Fibrous Feeds for Functional Fowls

Robin Kalmendal
Faculty of Veterinary Medicine and Animal Science
Department of Animal Nutrition and Management
Uppsala

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Abstract

Fibres are not digested by fowls, but their impact on digestive physiology, nutrient metabolism and intestinal microflora can be substantial. Analytical shortcomings have historically contributed to different notions on the significance of fibres, but modern techniques now enable us to better discriminate between various fractions of fibre and relate them with the performance of poultry.

The four present studies examined relationships between different qualities and means of providing fibres, and the functionality of broiler chickens and laying hens.

In broiler chickens, increasing the dietary levels of insoluble fibres stimulated fat and protein digestion, but not in a dose-dependent manner. In the small intestine, these effects were mirrored in altered tissue morphology and luminal microflora composition. Moreover, addressing the negative effects of soluble fibres in wheat on digestion by adding a xylanase to the diet improved feed utilization. These effects were not mirrored by macroscopic or microscopic changes of the small intestine. Effects similar to those of the xylanase were achieved by adding a protease, but not a coccidiostat, to the diet. There were no signs of the effects of the two enzymes being additive.

In laying hens, supplementary roughage reduced beak-inflicted injuries and improved feed efficiency; the latter finding likely reflecting better plumage conditions. Increased dietary levels of fibres and fat reduced the liver fat and tended to improve feed efficiency; the latter result probably explained by beneficial effects of added fat on digestion. The diet with more fat and fibres also impaired the hygienic conditions in the stable, presumably due to the fibre fraction being mainly soluble in nature. Experimental lines of hens predisposed to perform severe feather pecking preferred a diet supplemented with spelt hulls more than corresponding hens not predisposed to perform severe feather pecking. This finding supported previous indications that injurious pecking correlates with the fibre content of the feed.

It was concluded that the significance of fibres in poultry nutrition largely depends on the quality of fibres. Whereas soluble fibres primarily acted detrimentally on the functionality of the birds, insoluble fibres tended to act in the opposite direction. In poultry nutrition experiments, however, it remains difficult to separate the effects of increasing amounts of fibrous feedstuffs from those of simultaneous dietary fat supplementation, nutrient dilution or ingestion of fibrous materials such as litter.

Keywords: broiler chicken, laying hen, fibre, nutrition, intestinal health, feather pecking

Author's address: Robin Kalmendal, SLU, Department of Animal Nutrition and Management, SE-753 23 Uppsala, Sweden

E-mail: robin.kalmendal@slu.se

In the field of observation, chance favors only the prepared mind.

Louis Pasteur (1854)

Contents

List of Publications

This thesis is based on the work described in the following papers, referred to by Roman numerals in the text:

I Kalmendal, R., Elwinger, K., Holm, L. and Tauson, R. (2011). High-fibre sunflower cake affects small intestinal digestion and health in broiler chickens. *British Poultry Science* 52(1), 86-96.

II Kalmendal, R. and Tauson, R. (2012). Effects of a xylanase and protease, individually or in combination, and an ionophore coccidiostat on performance, nutrient utilization, and intestinal morphology in broiler chickens fed a wheat-soybean meal-based diet. *Poultry Science* 91(6), 1387-1393.

III Kalmendal, R. and Wall, H. (2012). Effects of a high oil and fibre diet and supplementary roughage on performance, injurious pecking and foraging activities in two layer hybrids. *British Poultry Science* 53(2), 153-161.

IV Kalmendal, R., and Bessei, W. (2012). The preference for high-fiber feed in laying hens divergently selected on feather pecking. *Poultry Science* 91(8), 1785-1789.

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Abbreviations

AME_n	Apparent metabolizable energy, corrected to zero nitrogen retention
BP	Base pairs
CF	Crude fibre
CP	Crude protein
DM	Dry matter
FCR	Feed conversion ratio
HFP	High feather pecking (laying hens)
i-NSP	Insoluble non-starch polysaccharides
LB	Lohmann brown
LFP	Low feather pecking (laying hens)
LSL	Lohmann selected leghorn
NSP	Non-starch polysaccharides
PCNA	Proliferating cell nuclear antigen
s-NSP	Soluble non-starch polysaccharides
SLU	Swedish University of Agricultural Sciences
TRF	Terminal restriction fragment
t-RFLP	Terminal restriction fragment length polymorphism

1 Background

The stipulations of the domestic fowl (*Gallus gallus domesticus*) or poultry, in agriculture have undergone dramatic changes in the last 50 years. Much of the transformation seen can be described in terms of intensification, rationalisation and specialization of the production systems in which the birds are held.

While it would take approximately 100 days for a chicken of the 1950’s to reach a target weight of 1.8 kg, the equivalent time needed was reduced to 32 days some 50 years later (Havenstein *et al.*, 2003). It has been estimated that advancements in nutrition, management and veterinary practices explain some 40 to 50% of this development while the remainder would be attributed genetic selection (Robins & Phillips, 2011). Research has shown that breeding programmes have resulted in birds, which *per se*, are biologically more efficient in utilizing the nutrients in the feed (Schmidt *et al.*, 2009; Havenstein *et al.*, 2003).

The intensification of the genetic work in the 1950’s was followed by dramatic improvements at production level. During this period, commercial genotypes, or hybrids, were developed based on the crossing of genetic lines selected for either rapid growth and muscle accretion, *i.e.* the broiler chicken, or fast sexual maturation and high rates of egg laying, *i.e.* the laying hen.

Inevitably, new practices in parallel with large modifications of the gene expression profile affect the nutritional requirement of the bird. Thus, the feeding recommendations of commercial poultry are continuously being revised, and trends in poultry nutrition often reflect the progress made in genetic selection.

In the 21st century, the nutrition of broiler chickens and laying hens has become a highly specialized area. This means that the success of commercial poultry operations partly depends on the skills and knowledge present at the feed plant. Ultimately, the feed manufacturer must fulfil the nutritional demands and match the physiological and behavioural characteristics of the birds by carefully balancing raw materials and technical additives within a framework of economic, technological and environmental constraints. Failure to do so may not only reduce the performance, but also the health and welfare of the animals.

1.1 The functional fowl

In general, the term ‘functional’ refers to the ability of something to fulfil its role or purpose. For example, a certain egg laying hybrid can be considered functional in a new type of housing system if it meets the expectations on production performance, technical results and specific health parameters. In poultry operations, the birds’ performance is largely determined by the physiological and behavioural functionality of the birds.

There are several different biological aspects of functionality, but which specific aspects require the attention of managers, consultants and researchers depend both on the production system and the type of bird used.

The juvenility of broiler chickens, for example, may predispose them to intestinal disturbances (Petersen *et al.*, 1999). Not only is their fast growth rate highly dependent on the function of the digestive tract, but a dysfunctional digestion can also impair litter quality and foot-pad health, thereby threatening both animal welfare and the economic margins of the production (Bessei, 2006). Gut function is therefore a key issue in broiler chicken production.

In laying hens, however, nutritional factors which predispose to gut disorders may have a comparatively less dramatic impact. This relates to both a more mature bacterial flora in the gut and a generally lower stocking density in egg production systems. On the other hand, layers develop a larger behavioural repertoire which greatly influences the fate of the production. Two important behavioural aspects of functionality in laying hens include the nesting behaviour and abnormal pecking behaviours.

In modern production systems the functionality of poultry is influenced by several factors, including management, genetics and nutrition. The present thesis addresses the nutritional impact of fibres on different biological functions of broiler chickens and laying hens. The topic is not a novel one; the importance of fibres to the digestive function in broiler chickens was discussed in the scientific literature already some 60 years ago (O'Dell *et al.*, 1959; Davis & Briggs, 1947) and the role of fibres in the development of behavioural disorders in laying hens even earlier than that (Schaible *et al.*, 1947; Bearse *et al.*, 1940). However, changes in the way we keep and feed poultry, and developments in our understanding of the nature of fibres justify a revision of their role in poultry nutrition.

2 Introduction

Poultry do not produce the enzymes required to digest fibres present in the feed. This fact is reflected in the high degree of nutrient concentration and refinement of commercial feed compositions. Grains constitute the dominating ingredient of poultry feeds and the birds appear rather well suited for such diets as the capacity to digest *e.g.* wheat starch is typically very high (Hetland *et al.*, 2003; Svihus & Hetland, 2001). Therefore, it might be surprising that poultry should not be classified as granivores, but are generalist and consequently best described as omnivores (Klasing, 2005).

Even if fibres cannot be digested by broiler chickens and laying hens they are not inert. In fact, studying the digestive physiology of poultry may lead to the suggestion that fibres do have an important role to play. A brief look at some characteristic segments of the alimentary canal of poultry may justify such an assumption. In the text below, the different segments are indicated with letters (A-D) in the schematic illustration of Figure 1.

