on bird performance, behaviour and digestive function. In general, the results supported the findings from the previous experiments, that birds fed diets with a high-insoluble NSP content showed a reduction in cannibalism mortality. It was found that birds fed a high-fibre mash diet increased social pecking and exhibited a higher incidence of escape and freezing behaviour. The lower mortality in these birds suggested the high-fibre mash diet was effective in controlling cannibalism.

It was clearly shown that dim-light, high-insoluble NSP content in the diet and mash diets rather than pelleted diets had potential to reduce mortality due to cannibalism in laying hens. We therefore recommend that laying hens are reared under low-light conditions and are fed diets containing high-insoluble NSP in mash form. However, since the concentration of insoluble NSP is likely be influenced by diet composition, it is recommended that the insoluble NSP content of a diet be determined prior to formulation. A further study is needed to pursue the effect of gut microflora on cannibalism in laying hens, especially identifying the species of microorganisms that proliferate due to ingestion of NSP. Application of dim light during the pre-lay and laying period and different feed forms (mash vs crumble) may also be worthy of future study.

1. Introduction

Since the introduction of the Rispen's Marek's Disease vaccine, cannibalism has become the major cause of mortality in the Australian egg industry. Cannibalism is the result of birds pecking and tearing the skin off other birds' bodies. This behaviour usually leads to death of the pecked bird and can occur at any age and in any species. Once cannibalism has started, it can spread very rapidly through the entire flock causing very serious economic loss to the farmer, in addition to the injury of the birds and associated welfare concerns.

In Australian flocks, mortality from cannibalism in some strains ranges from 10 to 20% depending on the production system and management strategies (Cumming *et al*, 1998). Since the Australian egg industry has a flock size of approximately 10 million layers, 10% mortality during lay means an approximate loss of 10 million dollars annually.

Determining the exact reason for an outbreak of cannibalism is almost impossible since numerous factors seem to influence its occurrence. Some researchers consider that housing factors such as high stocking density (Cain *et al*.,1984), wire floor (Blokhuis and van der Haar,1989), hot weather or bright lighting (Scott *et al*., 1954), and inadequate feeding space (Robinson, 1979) cause a higher degree of feather pecking and/or cannibalism. Others believe that nutritional factors such as protein deficiency (Curtis and Marsh, 1992), mineral imbalance (Cooke, 1992), and insufficient dietary fibre (Esmail, 1997) are factors that exacerbate the incidence of cannibalism. Genetic factors are also considered to be involved in the outbreak of cannibalism, and genetic selection to reduce the incidence of cannibalism has been proposed.

Among the factors above, some researchers consider that a better environment or better management are the most important factors in preventing cannibalism. However, even with almost perfect management, cannibalism still occurs. Furthermore, it is not easy to control the environment. Genetic selection seems to offer a great possibility of preventing the occurrence of cannibalism; however, a long-term approach is required. Moreover, more experimental evidence must be gathered as to the likely overall success of concurrent selection for increased productivity and decreased cannibalistic behaviour (Craig, 1981).

Prevention of cannibalism through nutrition has not been evaluated. Although nutritional deficiencies are often difficult to detect since feed is changed regularly in layer operations, preventing cannibalism through dietary manipulation appears an attractive option. Some studies report prevention of cannibalism through dietary supplementation. Some protein sources were reported to result in the outbreak of cannibalism (Curtis and Marsh, 1992), while Cooke (1992) claimed that sodium deficiency was the cause of cannibalism. Changes in the source of protein may affect the sodium level of the diet. Esmail (1997) found that an increased retention of sodium and potassium occurred with inclusion of extra fibre in the diet. In addition, substitution of oat mill feed for corn prevented an increase in cannibalism. Wahlstrom *et al*. (1998) also found that the total mortality due to cannibalism in birds given oat-based diets was lower than in those given wheat-based diets.

NSP refer to all polysaccharide fractions except starch and types of dietary fibre, with the exception of lignin, either soluble or insoluble (Ebihara and Kiriyama, 1982, Englyst and Hudson, 1987). The effect of soluble or insoluble NSP in the gastrointestinal tracts of birds is well documented and the ingestion of soluble NSP increases intestinal digesta viscosity causing slower food transit time (Salih *et al*., 1990). Reduction in digesta transit time or gastric emptying is likely to induce a feeling of fullness or satiety and to shorten the individual meal periods (Read, 1992). While the soluble NSP reduced digesta transit time in the gastrointestinal tract, the insoluble NSP increased it (Roberfroid, 1993) and more rapid clearing of digesta is likely to stimulate birds to begin a new meal sooner after the last meal. Such changes in gut conditions and the time spent eating may modify the behaviour of birds especially with regard

to cannibalism. It has been postulated that the longer birds spend feeding the less time they are likely to spend in pecking behaviour. Whether this is true has yet to be confirmed.

The general objectives of this project were to identify both nutritional and husbandry factors that might contribute to increased cannibalism, and to develop strategies to minimise cannibalism.

2. Literature Review

2.1 Scope of the Review

This chapter is intended to provide background information based on the available literature on cannibalism in laying hens and the potential dietary fibre may have in reducing this behaviour. The review has been divided into two parts. Section 2.2 is a review of cannibalism in laying hens covering the definition and description of cannibalism, the causation and occurrence of cannibalism and the preventive factors that have been applied. Sections 2.3 to 2.6 deal with dietary fibre itself. This includes the general description of dietary fibre, its physicochemical properties and its physiological function in the gastrointestinal tract of birds, and its role in prevention of cannibalism in birds.

2.2 Cannibalism in laying hens

2.2.1 Definition and description of cannibalism

Cannibalism in poultry has been defined as the behaviour exhibited when birds start pecking and tearing off the skin or underlying tissues of another bird (Appleby *et al.*, 1992). The occurrence of cannibalism is usually associated with feather pecking, although cannibalism can occur independently (Allen and Perry, 1975; Savory, 1995).

Feather pecking, the behaviour of birds pecking or pulling out feathers of another bird (Blokhuis and Arkes, 1984), can trigger cannibalism when feather pecking results in severe damage or wounding to the skin (Hughes and Duncan, 1972; Savory, 1995). The presence of blood or wounds seems to exacerbate the cannibalism problem (Blokhuis and van der Haar, 1992). However, in this study when the blood was wiped off the attack ceased. Therefore, it was claimed that the wounding, not the feather pecking, was the primary cause of cannibalism (Allen and Perry, 1975). Moreover, the ability of birds to readily copy behaviour in such a way as to perform different activities synchronously in a group (Appleby *et al.*, 1992) accentuated the condition. The most commonly pecked area was on the back and wings and the dorsal surface or base of the tail (Allen and Perry, 1975).

Cannibalism that has occurred without being preceeded by feather pecking is known as 'vent peck' (Allen and Perry, 1975). This is the commonest pecking in laying hens, especially occurring at point of lay. Vent pecking appears to be initiated when a minor partial prolapse of the uterus occurs immediately after laying (Savory, 1995). This protrusion of the vent with its red colour can easily attract birds to peck. In this condition, the pecked bird may die from loss of blood. The result of vent pecking is more severe than the result of cannibalism caused by feather pecking because the intestine can sometimes extrude through the vent wound. The intestine is likely to be the subject of more pecking, and may be pulled out and ingested (Hughes and Duncan, 1972; Savory, 1995). Allen and Perry (1975) observed that of the 142 cannibalized birds, 19 had been pecked around the cloaca and partially eviscerated.

2.2.2 Occurrence of cannibalism

Cannibalism can occur in any age, species, strains and sex of fowl (Allen and Perry, 1975; Hughes and Duncan, 1972; Cain *et al*., 1984; Klemm *et al*., 1995). In addition, it can occur not only on birds that are raised in floor pens or loose housing systems, but also those raised in cages (Appleby *et al*., 1989).

Allen and Perry (1975) found that the first death of birds in cages from cannibalism occurred as early as 2 weeks of age and the incidence gradually decreased as the rearing period progressed. Johnsen *et al.* (1998), on the other hand, observed that cannibalism occurred between the fourth and seventh week of age and significantly more during the laying period. In a free-range flock, cannibalism was found to be most severe after 11 months of lay with most losses occurring during the final 8 weeks (Keeling *et al*., 1988).

Feather pecking or cannibalism can occur in any type of domestic fowl such as chickens (Johnsen *et al*., 1998), turkeys (Nixey, 1994), Japanese quails (Vestergaard and Hogan, 1992) or pheasants (Scott *et al*., 1954). In addition, it can occur both in female and male birds (Oden *et al*., 1999). According to Oden *et al*. (1999), male birds rarely showed aggressive behaviour, either towards the opposite sex or towards each other. Females, on the other hand, were seen to show aggressive pecks among themselves but less when males were included. However, the presence of males did not significantly reduce the incidence of pecking in large groups at high stocking density.

The incidence of cannibalism varies greatly among strains (Elliot, 1996; Ambrosen and Petersen, 1997). Level of aggression may also differ between lines (Savory, 1995). Brown-egg layers appear less likely to exhibit feather pecking and cannibalistic behaviour than white-egg layers (Elliot, 1996). However, Savory (1995) observed that feather pecking and cannibalism were now more common in modern brown hybrids. Hughes and Duncan (1972), moreover, found that one strain of chicken showed only slightly greater feather damage in larger-size cages than in smaller-size cages, while another strain had much greater damage in the larger-size cages. Bidermann *et al.* (1993) cited by Ambrosen and Petersen (1997), observed that mortality and production of birds were influenced by plumage condition. An intact plumage was associated with lower mortality. Since plumage condition varies among strains, the negative effect due to feather pecking or cannibalism also differs among strains of layers.

Cannibalism is more common in birds kept in floor pens than in cages. According to Appleby *et al*. (1989) this may be due to more birds being raised in loose housing systems or floor pens than in cages. Therefore, when pecked birds run through the flock trying to escape from attackers, this will attract other birds which will also attack them. Feather pecking that occurs in cages is less likely to develop into cannibalism (Elliot, 1996) except when there is an additional factor such as high stocking density (Appleby *et al.*, 1992).

Whether cannibalism occurs because of feather pecking or independently, the similarity is that the pecked bird usually makes little effort to escape despite the pain experienced (Appleby *et al*., 1992). Fraser and Broom (1997) presumed that the apathy of the pecked bird to escape could be because they have learned that previous attempts have proved useless. Another possibility is that the birds that are frequently attacked are smaller or weaker than other birds, or are known as low ranking birds (Appleby *et al*., 1992). For hens which are kept intensively in any large group, it is common that the submissive birds will be pecked continually by dominant birds.

2.2.3 Causation of cannibalism

The causes of cannibalism are not well understood, but the outbreak of cannibalism and/or feather pecking has been ascribed to a number of factors. High-stocking density pens increased the frequency of feather pecking in pheasants (Hoffmayer, 1969, Cain *et al*., 1984). Crowding and high-density pens had negative effects not only on production but also on welfare of birds (Adam and Jackson, 1970, Kato *et al*., 1991). Increasing mortality (Adam and Jackson, 1970, Kato *et al*., 1991) and decreasing egg production (Adam and Jackson, 1970) have been observed in pullets housed in a big flock. In addition, the effects were usually more pronounced for subordinate hens (Cunningham and Mauldin, 1996).

Blokhuis (1986) observed that a high stocking density will reduce the opportunity of birds to ground peck, and the latter is replaced by feather pecking. Domestic fowls exhibit the behaviour of pecking or scratching at the ground even when food is freely supplied in troughs (Blokhuis, 1986). Overcrowding will also suppress the habit of birds to clean their own faces by beak wiping which triggers the behaviour of pecking on each other's face that often leads to cannibalism (Appleby *et al*., 1992). Hansen and Braastad (1994) showed that birds reared in low-density groups ground pecked more frequently than birds raised in high-density groups. The lower incidence of feather pecking in birds raised on litter floors compared to that of those raised on slatted floors (Blokhuis and Arkes, 1984) confirmed this finding.

Vestergaard (1982) found that birds exhibited less feather-pecking behaviour when they were continually kept on suitable dustbathing substrate from an early age. In domestic fowl dustbathing occurs regularly (Vestergaard, 1982). Overcrowding suppresses dustbathing, and consequently increases the incidence of feather pecking. This finding was supported by Johnsen *et al.* (1998) who found that birds reared on a wire floor have higher mortality due to cannibalism than those reared on sand and straw. They indicated that this was due to the disturbance in the development of dustbathing behaviour. It was likely that feathers might be perceived as a dustbathing substrate.

Robinson (1979) observed that inadequate trough space per bird was the factor that caused cannibalism. The author found that with increasing numbers of birds/cage, birds could survive and perform well providing that feeding space is adequate. As birds tend to perform different activities synchronously in a group, birds will tend to eat together rather than independently (Appleby *et al*, 1992). Tolman (1964) noted that single chicks do not resume eating as quickly as the ones who have a companion who is still eating. Inadequate trough space per bird will increase the activity or competition during feeding time. During feeding time, dominant birds always exhibited longer feeding bouts and greater total feeding times than the lowest-ranking animals. Inadequate feeding space will lead to feather pecking. Therefore it is important not to increase activity or competition during feeding times (Savory, 1995). Meunier-Salaun and Faure (1985) studied different feeder designs and found that synchrony of feeding behaviour was low when feeding space was unpartitioned or the partitioned spaces adjacent.

Pecking behaviour increased when birds were exposed to fully lighted conditions or to hot weather (Scott *et al*., 1954). The presence of light highly increased the risk of pecking (Allen and Perry, 1975; Savory, 1995). Comparison of low and high intensity light (3 vs 30 lux, Kjaer and Vestergaard, 1999) revealed that severe pecks were 2-3 times more frequent in 30 lux and were associated with increased mortality. Areas of bright sunlight attracted large numbers of birds and stimulated activity (Savory, 1995). Nixey (1994) found that increasing day-length due to a changed lighting program might speed up sexual maturity and result in increased fighting and cannibalism among male turkeys. However, Sherwin and Kelland (1998) found that feather pecking and cannibalism in turkeys did not occur despite higher light intensities than conventional systems.

Several dietary deficiencies also appear to be causally related to feather pecking and/or cannibalism (Cooke, 1992; Curtis and Marsh, 1992; Ambrosen and Petersen, 1997; Esmail, 1997). A significant effect of reduced protein level has been found on mortality due to cannibalism and plumage condition (Ambrosen and Petersen, 1997). A low protein diet resulted in a high mortality rate and an unsatisfactory plumage condition. Curtis and Marsh (1992) found a similar result, but considered that it was due to changes in protein sources. Changes in protein source influence the flavour and palatability of the feed and reduced intake, leading to the outbreak of cannibalism. In contrast, Cooke (1992) claimed that mineral imbalance, especially sodium, was the cause of cannibalism. However, changes in protein source may well affect the sodium level of the diet. Esmail (1997) found that insufficient fibre in the ration was a factor that was associated also with increased incidence of cannibalism.

The form in which food is given may also influence the occurrence of cannibalism. Calet (1965) and Aerni *et al*. (2000) observed that feather pecking was high in birds receiving a pelleted feed. These authors reported a tendency of birds to cannibalize when fed on pellets, and to be much calmer when they were fed a mash diet. Disturbed birds are more likely to initiate pecking (Hughes and Duncan, 1972) and a negative correlation was found between nervousness score and feather score by Elliot (1996). It has been suggested that the more nervous the birds, the greater the feather damage and loss.

Elliot (1996), moreover, suggested that increased feather pecking in birds fed pelleted feed was due to boredom. Less feeding time will be spent by birds on a pelleted feed because of quicker satisfaction of energy and nutrient requirements due to faster intake of feed; as a consequence birds will try other activities which may eventually be able to lead to increased cannibalism (Esmail, 1997). Regular pecking and feather removal lead to vent pecking and cannibalism may be more likely in groups where activity levels are high (Savory and Mann, 1997).

2.2.4 Prevention of cannibalism

To find the exact reason for outbreaks in cannibalism is almost impossible. A combination of factors described above is usually involved. Savory (1995) considered effective prevention involved four categories: "pre-emptive intervention", environment, genetic variation, and nutrition.

2.2.4.1 Pre-emptive Intervention

Beak trimming is a process that has long been applied by poultry producers since it is believed to reduce the incidence of injury and death associated with feather pecking and cannibalism. It is the process of cutting off about one-third of the upper and lower mandible (Blokhuis and van der Haar, 1989). Although the use of beak trimming is often criticized on welfare grounds significant reductions in mortality as a result of beak trimming have been reported in many studies (Kuo *et al*., 1991, Cunningham, 1992).

Blokhuis and van der Haar (1989) suggested that the lower frequency of feather pecking in beak-trimmed birds might be due to the pain that occurred after trimming. The beak of birds is well-innervated (Gentle and Breward, 1986) and consists of both nociceptors and mechano-receptors (Breward, 1984). Beak-trimming will induce pain coming from the activation of specific nociceptors and from spontaneous nerve impulses originating from neuroma formation after trimming (Gentle, 1986). Recent work from Australia indicates that if beak trimming is done properly, the neuromas usually resolve (Glatz and Lunum, 2002).

Some behavioural studies observed that the pain resulting from trimming can also change the behaviour of birds, such as environmental pecking, feeding, drinking or preening (Kuo *et al*., 1991, Lee and Craig, 1991). One of the positive behavioural changes is a reduction in

cannibalistic activity (Kuo *et al*., 1991, Lee and Craig, 1991). Duncan *et al*. (1989) observed that pain caused by trimming could cause behavioural changes for at least 5 weeks after trimming. This may explain the finding of Blokhuis and van der Haar (1989), who found a reduction in feather pecking due to beak trimming during the rearing period but not during the laying period.

However, some studies have reported non-significant effects of beak trimming (Blokhuis and van der Haar, 1989, Lee, 1980) and Blokhuis and van der Haar (1989) suggested that feather pecking might even be enhanced by beak trimming. According to them, it is possible when the pain wears off or the beaks are re-grown, the opportunity for plumage damage and cannibalism is higher in beak-trimmed birds than in non beak-trimmed birds.

2.2.4.2 Environment

Reducing light intensity (Nixey, 1994) or introducing red lighting (Appleby *et al*., 1992; Savory, 1995) can reduce the occurrence of cannibalism. Reduction of light intensity will make it difficult for birds to see blood or wounds, therefore preventing them from performing pecking behaviours. However, this can only be applied in fully enclosed houses, not in partly or fully open-side houses such as strawyards (Appleby *et al*., 1992).

The use of mash feed instead of pelleted feed may also prevent the outbreak of cannibalism (Linberg and Nicol, 1994). Fine feed encouraged the hens to feed longer than coarse feed, consequently decreasing feather pecking rates (Walser *et al*., 1996). Time spent feeding was greater with mash than with pellets, as also observed by Savory and Mann (1997). Similar results may be obtained when birds are reared on litter floors compared with cages or wire floors (Blokhuis and van der Haar, 1989). Blokhuis (1988), cited by Blokhuis and van der Haar (1989), suggested that litter floors have a higher incentive value compared to wire floors since litter can act as a pecking substrate. Redirected ground pecking will take place when the relative incentive value of the ground is low (Bindra, 1978). Ensuring that there is no competition at feeders, drinkers and nest boxes, in other words, adequate feeding space, may also prevent the incidence of feather pecking and/or cannibalism (Savory, 1995). There was a positive relationship between available feeding space and time spent feeding in battery cages (Hughes and Black, 1977) and increased feeding space led to longer feeding times. Identification and removal of 'troublemaker' birds as soon as possible can prevent the increase of cannibalism (Savory, 1995).

2.2.4.3 Genetic Variation

Kjaer and Sorensen (1997) stated that 'one can evaluate performing feather pecking at any age, carry out selection and expect a change of performing feather pecking at all ages in the desired direction'. Cuthberson (1980) and Walser *et al*. (1996) agreed that selection of hens with low pecking rates could reduce feather pecking. The finding of Korte *et al.* (1999) that high- and low-feather pecking birds have different activities in terms of coping style, highlighted the feasibility of reducing the occurrence of cannibalism by genetic selection. Muir and Craig (1998) observed that by selecting using a kin selection method, a much less feather-pecking and cannibalistic strain of laying hen, with no reduction in productivity, had been developed.

2.2.4.4 Nutrition

Reduction in the occurrence of cannibalism through use of dietary supplements has been investigated. Some believe that increasing protein level can prevent the outbreak of cannibalism (Curtis and Marsh, 1992, Ambrosen and Petersen, 1997). According to Ambrosen and Petersen (1997), in diets high in protein, it is easier to obtain balanced amino acid composition than in diets lower in protein. The problem is each protein source has different amino acid composition, and therefore it is difficult to pinpoint the imbalanced amino acids.

Cooke (1992) recommended paying close attention to minerals, especially sodium, in relation to cannibalism. Partly agreeing, Esmail (1997) suggested that having moderate levels of fibre in the ration to improve utilisation of minerals could reduce incidence. Inclusion of oat hulls has been found to increase retention of sodium and potassium and substituting oat mill feed for corn reduced the incidence of cannibalism. This is similar to the finding by Bearse *et al* (1940), who found that oat hull fibre obtained by dilute acid digestion was the factor that reduced the occurrence of cannibalism. Based on their study, they recommended the inclusion of oat hull so as to produce a ration with 11 % fibre. Wahlstrom *et al*. (1998) also found that the total mortality due to cannibalism in birds given oat-based diets was lower than in those given wheat-based diets.

It appears from these studies that fibre plays an important role in dietary manipulation of cannibalism in birds.

2.3 The role of dietary fibre in prevention of cannibalism in birds

2.3.1 Description of dietary fibre

Dietary fibre has been described as the skeletal remains of plant cells in diets which are not digested by human digestive enzymes (Trowell, 1974). This definition was then expanded to include all polysaccharides and lignin which are not digested by the endogenous enzymes of man (Trowell, 1976). Because of the possibility that under some conditions starch is not completely digested, some scientists redefined dietary fibre as being comprised of non-starch polysaccharides plus resistant starch and lignin (Topping and Pant, 1993). The similarity of the definitions is that the polysaccharides that are not digested by human endogenous enzymes, also known as non-starch polysaccharides or non-α glucan (Cummings *et al*., 1997), are the major components of dietary fibre (Asp, 1995). Schneeman (1987), and Graham and Åman (1991), on the other hand, based on solubility, separated dietary fibres into two physicochemical groups. First, insoluble dietary fibre, which is mainly composed of cellulose, lignin and some hemicelluloses; and second, soluble dietary fibre such as pectins, gums, mucilages and other hemicelluloses. The chemical classification of dietary fibre can be seen in Table 1.

2.3.2 Non-starch polysaccharides (NSP)

Non-starch polysaccharides (NSP) according to Ebihara and Kiriyama (1982), and Englyst and Hudson (1987) refer to polysaccharides excluding starch and types of dietary fibre, with the exception of lignin, either soluble or insoluble. Included are pectic substances, hemicelluloses, celluloses and gums (guar) and mucilages (Southgate, 1995; Cummings *et al*., 1997). Cellulose, hemicellulose and pectic substances are known as plant cell wall NSP since they comprised 80-90% of the plant cell wall (Cummings *et al*. 1997). Resistant starch, theoretically, falls outside the NSP concept, but practically it depends on the method used to eliminate starch. Southgate (1995) divided NSP in plant foods into structural and non-structural polysaccharides. Table 2 illustrates major types of NSP in plants.

2.3.2.1 Cellulose (glucans)

Cellulose, consisting totally of 1→4 β-glucan, is the most plentiful and only truly fibrous component of the plant cell wall (Annison, 1993; Southgate *et al*., 1993). Individual chains are arranged in microfibrils held together by hydrogen linkages in a highly ordered structure (Aspinall, 1980; Eastwood, 1992). The microfibrils, characterized by crystalline regions, allow the molecules to pack very closely together which prevent the penetration of water molecules (Annison, 1993). Although cellulose is water insoluble, cellulose still has the property of taking up water (4 g water per g of cellulose) (Southgate *et al*., 1993). This explaines the ability of cellulose to increase faecal weight when added to the diet.

In cereal grains β-glucans are found, which are polysaccharides similar to cellulose. What differentiates β-glucans from cellulose is the presence of β 1→3 linkages (Edney *et al*., 1991).

Table 1 Chemical classification of dietary fibre (McPherson, 1985)

Fibre	Main chain	Side chain	Description
Polysaccharides			
Cellulose	Glucose	None	Main structural component of plant cell wall. Insoluble in concentrated alkali; soluble in concentrated acid.
Noncellulose			
Hemicellulose	Xylose Mannose Galactose Glucose	Arabinose Galactose Glucuronic acid Glucuronic acid	Cell wall polysaccharides containing backbone of 1-4 linked pyranoside sugars. Vary in degree.
Pectic substances	Galacturonic acid	Rhamnose Arabinose Xylose Fucose	Components of primary cell wall and middle lamella vary in methyl ester content. Generally, water-soluble and gel forming.
Mucilages	Galactose-mannose Glucose-mannose Arabinose-xylose Galacturonic acid-rhamnose	Galactose	Synthesized by plant secretory cells, prevent desiccation of seed endosperm. Food industry use, hydrophilic, stabilizer (e.g. guar).
Gums	Galactose Glucuronic acid-mannose Galacturonic acid-rhamnose	Xylose Fucose Galactose	Secreted at site of plant injury by specialized secretory cells. Food and pharmaceutical use (e.g. karaya gum)
Algal polysaccharides	Mannose Xylose Glucuronic acid Glucose	Galactose	Derived from algae and sea-weed. Vary in uronic acid content and presence sulphate groups. Food and pharmaceutical use (e.g. carrageenan, agar).
Lignin	Sinapyl alcohol Coniferyl alcohol p-Coumaryl alcohol	3-D structure	Noncarbohydrate cell wall component. Complex cross-linked phenyl propane polymer. Insoluble in 72% sulphuric acid. Resist microbial degradation.

Table 2 Major types of NSP in plant foods (Southgate, 1995)

Primary source	Major groups	Components present	Summary of structures	Distribution in foods
Structural materials of the plant cell wall	Cellulose		Long chain β-glucans	All cell walls
	Non-cellulosic polysaccharides	Pectic substances	Galacturonans	Mainly in fruits and vegetables
		Hemicelluloses	Arabinogalactans Arabinoxylans Glucurono-Arabinoxylans Glucuronoxylans Xylo-glucans β-glucans	 Cereals Cereals Fruits/vegetables Fruits/vegetables Cereals
Non-structural polysaccharides	Gums, mucilages		Wide range of heteropoly-saccharides	Seeds and fruits

2.3.2.2 Hemicelluloses

The term "hemicellulose" was coined by Schulze (1891), quoted by Choct (1991), who mistakenly thought that hemicellulose were precursors of cellulose. The term, thus, is not chemically accurate, but it is continuously used in food and feed industries today. Hemicelluloses are defined as those polysaccharides which are soluble in dilute alkali but insoluble in water (Southgate, 1993). The water-solubility of hemicelluloses is based on subsequent extraction with hot water at 85°C (Theander and Åman, 1979). The hemicelluloses comprise a series of heteroglycans, the largest group consisting of pentosans such as xylans and arabinoxylans; a second group consists of hexose polymers such as galactan (McPherson, 1985; Southgate *et al*., 1993). The acidic hemicelluloses containing galacturonic or glucuronic acids, form a third group of hemicelluloses. Hemicelluloses derived from pentose and hexose sugars may be intimately associated with cellulose (Anderson and Chen, 1979) through covalent linkage or hydrogen bonding (Stewart, 1986). The degree of bonding will determine hemicellulose solubility characteristics. The hemicelluloses are not digested in the small intestine but are hydrolyzed by microorganisms in the large intestine more readily than cellulose (Southgate *et al*., 1993).

Arabinoxylans and β-glucans are the main component of the hemicellulose fractions in cereal grains. The arabinoxylans generally consist of a main chain of β 1→4 D-xylopyranosyl with various substitutions and degrees of branching (Annison, 1993). Beside L-arabinose as a major branch component (Aspinall, 1970, Anderson and Chen, 1979), D-glucose, D-glucuronic acid and D-galactose may also be found either in side chains or as terminal units (Bailey, 1973). The level of arabinose indicates the solubility of arabinoxylans. The greater the levels of arabinose, the greater the solubility of arabinoxylans (Annison, 1993). The arabinoxylans or pentosans are found in cereals, with a particularly high level being present in rye (>10%) (Annison, 1993). The high amount of arabinoxylans in rye has been observed as responsible for its poor nutritive value to poultry (Antoniou *et al*., 1981).

The β-glucans with the composition of β 1→4 (70%) and β 1→3 (30%) are examples of hexosan hemicellulose fractions (Edney *et al*., 1991). The β 1-4 glucan alone is insoluble in water. The degree of β 1→3 linkages determines the solubility of β-glucans. The β-glucans

can become solubilized when the degree of β 1→3 linkages is higher than β1→4 linkages (Edney *et al*., 1991). The presence of the β 1→3 linkages introduces an irregularity in the chain which prevents them associating closely, thereby allowing water molecules to come between them (Annison, 1993). The presence of the β 1→3 linkages also reduces aggregation between β-glucan molecules. The β-glucans are found at high levels in barley and oats (2-10%) and at lower levels in other cereals (Fincher and Stone, 1986). Soluble β-glucans have been confirmed to cause deleterious effects in chickens fed barley diets (Campbell *et al*., 1989).

The xyloglucans are one example of hemicelluloses which are soluble in alkali. It is comprised of a β 1→4 D-glucan with α1→6 D-xylopyranosyl side chains which are occasionaly extended by β-galactopyranosyl or arabinosyl residues on substitution with the xylose (McNeil *et al*., 1984). The β 1→4 D-glucan main chain makes xyloglucans very similar in structure to cellulose; however, the presence of the side chains differentiates their properties (Selvendran and MacDougall, 1995). The xyloglucans have been found in a number of plants including soybean, mung beans, potatoes, runner beans and rice (Annison, 1993).

2.3.2.3 Pectic polysaccharides

Pectins are common to all cell walls as cementing substances in the middle lamellae (Kay and Strasberg, 1978). Annison (1993) described pectins as plant polysaccharides characterized by having α-(1→4)-D-galacturonic acid as a principal constituent with various amounts of α-(1→2)-L-rhamnosyl residues interspersed among the galacturonosyl residues. The short side branches of pectic substances consisted mainly of arabinose and galactose (Aspinall 1980). Since the short side branches are more easily hydrolysed than the main chain, commercial samples of pectins have usually lost the majority of these side chains (Aspinall 1970, Olson *et al*., 1987). The arabinogalactans, β-glucans and arabinoxylans are included in the pectin fractions (Southgate, 1993). Although pectins can be both insoluble and soluble, due to their branched nature most of the pectins are very water-soluble (Annison, 1993).

2.3.2.4 Gums and mucilages

Gums are the sticky materials that are extruded at the site of injury to plants (McPherson, 1985) or which are known as plant exudates and rarely occur in cereals (Annison, 1993). Gums are also derived as products from microbial biosynthesis. These gums are hydrophilic polymers consisting of monosaccharide units and derivatives, including neutral sugars, uronic acids and other acid groups which are linked glycosidically to form long chains (Klose and Glicksman 1972, McPherson, 1985). They also often contain at least a trace of protein (Annison, 1993). Gum arabic, which is characterised by containing a (1→3) and (1→6)-D-galactan as main chain with side chains containing arabinose, rhamnose and glucuronic acid (Aspinall, 1969), is one of the commercially known gums. Other commercial gums include mesquite gum, gum tragacanth, and karaya gum (Annison, 1993). Gum tragacanth has the ability to swell in water to give thick, viscous dispersions or pastes (Glickman, 1969). Increased level of gum tragacanth in poultry diets has been observed to cause a reduction in weight gain and feed-conversion efficiency which corresponded to increased digesta viscosity (Hartini, 1993). Inclusion of karaya gum, on the other hand, had only minor effects on broiler performance and digesta viscosity.

Mucilages, which are often called natural seed gums, are usually dispersed throughout the endosperm of dicotyledonous plant seeds, and their role is to protect seed endosperm from desiccation (McPherson, 1985). Mucilages are galactomannan polysaccharides. The proportion and distribution of galactose side chains vary between the gums and this affects both their physical and nutritional properties (Evans *et al*., 1992). Guar gum from the seed of *Cyanopsis tetragonolobus*, carob gum (locust bean gum) from *Ceratonia seliqua* and fenugreek gum from *Tigonella foenum-graecum* are classified as mucilages (Annison, 1993). The growth

inhibitory properties of guar gum when fed at a dietary level of 1% have been observed for broiler chickens (Hartini, 1993).

2.4 Physical properties of NSP

As plants mature, the matrix of the plant cell wall, which is comprised of cellulosic and non-cellulosic material, changes its composition percentage according to the plant stage. The structure of plant cell wall influences the physical and chemical properties of the individual NSP and these vary considerably between different polymers and different molecular weights of the same polymer (Choct, 1997).

Another factor that differentiates the physical properties among polysaccharides is the way the monomer units of polysaccharides are linked together (Morris, 1990). Different sugars often give polysaccharides with very similar physical properties if they are linked together in the same way. On the other hand, despite being built up from the same monomer units, polysaccharides can have different physical properties when the monomer units are linked together in different way. Cellulose and amylose, for example, are polysaccharides that are built up from the same monomer, glucose. However, since the monomer units are linked together in different ways, glucose in cellulose being linked through $\beta 1 \rightarrow 4$, while in amylose linked by $\alpha 1 \rightarrow 4$, cellulose and amylose have very different physical properties.

In addition, method of processing can also affect the physicochemical properties of dietary fibre (NSP) (Robertson and Eastwood, 1981). The physical properties of NSP most relevant to their physiological functions are their capacity to hold water and their ability to bind ions and polar molecules (Reiser, 1987; Oakenfull, 1993). Water-holding capacity of NSP has also been associated with the viscosity and solubility of various fibre sources (Schneeman 1986, Eastwood, 1992).

2.4.1 Water-holding capacity

Water-holding capacity has been defined as the "amount of water that can be taken up by unit weight of dry fibre to the point at which no free water remains" (Southgate *et al*., 1993). Since polysaccharides are hydrophilic molecules, both soluble and insoluble polysaccharides have the ability to hold water. However, the water-holding properties of cell-wall polysaccharides differ considerably depending on their type. Some are more hydrophilic than others (Kay and Strasberg, 1978). The formation of gels is the obvious proof of the ability of polysaccharides to hold water (Oakenfull, 1993). The capacity of fibre to hold water is determined by the physicochemical characteristics of the macromolecules and by the pH and electrolyte concentration of the surrounding medium (Eastwood and Mitchell, 1976). Coarse bran, for example, has greater ability to hold water than fine bran (Brodribb and Groves, 1978). Soluble fibre holds up to 10 times its own weight in water, whereas insoluble fibre 4-6 times its own weight in water (Kirwan *et al*., 1974, Bourquin *et al*., 1993).

2.4.2 Solubility

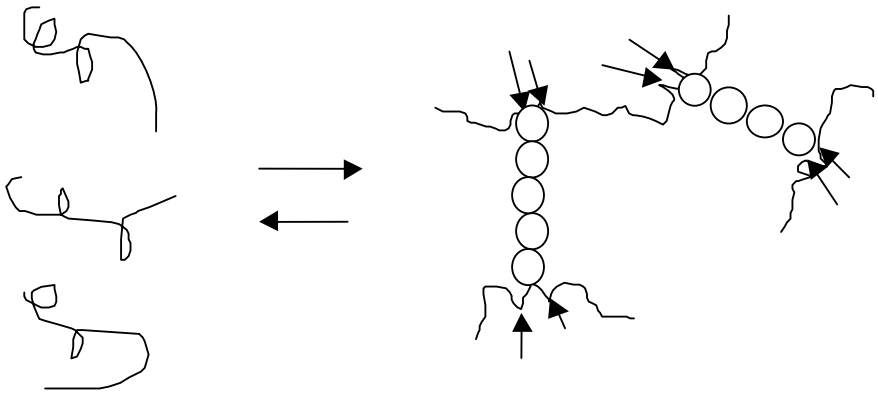
One characteristic that reflects the properties of NSP is their solubility. Solubility is the continuation process of 'swelling', the spreading of macromolecules due to the water coming into the polymer until they are fully extended and dispersed (Thibault *et al*., 1992). The macromolecules, then, are solubilised as random coils. Polysaccharides with irregularities in their backbone or branching will dissociate more easily than those with regular backbones such as cellulose (Morris, 1990; Thibault *et al*., 1992). Furthermore, the type of linkage between sugar residues also determines solubility. The more flexible the chain between sugar residues, the more soluble is the polysaccharide.

The polysaccharide charge is another important factor influencing solubility (Morris, 1990; Thibault *et al*., 1992). Many polysaccharides have charge groups which repel one another, so favouring solubilisation of the polymers. However, the presence of other solutes, such as salt or sugars, the ionic form of the charge and pH condition can alter the solubility of polysaccharides (Thibault *et al*., 1992). Pectic acids, for example, will not be soluble at acid pH. Finally, temperature is an important determinant of solubility. Temperature breaks the weak bonds and melts the ordered structure, so increasing the solubility of polysaccharides (Morris, 1990; Thibault *et al*., 1992). Soluble polysaccharides give rise to other properties such as gel formation and viscosity.

2.4.3 Gel formation

When water is entrapped, many polysaccharides swell to eventually form gels. Some polysaccharides can form gels rather than viscous solution, especially when the concentration is increased. Gels are an intermediate form between solid and liquid (Brosio, 1995). Polysaccharide gels are produced by cross-linking between adjacent molecules of a similar kind in a crystalline manner (junction zones) forming a continuous or interconnected network (Whistler and Daniel, 1985; Brosio, 1995) that holds water (Brosio, 1995) (Figure 1). The gel properties are the net result of the complex interactions between water, as a solvent, and the polymer network. The water maintains the integrity of the polymer network by influencing the nature and magnitude of the intermolecular forces; the polymer network holds the water, preventing it from flowing away (Brosio, 1995).

Figure 1 Schematic representation of the process of gel formation (Brosio, 1995)



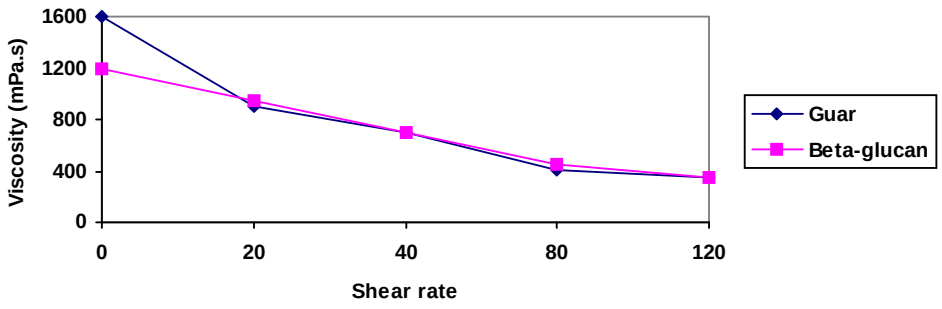
The junction zones are usually held together by weak and reversible intermolecular forces; hydrogen bonds, electrostatic forces, van der Waals forces and hydrophobic interactions (Brosio, 1995). These cross-linkages are not permanent but are free to continuously break and reform (Brosio, 1995). Although individually the intermolecular forces are weak, in gel formation many of the intermolecular forces operate cooperatively, forming stable cross-linkages, so making gels resistant to disruption. However, unlike viscous solutions, gels can be irreversibly disrupted by large shear forces (Read and Eastwood, 1992). The longer the segment in the junction zones usually the more stable and firm are the gels being formed (Whistler and Daniel, 1985).

2.4.4 Viscosity

Viscosity is related to the physical entangling of long-chains of polysaccharides as ribbons, which usually happens as their concentration increases (Morris, 1992). Most polysaccharides exist in solution in the form of 'random coils'. As the concentration of polymer solutions is increased, the individual coils interpenetrate one another to form an entangled network (Morris, 1990). Almost all water-soluble polysaccharides, such as guar gum and pectin, produce viscous solutions. The entanglement of polysaccharides, which generates viscosity, depends on the number of chains present, and the volume that individual coils occupy, which primarily depends on molecular weight (Morris, 1992). A strong relationship was found between digesta viscosity and the concentration of high molecular weight carbohydrates for chickens fed soluble NSP (Hartini, 1993; Bedford and Classen, 1992).

Viscosity can also be strongly dependent on the rate at which the liquid is stirred, known as 'the shear rate' (Morris, 1992; Oakenfull, 1993). At high shear rates, the apparent viscosity decreases since the rate of re-entanglement is slower than the rate of disentanglement. However, in dilute solutions, where the concentration is below the critical concentration or in other words 'below the onset of entanglement', viscosity is only slightly dependent on, or even independent of, the shear rate. Therefore, it is essential to quote the shear rate condition under which the viscosity is measured. Figure 2 shows variation of apparent viscosity with rate of shear for 1% of guar gum and barley β-glucans.

Figure 2 Variation of apparent viscosity with rate of shear for guar gum (1%) and barley β-glucans (1%).



Source: Oakenfull (1993).

Ingestion of viscous polysaccharides in chickens has been observed to reduce the rate of digesta passage (Salih *et al*., 1990), increase digestive tract microorganisms (Choct *et al*., 1996), and to have a negative impact on digestive enzyme activity (Hartini, 1993). Birds adapt to viscous digesta by increasing the pancreas and digestive organ size (Johnson and Gee, 1986, Hartini, 1993). Although the increase in gut viscosity is mostly related to soluble NSP, the possible degradation and solubilisation of insoluble NSP through acid digestion in the crop can also contribute greatly to the elevation of gut viscosity (Pettersson and Åman, 1989, Annison, 1993)

2.4.5 Binding to ions

Non-starch polysaccharides have been shown to have the ability to bind ions and other polar molecules. Reduced bioavailability of minerals after high dietary-fibre intake appeared to be due to binding of metal ions (Fernandez and Phillips, 1982; Schneeman, 1986). The major factor that determines the ability of polysaccharides to bind metal ions is the presence of free carboxyl groups and particularly the uronic acid content (Oakenfull, 1993). There was an

assumption that these ions are subsequently released as fibre is broken down in the colon. The finding that viscous soluble polysaccharides can also bind bile acids in the small intestine (Gallaher and Schneeman, 1986) is still questionable since both pectins and bile acids are negatively charged (Oakenfull and Sidhu, 1986).

2.5 Physiological functions of NSP

Physicochemical properties of individual NSP strongly influence their physiological activities and, as a result, not all NSP have the same physiological action (Southgate, 1995).

In the small intestine, physiological action of NSP is often associated with interference in absorption and metabolism of nutrients such as carbohydrates and lipids (Fernandez and Philips, 1982). In the large intestine, on the other hand, the physiological action is more associated with their ability to increase faecal bulk, accelerate transit time and increase the expulsion of gases (Oakenfull, 1993; Wisker *et al*., 1998). The latter is, furthermore, associated with enhanced microbial fermentation (Southgate, 1995). Figure 3 illustrates how the structural features of plant cell-wall material determine physiological effects.

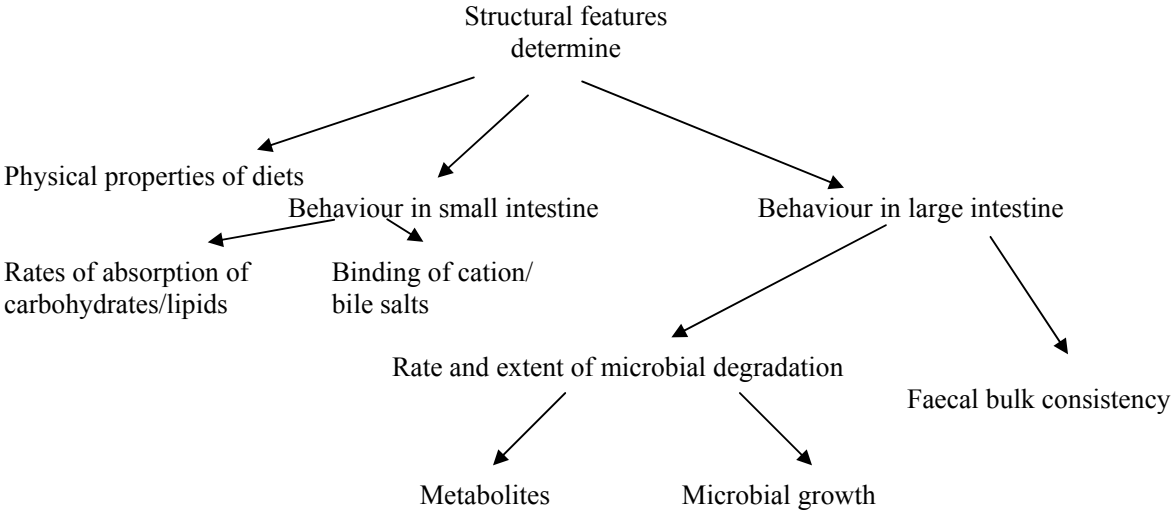


Figure 3 Illustration of how the structural features of plant cell wall materials determine physiological effects (Southgate, 1995).

2.5.1 Interfering in nutrient absorption and metabolism

The effect of soluble NSP on nutrient digestion and absorption is mediated via increased digesta viscosity (Lund *et al*., 1989, Choct and Annison, 1992). Nunes and Malmlof (1992) observed a decrease in glucose absorption and insulin production in pigs after guar gum ingestion. A similar reduction in absorption was also found for xylose and galactose (Jenkins *et al*., 1976; Lund *et al*., 1989).

Bakker *et al*. (1998) found that NSP negatively affected the apparent digestion of protein, fat and some minerals in pigs. Depression of starch, protein and fatty acids digestibilities due to addition of wheat pentosans has also been observed in intact and cecectomized broiler chickens (Choct *et al*., 1992). Feeding guar gum or konjac mannan also delayed absorption of both cholesterol and triglyceride from the small intestine of rats (Ebihara and Schneeman, 1989). Ingestion of NSP inhibited nutrient digestion as shown by the retarded growth of broiler

chickens fed wheat pentosan (Choct and Annison, 1992), guar gum and gum tragacanth (Hartini, 1993).

The mechanism by which NSP reduces nutrient absorption is very complex. Johnson and Gee (1981) have proposed that ingestion of viscous NSP will reduce the rate of digesta passage, resulting in a thickened intestinal unstirred water layer, consequently decreasing the diffusion rate of the available nutrients to the absorptive mucosal surface of the small intestine (Olson *et al*., 1987). Leeds (1979) and Schwartz *et al*. (1982), on the other hand, suggested that reduction in nutrient absorption was due to nutrients and digestive enzymes being trapped in gel matrix of viscous polysaccharides. This condition impairs the mixing of substrates with digestive enzymes in the small intestine. Trapping of bile acids has been suggested to reduce the recycling of bile via the enterohepatic circulation, so increasing the degree of excretion in the faeces (Ebihara and Schneeman, 1989). Read and Eastwood (1992), however, pointed out that impairing the mixing of substrate and increasing the intestinal water layer are two ways of describing the same thing. According to them, the concepts might be differed if viscous polysaccharides causing increase in intestinal water layer impaired the absorption of substrates that did not contain viscous polysaccharides.

A delay in gastric emptying is another possible factor that reduces nutrient absorption, especially carbohydrate (Wilmshurt and Crawley, 1980). After ingestion of meals, the stomach has a function to store and homogenize the meals, and then deliver the chyme at a steady rate into the small intestine for digestion and absorption. In the case of NSP ingestion, the water-holding capacity of NSP bind the fluid contents of the stomach, therefore causing slower delivery of nutrient to the absorptive site in the small intestine, and the result is the limitation of nutrient digestion and absorption. While insoluble fibres have been known to increase the speed of gastric emptying, soluble fibres reduce it (Delargy *et al*., 1995).

Reduction in gastric-emptying rate has been correlated with the feeling of fullness or satisfaction which is a signal to stop eating (Read, 1992). Zorrilla (1998), in addition, described satiety as "those processes that determine the length of time between meals". Distension of the stomach is believed to be one important signal to stop eating. Slowing gastric emptying will increase the duration of gastric distension which as a consequence extends the feeling of satiety, delaying the appearance of hunger. A correlation has been observed between echographic gastric emptying and sensations of satiety and hunger (Bergmann *et al*., 1992). However, Read (1992) suggested that “a true feeling of satiety” also requires the presence of nutrients in the small intestine.

2.5.2 Increasing faecal bulk consistency

Faecal bulk is the best-known physiological effect of dietary fibre in the large intestine. The ability of NSP to increase faecal bulk depends primarily on the source of fibre (Stephen and Cummings, 1980) and physicochemical properties of individual NSP (Oakenfull, 1993). In addition, it depends on the type and number of microorganisms in the large intestine (Cummings, 1984; Oakenfull, 1993) and transit time (Cummings, 1984). According to Stephen and Cummings (1980), fibres that resist breakdown by large intestinal microorganisms have a higher faecal-bulking capacity than more easily degraded types of fibre. Xanthan gum, carboxymethylcellulose and ispaghula are examples of fibres that have a great effect on faecal bulking. The highly lignified fibres such as wheat bran also appear to have a great effect on faecal bulking (Read and Eastwood, 1992). Lignin was assumed to protect fibre sources from extensive microbial degradation (Selvendran, 1984) and hence increase faecal output.

Increasing faecal weight is commonly related to the water-holding capacity of the NSP. However, the important water-holding capacity of NSP related to increased faecal weight is after exposure to large intestinal microorganisms (McBurney *et al*., 1985). Many NSP are

rapidly degraded by microorganisms to small molecular weight units that have no ability to retain water (Tomlin and Read, 1988b).

Another physical property of fibres that affects faecal bulking is solubility. This particular property relates to the fermentative processing of fibres in the colon. Insoluble NSP are only slightly fermented, therefore insoluble NSP serve almost entirely as bulking agents and shorten transit time (Roberfroid, 1993). On the other hand, soluble NSP are almost completely fermented by anaerobic microorganisms in the proximal colon in man. Therefore, soluble NSP seems to fall into a neutral category with regard to faecal bulk. However, some fermentable fibres do increase faecal bulk (Stephen and Cummings, 1980). This effect is due to fermentation of soluble NSP by caeco-colonic microbial flora which resulted in an increase of microbial biomass (Roberfroid, 1993; Choct *et al*., 1996). The microbial biomass, then, causes an increase in faecal weight. Tomlin and Read (1988a) observed that this effect is more likely accompanied by the viscous property of the NSP. Viscosity reduced the transit time through the gut (Salih *et al*., 1990) and consequently gave more time for gut-microorganisms to proliferate. Tomlin and Read (1988a) observed a smaller increase in faecal mass when the viscosity of the appropriate cultures was removed than when it was maintained or reduced.

A correlation between chemical structure of fibre and faecal output has also been found. Stephen *et al.* (1986) observed that faecal carbohydrate excretion increased with the addition of bran which was mainly due to increased amounts of cellulose and pentosan (arabinose + xylose). However, the mechanism is not known.

The physical form of NSP is another important factor that influences faecal bulk. Coarsely ground wheat bran is more effective in increasing faecal bulk than finely ground wheat bran (Kirwan *et al*., 1974). Large particles may be degraded more slowly, and therefore are more likely to survive on their way through the large intestine. These large particles may act by themselves and through their water-holding capacity (Stephen and Cummings, 1980). The effect of fibre on faecal weight, moreover, is dose-dependent (Stephen *et al*., 1986). Approximately 1g NSP increased faecal weight by 5 g/d. Table 3 shows the effect of fibre supplements on faecal bulk.

Table 3 Effect of fibre supplementation on faecal bulk (Schneeman, 1986)

Fibre supplement	% increase in faecal wet weight
Oat bran	15
Pectin	16-35
Guar gum	20
Cellulose	75
Wheat bran, coarse	80-127
Wheat bran, fine	24

2.5.3 Accelerating transit time or laxative action

Transit time and faecal weight were closely related (Stephen *et al*., 1986). It is known that products high in insoluble NSP such as wheat bran and rice bran appear to be the best laxative agents through increased faecal bulking (Topping and Pant, 1993). Coarse particle bran, moreover, which is effective in increasing faecal weight, is also well known as having a greater laxative action (Smith *et al*., 1981). The mechanism proposed is that the edge of large particles stimulates mucosal mechano-receptors in the colon, which then induces its secretion and propulsive motor activity (Tomlin and Read, 1988b).

However, Tomlin and Read (1988a) observed that supplementation of diets with NSP often stimulates laxative action without affecting faecal weight or increases faecal weight without affecting transit time. They, furthermore, suggested that the best laxative is fibre that undergoes some fermentation but retains sufficient of its complex structure to hold water and bulk the faeces. Short chain volatile fatty acids (VFA) produced by fermentation might additionally influence some laxative effect (Topping, 1991; Eastwood and Morris, 1992).

2.5.4 Microbial degradation

NSP are mainly fermented by microorganisms in the large intestine (Bakker et al., 1998) and caeca (van Barneveld, 1996). Feeding readily fermentable NSP to monogastrics increased proliferation of microorganisms in the digestive tract (Ratcliffe, 1991, Choct, 1997). This was by either providing fermentable materials (Ratcliffe, 1991, Choct, 1997), or changing the environment in which they live, for example by increasing viscosity (Hartini, 1993).

It has been observed that fermentation of NSP by anaerobic microorganisms in the large intestine yielded short chain VFA and various gases, such as hydrogen, methane, and carbon dioxide (Stephen and Cummings, 1980; Roberfroid, 1993; Nordgaard et al., 1995). Both microorganisms and host obtain clear benefits from the association. Short chain VFA, formed from microbial NSP breakdown, can provide energy not only for microorganisms to proliferate but also for the body as a whole (McNeil *et al*., 1984). Cummings and Macfarlane (1997), furthermore, indicated that 40-50% of the available energy of the carbohydrate in humans is represented by VFA. Butyrate is a principal source of energy for epithelial cells in the distal part of the small intestine (Macfarlane and Macfarlane, 1997). Propionate is metabolized by the liver, whereas acetate is metabolized by the muscle. Intestinal microorganisms also have a role in the synthesis of vitamins B and K and the metabolism of bile acids, other sterols and xenobiotics (Cummings and Macfarlane, 1997).

However, fermentation of NSP also has a negative effect. Large amounts of fermented NSP, for example, increased the empty weight of the hindgut. Since the intestinal tract is metabolically very active, the energy required for maintenance will be increased, leaving less energy for growth (Macfarlane and Macfarlane, 1997). Chicks fed diets rich in soluble NSP such as rye and pectin have been found to increase the number of microorganisms adhering to the lower part of the intestine (MacAuliffe and McGinnis, 1971; Untawale and McGinnis, 1979). It has been postulated that increased amounts of soluble NSP such as rye in the diets leads to the development of an undesirable microflora in chicken intestinal tracts (MacAuliffe and McGinnis, 1971). Choct *et al* (1992) indicated that increased activity of hindgut microorganisms is partially responsible for anti-nutritive effects of wheat pentosans in poultry.

2.5.4.1 Factors influencing development of gut microflora

According to Savage (1987), development of microorganisms colonizing the intestinal tract is believed to come from nutrition or environment factors which the author called 'allogenic factors'. In addition, it is also influenced by the native inhabitants or indigenous microorganisms which the author referred to as 'autogenic factors'. Included in allogenic nutritional factors are the nutrition coming from the host's diets/drugs and animal tissue. Abandoning the allogenic nutritional substrates opens a possibility for intestinal organisms to utilize a wide range of compounds as their carbon and energy sources (Allison *et al*., 1979; Varel *et al*., 1987). However, since many microorganisms still depend on their host's diet as their main source of nutrients, among the allogenic nutritional factors, the host's diet seems to be the most powerful factor, especially in regulating the type of microbial species that can be found and biochemical functions that takes place in the gastrointestinal (GI) communities, although the effect is species specific and depends on the communities located in the GI tract.

As Macfarlane and Macfarlane (1997) stated, the amounts and types of fermentation products formed by microorganisms depend on the relative amounts of each substrate available, the chemical structures and composition, in addition to the fermentation strategies (biochemical characteristics and catabolite regulatory mechanisms) and types of microorganisms participating in 'depolymerization' and fermentation of the substrates.

Some environmental factors such as temperature, pH, intestinal motility, and oxidation-reduction potential are also believed to affect development of GI tract microorganisms (Table 4).