The anterior segment of the digestive tract is characterized by an enlargement of the oesophagus, called the crop (A). The crop is used as a food storage organ and filling of the crop inhibits food intake (Richardson, 1970). It may well be that the fibre fraction of the feed is important to the sensation of satiety, as the quality of ingested fibres affects the rate of entry and time of retention in the crop (Vergara *et al.*, 1989).

Ingested items are either retained in the crop, or passed down directly to the next segment of the digestive tract. The anterior compartment of this segment is called the glandular stomach, or proventriculus, and constitutes the site for hydrochloric acid and pepsinogen secretion. The posterior compartment is named the ventriculus, or more commonly, the gizzard (B). In the gizzard, the particle size of ingesta is reduced by means of grinding, while the chyme is mixed with the secretions of the proventriculus. To function properly, however, the gizzard requires mechanical stimulation. Here, the excitatory effect of fibrous materials on the gizzard is well documented (Steenfeldt *et al.*, 2007; Hetland *et al.*, 2005; Rogel *et al.*, 1987).

Once ingested items have been ground to a critical size the particles are moved into the small intestine (C). The size of the fowl intestine has been adapted to flight and is therefore comparatively short. To compensate for this reduction in digestive capacity, poultry reflux digesta between various locations of the alimentary canal (Duke, 1988; Sklan *et al.*, 1978). Although data are somewhat limited, ingested fibres were shown to influence peristalsis in man (Cherbut *et al.*, 1994) and fibres present in the feed are believed to influence the gastroduodenal reflux of digesta in poultry (Hetland *et al.*, 2003).

Nutrients are digested and absorbed along the small intestine; but as previously indicated, poultry cannot degrade the fibre fraction of the feed. Instead, completely or partly undigested fractions of water soluble digesta particles, including fibres, are moved by means of anti-peristalsis into the pair of caeca (D). Coarser fractions of digesta are prevented from entering the caeca by a filter-like meshwork of villi stretching into the lumen (Björnhag, 1989). The caeca harbour large numbers of bacteria with the capacity to use the energy present in the fibres. Some metabolic end products from this fermentation, such as short chain fatty acids, can finally be absorbed and utilized by the bird.

In summary, it seems valid to assume that fibres exert a range of effects in the digestive tract of the fowl. However, to assess the significance of the fibre fraction of poultry feeds, it is necessary to understand the nature of the different fibre fractions; where they come from and what their features are.

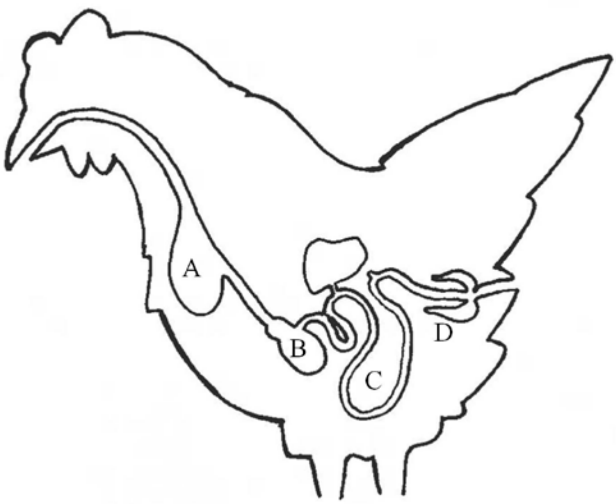

Figure 1. A schematic illustration of the gross anatomical structure of the digestive tract in fowls.

2.1 The fibre fractions of poultry feeds

Somewhat simplified, the fibre fraction of vegetable feed ingredients can be viewed as a large group of heterogeneous carbohydrates. Both in research and practice the rationale for a more precise determination and classification of fibre fractions has historically been a matter of confusion. The term ‘dietary fibre’ was first used by Hipsley (1953) and later defined by Trowell (1976) as “the residue of plant food resistant to hydrolysis by human alimentary enzymes”. From an analytical perspective, however, the story is more complicated.

Although the crude fibre analysis developed in the early 1800’s was soon discovered to be an incorrect estimator of the actual fibre content of the feed, it has long been the standard method for fibre determination (van Soest & McQueen, 1973). In the last two decades, however, the advent of more physiologically valid determinations has increased our understanding and appraisal of the significance of the fibre fraction in poultry nutrition (Smits & Annison, 1996).

What we often mean by ‘fibre’ may chemically be described as chains of sugar units. These chains can be identified following the removal of another sugar polymer, starch, in the feed sample. The fractions obtained may then be denoted non-starch polysaccharides (NSP) and they are commonly further characterized with respect to their solubility in water. Table 1 displays the fibre contents of some raw materials used in North European poultry feeds. For comparative purposes, the crude fibre content of each feedstuff is listed next to the contents of the soluble NSP (s-NSP) and insoluble NSP (i-NSP).

Table 1. *The fibre contents (% of DM) of some feedstuffs available in Northern Europe, ordered in falling crude fibre contents.*

	Crude fibre[1]	Soluble NSP[2]	Insoluble NSP[2]
Maize	2.5	0.9	6.6
Wheat	3.4	2.5	7.4
Peas	6.1	5.2	7.6
Barley	6.2	5.6	8.8
Soybean meal (44% CP)	7.9	6.3	9.2
Oats	12.1	4.0	11.0
Wheat bran	12.4	2.9	27.3
Rape seed meal	12.9	5.5	12.3
Lucerne meal	26.2	7.7	11.3
Sunflower seed meal	26.7	4.5	23.1
Oat hulls	31.2	<0.5	84.7

[1]Adapted from (NRC, 1994)
[2]From Knudsen (1997) except values of oat hulls which derive from Hetland and Svihus (2001) and sunflower seed meal which derive from Irish and Balnave (1993)

Hitherto, there have been few systematic comparative studies of the contents of crude fibre and NSP in common sets of feedstuffs, but practical experience suggest that the methods provide us with two divergent pictures. Although the values presented in Table 1 originate from different samples and laboratories, they can still tell us something about a lack of correlation between the analyses. As an example, it can be seen from the column of crude fibre that the fibre contents of lucerne meal and sunflower seed meal appear to be similar. When the NSP values are considered, however, it is clear that the contents and solubilities of the fibre fraction in these two feedstuffs are completely different. This indicates that the methodological shortcomings of the crude fibre analysis are substantial, particularly if we wish to consider the quality aspects of fibres. The significance of fibre quality to the physiological response of the bird will be addressed next.

2.2 The significance of fibre quality in poultry feeds

With little gradation, the fibre fraction of poultry diets was long considered of diluting or even anti-nutritive nature, as reviewed by Mateos *et al.* (2012). Thus, it has commonly been used as a negative coefficient in prediction equations of the nutritive value of feeds (Larbier & Leclerq, 1994). Today, following the development of more sophisticated methods of fibre determination, it is known that different fibre fractions have different properties and should consequently be viewed differently.

The solubility is an important feature of fibres as it largely determines their effect on production performance (Choct, 2002). More specifically this effect is primarily mediated by the actions of the NSP in the digestive tract. An overview of the different roles of s-NSP and i-NSP in the anterior digestive tract is given in Table 2.

Table 2. *A brief overview of the actions of soluble and insoluble non-starch polysaccharides (NSP) in the anterior digestive tract. Signs represent increases (+) or decreases (-).*

	Soluble NSP	Insoluble NSP
Water-holding capacity / viscosity	++	+
Gastrointestinal motility	-	+
Digesta transit time	+	-
Nutrient digestion	-	+
Bacterial growth substrate	++	+

The features in Table 2 should be viewed as the result of interactions between the constituents of the feed and the biology of the bird. In some cases these interactions are manifested on a gross anatomical level, sometimes they occur on a cellular or even molecular level. In general, it seems reasonable to suggest that s-NSP tend to act detrimentally on digestion whereas the negative effects of i-NSP are less pronounced, and in some cases, even beneficial to the bird. The underlying factors of such a difference will now be scrutinized.

2.2.1 Nutritional aspects of soluble non-starch polysaccharides

Whether fibres are soluble in aqueous solutions, including digesta, tells us something of how they interact with water. Chemically, the water-binding capacity of fibres reflects the frequency of bonds between the carbohydrate units, which in turn determines how much water can be trapped in the intermolecular spaces (Carré, 2002). To the birds, the water-binding capacity of the feed may have both positive and negative aspects. In systems where restricted feeding is practised, such as in broiler chicken parent stocks, a high water-binding capacity of the feed has been suggested to distend the digestive tract, thereby possibly increasing the sensation of satiety (Hocking *et al.*, 2004). On the other hand, water-binding fibres will increase the water consumption in birds (Langhout *et al.*, 1999) and in floor systems, an elevated moisture level of the litter implies an increased risk of reduced hygiene and foot-pad inflammation (Wang *et al.*, 1998).