Table 4 Some allogenic environment factors believed to influence indigenous microorganisms in the gastrointestinal tract (Source: Savage, 1987)

Factor	Area in tract	Parameter
Temperature	All	Body temperature (about 37^0C)
PH	Stomach, small intestine	Acidic, Neutral to alkaline
	Large intestine	Neutral to alkaline
Peristalsis	Stomach	Periodic; lumen may empty
	Small intestine	Periodic; rapid in upper areas, sluggish in distal areas
	Large intestine	Periodic, sluggish
Villous contraction	Small intestine	Adds to movement due to peristalsis
Oxygen	Stomach	From food, ingested air, blood (by diffusion?)
	Small intestine	From blood (by diffusion?)
	Large intestine	From blood (by diffusion?)
Oxidation-reduction potential[a]	Large intestine	Low, especially when microbiota present
Bile acids[a]	Small intestine	Detergent effects; bind to cells
	Large intestine	Detergent effects; bind to cells
Epithelial turnover[a]	All areas	Sloughing of cells necessitates replacement of adherent microbial cells
Mucous gel[a]	All areas	Layer of hydrated gel on all columnar epithelia
Host's diet[a]	All areas	Provides lumenal habitats
Host's drugs (e.g.antimicrobial)[a]	All areas	Influence characteristic of drug
Phagocytic cells	Crypts of Leiberkuhn, Peyer's patch epithelium	Destructive of microbial cells
Antibodies[a]	All areas	Precise effects in tract not known

[a]Factor known to be altered by microbial activities

There are numerous autogenic factors which are believed to affect conditions of microorganisms in GI habitats including the microorganism products, the nutrition of individual microbial strains, and the capacity of microorganisms to associate with the epithelial surface (Table 5).

Concentrations of certain sulfide acids produced in the large intestine by certain microorganisms, for example, have been known as important in limiting growth and even in killing transient microorganisms that enter large intestine habitats (Freter, 1983). VFA were

also reported as controlling transient population but some investigators disagreed with this finding (Freter, 1983).

Competition for nutrients, derived from their host or other ingesta, between the host cells and microbial cells, or between microbial cells and microbial cells, can influence the populations of microbial species that colonize intestinal habitats in birds (Freter, 1983). Furthermore, the capacity of microorganisms to associate with the epithelial surface, either by adhering to it or by forming a community in a mucous gel overlying the surface, is another important measure of the autogenic force, especially in responding to the environmental circumstances such as villous and peristaltic motility (Savage, 1984). The force was reported to control not only the composition of epithelial communities but also that of lumenal communities (Savage, 1987).

Table 5 Some autogenic factors that are believed to influence indigenous microorganisms in gastrointestinal tract (Source: Savage, 1987)

Factor	Area in tract	Parameter
Lactic acid	Stomach	Lowers pH (especially in neonates)
	Large intestine	Carbon and energy sources
Volatile fatty acids	Potentially all areas, but especially rumen and large intestine	Carbon and energy sources; growth inhibitors at low oxidation-reduction potentials
Hydrogen sulfide	Large intestine	Growth inhibitor for some microorganisms
Bacteriocins	All areas	Effects are unknown; considered potential only
Nutritional competition	All areas	Competition for nutrients of microbial cells with animal cells or microbial cells with each other
Nutritional synergy	Large intestine (rumen)	Synergy among microbial cells
Association with epithelial or particulate surfaces	All areas	Microbial cells can colonize areas where peristalsis or villous contraction moves contents more rapidly than microbial cells can multiply; provides reservoir of inoculants for digesta

The fact that the growth rate of conventional chicks was poorer than the germ-free chicks suggested the use of energy by gut microflora (Muramatsu *et al*., 1994). Inclusion of arabinoxylan, the known viscous agent in wheat, caused poorer gain per MJ intake which also suggested the energy cost of the gut microflora (Choct *et al*., 1995).

The viscous condition in the intestine can affect the rate of digestion, which can markedly influence the partitioning of energy between the birds and the microorganisms. The tendency of reducing intestinal viscosity as birds get older is offset by the fact that the older the birds the greater the microorganisms colonized in the gut (Barnes *et al*., 1972). Therefore, the slower was the rate of host digestion, the greater the opportunity of ingested nutrients to be metabolized by microorganisms.

The fermentation of substrates by microorganisms resulted in production of VFA (acetate, propionate and butyrate) and other metabolic products such as hydrogen, methane, and carbon dioxide (Stephen and Cummings, 1980; Roberfroid, 1993; Nordgaard *et al*., 1995). These fermentation products may affect directly or indirectly the digestive system. The pH reduction due to increased production of VFA (Savage, 1987), for example, has caused reduction in the production of 7 α-dehydroxylation of bile acids. As a result, the conversion of primary bile acids to secondary bile acids is also reduced (Aries and Hill, 1970). Secondary bile acids have been implicated as carcinogens. On the other hand, decreasing pH was also reported to cause a decrease in pancreatic secretion (Garcia *et al*., 1990).

Short-chain VFA production has also been reported as the signal for increased mucosal growth (Furuse *et al*., 1991). Increasing microbial colonization due to large intestinal obstruction will enhance the concentration of polyamines. Production of polyamines, a putatively toxic metabolite (Macfarlane and Macfarlane, 1997) by lumenal microorganisms increases the intestinal mucosal growth rate (Osborne and Seidel, 1989). An increase in gizzard size was found due to an ingestion of high levels of dietary fibre (Schneideler *et al*., 1998). A change in gut morphology was observed due to ingestion of viscous polysaccharides (Jaroni *et al*., 1999). Johnson and Gee (1986) found an increase in caecal weight due to an ingestion of soluble NSP. For birds that can cope well, the effect of stimulating intestinal growth unnecessarily would only reduce feed efficiency (Bedford, 1996).

Clinical or subclinical infections can affect the efficiency of host digestion. The GI tract ecosystem is open to the environment of the host; consequently transient microorganisms can enter some parts of the GI tract. Although transient microorganisms usually just pass through the tract, under certain conditions they may colonize and persist for some time in a habitat. Some microbial strains, either indigenous or transient, were able to cause enteric disease (Savage, 1987). Disease will lower the immune response of birds, leading to increased maintenance cost and subsequently reduced feed efficiency (Bedford, 1996).

2.6 The role of NSP in prevention of cannibalism

There is only limited documentation available on the role of NSP directly involved in the prevention of cannibalism. Nutritional studies on feather pecking and cannibalism, in fact, was carried out more than 60 years ago. Inclusion of oats was found to reduce the occurrence of cannibalism (Bearse *et al*., 1940) in layers. However, the mechanism was not determined. A similar study was carried out using pheasants by Scott *et al* (1954). They demonstrated that rations containing a high level of ground or pulverized oats tended to decrease the incidence and severity of feather pecking under fully lighted conditions and produced excellent results when direct sunlight was excluded from the brooder house. However, the mechanism, again, was not determined. No other studies had been done on the role of fibre in feather pecking and cannibalism, until a few years ago (Esmail, 1997; Wahlström *et al.*1998). Esmail (1997) reported that substituting corn with oat mill reduced the incidence of cannibalism. Wahlström *et al* (1998) also found that the incidence of cannibalism was lower when feeding an oat-based diet. Wahlström *et al* (1998) suggested that the severe outbreak of cannibalism and feather pecking in their experiment was probably due to sodium deficiency. It was proposed that inclusion of oat hulls might increase the retention of sodium and potassium (Esmail, 1997).

Fibre consists of soluble and insoluble portions which exert markedly different effects on nutrient utilization and gut physiology in animals. In monogastrics, ingestion of soluble NSP was known to have a negative impact on bird performance. With their ability to increase digesta viscosity in the gut, ingestion of soluble NSP is believed to affect nutrient metabolism either by impairing the mixing of substrate with digestive enzymes or by preventing diffusion of nutrients to the absorptive mucosal surface of the gut (Olson *et al*., 1987). The increase in gut viscosity was also reported to slow down the digesta transit rate (Salih *et al*., 1990), which in

turn may create the feeling of fullness to the bird (Read, 1992). The further effect of increased gut viscosity is the proliferation of fermentative microorganism in the small intestine, which changes the gut ecosystem in a detrimental manner (Choct *et al*., 1996).

Insoluble NSP, on the other hand, have little or no effect on nutrient utilisation in monogastric animals. In monogastrics, inclusion of insoluble NSP serves almost entirely as bulking agents and to shorten transit time (Roberfroid, 1993). Wheat bran was reported to have a profound effect on faecal bulking (Selvendran, 1984) and on maintaining normal motility of the gut in humans (Stephen and Cumming 1979). These effects are commonly related to their ability to absorb a large amount of water (Stephen and Cumming 1979).

There was no such study reported on the properties of soluble or insoluble NSP in chickens with respect to the occurrence of feather pecking and cannibalism. It was evident from the results obtained by previous authors (Bearse *et al*., 1940; Scott *et al*., 1954; Esmail, 1997; and Wahlström *et al*., 1998) that inclusion of fibre had an effect on feather pecking and cannibalism. The problem is all the conclusions drawn from previous research seem based on 'the crude fibre level' of the diet which represents only a small portion of the 'fibre or NSP' content of the diet. Diet composition can affect the concentration of soluble and insoluble fibre of a diet. The properties of soluble or insoluble NSP can be minimized by the presence of other cell wall components of grains in the gut (Choct 1997). Based on the properties of soluble and insoluble NSP, there were two hypotheses that could be drawn with regard to the occurrence of feather pecking and cannibalism on laying hens fed dietary fibre. First, diets high in insoluble NSP, such as oat hulls, will speed up the digesta transit time as oat hulls serves as a bulking agent. As a result birds will feel hungry quicker and therefore will spend more time eating and less pecking. Second, diets high in soluble NSP will cause birds to feel full, the lag time between feeding bouts can trigger the birds to do other activities such as pecking which may lead to cannibalism. To prove these hypotheses, a series of experiments using different dietary fibre sources was designed. Several diet types were applied in the experiment to observe whether or not diet composition affects the concentration of soluble and insoluble NSP in a diet, which would lead to different cannibalism and behaviour outcomes.

3. General Materials and Methods

3.1 Rearing trial

Trials were conducted at the pullet-rearing shed at Kirby Research Station, University of New England, Armidale, NSW, Australia.

The shed was divided into two compartments. One compartment had dark curtains and the other had conventional curtains. All compartments had dimmable lights with timers.

For the first laying-year experiment, each compartment was divided into two pens using wire mesh. Day-old commercial ISA Brown chicks, half of which were beak-trimmed, were obtained from a local hatchery (Baiada hatchery in Kootingal, NSW). The birds were divided into 4 groups and allocated to a 2 x 2 factorial array design, i.e. beak-trimmed and untrimmed under bright and dim light intensity.

For the second laying-year experiment, day-old commercial ISA Brown chicks were obtained that were not beak-trimmed. The birds were only divided into two groups and reared under bright or dim light.

For dim light rearing, the light intensity was set out at 5 lux and for bright rearing, the light intensity was set out at 60-80 lux. The lighting program was applied according to the Management Guidelines of ISA Brown Commercial Layers. The shed had automatic feeders and drinkers. The birds were held in this research station for 15 weeks.

Commercial vaccinations were given at day old in the hatchery and thereafter (Fowl Pox, IB, ILT, EDS and AE) on farm. Beak trimming (when applied) was done at 10 weeks of age.

During the rearing period, birds were fed commercial starter mash (Ridley Agriproducts). Feed and water were given *ad libitum* through automatic feeders and drinkers.

3.2 Pre-lay and lay trial

A conventional layer house at Laureldale Research Station, University of New England, Armidale, NSW, Australia was used for experiments using birds more than 15 weeks of age.

At 15 weeks all birds from Kirby Research Station were transferred to 5-bird cages (24”width x 24”depth x 16”height) in a conventional layer house at Laureldale Research Station (7km distance). There was a total of 576 cages in 4 paired-rows, giving a total of 8 rows. Two paired rows, containing 76 cages per row, were situated on either side of the layer house. The remaining 2 paired-rows, containing 68 cages per row, were located centrally. Feed troughs were placed outside the cages and an automatic drinker was located inside the cage at the top back.

For the first laying year experiment, the birds from each group, i.e. beak-trimmed and untrimmed birds under bright or dim rearing were distributed randomly and evenly across the house. Thus, each row contained 4 groups of birds, i.e. beak-trimmed and untrimmed birds under bright and dim light, respectively.

For the second laying year experiment, each row only contained 2 groups of birds from bright and dim rearing.

In the laying shed birds were held under a natural light regime supplemented by artificial light (>80 lux) to a total of 16h/day. The birds were allowed to adjust to the new environment for 2 weeks. During this period they were fed a standard grower diet (Ridley Agriproducts) and at 16 weeks of age behavioural observations were made to record the effect of rearing conditions on behavioural patterns.

Experimental diets, formulated according to commercial specifications and produced at a commercial mill (Ridley AgriProducts, Tamworth, NSW) were given at 17 weeks of age. However, due to the unavailability of certain ingredients, in the second year layer trial, experimental feeds were given at 20 weeks of age. The overall layout of housing conditions and experimental diets given in the first and second year experiments can be seen in Appendices 1 and 2, respectively. Feed and water were given *ad libitum*. Birds that died during the experiment were post-mortemed and the cause of death was established.

3.3 Behavioural observations

Behaviours and feather score of sub groups of birds were systematically recorded in some of the experiments. Behavioural observations were applied on birds in the cage and outside the cage (an open-field test). In addition to those observations, a colour-object pecking test was also performed to observe bird preference to certain colour.

The behavioural observations for birds in the cage included social pecking, feeding, moving, preening and drinking. Escape or 'freeze' behaviour in birds being pecked was also recorded.
Description of behavioural observations observed for birds in the cage:

- Social pecking: pecks directed to other birds. Every peck was counted as one occurrence.
- Feeding: every peck made into the feed trough.
- Moving: the birds walk around in the cage.
- Preening: the birds stroke their back or their wings with their beak in either sitting or standing positions.
- Drinking: birds peck into the drinker's nipple.
- Escape: birds run to avoid being peck by other birds.
- Freezing: birds were frightened to move.

An open-field/latency test (behavioural observation applied outside the cage)

An open-field/latency test was used to determine the degree of fearfulness of birds (Jones *et al*., 1995, Lindberg and Nicol, 1996). A bird was captured and placed in the corner of a square open field measuring 2x2m. The behaviour pattern measured was the latency to the first step (duration of freezing), indicating that birds have no more fear. The threshold observation time was set 300 seconds per bird.

A colour-object pecking test

The test is performed to identify the preference of birds to certain colours based on the statement that a red colour easily attracted birds to peck (Savory, 1995). Three different colours of rice grains (dyed brown, red, and natural white) which were glued to a board were used as pecking objects. The board was placed over the feed trough of each cage for 10 min and the number of pecks at each colour grain was recorded.

Feather score

Feather score test was carried out using the Tauson's scale (Tauson *et al*., 1984). A bird with perfect plumage in the neck, breast, back, wing and tail area had a maximum of 20 points, whereas a bird with very bad plumage had a minimum of 5 (five) points.

3.4 Variables measured

3.4.1 Gastrointestinal weight

At the end of the trial, 2 birds per treatment were weighed and killed by cervical dislocation. After the bird was killed, the digestive tract, including the gizzard, was removed immediately and then cut into segments, viz gizzard, crop, jejunum, ileum, and caecum. The gut sections were weighed prior to and after removal of digesta. The empty section weights were expressed as a percentage of body weight (%BW). The digesta from gizzard, crop, jejunum, ileum and caeca were individually stored to measure the variables below.

3.4.2 Volatile fatty acids (vfa)

3.4.2.1 Caecal VFA determination

Three gram of samples were diluted with 3mL of 0.1M H_2SO_4 and thoroughly mixed. The sample was centrifuged (12,000 *g*, 15min). To an aliquot of 1mL supernatant, 0.1mL of a reference volatile fatty acid (caproic acid) was added. The volatile fatty acids were distilled using Thundberg tubes. The concentration of VFA was quantified using a Hewlett Packard 427 GLC (Gas Liquid Chromatograph).

3.4.2.2 Ileal VFA determination

Ileal (from Meckel's diverticulum to ileocecocolic junction) digesta was collected and stored on ice before VFA determination. Two grams of fresh digesta were centrifuged (12,000 *g* , 15min). To an aliquot of 1mL supernatant, 0.1mL of a reference volatile fatty acid (caproic acid) and 0.1mL of 0.5 M H_2SO_4 was added. The VFA was distilled using Thundberg tubes. The distillate was treated with 2 drops of 10 M NaOH and concentrated to dryness in a vacuum desiccator. The VFA salts were dissolved in 0.1mL of 0.5M H_2SO_4 and 0.05mL isocaproic acid and determined using a Hewlett Packard 427 GLC.

3.4.3 Viscosity

Jejunal (end of duodenum to Meckel's diverticulum) and ileal (from Meckel's diverticulum to ileocecocolic junction) digesta were individually collected and stirred. Approximately 2g (wet weight) of the digesta were centrifuged at 12,000 *g* for 15min. The supernatant was withdrawn and viscosity (in centipoise, cps=1/100 dyne sec x cm^{-2}) was determined using a Brookfield DVIII viscometer maintained at 25^{o}C with a CP40 cone and a shear rate of 5-500 s^{-1}.

3.4.4 Non-starch polysaccharides

3.4.4.1 Sample preparation

Samples either from diets, jejunum, ileum or faeces were freeze-dried and ground through a 0.5mm screen before NSP analyses. Approximately 200 mg of sample was placed in a screw-capped culture tube and 10mL of hexane added. The mixture was sonicated for 15min and was then centrifuged (2,000 *g* at 20^{o}C, 15min) to remove fat. The residue was extracted with 5mL of 80% ethanol to remove free sugars and oligosaccharides. After centrifuging, the supernatant

was collected for free sugars determination as described later in this chapter. The insoluble residue was dried to a slurry using nitrogen before incubating at 100°C for 30min to gelatinase starch. Starch was removed enzymatically by incubation in 10mL of 0.1M acetate buffer (pH5.0) with 50μL of α-amylase (E.C.3.2.1.1. heat-stable amylase, Sigma Aldrich) at 95°C for 30min. After equilibration to 55°C, 50μL of amyloglucosidase was added. After incubation for 16h, the mixture was centrifuged at 2,000 *g* for 30min. An aliquot of supernatant was withdrawn for soluble NSP measurement and residue was kept for insoluble NSP determination. Hydrolysis, reduction and acetylation of the samples were carried out based on the Uppsala method (Theander and Westerlund, 1993).

3.4.4.2 Soluble NSP determination

To an aliquot of 4mL supernatant, 16mL of absolute ethanol was added. The mixture was centrifuged at 2,000 *g* for 20min. The residue was precipitated using 80% ethanol and centrifuged at 2,000 *g* for another 20min. The first step was repeated once more. The precipitate was then dried under nitrogen stream at 40°C and 1mL of 2M Trifloroacetic acid added. The mixture was hydrolysed at 125°C for 1h. After cooling, precisely 50μL of internal standards (allose, 4 mg/mL) was added. The mixture was evaporated to dryness using nitrogen at 40°C. The dry residue was recovered in 0.2mL of distilled water and the monosaccharides were reduced by treatment with 0.3mL $NaBH_4$ (50mg sodium per mL 3M NH_4OH) at 40°C for 1h after making the mixture slightly alkaline with a drop of 3M NH_4OH. Excess amounts of $NaBH_4$ were decomposed with glacial acetic acid. The alditol acetate derivates were acetylated by addition of acetic anhydride (5mL) in the presence of 0.5mL 1-methylimidazole. The excess amount of acetic anhydride was decomposed by adding 8mL of distilled water, and the alditols were extracted with 3mL of dichloromethane. The clear layer was transferred into a vial and was dried using nitrogen. To the dried residue was added 1mL of ethyl acetate and 1mL of distilled water and the mixture centrifuged (2,900 *g*, 5min). The volatile alditol derivatives of monosaccharides were analysed using a Varian 3400 gas chromatograph equipped with a Varian series 8200 auto-sampler, a capillary column (BPX70, 25 m, 0.32mm, SGE International, Australia), and a flame ionisation detector (FID) set at 280°C. During analysis, the column was held at 195°C for 1min and then raised by 5°C/min to 225°C and held for 4min. The levels of polysaccharides were calculated from the levels of the component sugars using a polymerisation factor of 0.88 for pentoses (ribose, xylose and arabinose), 0.9 for hexoses (mannose, galactose and glucose), 0.89 for deoxysugars (fucose and ribose) and 0.91 for rhamnose (Theander and Westerlund, 1993).

3.4.4.3 Insoluble NSP determination

The insoluble residue was washed with distilled water (5mL), centrifuged (2800 rpm, 15min) and the supernatant was discarded. The washing step was repeated to make sure that the glucose released from starch digestion was completely removed. Acetone (2mL) was added and the mixture was centrifuged at 2,800 *g* for 15min. The residue was dried under nitrogen. The mixture was first hydrolised by adding precisely 1mL of 12M H_2SO_4 at 35°C for 1h followed by distilled water (11mL) at 100°C for 2h. After cooling, the mixture was centrifuged at 2,800 *g* for 15min to sediment insoluble materials. To an aliquot of 0.8mL supernatant, 0.20mL of 28% ammonium and precisely 50 uL of allose (4mg/mL) were added. The mixture was dried in a vacuum rotary evaporator (16h at 40°C). Reduction and acetylation were carried out using the same procedure as described earlier in the soluble NSP determination.

3.4.4.4 Free sugars determination

The supernatant was evaporated under nitrogen and hydrolysed with 3mL of 1M H_2SO_4 for 2h at 100°C. After cooling, the hydrolysate was centrifuged at 2,800 *g* for 15min to sediment insoluble materials. To an aliquot of 0.4mL supernatant, 0.10mL of 28% ammonium and precisely 50uL of allose (4mg/mL) were added. The mixture was dried using nitrogen at 40°C before going into the reduction and acetylation procedure as described earlier in this chapter.

3.4.5 Starch

Starch contents were determined using Megazyme total starch kit (AA/AMG 9/97) based on the method developed by McCleary *et al*. (1994). This method is recognised by the AOAC (AOAC method 996.11) and the AACC (AACC method 76.13). Samples were finely ground through 0.5 mm screen and were weighed accurately 100 mg. Weighed samples were placed in a glass tube and wetted with 0.2mL of aqueous ethanol (80% v/v). 3mL of thermostable α-amylase in MOPS buffer (sodium salt, Sigma M9381; 50mM, pH 7.0) was immediately added and the samples were mixed well and incubated in a boiling water bath for 6min. The tube was placed in a water bath at 55°C. Sodium acetate buffer (4mL, 200mM, pH 4.5) and amyloglucosidase (0.1mL, 20 U) were added and the mixture was incubated for 30min. Glucose was determined colorimetrically after incubating an aliquot (0.1mL) with 3mL of GOPOD reagent (Megazyme) at 50°C for 20min and reading the absorbance at 510nm against reagent blank (glucose oxidase assay).

3.4.6 Alkane

Samples were oven- or freeze-dried and ground through a 0.5mm screen before alkane analyses. Approximately 200 to 400mg of sample were placed in a 30mL culture tube and 50μL of internal standard (1mg $C_{34}H_{70}$ per mL dodecane; kept at 30°C warm water to prevent crystallising), 4mL absolute ethanol and 200μL of 7.5M KOH added. The mixture was vortexed and placed in a heating block at 90°C for 1h with stirring. After cooling, 3mL n-heptane was added. The tube was placed in the warm water and 2mL of warm water were added. After centrifuging at 25°C (1000-1500 rpm) for 5min, the top phase was extracted with a Pasteur pippette into an 8mL vial. The extraction with 3mL n-heptane was repeated. The top phase was decanted and added to the first extract. The extract was filtered through a Silicon Gel 60 into another 8mL vial. 1mL n-heptane was eluted into the vial 3 times before the extract was evaporated to dryness using nitrogen at 90°C. Once dry, 1mL of n-hexane was added. The amount of alkane (μg) was determined using Gas Chromatography.

Digestibility coefficients (DC) of nutrients (starch, NSP) were calculated using the following equation:

$$DC = 1- \{(\text{digesta nutrient}/\text{digesta alkane})/(\text{diet nutrient}/\text{diet alkane})\}$$

3.4.7 Nitrogen

The nitrogen content of diets was determined using LECO FP-2000 automatic analyser (Leco® Corporation, St. Joseph, MI). The protein contents were calculated by multiplying by a factor of 6.25.

3.4.8 Dry matter

The dry matter (DM) content was determined gravimetrically following drying at 105^{o}C for 24h or drying at 80^{o}C for 72h.

3.4.9 Gross energy

Gross energy contents of diet and excreta were determined using a Parr isoperibol bomb calorimeter (Parr Instrument Company, Moline, IL) at the PPPI or a DDS 500 isoperibol calorimeter (Digital Data System Ltd, CP 500, Northcliff, South Africa) at UNE.

3.4.10 Minerals

Samples were prepared for mineral analyses using a Milestone ETHOS PLUS Microwave digestor with HPR-1000/10S rotor. A TFM vessel was placed on the balance plate and tared, and 0.5g of sample was weighed. 8mL nitric acid (65%) and 2mL hydrogen peroxide (30%) were added; the solution was then swirled to homogenise it. The vessel was closed and introduced into the rotor segment, then tightened using the torque wrench. The segment was inserted into the microwave cavity and connected to the temperature sensor. The microwave was run until completion ie. 2min@85^{o}C, 5min @ 145^{o}C, 23 min @ 200^{o}C all at 1000W power setting. The rotor was cooled by air until the solution reaches room temperature. The vessel was opened and the solution transferred to a marked 50mL volumetric flask and filled to volume with deionised water. Multi element analysis was carried out using a Varian VISTA MPX Radial Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES).

3.5 Statistical analyses

The behaviour data (pecking, preening, drinking, feeding, escaping and freezing) were analysed using a Generalised Least Squares Program (Gilmor, 1990). Others were analysed using the general linear model procedure for ANOVA appropriate for factorial design (Statgraphics, STSC Inc., Rockville, MD). After a significant F test ($P<0.05$), Duncan's multiple-range test was used to inspect differences among group means (Duncan, 1955).

3.6 Ethical consideration

All experiments were approved by the Animal Ethics Committee at UNE under Animal Research Authority No's AEC990072, AEC2000/0062, AEC01/005 and AEC02/050. All work complied with the "Code of Practice for the Use of Animals for Scientific Purposes" issued by the Australian Bureau of Animal Health (National Health and Medical Research Council, 1990).

4. Effect of light intensity during rearing, beak trimming and dietary fibres on cannibalism mortality and performance of ISA Brown laying hens (first laying year)

4.1 Introduction

High light intensity and lack of beak trimming are the two prominent factors that induce the onset of cannibalism in imported strains of layers. Bright light can stimulate the activity of the birds, which in turn may lead to an increase in cannibalistic behaviour. Kjaer and Vestergaard (1999) found that severe pecks were 2-3 times more frequent in 30 lux than in 3 lux. Reducing light intensity (Nixey, 1994) can reduce the occurrence of cannibalism. Sherwin and Kelland (1998), however, did not see any cannibalism despite light intensity higher than conventional.

Beak trimming is a process that has long been applied by poultry producers because it is believed to reduce the incidence of feather pecking and/or cannibalism. Blokhuis and van der Haar (1989) suggested that the lower frequency of feather pecking in beak-trimmed birds might be due to the pain that occurrs after trimming. However, some studies have reported a non-significant effect of beak trimming (Lee, 1980, Blokhuis and van der Haar, 1989). They suggested that when the pain wore off or the beak had re-grown, beak-trimmed birds would be more prone to cannibalism than untrimmed birds.

There is little information available on prevention of cannibalism through nutritional manipulation. Some believe that increasing mineral levels, especially sodium, could reduce the incidence of cannibalism, but others have argued that an increase in fibre level is needed to reduce the incidence of cannibalism (Bearse *et al*., 1940, Esmail, 1997, Wahlstrom *et al*., 1998) possibly as a result of increased retention of minerals. Esmail (1997) found that increased dietary fibre reduced the cannibalism mortality in a dose-dependent manner and observed that no cannibalism mortality occurred with the inclusion of 18% oat offal in diets.

Fibre or NSP, either soluble or insoluble, had markedly different effects on the gastrointestinal tract. While ingestion of soluble NSP in broiler chickens was believed to increase digesta viscosity, ingestion of insoluble NSP was reported to increase the rate of digesta passage in the gut (Salih *et al*., 1991, Choct *et al*., 1996). Addition of enzymes to a soluble NSP-based diet was believed to alleviate the anti-nutritive effect of soluble NSP. Whether these properties of NSP influence the outbreak of cannibalism has not been determined.

In order to establish which of the factors above is most effective in alleviating the occurrence of cannibalism, it was necessary to include all the factors in an experiment as an important initial step. The present study was designed to examine the effect of light intensity during rearing, beak trimming and dietary fibres on cannibalism mortality and performance of cage-housed hens.

4.2 Materials and Methods

4.2.1 Birds

A total of 2880 commercial strain ISA Brown chickens was used in this study. The management during the rearing, pre-lay and lay period is described in Chapter 3 (Section 3.1 and 3.2). At 10 weeks of age, half of each of the rearing groups was beak-trimmed (upper beak 1 cm, lower beak 0.5 cm) and at the end of 15 weeks, behaviour observations, as described in Chapter 3 (Section 3.3), were conducted. The birds were allocated to one of four pelleted-layer diets at 17 weeks of age. Two groups of 90 birds each were assigned to each dietary treatment.

4.2.2 Feeds

The diets used in the experiment were: a commercial wheat-based diet, a millrun-based diet, a barley-based diet and a barley+enzyme diet. The enzyme product used was a β-glucanase-based glycanase (Biofeed Beta, Novozymes, Australia) which was added at 300g per tonne during feed mixing. The β-glucanase and the xylanase are very effective in partially degrading NSP to smaller polymers so reducing their ability to form highly viscous digesta. Since in mature barley grains the concentration of arabinoxylans and β-glucans is similar, the use of the β-glucanase in this experiment was just a matter of availability. All the diets were formulated to be isonitrogenous and approximately isocaloric according to commercial specifications and were produced at a commercial mill (Ridley AgriProducts, Tamworth, NSW). Feed and water were available *ad libitum* throughout the experiment. Nutrient composition of each diet for pre-lay and lay periods is given in Table 6 and 7, respectively.

4.2.3 Variable measurement

The following variables were measured in three recording periods, viz. rearing period (day-old to 15 weeks), pre-lay (17 to 20 weeks) and early lay (21-24 weeks).

4.2.3.1 Rearing period

During the rearing period, body weights as well as mortality data were recorded. At the end of the rearing period, behavioural data from latency and pecking tests were recorded. In addition, feather score was determined.

4.2.3.2 Pre-lay and lay-period

In the pre-lay period, weekly feed intake and daily mortality were recorded. To prevent large imbalances in the number of birds per cage due to treatment differences in mortality, the dietary treatments were crossed over at the end of week 20; the wheat diet being swapped with the millrun diet and the barley diet swapped with barley+enzyme diet. In the early-lay period, feed intake and egg production were recorded weekly, but mortality was recorded daily. For welfare reasons, birds that were severely cannibalized were removed to another house and recorded as "dead". Post-mortem examination was carried out on birds that died during the experiment.

Results were analysed statistically using analysis of variance. Data not normally distributed were log transformed before analysis.

Table 6 Composition of experimental diets in the pre-lay period (g/kg)

Ingredients	Wheat diet	Millrun diet	Barley diet	Barley+enzyme diet
Wheat 12.5%	730.05	-	-	-
Mung beans	10	-	-	-
Millrun 15.5%	-	320	-	-
Sorghum	-	482.85	-	-
Barley	-	-	759.2	759.2
Oil	-	35	21	21
Meat meal 50%	73	110	110	110
Soybean meal 48%	-	9	47	47
Soy flour	-	3	27	27
Cottonseed meal 37%	3.5	-	-	-
Rice pollard	44	-	-	-
Limestone	39	30	30	30
Rockphos	2	-	-	-
Kynofos	1.8	-	-	-
Salt	1.5	1.1	1.4	1.4
Choline chloride 75%	0.15	0.35	-	-
DL-methionine	0.9	2.2	0.9	0.9
L-Lysine	0.6	3	-	-
Layer/pullet premix	2	2	2	2
Synthetic yolk color premix	1.5	1.5	1.5	1.5
Chemical composition[a]				
ME, kcal/kg	2868	2815	2818	2818
Crude Protein	160	160	160	160
Fat	32	73.3	53.1	53.1
Fibre	29.3	43.4	51.6	51.6
Methionine	3.2	4.3	3.2	3.2
Lysine	7.2	8.6	7.6	7.6
Calcium	25.0	25.0	25.1	25.1
Nonphytate Phosphorus	5.0	5.9	5.8	5.8
Enzyme[A]	None	None	None	Yes

[A]commercial β-glucanase added at 300g/tonnes (Novozymes Pty Ltd, Australia)
[a]Calculated from the nutrient composition of the ingredients

Table 7 Composition of experimental diets in the lay period (g/kg)

Ingredients	Wheat diet	Millrun diet	Barley diet	Barley+enzyme diet
Wheat	672	-	-	-
Millrun	-	320	-	-
Sorghum	-	423.10	-	-
Barley	-	-	646.75	646.75
Oil	-	35	22.5	22.5
Meat meal	100	105	105	105
Mung beans	67.5	-	-	-
Soybean meal	-	50	70	70
Cottonseed meal	27	-	40	40
Rice pollard	67.5	-	52.5	52.5
Limestone	60	57.5	57.5	57.5
Salt	1.0	1.1	1.25	1.25
Choline chloride	0.3	0.3	0.3	0.3
DL-methionine	0.75	1.5	0.7	0.7
L-Lysine	0.45	3	-	-
Layer/pullet premix	2	2	2	2
Synthetic yolk color premix	1.5	1.5	1.5	1.5
Enzyme[A]	None	None	None	Yes
Chemical composition				
ME, kcal/kg	2786	2696	2698	2698
Crude Protein	170	170	170	170
Fat	38.0	71.1	57.4	57.4
Fibre	31.9	44.0	56.3	56.3
Methionine	3.2	3.8	3.2	3.2
Lysine	7.5	9.6	1.1	1.1
Calcium	35.1	34.8	34.9	34.9
Nonphytate Phosphorus	5.4	5.7	5.6	5.6

[A]commercial β-glucanase added at 300g/tonnes (Novozymes Pty Ltd, Australia)

4.3 Results

4.3.1 Rearing period: day-old to 15 weeks

From as early as 5 weeks of age, birds reared under bright light started to chase each other and at 6 weeks of age, two birds were cannibalized. The pecking behavior intensified as the birds aged. The birds reared under bright light were chasing each other with particularly aggressive individuals identified. Many birds started to develop feather-sucking behaviour, and eventually pecking. The pecking was often directed to the back and bottom part of the birds. By 10 weeks of age, four birds were cannibalized. There was no such activity among the birds kept under dim light.

The average body weight of birds reared under bright light, regardless of the age, was heavier than that of those reared under dim light. Thus, the average body weights of the birds reared under bright or dim light were: 902g vs. 858g, respectively, at 9.5 weeks of age. Between 14 and 15 weeks of age, this difference remained for the beak-trimmed birds, when the average body weights of the birds reared under bright or dim light were: 1412g vs. 1331g for the beak-trimmed birds, and 1455g vs. 1468g for the untrimmed birds, respectively.

In the pecking test, untrimmed-birds significantly did more pecking at objects offered compared to beak-trimmed birds ($P<0.01$). The mean total pecks for untrimmed-birds and beak-trimmed birds were 17.97 and 8.13, respectively. Birds preferred to peck white/natural grain (53%) rather than grains of other colours ($P<0.05$). The red (blood) colour was least pecked (8%). Feather score for beak-trimmed and untrimmed-birds were 14.9 and 14.6, respectively, and were significantly different ($P<0.01$). The tail was the area that was most highly pecked. Birds reared in dim light tended to have a higher ($P<0.08$) feather score (14.8) than birds reared in bright light (14.6). There were no significant differences in latency tests between groups ($P>0.05$).

The effect of the dark environment on the behaviour of the birds contrasted sharply with that of the birds reared under bright, natural light when they were transferred from the rearing house to the layer house at 15 weeks of age. The birds that had been under 5 lux were calm and docile, whereas those reared under bright light were noisy and easily frightened.

4.3.2 Pre-lay period: 17 to 20 weeks

At 17 weeks of age, the first eggs were seen, and cannibalism also started to occur. Birds were often cannibalized when they were in the process of lay although prolapse was not always associated with cannibalism.

Beak trimming (B) profoundly ($P<0.01$) affected mortality during the pre-lay period with cannibalism occurring predominantly in untrimmed birds. Cannibalism mortality was only 0.14% in trimmed birds, but was 13.4% in untrimmed birds (Table 8). There was also a significant ($P<0.01$) diet (D) x B interaction, with the untrimmed birds fed the commercial wheat diet having the highest mortality. Contrary to the result found during the rearing period, in this period birds reared under dim light showing greater aggression (8.1% vs. 5.4% mortality for dim vs. bright reared birds, respectively).

Diet (D), beak trimming and light regimen (L) all influenced ($P<0.05$) feed intake with a significant D x L interaction ($P<0.01$). The beak-trimmed birds ate less (88.2 g vs. 92.6g), but birds reared under dim light consumed more feed than those reared under bright light (93.3g vs. 87.5g). Birds reared in dim light and fed the millrun diet had a higher feed intake ($P<0.01$) than all the other treatments (Table 8).

4.3.3 Early lay period: 21 to 24 weeks

It is commonly believed that once an outbreak of cannibalism occurs, it is difficult, and often impossible, to stop it. Therefore, we swapped the most preventative diet (millrun) with the least preventative one (wheat) and the barley diet with the barley+enzyme diet to see if cannibalism mortality pattern would change with diet. Within a week of changing to the millrun diet, mortality due to cannibalism dropped to a low level in birds that had been on the wheat diet, but those previously on the millrun diet (now on the wheat diet) had an increased incidence of cannibalism (Figure 4). There was little change between the other two diets (Figure 4). Four weeks after the cross over, the total mortality due to cannibalism reached 37.7% in the untrimmed birds, whereas it was only 0.77% in the beak-trimmed birds (Table 8).

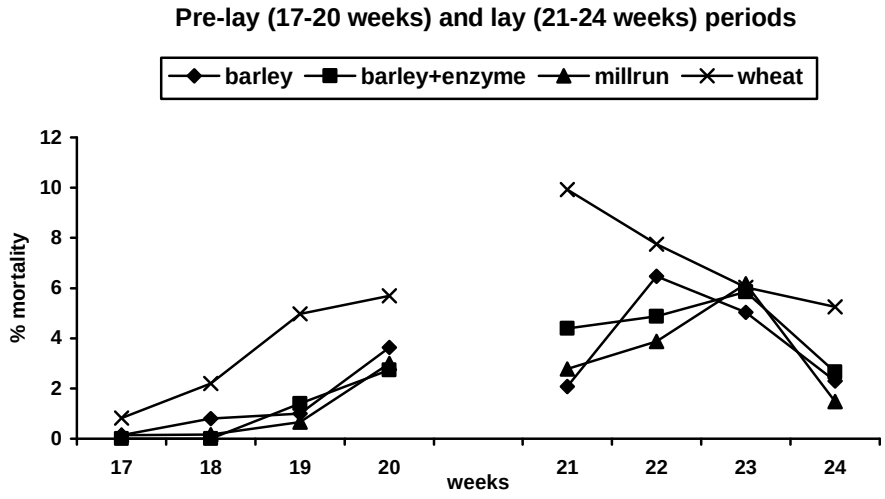


Figure 4 Mortality in ISA Brown layers in response to different diet composition during pre-lay and lay periods in a weekly basis.

Table 8 Means for mortality, feed intake, and egg production in ISA Brown layers in response to beak trimming, light level during rearing and diet composition

	Pre-lay period (17-20 weeks)		Early-lay period (21-24 weeks)			
Treatments	Total mortality (%)	Feed intake (g/d)	Total mortality (%)	Feed intake (g/d)	Egg prod. (%)	Feed: Egg ratio
Beak trimming (B)						
Beak-Trimmed	0.14[a]	88.2[a]	0.77[a]	102.0	58.9[a]	3.94
Untrimmed	13.50[b]	92.6[b]	37.68[b]	107.3	66.6[b]	3.39
P<	0.01	0.05	0.01	0.05	0.01	0.05
Diet (D)						
Commercial	13.69[b]	84.5[a]	28.94[b]	100.6[a]	61.2	3.85
Millrun	3.95[a]	99.3[b]	14.29[a]	101.4[a]	64.1	3.28
Barley	5.54[a]	88.5[a]	15.87[a]	108.9[b]	63.2	3.76
Barley+Enzyme	4.11[a]	89.4[a]	17.77[a]	107.8[b]	62.4	3.79
P<	0.01	0.01	0.01	0.05	NS	NS
Light regimen during rearing (L)						
Dim	7.97	93.3[b]	17.52	107.1 [b]	70.7[b]	2.95
Bright	5.68	87.5[a]	20.92	102.3 [a]	54.8[a]	4.39
P<	NS	0.01	NS	0.05	0.01	0.01
Interaction			P <			
B x D	0.01	NS	0.01	NS	NS	0.05
B x L	NS	NS	NS	NS	NS	NS
D x L	NS	0.01	NS	NS	NS	NS
B x D x L	NS	NS	NS	NS	0.05	0.01

Within column groupings, treatment means with different superscripts are significantly different (P<0.05). NS: not significant.

Light condition during rearing, beak trimming and diet all influenced (P<0.05) feed intake: untrimmed birds > trimmed birds; dim-light-reared birds > bright-light-reared birds; the barley and barley+enzyme diets > wheat diet and millrun diet (Table 7). It is not clear why birds fed the barley diet with or without enzyme ate more during this period.

The average egg production for weeks 21-24 reached 70% and was markedly influenced (P<0.01) by light condition during rearing (70.7% vs. 54.8% for dim- and bright-reared birds, respectively) and by beak-trimming (58.9% vs. 66.6% for trimmed and untrimmed birds, respectively) (Table 7). Diet did not affect egg production.

4.4 Discussion

Conditions during rearing greatly influenced pecking behaviour. The effect of lighting conditions on the behaviour of birds was confirmed. Kjaer and Vestergaard (1999) observed that floor rearing of pullets in dim light (3 lux) reduced the incidence of cannibalism and the result confirms this effect. It has been postulated that low light impairs the ability of the birds to identify environmental cues during early development (Kjaer and Vestergaard, 1999), but it could also impede the birds' ability to go to the feeders or suppress their appetite through feedback mechanisms. As the effect was more pronounced in the beak-trimmed birds, the suppression is probably more to do with the former than the latter.

In the current study, birds were beak-trimmed at 10 weeks of age to impose extra stress on them. The concern was that due to the sporadic nature of cannibalism outbreaks in birds, additional stress and disturbance would be required to predispose the birds to cannibalistic behaviour later in life. Effects of beak trimming were observed in the pecking test applied with untrimmed-birds showing higher number of peck at objects offered than beak-trimmed birds. It is likely that the pain caused by beak trimming is the reason of the lower pecking in beak-trimmed birds. This view is shared by Duncan *et al* (1989), who proposed that pain caused by beak trimming was the most probable cause of the behavioural changes including pecking in 16-week-old Brown leghorn hens. Blokhuis and van der Haar (1989) also demonstrated that beak trimming reduced the average level of pecking at objects during rearing. The authors suggested that pain was a possible factor that may cause the lower frequency of pecking behaviours.

The birds preferred pecking white as opposed to red objects, which is contrary to the popular belief that cannibalistic birds are specifically attracted to the red colour of blood. It is suggested that objects which reflect light, not the colour, might attract birds to peck.

Contrary to the hypothesis that birds reared under dim light will carry their calm, non-aggressive behaviour throughout their life (Kjaer and Vestergaard, 1999), in this study we tended to see the opposite, with birds reared under dim light showing greater aggression during the pre-lay period. It is hypothesized that a sudden change of environment from dark to bright natural day lighting could make the birds more prone to stress than keeping them under the same natural environment throughout their life.

Diet had a significant effect on the incidence of cannibalism in the present study. Birds fed the millrun diet, the barley diet or the barley+enzyme diet had much lower levels of mortality due to cannibalism than those hens fed a 'commercial' (wheat) diet. The finding confirms the report of Esmail (1997) that a diet insufficient in fibre will induce increased levels of pecking leading to cannibalism. However, the mechanism is not known. It has been reported that fibres with different physicochemical properties were likely to induce different physiological changes in the gastrointestinal tract of animals (Southgate, 1995). The millrun diet contained 30% millrun (2 parts wheat bran, one part wheat pollard), which contains up to 40% NSP. The NSP represent the bulk of "fibre" and are poorly digested by birds (Choct and Annison, 1992). Diets high in insoluble fibre may increase the rate of digesta passage in the small intestine, causing birds to feel hunger more quickly. Thus, it is possible that birds fed the millrun diet may spend longer eating and consequently less time pecking. Hughes and Black (1977) observed that the longer the birds spent eating, the lower their mortality due to cannibalism. The higher intake of birds reared in dim light and fed the millrun diet than all the other treatments supports the hypothesis.

Birds fed the barley and the barley+enzyme diets had lower cannibalism mortality than the control birds. It was postulated that ingestion of soluble dietary fibre would increase gut viscosity. Enzyme supplementation, then, was supposed to overcome the viscosity problem, thereby reducing the 'gut fullness', which in turn may lead to the development of cannibalistic behaviour or increase feed intake. In fact, mortality due to cannibalism remained low in birds

fed the barley diet with or without enzyme supplementation. The activity of the enzyme after pelleting and viscosity of gut contents in birds fed two soluble fibre diets has yet to be determined.

In the lay period, the total mortality due to cannibalism reached 37.7% in the untrimmed birds, whereas it was only 0.77% in the beak-trimmed birds. This reinforces the conclusion that beak-trimming is the most effective method of controlling cannibalism in birds kept in conventional houses. The lower mortality from cannibalism in beak-trimmed birds than in untrimmed-birds highlights the difficult welfare dilemma. On the one hand, beak trimming causes pain and discomfort to the bird but on the other hand, it is highly effective in reducing cannibalism. However, a significant reduction of feed intake in beak-trimmed birds found in our experiment emphasized the possibility that trimmed birds felt some pain after trimming, which in turn was affecting their feed intake. In addition, trimming has been suggested to reduce the sharpness of the beak and the accuracy with which the bird can peck (Appleby *et al*., 1992). As a result, to achieve the same intake as untrimmed birds, more pecking frequency while feeding may be needed by the beak-trimmed birds.. Pain resulting from trimming can change behavioural factors such as environmental pecking, feeding, drinking or preening (Kuo *et al*., 1991, Lee and Craig, 1991).

This experiment provides evidence that, under commercial conditions, the use of diets high in fibre, especially insoluble fibre, may reduce the incidence of cannibalism, and therefore may be used as an alternative to beak-trimming in some production systems. The effect of the high-fibre diets is large for non-beak-trimmed birds but further study is needed to confirm that there are positive long-term reductions in cannibalism due to beak trimming.

5. Effect of soluble and insoluble NSP from cereal grain products on digestive function of laying hens

5.1 Introduction

The effect of soluble and insoluble NSP on the gastrointestinal tract is well known. In the small intestine, physiological action of NSP is often associated with interfering with nutrient absorption and digestion (Fernandez and Philips, 1982), consequently reducing body weight. These effects are mostly related to soluble NSP, with a major portion of the effect being mediated via increased digesta viscosity (Choct and Annison, 1992). Johnson and Gee (1981) suggested that ingestion of viscous NSP will reduce the rate of digesta passage, resulting in a thickened intestinal unstirred-water layer, consequently decreasing the diffusion rate of available nutrients to the absorptive mucosal surface of the small intestine (Olson *et al*., 1987). In the large intestine, on the other hand, the physiological action of NSP is associated more with their ability to increase rate of digesta passage, faecal bulk consistency and to increase the expulsion of gases (Oakenful, 1993, Wisker *et al.*, 1998). These particular properties relate to the fermentative process of fibres in the large intestine. Insoluble NSP are not well fermented, and therefore serve almost entirely as bulking agents and shorten transit time (Roberfroid, 1993). However, some fermentable fibres have been found to increase faecal bulk. This effect was due to fermentation of soluble NSP by caeco-colonic microflora which resulted in increased microbial biomass (Roberfroid, 1993, Choct *et al*., 1996). The microbial biomass, then, caused an increase in faecal weight.

Physiological function of NSP is strongly influenced by the physicochemical properties of individual NSP, which in turn is influenced by the cell wall structure in which they are embedded, to the way the monomer units link together and the method of processing used. Consequently, not all NSP have the same physiological action.

The results from the previous experiment (Chapter 4) demonstrated that a diet high in fibre content reduced the incidence of cannibalism. There is only limited information available on the effect of dietary fibre on digestive function. Increasing fibre level in the diet has reduced mortality due to cannibalism in a dose-dependent manner (Esmail, 1997). However, the author was not sure about the mechanism. He suggests that the reduction in cannibalism mortality may be related to the increase in feed intake and the time spent feeding or the increased utilisation of minerals, especially sodium and potassium.

The current study investigated the effects of dietary fibres used in the previous experiment (Chapter 4) on the digestive function and performance of laying hens and energy utilization from the diet. In particular, emphasis was placed on the rate of digesta passage in the small intestine, the viscosity of intestinal digesta and the composition of NSP in the jejunum and ileum of laying hens.

5.2 Materials and Methods

ISA Brown laying hens (n = 32) at 42 weeks of age were used in this experiment. The same four diets (a commercial wheat-based; millrun-based; barley-based, and barley+enzyme diet) as in the previous experiment were used (for diet composition see Table 7). Eight birds per diet were kept in individual cages and fed the experimental diets for 7d. On d8, all birds were fasted for 2h and given 10g of their diet containing a digestibility marker (400mg/kg alkane $C_{36}H_{74}$). After two hours, excreta were collected every 30min for 8h, alkane concentrations were

determined and digesta transit time calculated. The birds were fed their respective diets for a further 7d. Apparent Metabolizable Energy (AME) bioassay was conducted from d4 to d7. During these four days feed intake was measured and all excreta voided were collected daily. The excreta were dried in a fan-forced oven at 80°C for 36h and excreta from each replicate were pooled over the collection period for determination of gross energy (GE). The moisture content of excreta voided was measured. The AME of diets was calculated using the following equation:

AME Diet= {(g feed eaten x GE feed)-(g excreta voided x GE excreta)}/g feed eaten

At the end of 7d birds were killed by cervical dislocation. The body cavity was opened and gastrointestinal tract, including gizzard, was removed and weighed prior to and after removal of digesta. The gastrointestinal weight measured included gizzard, jejunal, ileal and caecal weight. Gross energy, alkane, total starch in the jejunum and ileum, nitrogen, minerals, free sugars and NSP, jejunal and ileal viscosity, and ileal and caecal VFA were measured. The analyses were performed as described in Chapter 3. Body weight, feed to egg ratio and egg production were also recorded.

The data were analysed by ANOVA to determine significance of the main effects (type of diets).

5.3 Results

5.3.1 Chemical composition and amount of free sugars, insoluble and soluble NSP in the experimental diets

The composition of the experimental diets is shown in Table 9.

Table 9 Measured chemical composition of the experimental diets

	Wheat	Millrun	Barley	Barley+enzyme
Free sugars (g/kg DM)**	10.1[bc]	4.5[a]	11.1[c]	9.9[b]
Insoluble NSP (g/kg DM)**	67.2[a]	115.6[b]	112.5[b]	82.4[c]
Soluble NSP (g/kg DM)**	5.0[a]	6.5[b]	20.3[d]	8.6[c]
Crude protein, %	18	18	17	17
Minerals:				
Ca, %	3.50	3.51	3.45	3.54
K, %	0.76	0.88	0.79	0.79
P, %	0.92	1.04	0.91	1.00
Mg, %	0.20	0.24	0.18	0.20
S, %	0.22	0.21	0.21	0.23
Na, μg/g	976.1	1039.4	1243.2	1357.7
Mn, μg/g	130.6	152.4	113.6	122.4
Zn, μg/g	80.1	89.8	81.8	83.9
Al, μg/g	201.2	138.7	158.7	175.2
Cu, μg/g	16.8	19.6	19.0	22.7
Fe, μg/g	164.8	239.2	209.3	247.7
B, μg/g	1.6	2.0	13.9	2.7
Mo, μg/g	2.4	3.0	2.5	4.5

[a-d]values in a row with different superscripts differ significantly (**P<0.01).

The barley diet had the highest soluble NSP content (P<0.01), but its insoluble NSP content was not significantly different from that of the millrun diet. Both diets had higher insoluble NSP

concentrations than the wheat and barley+enyme diets (P<0.01) and the barley diet had a higher soluble NSP concentration than all other diets. Addition of the enzyme to the barley diet greatly reduced (P<0.01) its soluble and insoluble NSP contents.

The constituent components of free sugars and NSP are shown in Table 10. The main sugars present in the diets were arabinose, xylose and glucose.

5.3.2 Effects of diets on bird performance

Compared to the wheat diet, feeding the barley diet significantly depressed body weight by 10.4% (Table 11). Enzyme supplementation to the barley diet tended to increase body weight. Feed intake, feed to egg ratio, and egg production were not significantly different between diets.

Table 10 Constituent components of free sugars and NSP in the experimental diets (g/kg DM)

Sugar	Wheat diet	Millrun diet	Barley diet	Barley+enzyme diet	P-value
Insoluble NSP					
Rhamnose	0	0.3	0.3	0.3	NS
Fucose	0.0[a]	0.2[b]	0.3[b]	0.3[b]	<0.01
Ribose	0.2 [ab]	0.3[bc]	0.3[c]	0.2[a]	<0.05
Arabinose	17.2[a]	29.6[c]	18.9[b]	17.0[a]	<0.01
Xylose	24.9[a]	44.7[c]	44.0[c]	30.7[b]	<0.01
Mannose	1.3	2.7	2.3	1.8	NS
Galactose	2.4[a]	6.1[b]	6.2[b]	5.3[b]	<0.01
Glucose	29.6[a]	46.4[b]	54.1[d]	37.3[b]	<0.01
Total	67.2[a]	115.6[c]	112.5[c]	82.4[b]	<0.01
Soluble NSP					
Rhamnose	0.0[b]	0.1[c]	0.0 [a]	0.0[a]	<0.01
Fucose	0.0	0.0	0.0	0.0	NS
Ribose	0.0	0.0	0.0	0.0	NS
Arabinose	1.5[d]	1.5[c]	0.9[b]	0.3[a]	<0.01
Xylose	2.0[c]	2.1[c]	1.0[b]	0.3[a]	<0.01
Mannose	0.0[a]	0.3[c]	0.3[c]	0.1[b]	<0.01
Galactose	0.8[c]	1.5[d]	0.5[b]	0.1[a]	<0.01
Glucose	1.3[a]	1.9[b]	19.9[d]	8.9[c]	<0.01
Total	5.0[a]	6.5[b]	20.3[d]	8.6[c]	<0.01
Free sugars					
Rhamnose	0.0	0.0	0.0	0.0	n.a
Fucose	0.0	0.0	0.0	0.0	n.a
Ribose	0.0	0.0	0.0	0.0	n.a
Arabinose	0.2	0.2	0.1	0.2	NS
Xylose	0.1	0.0	0.0	0.0	NS
Mannose	1.2	1.0	1.8	1.8	NS
Galactose	1.7[b]	1.0[a]	2.4[c]	2.5[c]	<0.01
Glucose	6.9[c]	2.5[a]	6.8[c]	5.5[b]	<0.01
Total	10.1[bc]	4.5[a]	11.1[c]	9.9[b]	<0.01

[a-d]values in a row with different superscripts differ significantly (P<0.01). NS: not significant, n.a: not available.

Table 11 Feed intake, feed:egg ratio, egg production and body weight of laying hens fed different dietary fibres

Diet	Feed intake (g/bird/day)	Feed:egg ratio	Egg production (%)	Body weight (g/bird)
Wheat	164.8	2.4	89	2266[b]
Millrun	168.8	2.4	83	2131[ab]
Barley	155.4	2.2	83	2031[a]
Barley+enzyme	155.3	2.2	76	2133[ab]
P-value	NS	NS	NS	<0.05

[ab]values within a column with different superscripts differ significantly (P<0.05). NS: not significant.

5.3.3 Effect of diets on AME, excreta moisture and digesta viscosity

There were significant differences (P<0.01) in the AME of diets (Table 12). The millrun diet had the highest AME, whereas the barley diet had the lowest. Addition of enzyme to the barley diet increased the AME.

Birds fed the barley diet showed lower excreta moisture (P<0.05) than those fed wheat and millrun diets. Enzyme addition to the barley diet slightly reduced the moisture content of the excreta.

Birds fed the wheat diet had the highest digesta viscosity (P<0.01) both in the jejunum and ileum (Table 12). Birds fed the barley diet had markedly higher digesta viscosity in the jejunum than birds fed the millrun diet but their digesta viscosity in the ileum was not different (P<0.01). Enzyme addition to the barley diet reduced jejunal digesta viscosity (P<0.01).