In fibres with low frequency of intermolecular bonds, such as in pectic substances, less water is entrapped in cavities between the molecules. Instead, more water is directly associated with the many saccharide units due to their hydrophilic nature (Carré, 2002). Thus, fibres characterized by long-stretched chains of sugar units can associate with large amounts of water, resulting in increased viscosity. Here, it is worth noting that the physiochemical characteristics of a feed may not always be static. For example, it is likely that changes in the degree of polymerisation of the fibres explain the increased feed viscosity often seen in response to high pelleting temperatures (Cowieson *et al.*, 2005).

The viscosity-inducing properties of s-NSP are believed to be a key factor in their detrimental effect on feed utilization (Langhout *et al.*, 1999; Smits & Annison, 1996). In humans, ingestion of water-binding fibres reduce the rate of glucose absorption by altering the motility pattern of the small intestine (Cherbut *et al.*, 1994) and similarly, high viscosities are believed to hinder the convection of digesta with the intestinal epithelium of poultry. Because the breakdown and uptake of nutrients in the digestive tract relies on the ability of digestive enzymes and their substrates and end-products to move freely in the gut lumen, elevated viscosities have been associated with impaired feed utilization. This effect is particularly evident in fat digestion, as the step of emulsification requires rigorous mixing of digesta (Bedford, 2002).

Interestingly, the magnitude of the negative effects of s-NSP on feed utilization is mediated by the bacteria present in the digestive tract of the bird (Maisonnier *et al.*, 2003; Langhout *et al.*, 2000). Many of the >640 bacterial species residing in the gut have the capacity to use fibres as substrates for growth, but they also compete with the host for other nutrients such as nitrogenous compounds, fats and minerals (Apajalahti *et al.*, 2004). Although some bacteria are believed to benefit the host, many species do not, and the competition for nutrients implies that any factors hindering an efficient feed digestion and utilization of the bird are likely to promote the microflora. For this reason, reductions in bird performance due to high levels of s-NSP in the feed are often mirrored in quantitative and qualitative changes of the bacterial community (Langhout *et al.*, 1999).

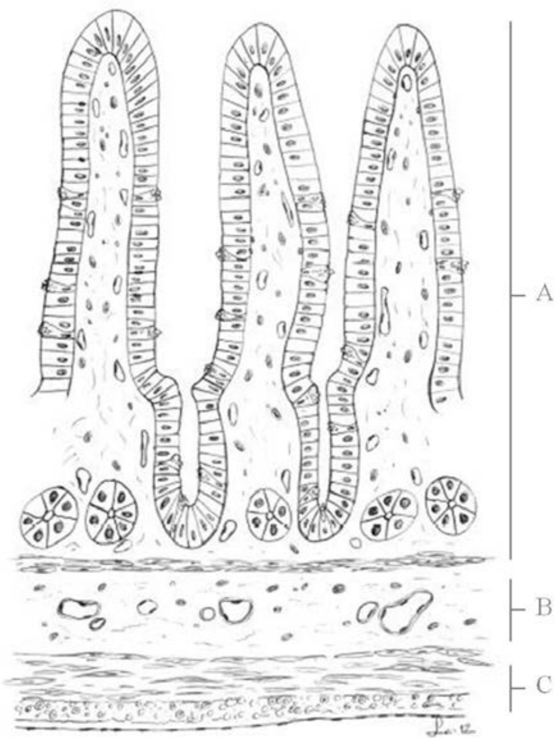

Figure 2. Illustration of the intestinal wall. The mucosa (A) is ordered in finger-like protrusions, called villi. The villi are lined with epithelial cells which are continuously sloughed off into the gut lumen and regenerated in the crypts of Lieberkühn, embedded in the lamina propria. A section of submucosa (B) serves as the interface to the muscular layers (C) which facilitate gut peristalsis.

To the birds, the negative impact of s-NSP is not only manifested in disadvantageous allocations of nutrients to bacterial growth in the gut lumen, but can also be expressed as damage to the intestinal mucosa. Serving as the interface between the constituents of digesta and the underlying mucosa, the epithelial cells must facilitate an efficient absorption of digestion derivates while preventing microbes from damaging or infiltrating the tissue. To increase the absorptive area the intestinal mucosa is ordered in finger-like protrusions into the lumen, called villi. At the villus tip epithelial cells are continuously sloughed off and replaced by migrating cells formed in the crypts of Lieberkühn at the villus base. A schematic illustration of the intestinal wall is shown in Figure 2.

It is sometimes assumed that the length of villi reflects their absorptive capacity, and studies have demonstrated that s-NSP have a deleterious effect on villus height (Teirlynck *et al.*, 2009; Viveros *et al.*, 1994) although results are not consistent (Iji *et al.*, 2001; Langhout *et al.*, 1999; Rolls *et al.*, 1978). Others have found that s-NSP increase the depth of the crypts, which possibly would indicate an increased rate of cell renewal, and indirectly, more damage of the villus tip (Wu *et al.*, 2004b). In general, divergences in the literature indicate that the effects of s-NSP on mucosal morphology are not clear-cut.

Today, the detrimental effects of s-NSP in the feed are largely alleviated by the routine use of fibre-degrading enzymes in the feed. The efficacy of many mono- and multicomponent enzyme products is well documented, but their mode of action has been a matter of debate. In essence, the question has resolved around whether the benefits of the enzymes should be attributed their capacity to reduce the viscosity of the digesta, or their capacity to release nutrients encapsulated in the fibre matrix of the grain cell walls, or both (Bedford, 2002).

2.2.2 Nutritional aspects of insoluble non-starch polysaccharides

As previously mentioned i-NSP share some physical features with s-NSP, but the negative effects exerted by i-NSP are much less severe. For example, i-NSP may display some water-holding capacity, but their viscosity-inducing properties are relatively low (Smits & Annison, 1996). Although i-NSP are less rapidly fermented by the microflora, they are not inert, but may indeed interact with the constituents of the digesta. For example, i-NSP were shown to bind bile salts (Kritchevsky & Story, 1974) and influence plasma, liver and egg cholesterol in laying hens (McNaughton, 1978). In theory, these effects may partially be mediated by the intestinal microflora as i-NSP tend to favour certain bacterial species (Baurhoo *et al.*, 2007) some of which display a capacity to deconjugate bile salts (Guban *et al.*, 2006).

Further, depending on the pH, some NSP may associate with cations (Smits & Annison, 1996). Although certain fibres may improve mineral retention in broiler chickens (Ortiz *et al.*, 2009) the effects of different fibres on different minerals are equivocal (van der Aar *et al.*, 1983). Overall, there is a scarcity of *in vivo* experiments on the effects of i-NSP on the uptake of *e.g.* calcium and other nutritionally important minerals in poultry.

In contrast, the suggestion that i-NSP may stimulate the digestion of macronutrients and benefit bird welfare has gained increasing attention in the scientific community. The majority of hypothesized explanations for these observations relate to the effects of i-NSP on gastrointestinal function.

Whereas water-soluble particles dissolve in the intestinal fluids and rapidly pass the anterior digestive tract, i-NSP are retained for a longer period of time in the crop and gizzard (Vergara *et al.*, 1989; Ferrando *et al.*, 1987). Increased retention time of digesta in the anterior digestive tract may alleviate problems with over-consumption of feed in broilers (Svihus *et al.*, 2010) probably as feed intake is partially controlled by distension-sensitive receptors in this section of the alimentary canal (Duke, 1988).

Numerous studies have demonstrated that i-NSP stimulate gizzard development (Jiménez-Moreno *et al.*, 2010; Hetland & Svihus, 2007; Hetland *et al.*, 2005; Hetland *et al.*, 2004; Hetland *et al.*, 2003; Hetland & Svihus, 2001; Riddell, 1976) and reduce the occurrence of spontaneous gizzard erosion and ulceration (Kaldhusdal *et al.*, 2012). The notion that gizzard development is important to digestion derives from several experiments. For example, gizzard size correlated with the efficiency of mechanical degradation of raw potato starch granules (Rogel *et al.*, 1987) and broiler chickens selected for high utilization of a wheat-based diet displayed increased gizzard weights (de Verdal *et al.*, 2010).

The effects of i-NSP on gizzard function are not restricted to the control of feed intake and digesta particle size, but probably involve the regulation of digesta flow further down the alimentary canal. Ingestion of i-NSP increased the contents of bile acids and amylase activity in digesta, hypothetically via an increased gastroduodenal reflux (Hetland *et al.*, 2003). An increased frequency of small intestinal reflux has been suggested to facilitate the digestion of nutrients (Duke, 1997). In addition to the aforementioned effects of i-NSP on gizzard grinding capacity, the elevated contents of bile acids in digesta reported by Hetland *et al.* (2003) may help explaining the increased digestibilities of *e.g.* fat in young broiler chickens fed i-NSP rich hulls derived from soybeans and oats (Jiménez-Moreno *et al.*, 2009a).