Table 12 AME, excreta moisture and jejunal and ileal viscosity of laying hens fed different dietary fibres

Diet	AME (MJ/kg DM)	Excreta moisture (%)	Jejunal viscosity (mPa.s)	Ileal viscosity (mPa.s)
Wheat	14.0[b]	78.7[b]	3.2[b]	9.5[b]
Barley	13.3[a]	75.9[ab]	3.0[b]	4.8[a]
Millrun	14.8[d]	78.7[b]	2.3[a]	5.6[a]
Barley+enzyme	14.3[c]	70.6[a]	2.0[a]	3.5[a]
P-value	<0.01	<0.05	<0.01	<0.01

[a-d] values within a column with different superscripts differ significantly (P<0.05).

5.3.4 Effect of diets on jejunal and ileal starch digestibility and VFA concentration in the ileum and caeca

In the ileum, birds fed wheat and millrun diets had a higher VFA concentration (P<0.05), whereas in the caeca, the VFA concentration in birds fed the barley and millrun diets was not significantly different, but was lower (P<0.01) than the other diets. Addition of enzyme to the barley diet increased caecal VFA concentration nearly two-fold (Table 13).

Table 13 Jejunal and ileal starch digestibility and VFA concentration in the jejunum and ileum of laying hens fed different dietary fibres

Diets	Ileal VFA (μmol/bird)	Caecal VFA (μmol/bird)	Jejunal starch digestibility coefficient	Ileal starch digestibility coefficient
Wheat	82.0[a]	2820.3[b]	0.90	0.92[a]
Millrun	80.2[a]	2085.2[a]	0.82	0.96[ab]
Barley	29.4[b]	1784.7[a]	0.96	0.98[b]
Barley+enzyme	28.1[b]	3491.1[c]	0.97	0.99[b]
P-value	<0.05	<0.01	<0.07	<0.05

[ab]mean values within a column with no common superscripts differ significantly (P<0.05)

Diet had a significant effect (P<0.05) on the digestibility of starch in the ileum. Birds fed the barley and barley+enzyme diets had the highest starch digestibility in the ileum, whereas those fed the wheat diet had the lowest (Table 13). There was only a tendency for the barley diets to be higher for the digestibility of starch in the jejunum (P<0.07).

The concentration of acetic acid in the ileum was higher in birds fed the wheat and millrun diets (P<0.05) (Table 14). In general, birds fed the wheat or the barley + enzyme diet had higher concentrations of acetic, propionic and butyric acids in the caeca (P<0.01). Addition of enzyme to the barley diet increased the concentration of acetic, propionic and butyric acids in the caeca nearly two-fold whereas the effect in the ileum was inhibited (Table 13).

Table 14 The concentration of VFA in the ileal and caecal digesta of laying hens fed different dietary fibres

	Ileal VFA composition (μMol/bird)			Caecal VFA composition (μMol/bird)		
Diet	Acetic	Propionic	Butyric	Acetic	Propionic	Butyric
Wheat	80.3[b]	1.15	0.22	1415.8[c]	768.3[b]	438.7[b]
Millrun	77.6[b]	2.02	0.16	1110.5[b]	594.2[ab]	256.2[a]
Barley	26.7[a]	1.54	0.40	961.7[a]	470.1[a]	229.7[a]
Barley+enzyme	26.2[a]	1.43	0.16	1369.0[c]	1402.1[c]	648.8[c]
P-value	<0.05	NS	NS	<0.01	<0.01	<0.01

[a-c]mean values within a column with no common superscripts differ significantly (P<0.05). NS: not significant

5.3.5 Effect of diets on NSP levels in jejunal and ileal digesta

There was no significant different in the concentration of free sugars and NSP in the jejunum (P>0.05), but the concentration of insoluble (P<0.05) and soluble NSP (P<0.01) in the ileum was significantly affected by diet (Table 15). The wheat diet had a lower insoluble NSP concentration in the ileum (P<0.05), whereas the concentration of soluble NSP was higher in the barley diet (P<0.01).

Table 15 Amounts of free sugars, insoluble and soluble NSP (g/kg DM) in jejunal and ileal digesta of laying hens fed different dietary fibres

	Jejunum			Ileum		
Diet	Free sugars	Insoluble NSP	Soluble NSP	Free sugars	Insoluble NSP	Soluble NSP
Wheat	20.0	70.1	18.3	20.2	134.1[a]	26.4[a]
Millrun	18.6	132.6	13.5	26.6	218.0[b]	19.0[a]
Barley	20.7	94.3	33.9	18.4	199.1[b]	51.0[b]
Barley+enzyme	9.3	60.4	15.8	23.7	200.1[b]	30.5[a]
P-value	NS	NS	NS	NS	<0.05	<0.01

[ab]values within a column with different superscripts differ significantly (P<0.05). NS: not significant

The sugar profiles in the jejunal digesta showed that the barley diet had more free rhamnose (P<0.05), and both the barley and barley + enzyme diets had more arabinose (P<0.05) compared with the other diets. The barley diet also had higher levels of soluble mannose and glucose, whereas the millrun gave the highest level (P<0.05) of insoluble arabinose (Table 16).

A detailed analysis of free sugars, soluble and insoluble NSP in the ileal digesta showed that the wheat diet had the lowest concentration of insoluble xylose (P<0.05), galactose (P<0.01) and glucose (P<0.05) in the ileum (Table 17). The barley diet had the highest concentration of soluble mannose (P<0.01) and glucose (P<0.01) in the ileum (Table 17).

5.3.6 Effects of diets on jejunal and ileal NSP digestibility

Only the digestibility of insoluble NSP in the ileum was found different (P<0.05) with birds fed the barley+enzyme diet having a lower insoluble NSP digestibility compared to other diets. Addition of enzyme to the barley diet reduced the digestibility of insoluble NSP in the ileum (P<0.05) (Table 18).

5.3.7 Effect of diets on faecal digestibility and NSP concentration in the faeces

Diet affected the digestibility of free sugars (<0.01) and soluble NSP (<0.05) in the faeces. Birds fed the barley diet had a higher soluble NSP digestibility than those fed other diets. Addition of enzyme slightly reduced the soluble NSP digestibility. Digestibility of free sugars in the faeces was higher in birds fed the millrun diet than those fed the wheat diet, but was not different from birds fed the barley or barley+enzyme diets (Table 19).

Significant differences were found in the faecal concentrations of free sugars (P<0.01) and insoluble NSP (P<0.01) (Table 19). Birds fed the wheat diet had the highest level of free sugar (P<0.01), but the lowest level of insoluble NSP (P<0.01) in their faeces. Addition of enzyme to the barley diet slightly increased the amount of free sugars in the faeces.

A detailed analysis of free sugars and insoluble NSP in faeces showed that birds fed the wheat diet had the highest concentration of arabinose, xylose and mannose in the free sugars. The concentrations of insoluble fucose, xylose, galactose (P<0.01) and rhamnose (P<0.05), however, were lower than other diets (Table 20).

Table 16 Neutral sugar composition in the jejunal digesta of laying hens fed different diets (g/kg DM)

				Free sugars				
	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose
Wheat	0.00[a]	0.16	0.12	0.28[a]	0.59	1.51	2.20	15.2
Millrun	0.02[a]	0.15	0.16	0.26[a]	0.62	0.17	2.31	13.9
Barley	0.12[b]	0.18	0.26	0.49[b]	0.68	1.46	5.08	12.4
Barley+enzyme	0.00[a]	0.19	0.10	0.56[b]	0.60	0.93	2.42	4.45
P-value	<0.05	NS	NS	<0.01	NS	NS	<0.07	NS
				Soluble NSP				
	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose
Wheat	0.17	1.03	0.19	5.06	6.03	0.59[a]	4.13[b]	3.64[a]
Millrun	0.14	0.86	0.10	2.87	4.30	0.56[a]	2.47[a]	4.10[a]
Barley	0.19	1.29	0.12	3.98	4.36	1.20[b]	3.75[b]	23.3[b]
Barley+enzyme	0.15	1.28	0.11	1.89	1.53	0.66[a]	2.95[ab]	9.39[ab]
P-value	NS	NS	NS	NS	NS	<0.05	<0.05	<0.05
				Insoluble NSP				
	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose
Wheat	0.34	0.97	0.62	16.5[a]	25.1	3.21	4.50	27.5
Millrun	0.37	0.96	0.71	37.0[b]	50.0	3.18	6.04	51.0
Barley	0.35	1.43	0.39	16.6[a]	36.0	3.78	6.72	40.7
Barley+enzyme	0.28	1.41	0.81	11.3[a]	21.6	3.19	5.13	24.2
P-value	NS	NS	NS	<0.05	NS	NS	NS	NS

[ab]values within a column with different superscripts differ significantly (P<0.05). NS: not significant.

Table 17 Neutral sugar composition in the ileal digesta of laying hens fed different diets (g/kg DM)

				Free sugars				
	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose
Wheat	0.02	0.13	0.06	0.45	0.65[ab]	1.77	2.80	14.4
Millrun	0.00	0.00	0.00	0.20	0.14[b]	1.74	2.53	22.0
Barley	0.08	0.06	0.06	0.66	0.28[a]	1.94	4.50	10.8
Barley+enzyme	0.09	0.04	0.04	1.08	1.24[b]	1.71	5.11	14.4
P-value	NS	<0.06	NS	NS	<0.05	NS	NS	NS
				Soluble NSP				
	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose
Wheat	0.22	1.13	0.12	7.85	9.42	0.74[a]	5.19	5.17[a]
Millrun	0.11	0.98	0.06	4.15	5.84	0.81[a]	3.28	6.14[a]
Barley	0.18	1.06	0.06	5.94	6.48	1.53[b]	4.30	37.5[c]
Barley+enzyme	0.20	0.87	0.07	5.06	4.42	1.14[ab]	4.43	18.0[b]
P-value	NS	NS	NS	NS	<0.09	<0.01	NS	<0.01
				Insoluble NSP				
	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose
Wheat	0.54	0.76	0.24[b]	33.8[a]	54.9[a]	4.04	5.95[a]	50.8[a]
Millrun	0.33	0.68	0.00[a]	63.6[b]	99.3[b]	3.28	8.03[ab]	70.6[b]
Barley	0.55	0.88	0.07[a]	38.3[a]	84.6[b]	5.00	10.3[b]	84.3[b]
Barley+enzyme	0.60	0.82	0.11[ab]	37.8[b]	85.0[b]	4.61	10.3[b]	86.0[b]
P-value	NS	NS	<0.05	<0.01	<0.05	NS	<0.01	<0.05

[a-c]values within a column with different superscripts differ significantly (P<0.05). NS: not significant.

Table 18 Digestibility coefficients of free sugars, insoluble and soluble NSP in jejunal and ileal digesta

	Jejunum			Ileum		
Diet	Free sugars	Insoluble NSP	Soluble NSP	Free sugars	Insoluble NSP	Soluble NSP
Wheat	-0.31	0.23	-1.72	0.38	0.28[b]	-1.65
Millrun	0.43	0.04	-0.80	0.65	0.34[b]	-0.01
Barley	-0.59	0.02	-0.96	0.58	0.35[b]	0.05
Barley+enzyme	0.47	0.16	-1.20	0.53	0.01[a]	-0.44
P-value	NS	NS	NS	NS	<0.05	NS

[a-b]values within a column with different superscripts differ significantly (P<0.05). NS: not significant.

Table 19 Digestibility coefficients of free sugars, insoluble and soluble NSP in the faeces and amount of free sugars, insoluble and soluble NSP concentration in faeces of laying hens fed different dietary fibres

	Faecal digestibility			Faecal concentration (g/kg)		
Diet	Free sugars	Insoluble NSP	Soluble NSP	Free sugars	Insoluble NSP	Soluble NSP
Wheat	0.93[a]	0.43	0.47[a]	4.1[b]	186.2[a]	13.3
Millrun	0.97[b]	0.26	0.39[a]	2.7[a]	239.7[b]	11.7
Barley	0.95[ab]	0.42	0.83[b]	3.1[ab]	226.5[b]	11.5
Barley+enzyme	0.95[ab]	0.24	0.66[ab]	3.8[b]	233.2[b]	11.1
P-value	<0.01	NS	<0.05	<0.01	<0.01	NS

[ab]values within a column with different superscripts differ significantly (P<0.05). NS: not significant.

Table 20 Neutral sugar composition in the faeces of laying hens fed different diets (g/kg DM)

				Free sugars				
	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose
Wheat	0.00[a]	0.07	0.05	0.99[b]	1.15[b]	0.31[b]	0.60	0.90[a]
Millrun	0.05[b]	0.01	0.02	0.49[a]	0.63[a]	0.16[a]	0.48	0.76[a]
Barley	0.14[c]	0.00	0.01	0.72[ab]	0.65[a]	0.21[a]	0.54	0.84[a]
Barley+enzyme	0.13[c]	0.05	0.02	0.68[ab]	0.62[a]	0.24[ab]	0.61	1.39[b]
P-value	<0.01	NS	NS	<0.01	<0.01	<0.05	<0.06	<0.05
				Soluble NSP				
	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose
Wheat	0.51[b]	0.36	0.31	3.28	4.58[c]	0.57	2.65	2.90
Millrun	0.42[a]	0.40	0.34	2.67	4.01[bc]	0.47	2.13	2.83
Barley	0.41[a]	0.31	0.34	2.41	2.60[ab]	0.49	2.12	4.29
Barley+enzyme	0.45[ab]	0.45	0.38	2.45	2.06[a]	0.62	2.77	3.44
P-value	<0.05	NS	NS	NS	<0.05	NS	NS	<0.06
				Insoluble NSP				
	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose
Wheat	1.25[a]	0.58[a]	0.45[a]	48.0[a]	73.3[a]	4.56[b]	7.84[a]	73.6
Millrun	1.37[ab]	0.80[b]	0.47[a]	80.7[b]	108.5[c]	3.43[a]	10.1[b]	65.1
Barley	1.53[b]	0.91[c]	0.43[a]	45.2[a]	99.1[b]	4.37[b]	11.1[bc]	92.2
Barley+enzyme	1.49[b]	1.03[d]	0.60[b]	46.8[a]	102.4[bc]	4.57[b]	12.3[c]	93.3
P-value	<0.05	<0.01	<0.05	<0.01	<0.01	<0.01	<0.01	NS

[a-c]values within a column with different superscripts differ significantly (P<0.05). NS: not significant

5.3.8 Effect of diets on gastrointestinal weight

Only the caecal weight relative to body weight was affected by diet, with birds fed the barley diet having the heaviest caeca (P<0.05) (Table 21). Enzyme addition had no effect.

Table 21 Relative gizzard, jejunal, ileal and caecal weight (%BW) of laying hens fed different dietary fibres

Diets	Gizzard	Jejunum	Ileum	Caeca
Wheat	1.32	1.15	0.93	0.53[a]
Millrun	1.29	1.22	0.91	0.50[a]
Barley	1.46	1.21	0.93	0.61[b]
Barley+enzyme	1.44	1.05	0.81	0.52[a]
P-value	NS	NS	NS	<0.05

[ab]values within a column with no common superscripts differ significantly (P<0.05).

5.3.9 Effect of diets on the rate of feed passage

The pattern of alkane excretion among diets is shown in Figure 4. Digesta transit rate was faster (between 3-3.5h) in birds fed the millrun, barley and barley+enzyme diets than in birds fed the wheat diet (4-4.5h). The maximum excretion of the marker from birds fed the millrun diet occurred in a very narrow time frame about 3-4 hours post dosing, whereas it was scattered over a wider window (3-5h after dosing) for those fed the wheat diet.

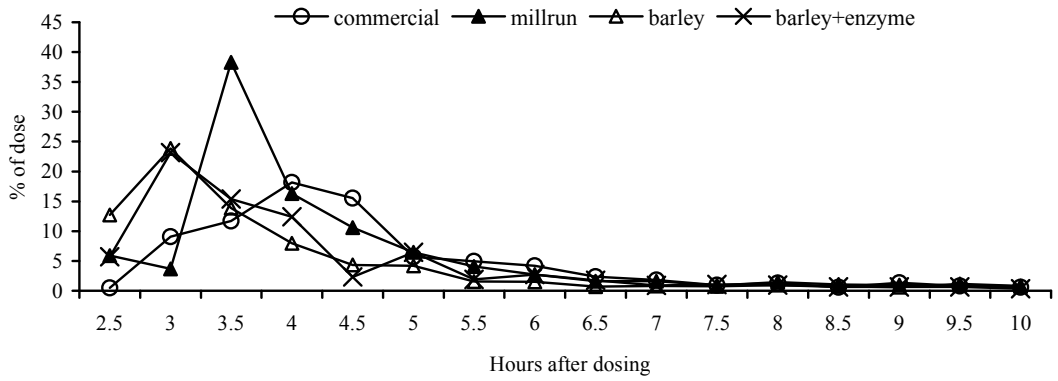


Figure 5 Alkane excretion per 30-minute sampling period expressed as a percentage of the total dose administered during an 8-h complete collection digestion trial.

5.4 Discussion

The level of NSP in dietary fibre depends not only on the chemical structure of the fibre itself but also on the variety of grains and the processing method applied. The chemical structure of NSP and their association with the rest of the cell wall, in turn, influences the physical and chemical properties of the individual NSP (Choct, 1997).

Analyses of the diets used in the experiment showed that the barley diet had a higher soluble NSP content than other diets, whereas its insoluble NSP content was also higher than but not different from the millrun diet. Detailed analyses on the monosaccharides profile showed that

the main sugar present in the soluble fractions of the barley diet was glucose, indicating that most of soluble NSP in the barley diet was β-glucans, as reported by an earlier researcher (Choct, 1997). The main sugars in the insoluble fraction in all diets were glucose, xylose, arabinose and galactose, suggesting that most of the insoluble NSP in the diets were insoluble glucans and insoluble arabinoxylans with galactose as side chains. Addition of enzyme to the barley diet greatly reduced its soluble and insoluble NSP concentration, suggesting the possible depolymerisation of the β-glucans by the enzyme. The improvement of nutritive value of feed ingredients due to the use of exogenous feed enzymes in cereal-based diets has been known for some years (Classen and Campbell, 1990, Annison and Choct, 1993).

An increased level of insoluble NSP in a diet shortens digesta passage rate (Roberfroid, 1993) and the results from this study support this finding. High levels of insoluble NSP in the millrun and barley diets increased the rate of digesta passage. The results from the previous experiment (Chapter 4) showed that birds fed the millrun, barley and barley+enzyme diets had lower cannibalism mortality than birds fed the wheat diet. It has been proposed before that ingestion of these diets may increase the rate of digesta passage, causing birds to feel hunger more quickly; consequently birds may spend a longer time eating and less time pecking. Ingestion of the wheat diet, on the other hand, may induce the feeling of fullness. The idle time between feeding bouts can result in increased pecking. The fact that birds fed the millrun and barley diets had a higher digesta passage rate than those fed the wheat diet confirmed the mechanism proposed.

It is documented that both the quality and quantity of NSP determine their anti-nutritive effects (Choct *et al*., 2003), and in this case, the β-glucans present in the barley appeared to be of low molecular weights. Furthermore, the insoluble NSP level in the intestine of birds fed the barley diets was higher than birds fed other diets, indicating that the β-glucans in this particular barley sample were less soluble than usual. Such variation is quite normal for barley varieties or even the same variety grown in different areas (Wood, 2000).

A previous study in rye-fed birds observed that cell-wall carbohydrates continue to be released during transit through the small intestine (Bedford and Classen, 1992) resulting in increased digesta viscosity. A similar condition seems to have occured in wheat-fed birds in this study. Depolymerisation of cell wall NSP in birds fed the wheat diet probably occurred throughout the intestine, resulting in increased jejunal and ileal viscosity.

Although birds fed the wheat diet had high digesta viscosity, they had a higher body weight than birds fed other diets. There were two possibilities that could be suggested in this case. First, birds could tolerate the increase in digesta viscosity. Gut viscosity, when reduced below approximately 10cP, will not depress performance (Cowan, 1995) and birds can tolerate the small increase in gut viscosity (Choct and Annison, 1992). If this is the case, then it can be postulated that the slower the digesta transit time the better the digestion of nutrients in the gut. The longer the feed is exposed to the digestive processes in the gut, the more complete is its digestion (Choct, 1997). The second suggestion is the increase in digesta viscosity might stimulate the bacterial fermentation, resulting in increased production of VFA. In fact, the production of ileal and caecal VFA, especially acetic acid, was high in birds fed the wheat diet. The increased production of acetic acid, which is mainly metabolized by the muscle (Macfarlane and Macfarlane, 1997), could provide energy to the birds, resulting in improved body weight. Although the energy contribution via caecal fermentation is less effective than absorption of glucose in the upper intestine, VFA as the end product of bacterial fermentation can provide some energy to the birds (Carré *et al*., 1995). However, it should be remembered that the amounts and types of fermentation products formed by colonic bacteria depend on the relative amount of each substrate available, the chemical structure and the compositions of the substrate (Macfarlane and Macfarlane, 1997).

A higher body weight of birds fed the millrun diet than those fed the barley diet may be due to its higher AME value. High levels of soluble NSP in wheat have caused poor ME values (Annison, 1991, Choct *et al*., 1995). Birds fed the millrun diet did not have high digesta viscosity, indicating that the NSP present in millrun had no viscous properties. Instead, the millrun diet acted as a bulking agent due to its ability to hold water, which was shown by its higher excreta moisture. Increasing the rate of digesta passage will give less time for microorganisms to ferment the substrates, thereby increasing absorption of energy as monomeric sugars (Carré *et al*., 1995, Choct, 1997). Addition of enzyme to the barley diet increased body weight and AME; this was probably due to elimination of fermentative substrate, and hence any microbial influence on the digestive tract of the birds (Choct *et al*., 1996, Brenes *et al*., 1993). The monosaccharide profile showed a reduced concentration of soluble mannose, galactose and glucose in the jejunum due to enzyme addition.

Data on jejunal and ileal digestibility showed that the addition of enzyme to the barley diet reduced the ileal insoluble NSP digestibility in birds. It is postulated that enzyme addition largely eliminated the fermentative organisms in the small intestine, reducing the utilization of the insoluble NSP. The digestibility of NSP by exogenous enzymes depends on the type of enzymes used (Choct *et al*., 2003). In addition, Pettersson and Lindberg (1997) suggested that other factors in addition to hull, fibre and beta-glucan content are of importance for ileal and hind-gut dietary components digestion in barley. Different fibre sources have different effects, depending on chemical structure and on association with the rest of cell-wall components, which all have an effect on the overall NSP digestibility. Insoluble NSP are slightly fermented (Roberfroid, 1993).

The faecal digestibility of soluble NSP ranged from 39 to 83%. The highest was in barley-fed birds and the lowest was in millrun-fed birds. Longstaff and McNab (1989) showed that NSP digestibility increased with increased solubility of NSP. In humans, soluble NSP were almost completely fermented by anaerobic microorganisms in the proximal colon (Roberfroid, 1993), which enhanced microbial biomass and consequently increased faecal weight. Increasing faecal weight is commonly related to the water-holding property of the NSP. The high excreta moisture found in barley-fed birds confirmed the statement. Addition of enzyme reduced the faecal digestibility. This was probably due to the elimination of fermentative substrate (Choct *et al*., 1996).

Birds fed the barley diet had the heaviest caecal weight. An increase in caecal weight was also observed in rats fed guar gum (Johnson and Gee, 1986). The authors suggested that the consumption of gel-forming polysaccharides could stimulate mucosal-cell proliferation in which the mechanism underlying this response appears not to be entirely dependent on physical properties of digesta. Enzyme addition to the barley diet increased caecal VFA concentration nearly two-fold, whereas the effect on the ileum was inhibited. Choct *et al.* (1996, 1999) found a similar result, the addition of xylanase to a sorghum-based diet supplemented with isolated wheat arabinoxylans increased caecal VFA levels three-fold. The authors suggested that smaller NSP molecules produced by the enzyme in the jejunum and ileum could enter the caeca, where they were rapidly fermented.

In conclusion, soluble and insoluble NSP have a profound effect on the digestive function of birds; however, their action may be modified by an interaction with other cell-wall components. The presence of enzyme improved performance by reducing intestinal digesta viscosity. Insoluble NSP with their properties to shorten transit time seem to have potential for reducing cannibalism mortality. However, since individual NSP have different physicochemical properties, the result with one fibre may not hold true for another. Further study is needed to confirm that there is a reduction in cannibalism mortality due to inclusion of insoluble NSP from any fibre source.

6. Effect of fibre sources on performance and cannibalism mortality in laying hens

6.1 Introduction

The results from Chapter 5 showed that insoluble NSP, with their properties to increase the rate of digesta passage, are probably the reason behind the beneficial effect of high fibre diets on cannibalism birds.

The structure of plant cell walls influences the physical and chemical properties of the individual NSP and these can vary quite considerably between different polymers and different molecular weights of the same polymer (Morris, 1990). Furthermore, the way the monomer units of polysaccharides link together also could differentiate the physical properties among polysaccharides. Different sugars can often give polysaccharides with close similar physical properties if they are linked together in the same way. On the other hand, despite being built up from the same monomer units, polysaccharides can have different physical properties when the monomer units are linked together in different ways. Method of food processing, in addition, can also affect the physicochemical properties of dietary fibre (NSP) (Robertson and Eastwood, 1981).

Since physicochemical properties of individual NSP strongly influences the NSP's physiological actions, not all NSP have the same physiological action (Southgate, 1995) and therefore may not impact on behaviour in the same way. The aim of this study is to investigate the effect of fibre sources on performance and cannibalism mortality.

6.2 Materials and Methods

ISA Brown laying hens (n = 540, at 45 weeks) which were randomly allocated to cages with 5 birds per cage in the previous study were used in the current experiment. Six different diets: wheat, rice hulls, protein meal, millrun, manno-oligosaccharides (MOS) and bentonite diets were offered for duration of 8 weeks. Bentonite was included as a non-nutritive "filler", to test the effect of nutrient dilution on cannibalism. Diets were formulated according to commercial specifications and were produced at a commercial mill (Ridley AgriProducts, Tamworth, NSW). The composition of the diets is presented in Table 22. Feed and water were available *ad libitum* throughout the experiment. The variables measured were soluble and insoluble NSP, protein and mineral contents of diets, body weight, feed intake, egg production and percentage of birds alive. The measurement of these variables was as described in Chapter 3.

Data obtained were analysed statistically using analysis of variance.

Table 22 Ingredients of the experimental diets (g/kg)[1]

Ingredients	Wheat	Rice hulls	Protein meal	MOS	Millrun	Bentonite
Wheat 12.5%	707.5	607.5	683.1	706	-	671.6
Millrun 15.5%	-	-	-	-	320	-
Sorghum 9.5%	-	-	-	-	482.9	-
Meat meal 50%	100	100	-	100	110	95
Sunflower meal 30%	50	50	68.5	50	-	47.5
Cottonseed meal 37%	5	5	-	5	-	5
Soybean meal 48%	8.5	8.5	110	8.5	9	8
Rice pollard	63	63	37.5	63	-	60
Rice hulls 2%	-	100	-	-	-	-
Soy flour	-	-	-	-	3	-
Oil	-	-	10	-	35	-
Limestone	59.5	59.5	75	59.5	30	56.5
Kynofos	-	-	10	-	-	-
Salt	1	1	1.25	1	1.1	1
Choline chloride 75%	0.3	0.3	0.3	0.3	0.35	0.3
DL-methionine	0.6	0.6	0.9	0.6	2.2	0.6
L-Lysine	1.1	1.1	-	1.1	3	1
Layer premix	2	2	2	2	2	2
Pigment	1.5	1.5	1.5	1.5	1.5	1.5
Bentonite	-	-	-	-	-	50
MOS	-	-	-	1.5	-	-
Chemical composition[a]						
ME, kcal/kg	2770	2483	2715	2723	2815	2631
Crude protein	170	164	165	173	160	166
Fat	36.9	35.4	32.7	36.6	73.3	35.1
Fibre	36.4	74.2	39.7	36.0	43.4	34.6
Methionine	3.2	3.3	3.5	3.2	4.3	3.1
Lysine	7.4	7.3	6.8	7.6	8.6	7.2
Calcium	35.0	32.0	30.8	32.0	25.0	30.4
Nonphytase Phosphorus	5.4	4.8	3.5	5.0	5.9	4.7

[1]Diets were formulated and produced by Ridley Agriproducts Pty Ltd.,Tamworth
[a]Calculated from the nutrient composition of the ingredients

6.3 Results

6.3.1 Chemical composition and amount of insoluble and soluble NSP in the experimental diets

The chemical composition including NSP levels of diets is presented in Table 23. Rice hull and millrun diets had a higher insoluble NSP content than other diets. Soluble NSP content among diets, on the other hand, were similar, with the exception of rice hull which was low (2%). Protein levels ranged from 18% to 20%, with the highest in MOS diet (20%), the lowest in rice hull, protein meal and millrun diets (18%), and wheat and bentonite were intermediate (19%).

Table 23 Composition of the experimental diets (DM basis)

Diet	Insoluble NSP	Soluble NSP	Protein	Ca (%)	P (%)	Na (μg/g)	K (%)
Wheat	77.0	8.56	19	3.6	0.96	1142.2	0.6
Rice Hulls	114.7	2.11	18	3.8	0.85	1363.0	0.6
Protein Meal	84.9	5.65	18	3.8	0.64	786.9	0.8
Bentonite	80.4	7.99	19	3.5	0.86	1178.2	0.6
Millrun	115.6	6.47	18	3.5	1.04	1039.4	0.9
MOS	82.0	8.74	20	3.3	0.94	1548.8	0.6

Sodium concentration was found to be low in the protein meal diet and high in the rice hull and MOS diets. The concentrations of sodium in other diets were similar. Potassium concentration was between 0.6% and 0.9% with the highest concentration found in millrun diets.

The constituent sugars of NSP are shown in Table 25. The main sugars present in the insoluble fraction were arabinose, xylose and glucose. The concentration of sugars present in the soluble fraction is low and mainly arabinose and xylose (Table 25).

6.3.2 Effect of diets on performance, percentage of birds alive and excreta moisture

Results on performance, percentage of birds alive and excreta moisture are summarized in Table 24. Diet significantly influenced ($P<0.05$) feed intake. Birds given the bentonite diet significantly eat more compared to those given the other diets. Birds on millrun diet had the lowest intake, but their egg production compared to those fed other diets, with the exception of protein meal diet, was higher ($P<0.01$). Birds fed the wheat and MOS diets had the lowest egg production. The effect of diets on percentage of birds alive only approach significant ($P<0.08$). Birds fed the rice hull diet tended to have higher percentage of birds alive (78%) than those fed other diets, whereas birds fed the protein meal diet tended to have the lower percentage of birds alive (56%).

Table 24 Feed intake, body weight, % hen day egg production (HDEP), % birds alive and excreta moisture of birds fed different fibre sources

Diet	Feed intake (g/bird/day)	Body weight (g/bird)	HDEP (%)	Birds alive (%)	Excreta moisture (%)
Wheat	137^{ab}	2227	58^{a}	65	73.7
Rice hulls	150^{bc}	2148	59^{ab}	78	75.0
Protein Meal	148^{bc}	2149	63^{b}	56	75.6
Bentonite	153^{c}	2177	62^{b}	59	70.8
Millrun	125^{a}	2076	63^{b}	68	75.7
MOS	138^{abc}	2370	56^{a}	67	73.6
P-value	<0.01	NS	<0.01	<0.08	NS

Table 25 Amounts of insoluble and soluble NSP in diets

				Insoluble NSP				
Diets	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Glactose	Glucose
Wheat	0.3	0.2	0.2	19.4	30.0	2.4	4.4	29.7
Rice Hulls	0.3	0.2	0.2	19.2	39.9	1.5	4.5	63.1
Protein Meal	0.6	0.5	0.3	20.4	28.2	2.5	7.9	35.1
Bentonite	0.3	0.2	0.2	20.5	29.5	2.4	5.0	32.3
Millrun	0.2	0.3	0.3	30.4	40.9	2.3	5.8	43.8
MOS	0.2	0.0	0.2	20.5	34.1	1.7	3.3	32.5
				Soluble NSP				
	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Glactose	Glucose
Wheat	0.07	0.07	0.03	2.61	3.09	0.42	1.71	1.64
Rice Hulls	0.03	0.00	0.00	0.64	1.05	-0.01	0.24	0.45
Protein Meal	0.08	0.06	0.00	1.70	2.27	0.10	1.00	1.16
Bentonite	0.06	0.06	0.03	2.39	3.06	0.37	1.56	1.46
Millrun	0.06	0.06	0.03	1.46	1.95	0.43	1.09	1.78
MOS	0.07	0.06	0.26	2.94	3.30	0.29	1.42	1.51

6.4 Discussion

It is clear from the literature that grain species do differ in the level of NSP which in turn influences the physical and chemical properties of the individual NSP and that these can vary quite considerably between different polymers and different molecular weights of the same polymer (Morris, 1990). Analysis of the monosaccharide profile for diets in this experiment showed that the main sugars present in the insoluble fraction are arabinose, xylose and glucose, suggesting that the most insoluble NSP are insoluble arabinoxylan and insoluble glucan (Table 23).

The results from this study showed that birds fed the rice hull and millrun diets tended to have higher percentage of birds alive than those fed the other diets. This indicates that high-insoluble NSP content does have a potential in reducing cannibalism. The numerically higher percentage of birds alive of birds fed the rice hull than those fed the millrun diets suggest that different polysaccharides elicit different physiological function in the gut. It has been suggested that the way the monomer units of polysaccharides linked together differentiates the physical properties among polysaccharides (Morris, 1990). Another interesting finding is the total ineffectiveness of the inert filler, bentonite, on cannibalism. Obviously, birds fed the bentonite diet ate a great deal more to compensate for the energy dilution caused by inclusion of 5% bentonite, but it probably did not produce the effect of increased digesta transit like the insoluble NSP present in rice hull and millrun.

The reason that diets did not give a strong effect on percentage of birds alive may also be due to birds in the current study were the same birds used in the previous experiment without any changing in bird density. Thus, it is possible that during this time birds have learned to recognise their flock mates individually, resulting in clear and stable dominance relationships and an associated reduction in aggression and pecking.

Increased cannibalism mortality was postulated due to deficiency in protein level by Ambrosen and Petersen (1997). Protein analysis in the present study showed that the MOS diet had the highest protein level (20%), followed by wheat and bentonite diets (19%) and the lowest was protein meal, rice hull and millrun diets (18%). The protein meal diet had the lowest percentage of birds alive whereas the rice hull diet had the highest percentage of birds alive. These results suggest that the low protein level was not a factor contributing to an increased incidence of pecking.

Low sodium and potassium were reported to have increased mortality due to cannibalism (Cooke, 1992). In the present study, it was found that the protein meal diet had the lowest content of sodium and birds fed that diet had the lowest proportion of birds alive. Thus, it seems that the lower sodium concentration may influence the occurrence of cannibalism. However, the fact that birds fed the rice hull and millrun diets had higher percentage of birds alive than those fed the MOS diet, which had the highest sodium level, suggests that there are other factors involved. Again it suggests that insoluble NSP is one important factor in reducing cannibalism in birds as seen with the rice hull and millrun diets. Potassium content of the rice hull and millrun diets were not lower or higher than other diets, suggesting that potassium is unlikely to be involved in changing the incidence of cannibalism.

In conclusion, a dominance hierarchy will be exhibited in a group of birds that have been together for some time, due to individual recognition, and stable dominance groups may have reduced the variation in aggressive behaviour and the impact of diet. The higher percentage of birds alive in the rice hull and millrun diets, although only approach significant still indicates that insoluble NSP has a potential effect in reducing cannibalism mortality.

7. Effect of feed form and fibre levels on behaviour of laying hens

7.1 Introduction

The results from previous experiments (Chapters 4 and 6) suggest that diets high in insoluble NSP are effective in reducing mortality due to cannibalism. Furthermore, the effect of insoluble NSP on the rate of digesta passage seems to affect the birds' behaviour which in turn may result in reduced cannibalism mortality. The rice hull treatment in the previous experiment (Chapter 6) had the highest percentage of birds alive and the protein meal diet the lowest. Rice hulls contained a higher insoluble NSP than the protein meal diet whereas the soluble NSP level of both diets was low. Until now there have been no studies on the effect of fibre levels on the behaviour of laying hens.

It has also been reported that the form in which feed is given can influence the occurrence of cannibalism. A study by Lindberg and Nicol (1994) showed that a mash diet greatly reduced the likelihood of an outbreak of cannibalism, while a pelleted diet, on the other hand, increased the incidence of feather pecking and cannibalism. Birds fed pelleted diets were thought to spend less time feeding and therefore increased pecking. Pelleted feed has been reported to provoke nervousness in the birds and nervous birds are more likely to start pecking, while birds fed mash diet will stay perfectly calm. Fine feed encouraged birds to feed longer than coarse feed, consequently decreasing feather-pecking rates (Walser *et al*., 1996).

The present experiment aimed to examine the effects of fibre level and feed form on the behaviour of laying hens based on the hypothesis that a diet low in fibre will reduce feeding time and increase the incidence of pecking and associated behaviours.

7.2 Materials and Methods

ISA Brown birds (n = 72) at 70 weeks of age were used in this experiment. Birds used were those from the previous experiment. The design was 2 x 2 factorial (2 fibre levels vs 2 feed forms) with 6 replicates of 18 birds per treatment. Diets used included a diet containing rice hulls, which represented a diet with a high fibre level (H), and a protein meal diet, which represented a diet with a low fibre level (L), and mash (M) vs pelleted (P) diet forms. All diets were formulated and produced at a commercial mill in Tamworth. Ingredients and nutritional composition of experimental diets can be seen in Table 22. At the beginning of the experiment, two birds, which were long-term residents, were allocated to each cage and an additional 'unfamiliar' bird was added just prior to the commencement of behavioural observations to induce a disturbance to socia1 structure. Scanning observations were done in two sessions, in the morning and in the afternoon (a.m. vs p.m.) with three replications at hourly intervals per session. Each cage was observed for 5 min with instantaneous recordings made every 30 s. Behaviours recorded (yes or no) included: pecking of other birds (social pecking), feeding, moving, preening and drinking. Escape or 'freeze' behaviour in birds being pecked was also recorded.

A 'coloured-object pecking' test was also performed on birds from each treatment at the start and end of the experiment (4 wks later) to determine the frequency of pecking oriented toward red (blood) or feather (white/brown) colours. The application and measurement of behavioural observations and a pecking test were as described in Chapter 3. Apart from the periods when this test was conducted feed and water were provided *ad libitum*. In addition to the behavioural observations and pecking test, feed intake, egg production and feed to egg ratio were recorded. Mortality data were also recorded.

For behavioural observations, a generalized linear model using a binomial distribution was used to analyse the proportion of birds involved in each behaviour and to determine the effects of diet (D), feed form (F), time of day (a.m. vs p.m.) and interactions. Data from the pecking test and the production measurements were analysed using least squares analyses of variance procedures.

7.3 Results

7.3.1 Effect of treatments on behavioural observations

Birds given the high-fibre diet spent more time feeding than birds given the low-fibre diet ($P<0.01$). An interaction showed that birds fed low-fibre pelleted diets had the lowest feeding time ($P<0.01$) compared to low-fibre mash and the high-fibre diet (Table 26). Feeding frequency was higher during the afternoon observations ($P<0.01$) but there were no interactions found between fibre level and feed form.

Social pecking behaviour of the birds was not influenced by diet or time of day but was influenced by feed form ($P<0.01$), with pecking incidence being higher for the pelleted diets. A significant interaction ($P<0.01$) between diet and feed form showed that there was a difference in social pecking frequency between feed form in birds fed low fibre diets, with pelleted diets causing higher pecking frequency than mash. A significant difference ($P<0.01$) in pecking frequency for the two feed forms was also observed in birds fed high fibre diets, but the effect was reversed (Table 26).

A similar result to the above was observed in escape behaviour. An interaction ($P<0.01$) showed the highest escape behaviour in birds fed low-fibre pelleted diets, followed by high-fibre mash diet and finally high-fibre pelleted and low-fibre mash diets (Table 26). The incidence of escape behaviour was higher ($P<0.01$) during the morning but again there was no interaction between time of day and treatment.

Freeze incidence was low but influenced by diet ($P<0.01$) and feed form ($P<0.05$). An interaction ($P<0.01$) was due to a similar relative response to the four treatments as observed for escape behaviour, although the overall effect of diet was different, with higher freeze behaviour on high-fibre diet (Table 26).

Table 26 Mean percentage of cages exhibiting a behaviour in any minute of observation for different diets and feed form

	Feeding	Social Pecking	Escape	Freeze	Drinking
Diets					
Protein meal (L)	4.7[a]	7.4	7.6[a]	1.1[a]	1.9
Rice hulls (H)	6.8[b]	8.3	4.8[b]	3.0[b]	2.1
P-value	<0.01	NS	<0.01	<0.01	NS
Diet Forms					
Mash (M)	6.2	6.7[a]	4.8[a]	2.3[a]	1.6[a]
Pellet (P)	5.3	9.0[b]	7.7[b]	1.8[b]	2.3[b]
P-value	NS	<0.01	<0.01	<0.05	<0.05
Interaction					
L x M	16.2[b]	10.6[a]	7.2[a]	1.2[a]	5.4[a]
L x P	9.4[a]	32.0[c]	23.7[b]	4.9[b]	5.1[a]
H x M	16.0[b]	26.8[c]	16.0[b]	12.6[c]	4.5[a]
H x P	17.3[b]	19.5[b]	9.1[a]	5.7[b]	7.4[b]
P-value	<0.01	<0.01	<0.01	<0.01	<0.05

[a-c]values within a column with no common superscripts differ significantly (P<0.05). NS: not significant.

Preening behaviour incidence was low (mean 1.8%). Moving behaviour was not influenced by main treatments (fibre level vs feed forms), while drinking frequency was higher during the afternoon (P<0.01) and higher (P<0.05) on the pelleted high-fibre diets than on the other dietary treatments (Table 26).

In the pecking test, birds preferred to peck white grains (43%) than grains of other colours (P<0.01). The red (blood) colour was least pecked (26%). Birds given the low-fibre diets tended (P=0.1078) to peck the white grains more frequently (48%) than birds given the high-fibre diets (37%). Total number of pecks was unaffected by diet (28.8 vs 28.1) but was higher for birds fed pellets rather than mash (35.0 vs 21.9, P<0.08).

7.3.2 Effect of treatments on feed intake, feed to egg ratio and egg production

Feed intake was significantly affected by diet (P<0.01) but not by feed form. Birds on high-fibre diets consumed more (140 g) than birds fed low-fibre diets (120 g). No significant difference among diets was observed for feed-to-egg ratio. The percentage of hen-day egg production for low-fibre pellets, low-fibre mash, high-fibre pellets and high-fibre mash were 61%, 67%, 73% and 73%, respectively, and were not significantly different.

7.3.3 Effect of treatments on mortality

The rates of mortality during the experiment in low-fibre pellets, high-fibre pellets, low-fibre mash and high-fibre mash were 19%, 12%, 11% and 6%, respectively, and were not significantly different from one another.

7.4 Discussion

The significant interaction between diet and feed form found in almost all behavioural observations underlines the previous findings that dietary characteristics and feed form appear to influence the behaviours of laying hens. Fibre content had no effect on feeding behaviour of layers given mash diets, but in protein meal diets, birds given pellets spent significantly less

time feeding than when given a mash diet. The differences in total feed intake supported the finding. This confirms our original hypothesis that a more energy-dense (MJ/kgDM) diet would lead to a lower feeding time as fibre increases the bulk of the diet. A longer time spent feeding by birds fed pelleted high-fibre diets may develop as a response to increased fibre concentration over a period of time.

With social pecking frequency, the highest pecking was observed in birds fed low-fibre pellets. This result corresponds very well with a popular belief that birds fed pelleted diet will spend little time feeding and consequently will increase pecking. On the other hand, a comparable response of higher pecking behaviour was observed for birds given the high-fibre mash diet. This indicates that birds on high fibre diets may not readily satisfy their energy and nutrient requirements when given their feed in mash rather than in pelleted form and so increase social pecking behaviour. However, further studies are needed to clarify the effect of diet sources and diet form on pecking frequency and time. The implication for management of cannibalism by manipulation of dietary-fibre level is that there is a fine balance between increasing birds' feeding time to reduce social pecking whilst still meeting their nutritional requirements.

Escape and freeze behaviours are considered to be responses to social pecking behaviour (Appleby *et al*., 1989). In this experiment, escape incidence essentially mirrored pecking behaviour incidence. The high social pecking behaviour found in birds fed high-fibre mash diets did not end up with high mortality rate, which was in contrast with the low-fibre pelleted diets. The high incidence of freezing behaviour in birds fed the high-fibre mash diet might save the birds from being pecked further. It is possible that the disturbance caused by escape behaviour triggers pecking behaviour, as disturbed birds are more likely to initiate pecking (Hughes and Duncan, 1972).

There is no clear explanation for the treatment differences in the other behaviours recorded. Preening behaviour has been considered to be an indicator of social stability (Glatz, 2000). The very low incidence of preening behaviour (1.8%) and variability in incidence within a day suggest that more frequent observations would be needed before preening could reliably be used as an indicator of social stability. Furthermore, there was no evidence that either fibre levels or diet form altered the birds' colour preference. The birds preferred pecking white as opposed to red objects, which is contrary to a popular belief that cannibalistic birds are specifically attracted to the red colour of blood.

This study indicates that fibre level and feed form in which a diet is fed (mash vs pellets) can influence the pecking behaviour of laying hens. The incidence of feeding, pecking, escape and freeze behaviours may all be useful indicators of factors leading to the onset of cannibalism. Changes in feed form can alter the incidence of social pecking behaviour and to a degree this is associated with changes in the proportion of time spent feeding. More studies are needed to further quantify the relative effect of diet sources and feed form on pecking and cannibalism, but initial indications are that feed form is of practical significance only when birds are given low-fibre diets.

8. Effect of light intensity during rearing and dietary fibres on cannibalism mortality and performance of ISA Brown laying hens (second laying year)

8.1 Introduction

The results from the previous year (Chapter 4) demonstrated that the dim light conditions during the rearing period reduced mortality due to cannibalism, which is in agreement with the finding by Kjaer and Vestergaard (1999). These authors suggested that the reduction in cannibalism mortality of birds reared under dim light resulted in the development of gentle pecking behaviour which persisted throughout their life.

Beak trimming was found to drastically reduce cannibalism mortality (Chapter 4). Although there are concerns that beak-trimming may lead to chronic pain, it is the best method of controlling cannibalism in a production system where opened-sided houses with natural day lights are commonly used.

Diets high in fibre (insoluble NSP) content have the potential for reducing cannibalism (Chapter 5) and it was observed that ingestion of a high-insoluble NSP diet decreased the incidence of cannibalism by increasing the rate of digesta passage, which in turn caused birds to spend longer time eating and consequently less time pecking or cannibalizing. However, the results from Chapter 7 showed that the effect was only evident when high-fibre diets were given in pelleted form.

To determine if these effects also apply when other fibre sources are used, a similar experiment was carried out using a diversity of fibre sources. The experiment aimed to investigate the effect of light condition and dietary fibres on cannibalism mortality and performance of cage-housed hens.

8.2 Materials and Methods

8.2.1 Birds

Two experiments (Trials 1 and 2) were carried out. A total of 1344 day-old ISA Brown chickens was used in both trials. The management of birds during rearing, pre-lay and lay period was as described in Chapter 3. At the end of the rearing period (16 weeks), behavioural observations were determined. At 17 weeks of age, 1320 birds were allocated randomly into cages with 5 birds per cage (Trial 1), whereas 24 birds, regardless of the rearing condition, were kept in individual cages in aimed to observe further effect of experimental diets (Trial 2). Different from the previous year, in this study at 17 weeks of age birds were not offered the experimental diets as manufacturing problems delayed delivery of the diet. Instead, birds were given a commercial pre-lay diet.

At 20 weeks of age, birds in both Trials 1 and 2 were offered one of six pelleted-layer diets for 8 weeks. The design for Trial 1 was 2 x 6 factorial (2 rearing conditions vs 6 diets), whereas the design for Trial 2 was a Completely Randomized Design (CRD) with diet as a main effect.

8.2.2 Feeds

The diets used in the experiment were: a commercial (wheat) diet, oat-based, rice hull-based, millrun-based diets, and a commercial (wheat) diet plus 0.2% manno-oligosaccharides (MOS) or 0.2% fructooligosaccharides (FOS). All diets were formulated according to commercial specifications and were produced at a commercial mill (Ridley AgriProducts, Tamworth, NSW). Feed and water were available *ad libitum* throughout the experiment. Nutrient composition of each diet is given in Table 27.

Table 27 Composition of experimental diets (g/kg)

Ingredients	Wheat	Oats	Rice hulls	Millrun	MOS	FOS
Wheat 12.5%	426	50.5	386.6	291.2	426	426
Sorghum 10.5%	250	250	250	250	250	250
Oats whole feed	-	400.3	-	-	-	-
Rice hulls	-	-	75	-	-	-
Millrun	-	-	-	200	-	-
Manno-oligosaccharides	-	-	-	-	2	-
Fructo-oligosaccharides	-	-	-	-	-	2
Rice pollard 13%	50	-	-	-	50	50
Meat meal 55%	87.5	100	100	100	87.5	87.5
Blood meal	-	7	4.2	-	-	-
Oil	-	10	20	15	-	-
Soybean meal 48%	33	15.5	31.1	7	33	33
Sunflower meal 30%	50	-	-	-	50	50
Cottonseed meal 37%	-	10	9	-	-	-
Soy flour	5	13	40.2	54	5	5
Limestone	85	71.5	73	75.5	85	85
Rockphos	5	5	3.6	-	5	5
Kynofos	0.5	-	-	-	0.5	0.5
Sodium bicarbonate	1.5	1.5	1.6	1.5	1.5	1.5
Choline chloride 75%	0.3	0.3	0.3	0.3	0.3	0.3
DL-methionine	1.2	1.4	1.2	1.3	1.2	1.2
L-Lysine	1.5		0.7	0.7	1.5	1.5
Layer/pullet premix	2	2	2	2	2	2
Synthetic yolk colour premix	1.5	1.5	1.5	1.5	1.5	1.5
Chemical composition[a]						
ME, kcal/kg	2712.7	2700.6	2700.5	2700.5	2712.7	2712.7
Crude protein	170	170	170	175	170	170
Fat	36.1	59.8	54.1	57.8	36.1	36.1
Fibre	34.6	60.0	50.4	35.5	34.6	34.6
Methionine	3.5	3.5	3.5	3.5	3.5	3.5
Lysine	7.8	7.9	7.8	7.8	7.8	7.8
Calcium	41.9	38.1	38.0	38.0	41.9	41.9
Nonphytate Phosphorus	5.0	5.0	5.0	5.0	5.0	5.0

[a]Calculated from the nutrient composition of the ingredients

8.2.3 Variables measured

The variables measured in each of three recording periods, viz. rearing period, day-old to 15 weeks, pre-lay, 17 to 19 weeks and lay, 20-28 weeks.

8.2.3.1 Rearing period

During the rearing period, body weights as well as mortality data were recorded. Behavioural data for latency and pecking tests as well as feather score were recorded at the end of the rearing period (16 weeks).

8.2.3.2 Pre-lay period

In the pre-lay period, mortality and egg production were recorded daily and birds' behaviour was monitored.

8.2.3.3 Lay period

Trial 1

In Trial 1, the variables measured were feed intake, body weight, egg production, percentage of dirty eggs and total mortality. Feed intake and egg production were recorded weekly whereas mortality was recorded daily. The dietary concentrations of soluble and insoluble NSP, protein and minerals were also determined.

Trial 2

At the end of 28 weeks, plumage condition was scored before birds were weighed and killed by cervical dislocation. Variables measured included digesta viscosity, excreta moisture, and weights of gizzard, jejunum, ileum and caeca (%BW). In addition, soluble and insoluble NSP in jejunal and ileal digesta were measured.

All the measurements and analyses for both in Trials 1 and 2 were performed as described in Chapter 3. As in previous experiments, for welfare reasons, birds that were severely cannibalized were also removed to another house and recorded as “dead”.

Results were analysed statistically using ANOVA.

8.3 Results

8.3.1 Rearing period: day-old to 15 weeks.

As in the previous year, birds reared under bright light appeared more aggressive than those reared under dim light. At 8 weeks of age, two birds in bright rearing were cannibalized and by 10 weeks of age, three additional birds were cannibalized. There was no such activity observed in the birds kept under dim light.
The average body weight of birds reared under bright light, regardless of the age, was lower than that of those reared under dim light. The average body weights of the birds reared under bright or dim light were 1052g vs 1103g, respectively, at 11 weeks of age. At 15 weeks of age, this difference remained; the average body weights of the birds reared under bright or dim light were 1425g vs 1492g, respectively.

In the pecking test, birds preferred to peck white (45%) and brown grains (45%) ($P<0.01$). The red grains were least pecked (10%). Feather scores for bright and dim light were 13.9 and 14.3,

respectively, and not significantly different (P>0.05). The tail was the area that was most highly pecked (P<0.01). The latency time of birds reared under bright light tended to be shorter than of those reared under dim light (P<0.06). The latency time of birds reared under bright and dim light were 78 and 124 seconds, respectively.

At 15 weeks of age, birds were transferred from the rearing house to the layer house (7 km distance between the two houses). Similarly to the previous year, the birds reared under dim light appeared very calm, which contrasted sharply with birds reared under bright light. Those reared under bright light appeared very aggressive.

8.3.2 Pre-lay period: 17 to 19 weeks

The first eggs were seen at 17 weeks of age, when cannibalism also started to occur. Birds were often cannibalized when they were in the process of lay and cannibalistic behaviour of birds increased with increasing numbers of birds in lay. Birds reared under bright light showed more cannibalistic behaviour than those reared under dim light and at the end of the pre-lay period, when egg production reached 40%, the total cannibalism mortality reached almost 23% in birds which were reared under bright light, whereas it was about 9% for those reared under dim light.

Since it is almost impossible to prevent the cannibalism once it commences, to reduce the cannibalistic behaviour of birds, a light beak-trimming (tip off) was applied two days before the experimental diets were given.

8.3.3 Lay period: 20 to 28 weeks.

The soluble NSP content was higher (P<0.01) in the oat diet than in the other diets, which did not differ significantly (P>0.05). The insoluble NSP content was highest in the millrun and rice hull diets, and was lowest in the FOS diet (P<0.01) (Table 28).

Table 28 Soluble, insoluble NSP, protein, Na and K contents of the diets

Experimental diet	Soluble NSP (g/kg)	Insoluble NSP (g/kg)	Protein (%)	Na (µg/g)	K (%)
Wheat	6.04[a]	68.9[bc]	17	1074.1	0.66
Oats	10.4[b]	67.5[ab]	18	1233.7	0.63
Millrun	5.88[a]	93.4[d]	17	1089.6	0.67
Rice hulls	5.06[a]	92.5[d]	16	1103.8	0.58
MOS	5.24[a]	71.5[c]	17	1067.9	0.64
FOS	5.62[a]	64.9[a]	16	1498.7	0.61
P-value	<0.01	<0.01	n.a	n.a	n.a

[a-d]values within a column with no common superscripts differ significantly (P<0.05). NS: not significant, n.a: not available.

The constituent components of NSP in the diets are shown in Table 29. The main sugars present in the insoluble fractions were arabinose, xylose and glucose, whereas the concentration of sugar present in the soluble fraction was low and mainly arabinose, xylose, galactose and glucose.

Table 29 Constituent components of NSP in the experimental diets (g/kg DM)

	Wheat	Oats	Millrun	Rice hulls	MOS	FOS	P-value
Insoluble NSP							
Rhamnose	0.34[d]	0.00[a]	0.21[bc]	0.18[b]	0.31[d]	0.23[c]	<0.01
Fucose	0.25[c]	0.27[c]	0.25[c]	0.21[b]	0.27[c]	0.16[a]	<0.01
Ribose	0.18[d]	0.00[a]	0.23[e]	0.14[b]	0.18[d]	0.16[c]	<0.01
Arabinose	17.8[d]	12.9[a]	25.5[e]	16.1[bc]	17.2[cd]	15.0[b]	<0.01
Xylose	21.4[a]	22.1[a]	33.2[d]	28.5[c]	22.1[a]	24.5[b]	<0.01
Mannose	3.23[bc]	1.99[a]	2.33[a]	2.62[b]	3.23[bc]	3.68[c]	<0.01
Galactose	5.64[b]	5.39[a]	6.44[d]	7.13[c]	5.94[c]	5.37[a]	<0.01
Glucose	28.7[b]	33.2[d]	36.9[e]	49.1[e]	31.0[c]	23.9[a]	<0.01
Soluble NSP							
Rhamnose	0.08	0.06	0.06	0.06	0.05	0.07	<0.05
Fucose	0.06	0.05	0.06	0.05	0.05	0.06	NS
Ribose	0.04[b]	0.04[b]	0.03[a]	0.03[a]	0.03[a]	0.03[a]	<0.01
Arabinose	1.67[c]	1.02[a]	1.59[c]	1.29[ab]	1.25[a]	1.54[bc]	<0.01
Xylose	1.66[b]	0.64[a]	1.89[b]	1.35[ab]	1.65[b]	1.56[b]	<0.05
Mannose	0.39	0.44	0.34	0.36	0.46	0.39	NS
Galactose	1.44	1.20	1.15	1.23	1.18	1.34	NS
Glucose	1.44[a]	8.20[b]	1.50[a]	1.31[a]	1.23[a]	1.34[a]	<0.01

[a-e]values in a row with uncommon superscripts differ significantly (P<0.05). NS: not significant.