When considering the effects of i-NSP on nutrient utilization the importance of particle size should be emphasized. Since digesta must pass through the gastroduodenal junction of the gizzard, larger particles are retained for a longer time and consequently stimulate gizzard function more than fine particles do (Jiménez-Moreno *et al.*, 2010).

2.2.3 Effects of insoluble non-starch polysaccharides on abnormal pecking behaviours in laying hens

It remains to be elucidated to what extent the function of the digestive tract affects the behaviour of poultry. Of course, any nutritional shortcomings following impaired feed utilization may have consequences on how the birds act, but in the case of i-NSP, the reported effects cannot fully be explained by classical deficiencies.

Early research demonstrated that the fibre fraction of oat hulls, primarily containing i-NSP, may reduce mortality due to damages from abnormal pecking behaviour in young pullets (Bearse *et al.*, 1940). Such pecking behaviour, referred to as ‘cannibalism’, is often described as the final manifestation of the less severe, yet more common, behaviour of pecking and pulling of the feathers of conspecifics denoted ‘feather pecking’ in laying hen flocks (McAdie & Keeling, 2000). It should be noted, however, that cannibalism and feather pecking can be found in flocks separately and independent from each other.

The motivational background of feather pecking has been much debated. Although there seems to exist an association between feather pecking and the perception of feathers as dust bathing substrate (Vestergaard *et al.*, 1993) researchers today appear to agree that feather pecking should be viewed as redirected ground pecking (Savory, 1995). Basically, feather pecking and cannibalism can be induced by any potent stressor, including poor nutrition and management. Because the majority of injurious pecks is typically performed by a small number of hens, it is generally considered that the impact of feather pecking increases with group size, where individual peckers may cause damage to a larger number of conspecifics (Bilcik & Keeling, 1999). It has been demonstrated that the behaviour contains a heritable component (Kjaer *et al.*, 2001) and differs in severity between different egg laying hybrids (Kjaer & Sørensen, 2002).

Few practically applicable tools, other than beak-trimming, exist to prevent hens from beak-inflicted injuries (van Krimpen *et al.*, 2005). In addition, beak-trimming is not an option in certain production systems, or even legal in some countries, including Sweden, Norway and Finland. However, recent findings support the early notion of Bearse *et al.* (1940) that abnormal pecking behaviours may be alleviated by the use of i-NSP-rich ingredients in the feed (van Krimpen *et al.*, 2009; Hartini *et al.*, 2002; Wahlström *et al.*, 1998). It has been suggested that the positive effects of i-NSP on feather pecking may relate to a better functioning of the gut (van Krimpen *et al.*, 2009) or increased time of feeding, and thereby less time for feather pecking, due to reductions in specific gravity of the feed (van Krimpen *et al.*, 2008). Here, it should be noted that several confounding factors need consideration when studying the role of i-NSP in abnormal pecking behaviours. For example, hens prone to feather pecking tend to eat feathers (Harlander-Matauschek *et al.*, 2007; McKeegan & Savory, 1999) which exert some effects similar to i-NSP in the digestive tract (Harlander-Matauschek *et al.*, 2006).

In addition, i-NSP are present in many types of litter substrates, which are often consumed as well (Hetland & Svihus, 2007; Hetland *et al.*, 2005). Hence, an evaluation of the fibre fraction of feeds should preferably take into account the role of other fibre sources.

2.3 Other fibre sources than feed in poultry production

Fibres are not only present in the feed but are also abundant in litter substrates such as straw, wood shavings and saw dust. The fibres found in these materials are primarily insoluble in nature.

From 2012, all laying hens in the EU should have access to litter as they must be housed either in furnished cages equipped with a litter bath, or in aviaries and traditional floor systems. Although the use of feed as litter substrate in furnished cages is practised in many countries, materials derived from *e.g.* wood processing are more commonly used in Scandinavia.

It was reported that laying hens housed in furnished cages consumed between 4 and 11 g fibrous litter per day from the litter bath, depending on the substrate used (Hetland & Svihus, 2007). Referring to the previous reasoning on the effect of i-NSP on gizzard function, Hetland and Svihus (2007) found that hens presented with wood shavings in the litter bath more efficiently utilized the energy of the feed. Correspondingly, when laying hens housed in a floor system littered with wood shavings were compared with layers housed in barren cages, the former displayed a 57% increase in gizzard weight (Hetland *et al.*, 2005).

In certain poultry systems, such as organic or free-range production, birds should have access to a variety of fibrous materials outdoors and to roughages such as silage, fresh vegetables and fruits. Here, it is more difficult to generalize about the quality of fibres, but compared to grains there is commonly more s-NSP in fruits and vegetables (Englyst, 1989) and more i-NSP in grasses (Knudsen, 1997) on DM basis. It was shown that silage may constitute a significant proportion of the total dietary intake in laying hens, resulting in increased weight and lower pH of the gizzard, increased fill of the anterior digestive tract and less feather damage and mortality following abnormal pecking behaviours (Steenfeldt *et al.*, 2007). Here, it should be emphasized that the provision of foraging substrates may decrease abnormal pecking behaviours even if they are not consumed, *i.e.* by increasing the birds' occupation (Huber-Eicher & Wechsler, 1998). It is therefore difficult to judge whether the effect of *e.g.* litter and silages on feather damage and mortality in laying hens should be regarded as nutritional, behavioural, or both.

Although not easily quantified in practical situations, litter ingestion is also seen in broilers. When cage-reared broiler chickens were presented different litter substrates, the ingestion of litter constituted up to 6% of the total intake in the first week of life (Malone & Chaloupka, 1983). As with laying hens, the significance of litter consumption in broiler chickens should not be neglected. For example, small amounts of wood shavings added to concrete floors alleviated the problem with dilation of the proventriculi among broiler chickens in a study by Riddell (1976). At present, broiler chickens raised in modern production systems are typically kept on litter, composed of regionally available materials such as wood shavings, rice hulls, saw dust or straw (Robins & Phillips, 2011).

3 Objectives

The overall objective of this thesis was to study the significance of fibre intake to the functionality of poultry in different systems. More specifically, the experiments conducted were designed to study:

- the impact of fibre quality and quantity on performance, small intestinal function and nutrient digestion in broiler chickens

- the effect of various fibrous materials, including feed, roughage and litter, on production performance and abnormal pecking behaviours in different genotypes of laying hens

- the interrelationship between feed, intestinal mucosa and the bacterial flora in the digestive tract of the broiler chicken

4 Materials and methods

Three experiments (Study I-III) were conducted at Lövsta research facilities of SLU in Uppsala, Sweden, between 2009 and 2011. The fourth experiment (Study IV) was conducted at Unterer Lindenhof research station of University of Hohenheim, Germany, in 2011. For more detailed information on *e.g.* procedures, analyses and materials used in the studies please see specific papers (Paper I-IV).

4.1 Rationales of the studies

The experiments outlined below were designed to increase our general understanding of the significance of fibres in broiler chickens (Study I and II) and laying hens (Study III and IV). Cold-pressed sunflower cake was used to elevate the fibre content of the experimental diets in two of the studies (I and III). In Study I the sunflower seeds were not decorticated, whereas in Study III the seeds were partially decorticated. It was reasoned that because the quality and quantity of the fibre fraction in sunflower seed derived products can vary widely (Senkoylu & Dale, 1999) sunflower cake may serve as a model ingredient in applied experiments evaluating the different roles of fibres in poultry production. The two other studies (II and IV) involved diets almost exclusively based on ground wheat and soybean meal, and as such, represent single-sided feed compositions used in commercial feed production.

4.1.1 Study I

This first experiment was set up using increasing amounts (0-30%) of cold-pressed sunflower cake, in coccidiostat-free maize-based diets for broiler chickens during the mid-phase of growth. Here, production performance and intestinal function were the main outcome variables of interest. Maize was chosen as the main ingredients as it contains less s-NSP compared to *e.g.* wheat (see Table 1). Thus, ambiguous results due to variable contents of s-NSP in the experimental feeds could be avoided. The sunflower cake used contained large amounts of i-NSP as the seeds were not decorticated prior to oil extraction. Supplementing broiler chicken feeds with i-NSP rich fractions, such as oat hulls, has been evaluated previously (González-Alvarado *et al.*, 2007; Hetland *et al.*, 2003; Hetland & Svihus, 2001) but it is not fully clear whether such an approach may be similar to using large amounts of a feed ingredient naturally rich in i-NSP. For example, it may be that the i-NSP present in the sunflower seeds exert a stimulatory effect on intestinal function but that encapsulation of nutrients in the fibre matrix reduces their availability to the bird. Because the sunflower cake used in this particular trial contained relatively small amounts of s-NSP, it was hypothesized that the high levels of fibres would not detrimentally influence the milieu of the small intestine.