8.3.3.1 Effect of diets on NSP concentration in jejunal and ileal digesta

There was no significant difference in concentration of soluble NSP both in the jejunum and ileum (P>0.05), but the concentration of insoluble NSP in the jejunum and ileum was significantly affected by diets (Table 30). The oat diet had the highest insoluble NSP concentration in the jejunum (P<0.01) and ileum (P<0.05), but the latter was not different from that in the rice hull and millrun diets. The concentration of free sugars was not different in the jejunum and only tended to be different (P<0.06) in the ileum.

Table 30 Amounts of free sugars, insoluble and soluble NSP (g/kg DM) in jejunal and ileal digesta of laying hens fed different diets

	Jejunum			Ileum		
Diet	Free sugars	Insoluble NSP	Soluble NSP	Free sugars	Insoluble NSP	Soluble NSP
Wheat	48.4	143[bc]	14.3	8.34	198[ab]	12.7
Oats	15.6	314[d]	12.4	7.15	284[c]	24.2
Millrun	36.8	200[c]	21.3	10.4	242[bc]	25.2
Rice hulls	20.9	202[c]	11.0	7.91	230[abc]	24.7
MOS	70.2	113[ab]	11.6	5.00	177[a]	16.2
FOS	27.2	72.5[a]	12.6	26.7	178[a]	22.2
P-value	NS	<0.01	NS	<0.06	<0.05	NS

[a-d] values within a column with no common superscripts differ significantly (P<0.05). NS: not significant.

A detailed analysis of insoluble NSP in the jejunal and ileal digesta showed that the oat diet had the highest concentration of insoluble xylose (P<0.01) and glucose (P<0.01) in the jejunum (Table 31) and ileum (Table 32). The millrun diet had the highest concentration of insoluble arabinose (P<0.01) in both the jejunum and ileum. The FOS diet had the lowest concentration of insoluble arabinose, xylose and glucose in the jejunum and its concentration of insoluble

xylose and glucose was also lower in the ileum than that for most other diets. However, the concentration of free sugar glucose tended to be higher in the ileum for the FOS diet ($P<0.08$), followed by the wheat and millrun diets.

8.3.3.2 Effect of diets on bird performance

Light conditions and diet significantly affected feed intake ($P<0.01$) with a significant D x L interaction ($P<0.05$). The birds reared under dim light consumed more feed than those reared under bright light (105g vs 101g). Birds reared in dim light and fed the oat and rice hull diets had a higher intake ($P<0.01$) than all the other treatments (Table 33). Light conditions also affected body weight and total mortality. Thus, birds reared in dim light had a heavier body weight ($P<0.01$) and lower mortality ($P<0.01$) than those reared in bright light (Table 33).

For weeks 20 to 28 the average egg production reached 93% and was not influenced by diet or rearing conditions ($P>0.05$) (Table 33). The percentage of dirty eggs was higher in the MOS diet, and was lowest in the wheat and oat diet ($P<0.01$, Table 33).

8.3.3.3 Effect of diets on digesta viscosity, gastrointestinal weight and feather score

Diet did not affect digesta viscosity, excreta moisture, or relative ileal and caecal weights ($P>0.05$) (Table 34). The relative gizzard weight ($P<0.01$) was higher in birds on the oat diet and jejunal weight also tended ($P<0.08$) to be higher on the oat diet. The effect of diet on feather score approached significance ($P<0.09$). Birds fed the oat diet tended to have a better feather condition than those fed other diets (Table 34). Birds fed the wheat diet had the lowest total feather score.

Table 31 Neutral sugar composition in the jejunal digesta of laying hens fed different diets (g/kg DM)

				Insoluble NSP				
Diet	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose
Wheat	0.67	0.97	0.34	38.2[b]	49.5[ab]	4.60	9.06	57.5[ab]
Oats	0.48	1.12	0.24	35.1[b]	153.2[c]	3.00	10.59	149[d]
Millrun	0.45	0.71	0.17	58.1[c]	80.5[b]	3.88	8.16	73.5[bc]
Rice hulls	0.63	1.01	0.41	38.4[b]	73.4[b]	3.53	10.31	99.6[c]
MOS	0.53	0.81	0.27	28.4[ab]	39.6[a]	3.62	8.73	45.5[ab]
FOS	0.43	0.90	0.28	18.4[a]	23.0[a]	4.06	8.02	26.4[a]
P-value	NS	NS	NS	<0.01	<0.01	<0.06	NS	<0.01
				Soluble NSP				
Diet	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose
Wheat	0.95	1.38	0.76	3.24	3.06	1.30	2.52	2.90[a]
Oats	1.92	1.80	0.63	1.41	0.95	2.02	2.08	3.10[ab]
Millrun	1.45	2.18	0.79	4.84	5.67	1.77	2.86	4.35[b]
Rice hulls	1.36	0.96	0.48	2.27	2.49	0.87	2.26	2.29[a]
MOS	0.65	0.84	0.34	2.94	3.25	0.84	2.23	1.90[a]
FOS	0.11	0.71	0.18	3.66	3.51	0.67	3.42	1.95[a]
P-value	NS	NS	NS	NS	<0.07	NS	NS	<0.05
				Free Sugars				
Diet	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose
Wheat	0.04[a]	0.12[bc]	0.17	0.24[ab]	0.23[bc]	1.98[b]	2.80	42.8
Oats	0.00[a]	0.00[a]	0.00	0.09[a]	0.00[a]	0.52[a]	1.74	14.4
Millrun	0.00[a]	0.11[bc]	0.15	0.24[ab]	0.25[bc]	1.77[b]	2.38	31.9
Rice hulls	0.00[a]	0.05[ab]	0.07	0.22[ab]	0.18[b]	1.32[ab]	1.83	17.2
MOS	0.00[a]	0.12[bc]	0.18	0.34[bc]	0.38[c]	2.09[b]	3.73	63.4
FOS	0.31[b]	0.15[c]	0.24	0.53[c]	0.66[d]	2.01[b]	2.72	20.7
P-value	<0.01	<0.05	NS	<0.05	<0.01	<0.05	NS	NS

[a-d]values within a column with no common superscripts differ significantly (P<0.05). NS: not significant

Table 32 Neutral sugar composition in the ileal digesta of laying hens fed different diets (g/kg DM)

				Insoluble NSP				
Diet	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose
Wheat	1.0	1.6	0.8	57.4[c]	64.9[ab]	5.6	12.7	79.0[a]
Oats	0.0	1.4	0.6	29.6[a]	140.7[d]	3.1	10.4	141[c]
Millrun	0.7	1.4	0.7	70.4[d]	91.1[c]	5.9	13.1	89.3[ab]
Rice hulls	0.8	1.4	0.7	46.4[b]	78.5[bc]	4.5	13.6	113[b]
MOS	1.1	1.6	0.9	49.2[bc]	58.7[a]	4.8	13.7	68.8[a]
FOS	0.5	0.9	0.3	48.7[bc]	62.1[a]	4.1	12.2	72.5[a]
P-value	NS	NS	NS	<0.01	<0.01	<0.09	NS	<0.01
				Soluble NSP				
Diet	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose
Wheat	1.23	2.25	0.48	2.70	2.83	1.14	2.09	2.43[a]
Oats	2.04	4.77	1.32	2.06	1.28	2.68	3.71	9.15[c]
Millrun	1.37	2.83	0.63	5.85	7.28	1.90	3.85	4.63[b]
Rice hulls	1.82	2.97	1.64	5.29	5.65	1.83	5.08	3.47[ab]
MOS	0.22	0.88	0.17	4.54	5.32	0.64	3.60	2.83[ab]
FOS	1.45	2.58	1.67	5.04	5.40	1.96	3.86	2.97[ab]
P-value	NS	NS	NS	NS	NS	NS	NS	<0.01
				Free Sugars				
Diet	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose
Wheat	0.00	0.05	0.05	0.11	0.09	0.50	0.91	6.70
Oats	0.13	0.16	0.12	0.18	0.10	1.09	3.12	2.40
Millrun	0.00	0.11	0.08	0.29	0.22	1.65	2.44	5.68
Rice hulls	0.06	0.11	0.04	0.50	0.69	1.52	2.31	3.41
MOS	0.00	0.11	0.04	0.32	0.29	1.01	1.02	2.31
FOS	0.05	0.16	0.18	0.45	1.04	2.03	4.32	18.5
P-value	NS	NS	NS	NS	NS	NS	NS	<0.08

[a-d]values within a column with no common superscripts differ significantly (P<0.05). NS: not significant.

Table 33 Feed intake, body weight, egg production, percentage of dirty eggs and total mortality of laying hens fed different dietary fibres

Experiment Diet	Feed intake (g/bird/day)	Body weight (g)	Egg production (%)	Dirty eggs (%)	Total mortality (%)
Diet (D):					
Wheat	100[ab]	1900	91	6.37[a]	33
Oats	109[c]	2029	90	7.26[a]	29
Millrun	101[ab]	2025	85	9.71[ab]	28
Rice Hull	108[c]	1883	86	12.6[bc]	21
MOS	103[b]	1933	90	14.4[c]	24
FOS	99[a]	1991	93	10.3[ab]	26
P-value	<0.01	NS	NS	<0.01	NS
Light Rearing (L):					
Dim	105[a]	2024[a]	91	9.98	22[a]
Bright	101[b]	1897[b]	88	10.2	32[b]
P-value	<0.01	<0.01	NS	NS	<0.01
Interaction:			P-value		
D x L	<0.01	NS	NS	NS	NS

[a-c]values within a column with no common superscripts differ significantly (P<0.05). NS: not significant.

Table 34 Intestinal digesta viscosity, excreta moisture, relative gizzard, jejunal, ileal, caecal weight and feather score of laying hens fed different dietary fibres

Diet	Viscosity (mPa.s)	Excreta moisture (%)	Gizzard weight (%BW)	Jejunal weight (%BW)	Ileal weight (%BW)	Caecal weight (%BW)	Feather score
Wheat	3.0	73	1.40[a]	1.11	0.80	0.50	12.0
Oats	2.5	71	2.38[b]	1.26	0.88	0.52	15.1
Millrun	2.9	72	1.48[a]	1.21	0.92	0.44	14.3
Rice Hull	2.9	74	1.39[a]	1.16	0.88	0.50	14.5
MOS	2.7	77	1.15[a]	0.94	0.84	0.55	14.9
FOS	2.3	75	1.32[a]	0.96	0.74	0.48	13.9
P-value	NS	NS	<0.01	<0.08	NS	NS	<0.09

[a-b]values within a column with no common superscripts differ significantly (P<0.05). NS: not significant.

8.4 Discussion

The results of the present study clearly show that conditions of rearing influence the development of pecking behaviour. Birds reared under dim light were calmer and less aggressive compared to those reared in bright light. The birds carried their calm and non-aggressive behaviour throughout the experiment (28 weeks). This is in contrast with our previous results (Chapter 4), where the birds under dim light showed aggressive behaviour when moved to bright light during the pre-lay period. In the present study, birds were not exposed to stress due to beak trimming before they were moved to a laying house. A light beak-trimming (tip off) was applied to birds after they adapted to the new environment (bright light)

for a few weeks. Thus, it is possible that less exposure to stress resulted in birds coping better with sudden environmental changes.

Craig *et al*. (1983) found that fearful behaviour was associated with greater feather loss. Severely feather pecked birds were more fearful than birds with minor pecking damage (Hughes and Duncan, 1972). In the present study, birds reared in bright light had a higher mortality and a lower feather score than those reared in dim light, suggesting that birds reared in bright light were more fearful than those reared in dim light. However, the fact that birds reared in bright light tended to have a shorter latency time was not in agreement with the finding by Johnsen *et al*. (1998) who suggested that the more fearful the birds the longer the latency time (tonic immobility). Jones *et al* (1995) also demonstrated that chicks of the low-pecking lines showed less freezing time than high-pecking lines. In the present study, when the latency test was carried out, some birds from bright rearing showed a movement, but with a tendency to escape after that. An accidental error in recording this condition may influence the results obtained.

The interaction between rearing conditions and diets showed that birds reared in dim light and fed the oat and ricehull diets had a higher intake and a higher body weight than those reared in dim light and offered other diets. Birds reared in dim light were calmer than birds reared in bright light, indicating that less energy was used for movement. The high concentration of insoluble NSP in the jejunal and ileal digesta of birds fed the oat and rice hull diets may speed up the rate of digesta passage, resulting in birds feeling hunger quickly, and consequently increasing intake. However, the physical structure of the oat diet may also contribute to the increase in feed intake. Hetland and Svihus (2001) proposed that physical structure of oat hulls initiated the increase of gut capacity and rate of feed passage, consequently allowing increased feed consumption. The larger gizzard in birds fed the oat diet supported the effect of oats' physical structure.

Millrun-fed birds did not increase feed consumption despite the high concentration of insoluble NSP in the jejunal and ileal digesta. Although not statistically different, the concentration of soluble NSP in the jejunum and ileum of millrun-fed birds was higher than that in those fed other diets. Analysis of the monosaccharide composition of the NSP fractions in the ileum of millrun-fed birds revealed that the concentration of glucose in the free sugar fraction tended to increase, suggesting that there was partial depolymerisation of the NSP fraction present in millrun. It was possible that the depolymerisation process influenced the physiological function of insoluble NSP. Physicochemical properties of individual NSP strongly affected the NSP's physiological actions, therefore not all NSP have the same physiological action (Southgate, 1995).

NSP such as β-glucans and pentosans are well known for their effect in increasing digesta viscosity. This has been shown for partially purified NSP (Ikegami *et al*., 1990) as well as for compounds derived from normal feed ingredients such as wheat (Annison and Choct, 1991).

Analysis on the diets used in the experiment showed that the oat diet had a higher soluble NSP concentration than other diets, whereas the concentration of insoluble NSP was higher in the millrun and rice hull diets. The monosaccharides profile showed that the main sugar present in the soluble fractions of the oat diet was glucose, indicating that most soluble NSP in the oat diet were mainly soluble glucans. The main sugars present in the insoluble fractions of the millrun and rice hull diets were xylose, glucose and arabinose, suggesting that most insoluble NSP in those diets were insoluble arabinoxylan and insoluble glucan.

Diets did not have a significant effect on mortality. The previous study (Chapter 5) concluded that the incidence of cannibalism mortality increased in birds fed diets containing high soluble NSP due to increased digesta viscosity, resulting in a slower rate of digesta passage, which in turn induced the feeling of fullness. In the present study, we found that diet did not have a

significant effect on digesta viscosity. A detailed analysis of the monosaccharide profile of soluble NSP fraction in the jejunal and ileal digesta revealed that there was no significant release of soluble NSP into the digesta. Water-soluble fibre in oat bran has been reported to be not totally soluble in water (Anderson *et al*., 1980). The solubility of NSP depends on their chemical structure and association with the rest of the cell wall components and perhaps at an appropriate ratio between soluble and insoluble fractions, the anti-nutritive effect of soluble NSP might be minimized (Choct, 1997). Although not statistically different, it can be seen that birds fed the oat diet had a higher mortality than those fed other diets. This may be a result of the physical structure of the diet. A high concentration of insoluble xylose was observed in the jejunal and ileal digesta of birds fed the oat diet. Xylans are mainly found in the hull proportion and although the feed intake increased, the high proportion of hull might not have satisfied the birds' nutrient requirements, with the consequence that increased pecking led to cannibalism. The finding reinforces our previous hypothesis (Chapter 7) that there is a fine balance between increasing birds' feeding time to reduce pecking behaviour whilst still meeting their nutritional requirements.

It could have been expected that the concentration of insoluble NSP in the rice hull and millrun diets would have reduced mortality due to cannibalism. However, it was found that the type and amount of ricehull and millrun used in this experiment were very different (lower) to previous diets due to commercial manufacturing of the diets. The fibre (insoluble NSP) levels were not high and clearly the method of processing can affect the physicochemical properties of dietary fibre, which in turn will result in different effects on metabolic and physiological processes. In addition, the extreme fluctuation in the composition of millrun produced in different mills is probably responsible for the variable responses obtained in this series of studies. The ME value of millrun produced in Australia can vary from 6 MJ to 12 MJ/kg DM in chickens (unpublished data, UNE).

The percentage of dirty eggs was higher in birds fed the MOS and rice hull diets, but lower in birds fed the wheat and oat diets. Included in dirty eggs were eggs with blood stains, even if it was only a little spot. The blood was suspected to be the result of vent pecking and the lower percentage of dirty eggs in birds fed the wheat and oat diet could possibly be due to severe pecking, resulting in no pecked birds surviving, and consequently no dirty eggs were observed. On the other hand, birds fed the rice hull and MOS diets might experience some pecking, not severe but enough to cause a wound, as a consequence dirtying the eggs. It is possible that the insoluble NSP content in the rice hull and MOS diet reduced the pecking behaviour. The lower percentage of total mortality in birds fed the rice hull and MOS diets supported the hypothesis.

The results obtained in this experiment reinforced the findings from the previous year that dim light during rearing influences the development of pecking behaviour. Soluble NSP with their properties to increase digesta viscosity, and insoluble NSP with their properties to increase rate of digesta passage, definitely have an effect on the incidence of cannibalism. However, the actions of both soluble and insoluble NSP in the gut may be modified by their interaction with other cell wall components of grains. Furthermore, there is a fine balance between increasing birds' feeding time to reduce pecking whilst still meeting their nutritional requirements.

9. Different sources of NSP and feed form on cannibalism mortality, behaviour and digesta transit time in laying hens

9.1 Introduction

The use of mash diets is believed to reduce problems with feather pecking (Aerni *et al*., 2000) and these authors suggested that hens fed on mash increased the time spent feeding, and therefore spent less time pecking. In our experiment (Chapter 7) we found that a diet high in fibre, when given in mash form, increased social pecking behaviour and suggested that a high-fibre mash diet might not readily satisfy the nutritional requirements of birds. Normal growth can be secured on a pelleted ration which is higher in fibre (Esmail, 1997).

Results from Chapters 5 and 8 confirmed the hypothesis that high insoluble NSP content in diet with their properties of increasing the rate of digesta passage was effective in reducing cannibalism mortality. On the other hand, high soluble NSP content in the diet which increased digesta viscosity, resulted in increased pecking. To investigate further whether these suggestions hold true for different sources of NSP and feed forms, three auxiliary trials were designed.

9.2 Materials and Methods

9.2.1 Trial 1

A total of 825 ISA Brown laying hens at 37 weeks were used in this study. The birds used were those from the previous experiment. Birds were allocated randomly into cages with 5 birds per cage. Three different diets: a commercial (wheat) diet, a commercial (wheat) diet plus 2% guar gum (guar) or 4% lucerne meal (lucerne) in mash form were offered for a duration of 12 weeks. The commercial (wheat) diet used was the same diet used in the previous experiment (Chapter 8). Feed and water were available *ad libitum* throughout the experiment. The variables measured were soluble and insoluble NSP of diets, body weight, feed intake, egg production and total mortality.

A coloured-object pecking test was applied at the end of the experiment. The application and measurement of a pecking test were as described in Chapter 3.

Data were analysed using ANOVA.

9.2.2 Trial 2

ISA Brown laying hens (n = 72, at 54 weeks) were used in this experiment. The design was 2 x 2 factorial (2 fibre levels vs 2 feed forms) with 6 replicates of 18 birds per treatment. Diets used were a rice hull diet which represented a diet with higher fibre level (H), and a commercial (wheat) diet which represented a diet with low fibre level (L) and mash (M) vs pelleted (P) forms. The ingredients and nutrient composition of experimental diets can be seen in Table 27. The procedure to allocate experimental birds was the same as in Chapter 7 (Section 7.2), in which an additional 'unfamiliar' bird was added just prior to the commencement of behavioural observations. Scanning observations were done in two sessions, in the morning and in the afternoon (a.m. vs p.m.) with three replications at hourly intervals per session. The method of behavioural observation and measurement carried out was the same as that in Chapter 7 (Section 7.2). Each cage was observed for 5 min with instantaneous recordings made every 30 s, and

behaviours recorded (yes or no) were pecking of other birds (social pecking), feeding, moving, preening, drinking and escape or 'freeze' behaviour in birds being pecked.

A 'coloured-object pecking' test was also performed on birds from each treatment at the start and end of the experiment. The application and measurement of behavioural observations and a pecking test were as described in Chapter 3. During the experiment birds had free access to feed and water. Apart from the behavioural observation, feed intake, body weight, egg production, percentage of dirty eggs and total mortality were also recorded.

As in the previous experiment (Chapter 7), a generalized linear model using a binomial distribution was used to analyse the proportion of birds involved in each behaviour and to determine the effects of diet (D), feed form (F), time of day (a.m. vs p.m.) and interaction. Regression analysis was employed to assess relationships between social pecking behaviour and other behaviour parameters. Data from the pecking test and the latest measurements were analysed using ANOVA.

9.2.3 Trial 3

A total of 56 ISA Brown laying hens at 67 weeks of age were used in this experiment. Birds were put individually in cages and offered diets which differed in fibre levels and feed forms for 8 weeks. Millrun was used to represent a diet with higher fibre level (H), and commercial (wheat) diet for a diet with low fibre level (L) and mash (M) vs pelleted (P) forms. The ingredients and nutrient composition of experimental diets can be seen in Table 35.

At the end of 8 weeks, all birds were infused with a gelatine capsule containing a digestibility marker (80mg $C_{36}H_{74}$/capsule). Birds were killed at 7 different times after infusion, 30min, 60min, 90min, 120min, 150min, 180min, and 210min. Gastrointestinal tract was segmented into 6 parts: crop, gizzard, duodenum, jejunum, ileum and large intestine. Digesta were collected from each segment and alkane concentrations were determined. Rate of digesta passage through the gizzard and the small intestine was calculated as in Svihus *et al* (2002), which is the amount of marker infused substracted by the amount of marker anterior to the duodenum and the ileo-caeco-colonic junction.

Table 35 Composition of experimental diets (g/kg)[1]

Ingredient	High Fibre Diet (H)	Low Fibre Diet (L)
Wheat 10%	-	600
Sorghum	386.9	25.75
Millrun	320	50
Soybean meal 48%	102	150
Limestone	85	90
Meat meal and bone meal	68	20
Tallow	34	-
Vitamine	2	2
DL-methionine	1.45	1.2
Choline	0.35	0.35
Lysine	0.3	0.2
Cottonseed solvent	-	40
Dicalcium phosphate	-	15
Sunflower oil	-	3
Salt	-	2.5
Chemical composition[a]		
ME, kcal/kg	2631	2633
Crude Protein	170.6	170.6
Fat	69.1	22.0
Fibre	45.6	34.9
Methionine	3.50	3.39
Lysine	7.39	7.76
Calcium	38.7	38.8
Nonphytate Phosporus	5.04	5.03

[1]Diets were formulated and produced by University of New England, Armidale, NSW.
[a]Calculated from the nutrient composition of the ingredients

9.3 Results

9.3.1 Trial 1

The NSP composition of the diets is presented in Table 36. The soluble NSP content of the guar diet appeared higher than the other diets but the result was not significant. The content of insoluble NSP was similar between diets.

Table 36 Composition of experimental diets (Trial 1)

Diet	Free sugars	Insoluble NSP	Soluble NSP
Wheat	19.4	68.9	6.04
Guar	21.9	67.5	22.9
Lucerne	21.8	66.4	9.27
P-value	NS	NS	NS

The constituent components of NSP in the diets are shown in Table 37. The main sugars present in the insoluble fractions were arabinose, xylose and glucose, whereas the concentration of sugars present in the soluble fraction was low and mainly arabinose, xylose, galactose and glucose.

Table 37 Constituent components of NSP in the experimental diets (g/kg DM)

				Soluble NSP				
Diet	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose
Wheat	0.08	0.06	0.04	1.67	1.66	0.39	1.44	1.44
Guar	0.22	0.04	0.27	3.65	3.59	9.04	7.15	1.67
Lucerne	0.18	0.04	0.24	3.05	2.97	0.64	1.84	1.47
				Insoluble NSP				
	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose
Wheat	0.34	0.25	0.18	17.8	21.4	3.23	5.64	28.7
Guar	0.4	0.2	0.2	20.1	24.6	2.3	3.2	25.1
Lucerne	0.5	0.2	0.1	17.6	24.4	1.6	3.1	27.3
				Free sugars				
	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose
Wheat	0.0	0.0	0.0	0.26	0.07	1.74	2.42	14.9
Guar	0.08	0.10	0.10	0.28	0.16	0.07	2.91	18.25
Lucerne	0.13	0.34	0.10	0.37	0.18	0.90	3.05	16.93

9.3.1.1 Effect of diets on bird performance

Feed intake, body weight and egg production were affected by diet (Table 38). Birds on the guar diet had the lowest intake ($P<0.01$) and body weight ($P<0.05$) and the lowest egg production ($P<0.01$). Percentage of total mortality was not statistically different between diets.

Table 38 Feed intake, body weight, egg production and mortality of laying hens fed different diets

Diet	Feed intake (g/bird/day)	Body weight (g)	Egg production (%)	Total mortality (%)
Wheat	153[c]	2064[b]	83[b]	8.4
Guar	111[a]	1827[a]	71[a]	11.3
Lucerne	147[b]	2190[b]	86[b]	6.9
P-value	<0.01	<0.05	<0.01	NS

[a-c]mean values within a column with different superscripts differed significantly ($P<0.05$). NS: not significant.

9.3.2 Trial 2

Totals of soluble NSP were 6.04 and 5.06 g/kg DM for the low-fibre and the high-fibre diets, respectively, whereas the concentration of insoluble NSP content was 68.9 g/kg DM for the low-fibre diet and 92.5 g/kg DM for the high-fibre diet (Table 28). A detailed analysis of constituent components of NSP and free sugars can be seen in Table 29.

9.3.2 1 Effect of treatments on behavioural observations

The results in the present study were similar to that in the previous year (Chapter 7). Birds given a high-fibre diet spent more time feeding than birds given the low-fibre diet ($P<0.01$). An interaction showed that birds fed low-fibre pelleted diets had the lowest feeding time ($P<0.01$) compared to low-fibre mash and the high-fibre diet (Table 39). Birds fed high-fibre pelleted diets spent most of the time feeding in the morning ($P<0.01$).

Diet, form and time of day had a significant effect on social pecking behaviour ($P<0.01$), but the interaction between treatment and time of day only approached significance ($P<0.06$). Birds fed a low-fibre diet pecked more than those fed a high-fibre diet ($P<0.01$). Birds fed a mash diet also pecked more than birds fed a pelleted diet. Birds pecked more in the morning than in the afternoon ($P<0.01$, Table 39). Birds fed the low-fibre mash diet tended to peck more in the morning than in the afternoon.

Escape behaviour was affected by diet ($P<0.01$). Birds fed the low-fibre diet exhibited greater levels of escape behaviour than birds fed a high-fibre diet ($P<0.01$). There was also a tendency of escape behaviour in birds fed the mash diet ($P<0.08$) to be high. There was no interaction between time of day and treatment.

Freezing behaviour incidence was influenced by diet ($P<0.01$). Birds fed a low-fibre diet showed higher freezing behaviour than birds fed a high-fibre diet. An interaction between diet and form showed that birds fed the low-fibre mash diet had a higher freezing behaviour frequency than other treatments ($P<0.05$) (Table 39).

Diet did not have a significant effect on moving behaviour, but feed form significantly affected moving behaviour ($P<0.01$) with birds fed mash diets moving more than birds fed pelleted diets (Table 39).

Birds fed a high-fibre diet showed more preening behaviour than those fed a low-fibre diets ($P<0.01$, Table 39). An interaction between D x F showed that preening behaviour was more frequent in birds fed high-fibre pelleted diets ($P<0.01$) and an interaction between time of day and treatment showed that the preening behaviour of birds fed the high-fibre pelleted diet occured more frequently in the morning than in the afternoon ($P<0.05$). Drinking behaviour was also observed more in birds fed high-fibre diets than in those fed low-fibre diets ($P<0.01$, Table 39). An interaction between treatment and time of day showed that birds fed the high-fibre mash diet spent more time drinking in the afternoon ($P<0.05$).