4.1.2 Study II

Study II was designed to study the effects of a fibre degrading enzyme (xylanase) used to alleviate the negative effects of s-NSP in wheat. For comparative reasons it was tested alone or in combination with another, novel enzyme (a protease) in wheat-based diets for broiler chickens during a full growth period. Further, comparisons were made with a feed additive routinely used to control intestinal parasites in commercial production (*i.e.* a coccidiostat). All additives were included on top of a basal diet (control). The performance promoting effects of the two enzymes in question had already been demonstrated (Freitas *et al.*, 2011; Choct *et al.*, 2004; Engberg *et al.*, 2004) but the mechanisms by which they worked are not fully clear. The main questions of interest were whether the effects of the two enzymes would equal the growth promoting capacity of the coccidiostat (Elwinger *et al.*, 1998) and if their effects were additive, and whether their impact would be equally mirrored in the utilization of nutrients and morphology of the small intestine.

4.1.3 Study III

Study III combined the assessment of increasing the fibre and oil content of a feed using 26% cold-pressed partly dehulled sunflower seeds, with an evaluation of providing roughage (lucerne hay) to laying hens. Production performance and abnormal pecking behaviours were the main variables of interest. In this study the press cake from partially decorticated sunflower seeds contained a relatively large s-NSP to i-NSP ratio, which also made hygienic aspects including plumage cleanliness, faecal dry matter and the proportion of dirty eggs of concern. Another aspect of interest was whether the feed containing sunflower cake would influence fat deposition in the liver.

The provision of roughage to laying hens has been studied previously but with much different experimental designs (Hammershøj & Steenfeldt, 2012; Steenfeldt *et al.*, 2007; Hammershøj & Steenfeldt, 2005).

4.1.4 Study IV

This trial was designed to investigate previously indicated correlations between the fibre content of the feed and severe feather pecking behaviour in laying hens. Although earlier studies have demonstrated reduced levels of abnormal pecking behaviours following i-NSP supplementation of the feed (van Krimpen *et al.*, 2009; Hartini *et al.*, 2002; Wahlström *et al.*, 1998) it is not known whether hens prone to perform severe feather pecking behaviour prefer diets supplemented with raw materials characterized by large amounts of i-NSP.

Two genotypes divergently selected for severe feather pecking behaviour (see details below) were allowed to choose from a control diet based on wheat and soybean meal and the control diet wherein 8% spelt hulls replaced an equivalent amount of wheat. Spelt hulls contain large amounts of i-NSP (Escarnot *et al.*, 2011) and it was hypothesized that the hens performing severe feather pecking would prefer the fibre supplemented diet to a larger extent than hens not performing severe feather pecking.

4.2 Birds, housing and data acquisition

4.2.1 The broiler chicken experiments

Study I and II were performed using Ross 308 broiler chickens (SweHatch AB, Väderstad, Sweden) of mixed sexes. Titanium dioxide was included as an inert marker in all feeds to calculate coefficients of nutrient utilization.

On the day of hatch the chicks were randomly divided into groups of eight and raised in pens elevated from the floor, shown in Figure 3 below. The floor of the pens was placed on top of a wire mesh and covered with wood shavings. These floors were removable, whereby the chickens could be kept on the wire mesh and droppings collected underneath for calculations of total tract nutrient utilization and apparent metabolizable energy of feeds, corrected to zero retention of nitrogen (AME_n) on day 34 post-hatch (Study II). Feeds and water were distributed *ad libitum*, and experimental diets were never changed during the data collection periods. Performance figures were calculated as means per pen. Thus, the pen served as the experimental unit and treatments were replicated five (Study I) or eight times (Study II). On day 31 post-hatch in Study I, birds were euthanized and digesta were collected for estimations of ileal digestibility of nutrients, feed AME_n and enumeration of selected bacterial groups using traditional culturing methods.

Concentrations of some bacterial fermentation metabolites and pH were determined in jejunal digesta. Further, a histological examination of the jejunal mucosa was made using one randomly selected bird per pen. In Study II, three randomly selected birds per pen were sacrificed on days 35 and 36 for assessment of gross morphology of the small intestine and caeca.

Figure 3. The broiler chicken pens (left) and furnished cages for layers (right). Each cage was equipped with a perch, laying nest and litter bath, and housed eight hens of either LSL or LB.

Additional analyses from Study II

Not included in Paper II, the effects of the feed additives **(**xylanase, protease and a coccidiostat) in broiler chicken diets were studied with some additional techniques. On day 35 post-hatch one randomly selected chicken per pen was dissected following an intravenous overdose of pentobarbital (100 mg/ml). Samples were then collected for histological evaluation of the jejunal mucosa and the relative abundance of selected bacterial groups in intact segments of jejunum and caecum.

Studies of the jejunal mucosa

The techniques employed comprised a conventional histological evaluation and an immunohistochemical method to quantify cell proliferation in the mucosa. First, a segment of the jejunum was excised 10-15 cm anterior of Meckel's diverticulum and prepared for microscopic examination, including *e.g.* the assessment of villus height, as described in Paper I.

Secondly, another segment, next to the first, was excised and fixed in paraformaldehyde, dehydrated in increasing concentrations of ethanol and embedded in paraffin. Sections of 4 µm were cut and stained with the ab29 antibody (Abcam, Cambridge, UK) to proliferating cell nuclear antigen (PCNA). This is a protein required for replication and repair of DNA (Kelman, 1997). Staining was visualized with the Vectastain ABC kit (Vector

Laboratories, Inc. Burlingame, CA) using normal mouse antibody (IgG-AC: sc-2343; Santa Cruz Biotechnology, Inc. Heidelberg, Germany) as a negative control. Results were visualized with hydrogen peroxide.

Pilot study of the intestinal bacterial flora using molecular techniques

Intact segments of jejunum (~5 cm in length, excised in immediate proximity of the samples described above) and caecum were snap-frozen in liquid nitrogen and stored at -18ºC. Bacterial DNA was then extracted from digesta and mucous scraped off the luminal side of the tissue segments upon thawing, using the QIAamp Stool Mini Kit (QIAGEN AB, Sollentuna, Sweden). The supplier's instructions were followed, except that a step of bead beating was introduced prior to cell lysis. This step comprised two cycles of 45 s at speed 5.0 on a FastPrep instrument (MP Biomedicals, Solon, OH). Amplification of the 16S rRNA gene was performed using the bacterial primers Bact-8F (5´-AGAGTTTGATCCTGGCTCAG-3´) 5´-labelled with 6-carboxyflourescein (6-FAM) and 926r (5´-CCGTCAATTCCTTTRAGTTT-3´). Duplicate amplification products (25µL) were digested during 2 h at 37ºC with the *Hae*III restriction enzyme (GE Healthcare, Uppsala, Sweden). Separation of digested products (>50 and <1000 base pairs) was performed on a capillary sequencer (ABI 3730) and the generated t-RFLP profiles were processed with PeakScanner 1.0. The relative abundances of terminal restriction fragments (TRFs) were calculated (<0.5% excluded) and a small number of TRFs whose mean abundance differed markedly between treatments were selected and matched against an in-house dataset of TRFs and clone libraries obtained from the gastrointestinal tract of rat (Dicksved *et al.*, 2012) and pig (Liu *et al.*, 2012) and human (Dicksved *et al.*, 2008). When TRFs of different lengths matched a defined bacterial group, their relative abundances were summed. The abundances, expressed in proportions, were finally analysed with a Bonferroni-corrected pairwise Mann-Whitney test. Due to data lost during the PCR step the final number of jejunal samples, *i.e.* from individual birds, examined was as follows: control (n=8), coccidiostat (n=7), protease (n=7), xylanase (n=8) and protease+xylanase (n=7). The corresponding numbers for caecal samples were: control (n=5), coccidiostat (n=5), protease (n=6), xylanase (n=6) and protease+xylanase (n=6).

4.2.2 The laying hen experiments

No birds in the laying hen studies were beak-trimmed. Study III was conducted using layers of two commercial hybrids; Lohmann Selected Leghorn (LSL) and Lohmann Brown (LB) obtained from the breeder (Gimranäs AB, Herrljunga, Sweden) just prior to the onset of lay. The main outcome variables of interest were production performance and damage to the plumage and skin following abnormal pecking behaviours. The use of both LSL and LB was thought to increase the applicability of the results both nationally and internationally, as LSL was the dominant hybrid used in Sweden at the time being, and LB represented a more commonly used medium-heavy brown hybrid outside of Sweden. Besides, these hybrids had previously been shown to differ in, among other features, their feather pecking activity (Kjaer, 2000).

The hens were housed in groups of 100 in a three-tier aviary system with access to litter (wood shavings) indoors and hen-runs outdoors (Figure 4). This system has previously been described by Abrahamsson and Tauson (1998).

Figure 4. The three-tier aviary system for layers (left) with connecting outdoor hen-runs (right).

The aviary pen was used as the experimental unit, implying that treatments were replicated two times per hybrid. Production performance figures were calculated from 20 to 74 weeks of age. Candling of eggs was performed in representative samples at seven occasions with an interval of 5 to 10 weeks. Fresh faeces were collected at 55 and 73 weeks of age for DM determination. Feather pecking activity was studied indirectly by integument scoring at 40, 55 and 70 weeks of age (Tauson *et al.*, 2005).

To study the effects of increased dietary levels of fibres and fat on gross liver characteristics a small number of LB hens were kept in furnished cages equipped with a perch, laying nest and litter bath (Figure 3) described as 'Model B' by Wall and Tauson (2007).

In Study IV two unique White Leghorn genotypes divergently selected on severe feather pecking activity were used. The birds originated from a random bred control line established in 1970, which has formed the basis for a genetic selection programme on feather pecking activity since 1996 (Kjaer *et al.*, 2001). At the research station Unterer Lindenhof, University of Hohenheim, Germany, the selection criteria was the frequency of severe feather pecks in mixed groups of hens displaying high feather pecking behaviour (HFP) and low feather pecking behaviour (LFP). These hens were initially kept in groups of approximately 50 on a perforated plastic floor with no access to litter. Individuals were identified by number tags attached to their backs, and 20 hens performing the greatest number of severe feather pecks (HFP) and 20 hens not performing severe feather pecks at all (LFP) during a one-hour observation session were transferred to single cages. Two separate feed troughs containing the control feed and the feed containing spelt hulls were placed in the cage front. The hens were habituated to the new environment for 2 days, during which the feed troughs were alternately covered to facilitate a better distinction between the two feeds.

In Study IV individual birds were considered the experimental unit. Prior to the transfer of hens to the single cages plumage conditions were registered (Tauson *et al.*, 2005). Feed preference data were registered daily during two weeks and the left/right position of the feed troughs were alternated every second day to minimize effects of position preferences. Following the period of feed preference recordings, all birds were presented with a single feed trough containing control feed only. Plastic dishes perforated with 10 white downy feathers were mounted to the front grid of the cage and the number of feathers plucked from the dish was recorded. This procedure was repeated for five days.

5 Results

5.1 The broiler chicken experiments

In general, results from the broiler chicken trials (Study I and II) supported earlier notions that s-NSP hinder digestion and impair performance, whereas the effects of i-NSP are less negative, or even beneficial, to the birds.

The fibrous sunflower cake used in Study I contained a high ratio of i-NSP (317 g/kg DM) to s-NSP (43 g/kg DM). As illustrated in Table 3, the successive inclusion of the sunflower cake increased the content of i-NSP from a relatively low level in the control diet to a noteworthy high level in the diet containing 30% sunflower cake, whereas the contents of s-NSP were not markedly changed. The wheat used in Study II contained a low or ordinary amount of i-NSP (82 g/kg DM) and a low content of s-NSP (16 g/kg DM).

Table 3. *Contents (g/kg DM) of crude fibre (CF) and s-NSP and i-NSP in the diets used in Study I and II. Study I evaluated increasing amounts of sunflower cake (SC) in a coccidiostat-free diet based on maize, and Study II investigated the use of two exogenous enzymes and a coccidiostat added to a basal feed mainly containing wheat and soybean meal.*

	Study I				Study II
Fibre fraction	0% SC	10% SC	20% SC	30% SC	Basal feed
CF	23	54	80	110	29
s-NSP	13	18	11	15	20
i-NSP	69	97	119	146	81

5.1.1 Performance

In Study I birds fed 20% sunflower cake increased their weight gain and feed intake compared to the control, whereas the FCR was unaffected. Feeding 30% sunflower cake increased weight gain, but a larger coincidental increase in feed intake negatively affected the FCR. Performance data from birds fed 10% sunflower cake were not analysed due to technical errors during the trial.

The increased feed consumption in groups fed sunflower cake mirrored the lower AME_n values of their diets, determined by an ileal assay. Although the consumption of AME_n, methionine, lysine and threonine was equal across all treatments, differences in feed intake mediated a higher consumption of protein, fat and ash in birds fed 20% and 30% sunflower cake.

In Study II, there were no differences in weight gain or feed intake, but the addition of xylanase or protease, or their combination, to the diet improved the FCR on every weekly registration. However, data did not indicate that the effects of the two enzymes were additive. Enzyme treatments resulted in lower FCR compared to the diet containing coccidiostat in the first two weeks, but this effect was later diminished. Compared to the control feed, the use of the coccidiostat had no effects on performance.

5.1.2 Nutrient utilization

In Study I the apparent ileal digestibility of fat and retention of crude protein improved linearly with the inclusion of sunflower cake, whereas the opposite was found for gross energy, dry matter and ash.

In Study II both enzymes, and their combination, increased the total tract apparent digestibility of gross energy, starch and fat. This resulted in increased AME_n values of the respective diets. Large numerical increases were noted in the apparent retention of crude protein but differences were not significant. The effect of enzymes on the total tract apparent retention of lysine, methionine, cystine and threonine is shown in Table 4 (not in Paper II). Both enzymes, but not their combination, improved the retention of threonine. A similar picture was true for the total tract apparent retention of lysine and methionine.

Table 4. *The total tract apparent retention of four amino acids in Study II. Birds were fed a wheat-based feed without feed additives (CON) or this feed supplemented with a coccidiostat (NAR) or a xylanase (XYL) or a protease (PRO) or their combination (XYL+PRO). All figures are presented in %, with the coefficient of variation (CV). Within a row, values with different superscript letters differ significantly (P<0.05).*

	CON	NAR	XYL	PRO	XYL+PRO	CV (%)	P-value
Lysine	89.6	90.7	90.9	90.6	90.2	1.1	0.06
Methionine	93.0	93.7	94.2	93.8	93.6	0.8	0.05
Cystine	83.9	84.5	84.6	85.0	84.0	1.5	0.39
Threonine	85.6^{b}	87.1^{ab}	87.7^{a}	87.7^{a}	87.1^{ab}	1.4	<0.01

Adding the coccidiostat to the diet increased the retention of protein and amino acids numerically, but there were no significant effects on nutrient utilization or the feed AME_n value.

5.1.3 Intestinal morphology

In Study I a linear increase in the dry matter weight of ileal digesta was seen in response to the inclusion of sunflower cake in the diet. This was accompanied by a shortening of villi and a thinning of the muscular layers in jejunum. Further, microscopic evaluations showed that a some birds fed the control diet displayed an extensive lymphocyte infiltration of the lamina propria, and a blunting and irregular organization of villi. There were no differences in depths of the crypts of Lieberkühn or thickness of submucosa and muscularis mucosa.

In Study II, but not reported in Paper II, neither enzymes nor the coccidiostat affected crypt depth or thickness of the submucosa or muscularis layers of the jejunum. Villus height and the ratio between villus height and crypt depth tended to be affected by diet. Here, the shortest villi were observed in birds fed the coccidiostat. Selected microscopic observations of the jejunal mucosa from Study I and II are summarized in Table 5.

Table 5. *Jejunal characteristics in broiler chickens. Study I evaluated a coccidiostat-free maize-based diet with increasing proportions (0-30%) of a fibrous sunflower cake (SC). Study II compared a wheat-based feed without feed additives (CON) and this feed supplemented with a coccidiostat (NAR) or a xylanase (XYL) or a protease (PRO) or their combination (XYL+PRO). All figures are presented in µm with the coefficient of variation (CV). For each study separately, values within a row accompanied by different superscript letters differ significantly (P<0.05).*

	Study I				CV (%)	P-value
	0% SC	10% SC	20% SC	30% SC		
Villus height	1430	1375	1303	1203	12	0.20
Crypt depth	163	179	154	171	15	0.45
Thickness of muscularis layers	230^{a}	231^{a}	183^{b}	158^{b}	16	<0.01

	Study II					CV (%)	P-value
	CON	NAR	XYL	PRO	XYL+PRO		
Villus height	1145	1021	1145	1156	1178	10	0.08
Crypt depth	152	162	166	172	165	10	0.18
Thickness of muscularis layers	210	232	239	224	207	20	0.53
Villus proliferation[1]	88.3	86.4	86.8	86.0	87.0	28	0.79

[1]The proportional length of villi (%) stained with PCNA antibody

In Study II, a notably strong and extensive binding was seen between the PCNA antibody complex and the jejunal mucosa. The staining was most prominent in the crypt regions, and decreased in intensity along the villus axis. This necessitated the degree of staining to be expressed as proportional length of villi stained with PCNA, as illustrated in Figure 5 below.