Regression analysis showed a positive relationship between increasing social pecking behaviour and escape behaviour ($Y = 0.078 + 0.809X$, $r = 0.68$), freezing behaviour ($Y = 0.114 + 0.417X$, $r = 0.34$), feeding behaviour ($Y = 0.195 - 0.106X$, $r = 0.17$), moving behaviour ($Y = 0.194 - 0.053X$, $r = 0.13$), drinking behaviour ($Y = 0.167 - 0.097X$, $r = 0.07$), and preening behaviour ($Y = 0.165 - 0.123X$, $r = 0.07$).

Table 39 Mean percentage of cages exhibiting a behaviour in any minute of observation for different diets and feed forms

	Feeding	Social Pecking	Escape	Freeze	Moving	Preening	Drinking
Diets (D)							
Low-fibre (L)	7.79[a]	6.86[b]	4.23[b]	4.26[b]	20.5	1.07[a]	1.70[a]
High-fibre (H)	14.3[b]	3.80[a]	2.50[a]	3.06[a]	21.7	1.90[b]	3.16[b]
P-value	<0.01	<0.01	<0.01	<0.01	NS	<0.01	<0.01
Forms (F)							
Mash (M)	11.7	6.06[b]	3.70	3.66	26.3[b]	1.33	2.76[b]
Pellet (P)	10.5	4.60[a]	3.03	3.63	15.8[a]	1.63	2.10[a]
P-value	NS	<0.01	<0.08	NS	<0.01	NS	<0.06
Time							
Am	11.9[b]	5.93[b]	3.30	3.26	20.7	1.86[b]	2.20
Pm	10.3[a]	4.73[a]	3.43	4.03	21.4	1.10[a]	2.66
P-value	<0.05	<0.01	NS	<0.06	NS	<0.01	NS
Interaction D x F							
L x M	9.99[b]	7.69	4.63	4.80[c]	25.0	1.47[b]	2.43[b]
L x P	5.76[a]	6.03	3.83	3.70[b]	15.8	0.63[a]	0.97[a]
H x M	13.5[c]	4.43	2.76	2.60[a]	27.6	1.17[ab]	3.10[b]
H x P	15.2[c]	3.20	2.23	3.50[b]	15.7	2.60[c]	3.23[b]
P-value	<0.01	NS	NS	<0.05	NS	<0.01	<0.05
Interaction D x F x T							
P-value	<0.01	<0.06	NS	NS	NS	<0.05	<0.05

[a-c]mean values within a column with different superscripts differed significantly (P<0.05). NS: not significant.

9.3.2.2 Effect of diets and feed form on bird performance

Diet and form significantly affected percentage of total mortality (P<0.01). Birds fed a high-fibre diet had significantly lower mortality than those fed a low-fibre diet (P<0.01). Pelleted diets caused higher mortality than mash diets (P<0.01). An interaction showed that birds fed the low-fibre pelleted diet had the highest percentage of total mortality (P<0.05, Table 40).

Dirty eggs were higher in birds fed a low-fibre diet (P<0.01). An interaction showed that there was a tendency for a higher percentage of dirty eggs in birds fed low-fibre mash diet (P<0.07) (Table 40).

9.3.3 Trial 3

Soluble and insoluble NSP and free sugar contents of the diet as well as their constituents are presented in Table 41. The soluble NSP content was higher in the low-fibre diet than in the high-fibre diet. The insoluble NSP content was higher in the high-fibre diet than in low-fibre diet (Table 41).

The main sugars present in the insoluble fractions were arabinose, xylose, galactose and glucose. The main sugars present in soluble fraction were similar but the concentration was low.

Table 40 Feed intake, body weight, egg production, percentage of dirty eggs and total mortality of laying hens fed different diets and feed forms

	Feed intake (g/bird/day)	Body weight (g)	Egg production (%)	Dirty eggs (%)	Total mortality (%)
Diet (D):					
High-fibre (H)	138	2033	80	3.8[a]	13[a]
Low-fibre (L)	134	2106	81	10[b]	33[b]
P-value	NS	NS	NS	<0.01	<0.01
Form (F):					
Mash (M)	139	2062	82	8.8	17[a]
Pellet (P)	132	2076	78	5.1	31[b]
P-value	NS	NS	NS	<0.06	<0.01
Interaction:					
L x M	138	2111	82	13.8	22[bc]
L x P	131	2100	80	6.3	44[c]
H x M	141	2014	82	3.9	11[a]
H x P	134	2053	77	3.8	17[b]
P-value	NS	NS	NS	<0.07	<0.05

[a-c]mean values within a column with different superscripts differed significantly (P<0.05). NS: not significant.

Table 41 Soluble and insoluble NSP, free sugars and the constituent components of NSP and free sugars in the experimental diets

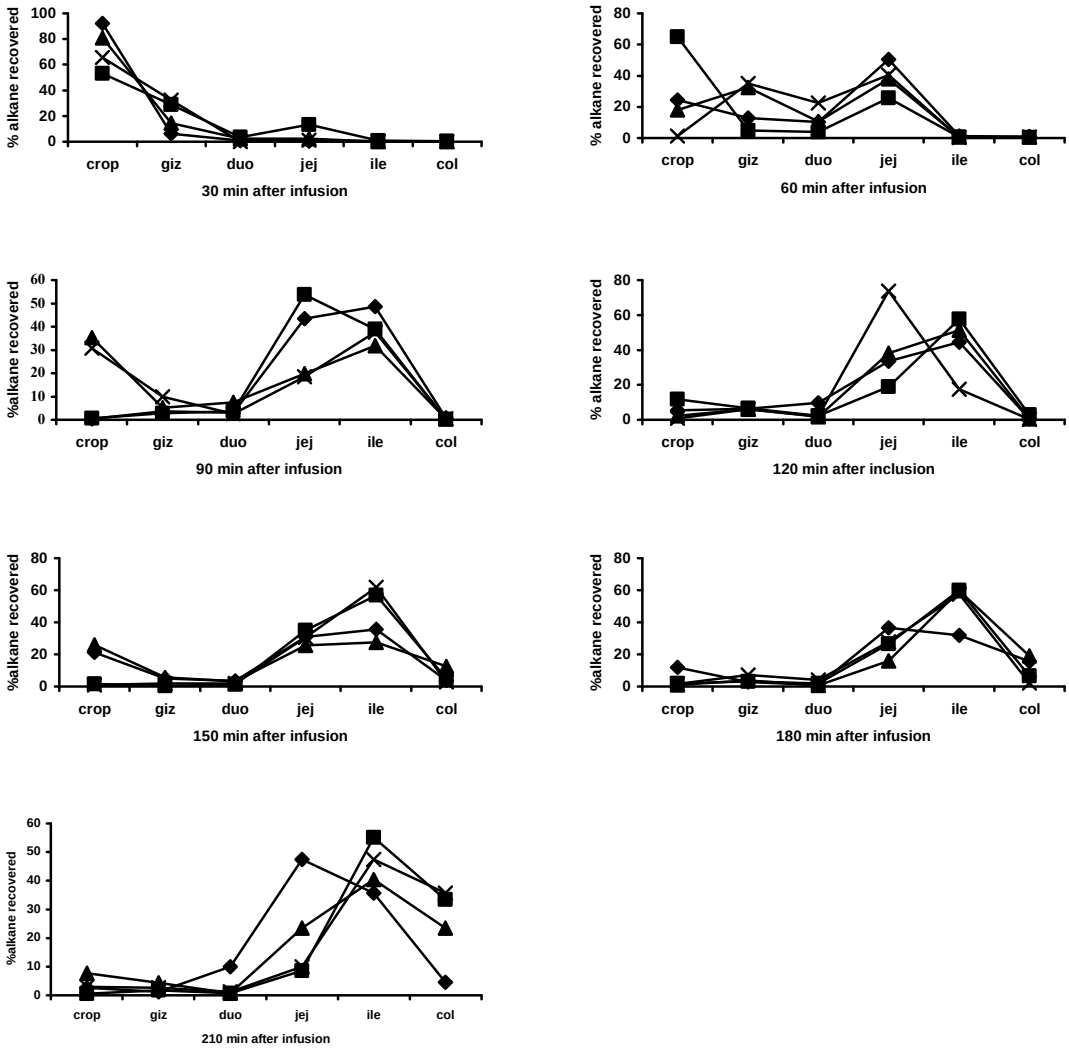
				Soluble NSP					
	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose	Total
Low-fibre	0.14	0.05	0.35	3.05	3.51	0.88	1.88	1.60	10.18
High-fibre	0.12	0.05	0.25	2.01	2.66	0.65	1.33	2.07	8.12
				Insoluble NSP					
	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose	Total
Low-fibre	0.61	0.64	0.21	23.58	30.70	1.60	8.85	30.97	86.35
High-fibre	0.61	0.49	0.24	42.23	56.68	1.71	8.14	52.81	144.64
				Free sugars					
	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose	Total
Low-fibre	0.20	0.04	0.10	0.39	0.18	1.14	5.77	21.78	29.58
High-fibre	0.16	0.15	0.07	0.36	0.29	0.84	4.32	19.78	25.96

9.3.3.1 Effect of diets on the rate of feed passage

The pattern of alkane excretion among diets is shown in Figure 5. Alkane excretion was presented as the percentage of total alkane recovered. At 30min after alkane infusion, the cumulative amount of alkane present was higher in the crop. However, in birds fed the high-fibre mash diet a considerable amount of alkane had already been found in the jejunum. At 60min after infusion, the alkane was found to have accumulated mostly in the gizzard and jejunum, but in birds fed the high-fibre mash diet, a high amount of alkane was found in the crop, instead of the gizzard. The cumulative amount of alkane in birds fed high-fibre pellets and low-fibre mash was found higher in the gizzard. The alkane content after 90 and 120min was mostly found in the jejunum and ileum. However, a considerable amount of alkane was found in the crop of birds fed the high-fibre pellets and low-fibre mash at 90 min after alkane infusion. At 150-180min post dosing, the maximum excretion of the marker from birds fed the high-fibre pellets, high-fibre mash and low-fibre mash diets occurred in the ileum, whereas it was scattered over a wider window for those fed the low-fibre pelleted diets. At 210min after alkane infusion, a high cumulative alkane concentration in birds fed the low-fibre pelleted diet was still present in the jejunum , whereas that in those fed other diets was found higher in the ileum and large intestine.

The estimated passage rate through the gizzard was significantly affected by diet ($P<0.01$) but not by form ($P>0.05$). However, there was a significant interaction between diet and form ($P<0.01$). Birds fed the low-fibre mash significantly had slower ($P<0.05$) passage rate through the gizzard than those fed the other diets which did not differ. Amount of alkane that has passed through the ileo-caeco-colonic junction in this study was referred to the amount of alkane present in the large intestine. Passage rate through the small intestine was significantly affected by form ($P<0.05$), but not by diet ($P>0.05$). Mash form significantly ($P<0.05$) had a faster passage rate through the small intestine than pellets. Birds fed the low-fibre pellets numerically had a slower passage rate through the small intestine compared to those fed the other diets. The order of passage rate through the small intestine from the fastest to the slowest was low-fibre mash, high-fibre mash, high-fibre pellets and low-fibre pellets.

Figure 6 Alkane concentrations in gut segments (crop, gizzard, duodenum, jejunum, ileum and colon) of birds fed different dietary fibres and feed forms (♦: Low-Fibre Pelleted diet, ■: High-Fibre Mash diet, ▲: Low-Fibre Mash diet, ×: High- Fibre Pelleted diet).



9.4 Discussion

In Trial 1, the amount of insoluble NSP in the lucerne diet was similar to that in the guar and the wheat diets, whereas the soluble NSP concentration was similar to that in the wheat diet. It was expected that the addition of 4% lucerne meal to the diet might increase the concentration of insoluble NSP. The method applied during the mixing of lucerne meal into the diet might contribute to the unexpected result obtained as lucerne was light and tended to separate from the rest of the diet. Obtaining a uniformly mixed sample was therefore difficult.

It was previously found that increasing the level of soluble NSP content in the diet decreased feed intake (Chapter 8). The results in the current experiments confirmed the previous findings. Feed intake, in general, decreased by increasing the level of soluble NSP content in the diet. The slower digesta passage caused by ingestion of a high-soluble NSP diet might have created the feeling of 'fulness' in the birds, which in turn affected feed intake. On the other hand, the high insoluble NSP diet led to higher feeding time as fibre increased the bulk of the diet. A negative correlation was found between increasing social pecking and increasing time spent feeding ($Y = 0.195 - 0.106X$, $r = 0.17$).

In this study, we also observed that birds fed the high-fibre pelleted diet spent more time feeding than those fed the low-fibre mash diet, a result which was similar to our previous findings (Chapter 7). This result again indicated that the increased time spent by birds fed high-fibre pelleted diets might develop as a response to increased fibre concentration over a longer period of time. Reduced feed intake seemed to relate to a reduction in body weight and egg production.

Percentage of total mortality decreased when insoluble NSP levels in the diet increased. The incidence of social pecking also decreased in birds fed a diet high in insoluble NSP. The results confirmed our hypothesis that birds fed a diet high in insoluble NSP tended to feel hunger more quickly and therefore would spend more time feeding and less pecking which leads to cannibalism. The fact that the rate of digesta passage was faster in birds fed the high-fibre diet accentuated the role of insoluble NSP in influencing the cannibalistic behaviour of birds. The interaction between diet and feed form showed that birds fed the low-fibre mash diet tended to increase social pecking. This is in contrast with our previous results (Chapter 7) where the birds fed the low-fibre mash diet showed the lowest social pecking behaviour. In the current study, we observed that the birds on this diet showed a higher tendency to escape when they were pecked, which was also in contrast with the finding in our previous experiment where the higher escape behaviour was found more in birds fed the pelleted diet. Thus, it was likely that the disturbance caused by escape behaviour triggers pecking behaviour, as reported by Hughes and Duncan (1972); disturbed birds are more likely to cause an onset of pecking. This finding is supported by a moderately strong relationship ($r = 0.68$) between these traits.

It was also found that birds fed the low-fibre mash diet showed a high incidence of freezing (fearful) behaviour. Increased social pecking behaviour was associated with increased incidence of freezing behaviour, although the poor relationship with freezing behaviour ($r = 0.34$) suggests that the effect of freezing behaviour is less important than the effect of escape behaviour on social pecking. This finding did not support our hypothesis that a high incidence of freezing behaviour would save the birds from being pecked further (Chapter 7), but reinforced the positive effect of high insoluble NSP and mash form in preventing cannibalism mortality.

The mortality in birds fed the low-fibre mash diet was lower than that in birds fed the low-fibre pelleted diet, suggesting that besides the high soluble NSP content, the form in which the diet was given had an influence on the cannibalistic behaviour of birds. In fact, the birds fed the low-fibre mash diet showed a lot of moving, which may also have helped them to avoid being

pecked. In Trial 1, the total mortality in birds fed the guar diet was higher than in birds fed other diets, but was not statistically different. We hypothesised that the lack of significant difference in this case was due to the fact that diets were all given in mash form, which was observed to reduce cannibalism mortality. The higher percentage of dirty eggs in birds fed low-fibre mash diet was hypothesised to be the result of the higher pecking behaviour.

In the present study, the results showed that passage rate through the gizzard was faster in birds fed the high-fibres and low-fibre pellets and slower in birds fed the low-fibre mash. The results support the findings of Almirall *et al* (1994), who found that large particles had a faster passage rate than small particles (Almirall *et al*., 1994). However, the fact that alkane content in the gizzard of birds fed the high-fibre pellets was higher than that of birds fed the other diets at 60 min after infusion, indicating that high-fibre pellets may be retained longer than other diets in the gizzard. It has been suggested that coarse feed particles or insoluble fibres tend to accumulate in the gizzard since it needs to be ground in a certain size before leaving the gizzard (Clemens *et al*., 1975; Hetland *et al*., 2002). However, after 30 min, a considerable amount of alkane in birds fed high-fibres was present in the duodenum and jejunum, indicating that once high-fibre emptied from the gizzard, it may pass through the gut quickly. The reason of slower passage rate in birds fed the low-fibre mash through the gizzard was unclear. At 90 min after infusion, birds fed the low-fibre mash had a higher amount of alkane accumulated in the crop. It was possible that chyme reflux happened in this stage, resulting in the reduction of digesta passage rate through the gizzard.

A high amount of alkane was also found in the crop of birds fed the high-fibre pellets at 90 min after infusion. There has been hypothesized that chyme reflux between the gizzard and the duodenum is increased by inclusion of insoluble fibre. Since at 60 min after infusion, there was no alkane accumulation in the crop of birds fed the high-fibre pellets, it was possible that there was a chyme reflux occurred at 90 min after infusion. The reflux that happened in birds fed the high-fibre pellets may reduce the rate of passage through the gizzard.

The effect of NSP was clearly shown when the diet reached the jejunum and ileum where the digestion and absorption of nutrients take place. The results showed that mash form significantly had a faster passage rate through the small intestine than pellets, and the high-fibre and the low-fibre mash diets appeared to accelerate the rate of digesta passage in the small intestine, whereas the low-fibre pelleted diet appeared to slow it. The results were in contrast with the findings of Svihus *et al* (2002), who observed a tendency of faster passage rate through the small intestine of birds fed diet supplemented with whole-wheat than those fed diet supplemented with ground-wheat. The slow passage rate observed in birds fed the low-fibre pellets was probably due to an increase in the thickness of the unstirred layer in the small intestine due to soluble fibre content of the diet, which in turn may induce a satiety sensation. The low-fibre mash diet did not slow the rate of digesta passage, suggesting the positive effect of diet form on cannibalism.

The findings in these studies indicate that type of NSP and the form in which a diet is fed influence the occurrence of pecking and cannibalistic behaviour in laying hens. The incidence of feeding, escape, freezing, moving, drinking and preening behaviours was in all cases found to be a useful indicator of factors triggering social pecking behaviour. High-insoluble NSP content in the diet, regardless of the sources, reduced the incidence of negative behaviours associated with cannibalism and to a degree this was associated with changes in the rate of digesta passage, which in turn affected the proportion of time spent feeding. Mash form has the potential to reduce cannibalism. However, it must be born in mind that high-fibre mash diets will only work if the diets are adequate in energy and nutrients. Otherwise, the converse may occur as the birds could stop continuous feeding even though they have not satisfied their nutrient requirements.

10. General Discussion

10.1 Diet composition

The prevention of cannibalism in laying hens through nutrition was the main focus in these studies, therefore the formulation of diets was an important aspect. The diets used in these studies were formulated according to commercial specifications with different cereal grains as sources of fibre. Chemical composition was calculated based on the nutrient composition of the ingredients, with fibre level as the priority. Some of the diets may possibly be criticised as not being isocaloric or isonitrogenous. Some of the diets with the same source of cereal grains used, such as the commercial (wheat) layer diet, have different ingredient compositions that may also be criticised as causing a biased result. It has been difficult to balance commercial relevance and scientific rigor in a highly applied project, such as this. However, analyses have been used to determine the content of soluble and insoluble NSP, protein and minerals in the diet which have been previously considered the important factors influencing the occurrence of cannibalism. Although most of the studies were primarily based on the effect of NSP contained in the diet, some studies also sought to observe the effects of protein and mineral contents on the occurrence of cannibalism in laying hens. The NSP were analysed for the diets according to the Uppsala method (Theander and Westerlund, 1993), and details of the method are outlined in this report. The accuracy of the analyses was checked based on the value obtained from the standard grain. The main sugars present in the NSP fractions of diets used in this study were similar, indicating that the different effects obtained may be due to differences in the concentration and/or the way the monomer units linked together.

10.2 Husbandry factors

Effects of husbandry factors on the prevention of cannibalism mortality revealed that dim light during rearing reduced the incidence of cannibalism (Chapter 4 and Chapter 8). In one experiment, it was observed that aggressive behaviour increased when birds moved from dim light rearing to natural light during the pre-lay period (Chapter 4), which was in contrast with the results obtained in Chapter 8. It is suggested that the stress due to beak trimming imposed on birds during the rearing period in the first trial (Chapter 4) influenced the development of aggressive behaviour. However, the mortality was still lower on birds reared in dim light than those reared in bright light. Kjaer and Vestergaard (1999) suggested that the reduction in cannibalism mortality of birds reared under dim light resulted in the development of gentle pecking behaviour which persisted throughout their life, and this needs to be confirmed.

Interestingly, when latency ('fear') tests were applied to the birds, birds reared in bright light tended to have a shorter latency time than those reared in dim light (Chapter 8). These findings are in contrast with the finding of Jones *et al* (1995) and Johnsen *et al*. (1998), who suggested that the high-pecker or the more fearful birds would have a longer latency time. Thus, it has been expected that birds reared under bright light would have a longer latency time than those reared under dim light. The latency test in this study was recorded based on the first step made by birds. A failure to differentiate between the first step which indicates that birds had no fear and the first step made by birds with a tendency to escape, can influence the results obtained. Although a carefull recording had been applied, such error could still happen. Thus, in the present study, it was likely that the first step recorded in some birds reared in bright light, which might represent the fear and insecurity to the new environment, was accidentaly recorded as ‘no fear’.

Increasing escape behaviour was found to be associated with an increase in social pecking behaviour (Chapter 7 and Chapter 9, Trial 2) and a high social pecking behaviour in birds reared under bright light is more likely to lead to cannibalism. A higher mortality and lower feather score in birds reared under bright light (Chapter 4 and Chapter 8) supported this hypothesis.

10.3 Effect of diet on cannibalism

The subsequent layer trials aimed to identify the factors that trigger the onset of cannibalism and to determine if nutritional changes could modify this. Therefore, the first layer trial was designed to examine a combination of factors that may relate to cannibalism. A commercial diet was used in every experiment as a 'control'. Birds on the commercial (wheat) pelleted diet exhibited significant cannibalistic behaviour, resulting in a high mortality. Beak trimming reduced the cannibalism mortality; however, the fact that a bird does experience pain after trimming and welfare concerns may prevent its widespread use.

Different types of cereal grains used in the diet had different effects on the gastrointestinal tract of birds. While some diets with low concentration of soluble NSP resulted in increased digesta viscosity, a similar concentration of soluble NSP in other diets did not increase digesta viscosity. This suggests that each cereal grain has its own physicochemical properties which affect its physiological function in the gut. However, the finding that a high soluble NSP content in a diet did not result in increased digesta viscosity, when the diet also had a high amount of insoluble NSP, suggests on interactive effect between soluble and insoluble NSP and other cell-wall components. As a consequence, the physiological function of each was minimized.

In this study, we found that a diet with a higher concentration of insoluble NSP had the largest effect in reducing cannibalism. It has been mentioned that there are interactive effects of insoluble, soluble and other cell-wall components in the gut and it seems likely that the lower the insoluble NSP concentration the less the impact of the diet in reducing cannibalism.

The findings in Chapter 6 demonstrate that the effect of high insoluble NSP concentration in reducing cannibalism was less obvious when given to birds that had already been together for some time. It is likely that such birds have learned to recognise their flock mates individually, resulting in stable dominance relationships and so called ‘social inertia’ (Craig, 1981). Such situations are likely to minimise the effect of diet.

Mash form was known to reduce the incidence of pecking leading to cannibalism (Aerni *et al*, 2001) and this was confirmed in this study. The increase of social pecking behaviour in birds fed high-fibre mash (Chapter 7 and Chapter 9, trial 2) did not result in high mortality, indicating the potential of insoluble NSP and the mash form in reducing the cannibalistic behaviour of birds. Increasing social pecking behaviour was associated with an increase in escape and freezing behaviour.

Studies on the rate of digesta passage showed that diets high in insoluble NSP content increased the rate of digesta passage, while soluble NSP decreased it. Soluble NSP have been known for their effect in increasing digesta viscosity which can cause a delay in digesta passage. The delay in digesta passage will create the feeling of 'fulness' in the birds, consequently birds will stop eating and do other activities such as pecking. Furthermore, increasing digesta viscosity may favour the proliferation of anaerobic microflora. Proliferation of some anaerobic organisms can lead to production of toxins (Choct, 1997) but it is not known whether the high number of anaerobic microorganisms present in the gut due to ingestion of soluble NSP may influence the cannibalistic behaviour of the birds. Insoluble NSP, on the other hand, are known to increase digesta passage, which in turn may cause birds to feel hungry more quickly and as a consequence birds will spend more time feeding and less time pecking. The results obtained in this study support this hypothesis.

It had been noted that a diet having a high fibre level did not always have a high insoluble NSP content. For example, the fibre levels of the rice hull diet in Chapter 6 and Chapter 8 were 7.4%

and 5%, respectively, but the insoluble NSP content in the first rice hull diet was lower (92.5g/kg DM) than that in the second rice hull diet (114.7g/kg DM). This reinforces the point that the same ingredient obtained from different places, at different times, or in different mills will differ in its chemical composition.

10.4 Conclusions

This series of experiments clearly demonstrated that dim light during rearing and high-insoluble NSP mash diets have a marked effect in reducing cannibalism mortality. While dim light suppresses the aggressive behaviour of birds, the potential effect of insoluble NSP lies in their properties of increasing the rate of digesta passage. The higher the insoluble NSP concentration, the better the effect they have on cannibalism. Whether this is due to less development of gut anaerobic microflora is not clear. The concentration of insoluble NSP in the diet depends on the diet composition. There is a need for future research to pursue the effect of gut microflora on cannibalism in laying hens, especially identifying the microorganisms that proliferate due to ingestion of non-starch polysaccharides. Application of dim light during pre-lay and lay period and different form (mash vs crumble) may be worthy of future study.

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Appendices

Appendix 1 Lay out of housing condition and distribution of experimental diets in the first laying year trial (Chapter 4)

Front view

	Wheat diet	Millrun diet	Barley diet	Barley + enzyme	Wheat diet	Millrun diet	Barley diet	Barley + enzyme			
B L I N D S	DBT	BBUT	DBT	BBUT	BBUT	DBT	BBUT	DBT	B L I N D S		
	DBUT	BBT	DBUT	BBT	BBT	DBUT	BBT	DBUT			
	BBT	DBUT	BBT	DBUT	DBUT	BBT	DBUT	BBT			
	BBUT	DBT	BBUT	DBT	DBT	BBUT	DBT	BBUT			

Rearing and beak conditions:

DBT: Dim rearing Beak-Trimmed
DBUT: Dim rearing Beak-Untrimmed
BBT: Bright rearing Beak-Trimmed
BBUT: Bright rearing Beak-Untrimmed

Appendix 2 Layout of housing condition and distribution of experimental diets in the second laying year trial (Chapter 8)

Front view

BLINDS											BLINDS
		BRIGHT WH	BRIGHT RH	BRIGHT RH	BRIGHT WH	DIM-WH					
	E	BRIGHT MR	BRIGHT MOS	BRIGHT MOS	BRIGHT OATS	DIM-OATS	E				
	M	BRIGHT OATS	BRIGHT FOS	BRIGHT FOS	BRIGHT MR	DIM-MR	M				
	P	DIM-WH	DIM-RH	DIM-RH	DIM-WH	BRIGHT WH	P				
	T	DIM-MR	DIM-MOS	DIM-MOS	DIM-OATS	BRIGHT OATS	T				
	Y	DIM-OATS	DIM-FOS	DIM-FOS	DIM-MR	BRIGHT MR	Y				
		BRIGHT RH	BRIGHT WH	BRIGHT WH	BRIGHT RH	BRIGHT RH					
	C	BRIGHT MOS	BRIGHT OATS	BRIGHT OATS	BRIGHT MOS	BRIGHT MOS	C				
	A	BRIGHT FOS	BRIGHT MR	BRIGHT MR	BRIGHT FOS	BRIGHT FOS	A				
	G	DIM-RH	DIM-WH	DIM-WH	DIM-RH	DIM-RH	G				
	E	DIM-MOS	DIM-OATS	DIM-OATS	DIM-MOS	DIM-MOS	E				
	S	DIM-FOS	DIM-MR	DIM-MR	DIM-FOS	DIM-FOS	S				

Rearing conditions:
BRIGHT: bright rearing, DIM: dim rearing.
Diets: WH: wheat diet, OATS: oat diet, MR: millrun diet, RH: rice hull diet, MOS: mannooligosaccharides diet, FOS: fructooligosaccharides diet.
EN.REFLIST