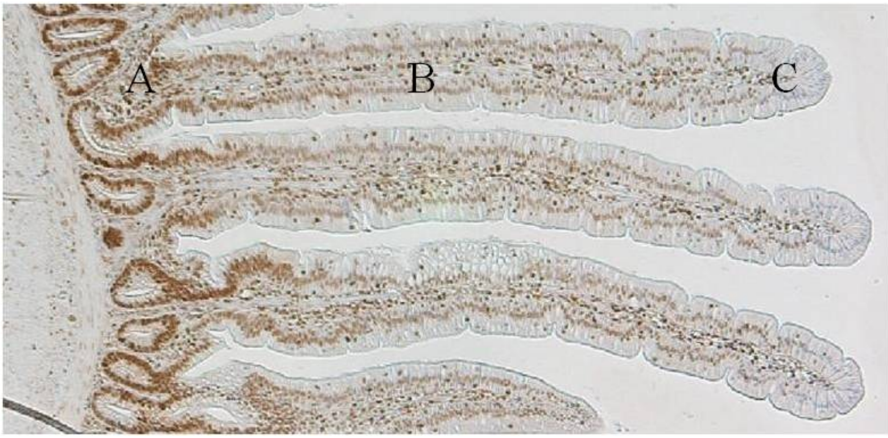

Figure 5. Jejunal mucosa from Study II stained with PCNA antibody and visualized with hydrogen peroxide (brown color) at 10 x magnification. The intensity of staining is extensive in the crypt region (A) and decreases along the villus axis (B) and is virtually absent at the top (C).

The quantifications of the degree of PCNA staining were characterized by large random variation terms and treatments did not differ significantly. Further, no feed additive in Study II affected the lengths or empty weights of duodenum or caecum, expressed in relation to body weight on day 35 and 36. However, the coccidiostat reduced the relative lengths of jejunum and ileum, and the combination of xylanase and protease reduced the relative length of ileum. The relative weight of jejunum was significantly lower in groups fed the protease compared to the combination of the xylanase and protease.

5.1.4 The bacterial flora of the intestinal tract

In Study I the jejunal bacterial community was influenced by increasing the amount of sunflower cake in the feed. Compared to the control diet the numbers of *Clostridium* spp. were significantly reduced in groups fed 20% sunflower cake and tended to be reduced in groups fed 30% sunflower cake. Moreover, increasing the proportion of sunflower cake in the diet mediated a linear reduction in the jejunal concentrations of acetic and propionic acid of jejunal digesta, but no corresponding effect on pH was seen.

In Study II both the protease and the coccidiostat affected the bacterial flora of jejunum and caeca (not in Paper II). Based on treatment differences in their mean abundances, a few TRFs were selected for analysis. These TRFs matched with *Lactobacillus* (63, 244, 323, 331 bp) and *Streptococcus* (264, 307 bp) in jejunal samples and *Lactobacillus* and *Clostridiales* (260 bp) in caecal samples.

In jejunum, the relative abundance of *Lactobacillus* was significantly lower in NAR and PRO compared to the other treatments. A similar, but opposite, trend was seen for *Streptococcus*. This is illustrated in Figure 6.

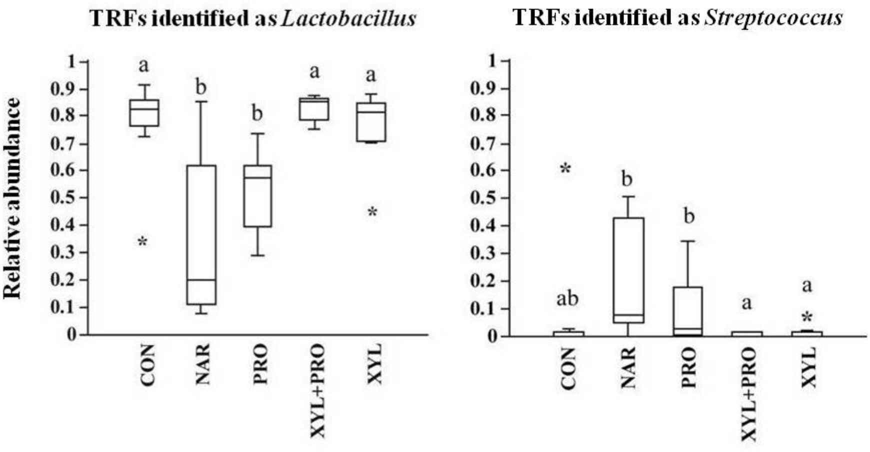

Figure 6. Relative abundance of TRFs identified as *Lactobacillus* (left) and *Streptococcus* (right) in jejunal samples. Boxes range from the 25% to the 75% quartiles. Median values are shown by horizontal lines within each box and ends represent maximum and minimum values. Stars indicate data outliers. Treatments not sharing common superscripts differ significantly (P<0.05).

The relative abundance of TRFs identified as *Lactobacillus* in caeca was significantly lower in PRO compared to all other treatments except NAR (Figure 7). Further, the TRF identified as *Clostridiales* was least abundant in NAR, whereas the protease generated a significantly higher relative abundance of this TRF compared to all other treatments, except the xylanase.

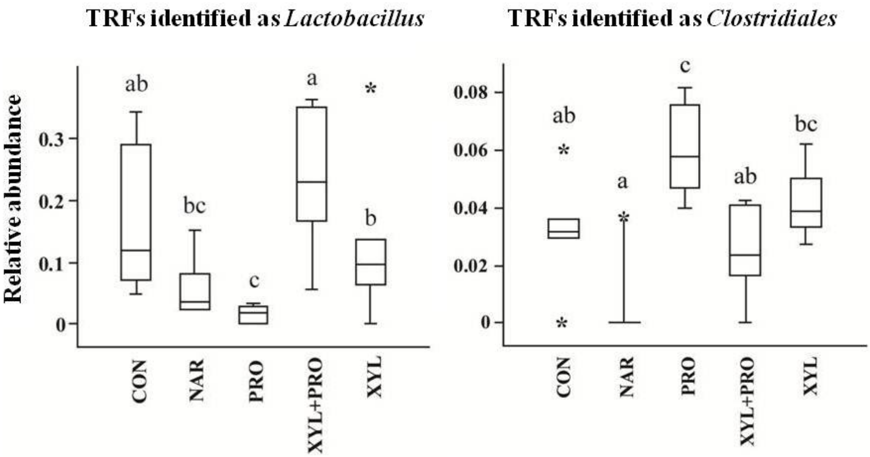

Figure 7. Relative abundance of TRFs identified as *Lactobacillus* (left) and *Clostridiales* (right) in caecal samples. Boxes range from the 25% to the 75% quartiles. Median values are shown by horizontal lines within each box and ends represent maximum and minimum values. Stars indicate data outliers. Treatments not sharing common superscripts differ significantly (P<0.05).

5.2 The laying hen experiments

The two experiments with laying hens (Study III and IV) showed large variation in performance and behaviour in both the commercial hybrids used (LSL and LB) and the experimental lines selected for high feather pecking (HFP) and low feather pecking (LFP) activity. Further, differences in the impact of various feeding strategies with relation to fibres were found.

In Study III the sunflower cake diet contained more both insoluble and soluble fibre fractions (113 g i-NSP and 30 g s-NSP/kg DM) in comparison with the control diet (96 g i-NSP and 22 g s-NSP/kg DM). The roughage was characterized by large amounts of insoluble fibres and smaller amounts of soluble fibres (433 g i-NSP and 35 g s-NSP/kg DM). Large fractions of insoluble, but no soluble, fibres were present in the wood shavings used as litter substrate (626 g i-NSP and 0 g s-NSP/kg DM). There were no analyses of NSP made in Study IV, but the control diet contained less CF (3.2%) compared to the diet containing spelt hulls (5.7%) on as-fed basis.

5.2.1 Performance

In Study III, providing hens with roughage significantly improved FCR, and tended to reduce the proportion of cracked eggs. The feed containing sunflower cake tended to improve FCR, but did not affect performance otherwise.

Although production performance was not a variable of interest in Study IV, it was noted that HFP hens weighed more and consumed more feed than LFP hens. An average low daily feed consumption in both lines (mean 81.2 g) was accompanied by very low rates of lay (mean 41%). There was a general preference for the control feed, but HFP consumed, on average, a significantly larger proportion of the spelt hulls supplemented feed than did LFP hens. As illustrated in Figure 8, this difference became established on day 3, but was successively decreased and finally diminished on day 14.

5.2.2 Hygiene and health

Feeding the sunflower cake diet in Study III lowered the DM of faeces. This was reflected in increased proportions of eggs contaminated with faeces and reduced foot-pad cleanliness. The faecal DM was also lowered following roughage supplementation, but this reduction had no substantial negative effects on the proportion of eggs contaminated with faeces or foot-health status.

The proportion of eggs laid outside the nest did not differ between dietary treatments. No effects of dietary treatment were seen on plumage cleanliness. Feeding the sunflower cake diet reduced the percentages of DM and fat in the livers of LB hens housed in furnished cages. This tended to be accompanied by reductions in fresh liver weight, total liver fat content and body weight.

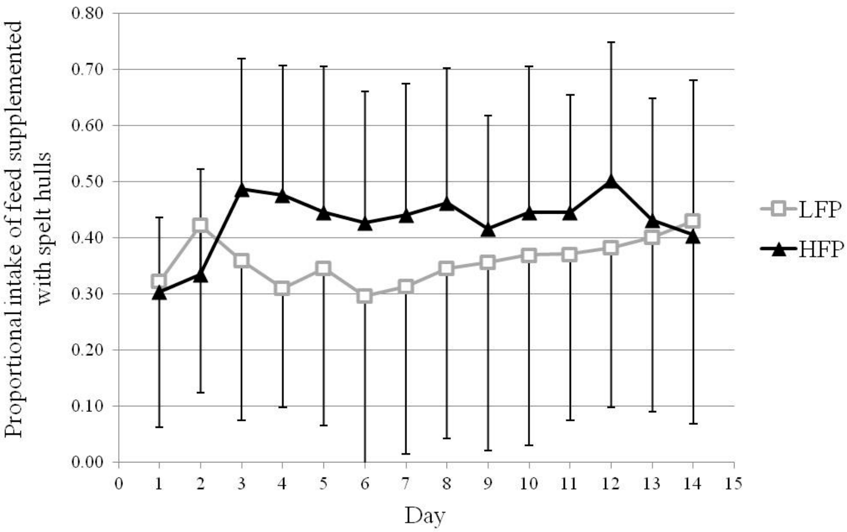

Figure 8. Daily proportional intake of feed supplemented with 8% spelt hulls in Study IV. LFP=low feather pecking hens, HFP=high feather pecking hens. Bars show standard deviations.

5.2.3 Foraging activities

In Study III all birds had access to outdoor hen-runs between 26 and 49 weeks of age. Compared to LSL, the LB hens were seen much more frequently outdoors and this was reflected in a steady decline in the amount of vegetation in their hen-runs. In contrast, more wood shavings were added to the floor in groups of LSL, as they continuously consumed their litter. In LB, groups fed the control diet were less frequently visiting the hen-runs. Roughage consumption averaged 2.9 g per hen and day, and groups provided with roughage tended to reduce their litter consumption.

5.2.4 Abnormal pecking behaviours

Feather conditions deteriorated with time in Study III. Although LSL had worse plumage condition than LB hens at 40 weeks of age, the difference disappeared with time and the feather conditions of the two hybrids were on average comparable.

Providing supplemental roughage reduced beak-inflicted injuries in the vent area and tended to improve feather conditions, but the diet containing sunflower cake did not affect injurious pecking. It was estimated that two thirds of the mortality in LB hens fed the control diet (10.2%) was due to cannibalistic pecking. Cannibalistic pecking was not seen in LSL groups.

In Study IV the feather condition of HFP was worse than in LFP hens at the time of transfer from the group housing system to the individual cages. Plumage conditions were negatively correlated with subsequent feed consumption (-0.53) and in statistical modelling, plumage condition was found to be a better predictor of feed consumption than genotype. The number of severe feather pecks delivered during the one-hour observation session in the group pens did not correlate with subsequent proportional intake of feed supplemented with spelt hulls. Following the period of feed intake registration, HFP pulled out more feathers from the plastic dish mounted to the cage front than LFP hens, but the number pulled out did not correlate with the previous proportional intake of spelt hulls supplemented feed or the number of severe feather pecks delivered during the observation in the group pens.

6 Discussion

The present experiments (Study I-IV) illuminate both positive and negative aspects of fibrous feeds in poultry nutrition. These results should be viewed in the light of differences between broiler chickens and laying hens in their sensitivity to s-NSP, and that the impact of i-NSP partly depends on *e.g.* feeding regimens and housing system used.

6.1.1 The functional broiler chicken

Performance

Compared to the control in Study I, birds fed 20% and 30% sunflower cake increased their feed intake by +9% and +17%, respectively. These increments perfectly mirrored the corresponding changes in the AME_n values of their feeds, estimated to -9% and -16% vs. control in the ileal assay, respectively. Although the methionine and lysine contents were fixed to the expected AME_n values, the CP was not. This mediated an increased CP intake in groups fed 20% and 30% sunflower cake, which limits the conclusions to be drawn from the results. Still, data suggest that broiler chickens may thrive on feeds far exceeding the crude fibre levels used in commercial practice, with the reservation that the fibres are insoluble in nature.

In Study II, both exogenous enzymes improved the FCR from 1.47 to 1.43 as calculated between day 1 and 34, but there were no indications of their effects being additive. Feed conversion ratios for full growth periods below 1.5 are quite rare, whereby the room for additional improvement following the use of either the xylanase or the protease may possibly be diminished. The lack of effect from the coccidiostat may reflect the very hygienic conditions in the experimental facilities. Overall, it seems that the effects of the feed additives studied may be different in commercial conditions.

Nutrient utilization

There was a positive linear response in the apparent ileal digestibility of CP and fat when including sunflower cake in the diet (Study I). However, scrutinizing the digestibility coefficients suggests that the trend is far from strong. Nevertheless, including 20% sunflower cake in broiler chicken diets clearly improved the digestibilities of CP and fat. The improved CP digestibility was unlikely due to differences in CP intake between treatments (Hurwitz *et al.*, 1972) but the improvement may be explained by the positive effects of i-NSP on nitrogen retention (Jiménez-Moreno *et al.*, 2009a). The improved fat digestibility is difficult to explain, but may reflect enhanced conditions for fat emulsification due to the suggested stimulatory effects of i-NSP on gut motility and bile contents in digesta (Hetland *et al.*, 2003).

In Study I, it should be borne in mind that increasing i-NSP contents were paralleled by increasing fat contents in the experimental diets containing sunflower cake. Similar to i-NSP, additional fat has been shown to increase the utilization of carbohydrates in laying hens (Mateos *et al.*, 1980) and fat digestion in broiler chickens (Nitsan *et al.*, 1997). With respect to the fibrous nature of the sunflower cake, the reductions in the digestibilities of DM, gross energy and ash were expected.

The two enzymes used in Study II improved apparent faecal digestibilities of gross energy, starch and fat to a similar degree. This was consequently reflected in elevated AME_n values of the feeds. A large numerical, but not significant, improvement in CP digestibility was seen, accompanied by significant, or close to significant, improvements in apparent digestibilities of lysine, methionine and threonine. Compared to ileal assays, determination of nitrogenous compounds in excreta is typically characterized by larger error terms (Zanella *et al.*, 1999). This difference can be explained by the presence of uric acid in excreta and bacterial fermentation in the hindgut.

The observation that the enzymes were not additive in their effects supports previous results with other combinations of enzymes (Ghazi *et al.*, 2003). Interestingly, Barekatain *et al.* (2012) recently evaluated the two enzymes used in Study II in diets containing sorghum distillers' dried grains with solubles (sDDGS). In that study, the xylanase improved the FCR and the protease increased both feed intake and body weight gain, but their effects were not additive. The authors discussed the possibility that "the lack of effect of protease on protein and amino acid digestibility in the control and 150 g/kg inclusion of sDDGS may suggest an effect other than simply improving degradation of protein which warrants further investigations".

In Study II it remains to be elucidated why the protease resulted in much similar results on digestion compared to the xylanase. It has been suggested that the positive effects of xylanases, particularly on fat digestion, may derive from their capacity to reduce viscosity (Bedford, 2002) but whether exogenous proteases affect digesta viscosity or other physiological parameters relevant to digestion is not fully understood.

There was no effect of the coccidiostat on nutrient utilization in Study II. Published data on the effect of coccidiostats on digestion in broiler chickens are scarce, possibly making the present findings unique in this sense. In swine, narasin decreased faecal nitrogen output, but not nitrogen retention (Wuethrich *et al.*, 1998) indicating that the coccidiostat may exert its growth promoting effect by alleviating endogenous nitrogen losses. Among the nutrients assayed in Study II, narasin exerted its numerically largest improvement in the retention of CP, and among the four amino acids analysed, the largest numerical improvement was seen in threonine. As reviewed by Kidd (2000) 61% of the dietary threonine is used by the gastrointestinal tract of piglets, and mucous typically contains large amounts of threonine. These observations may indicate that eventual growth promoting effects of narasin may derive from its capacity to spare the intestinal epithelium.

Intestinal morphology

Increasing the dietary inclusion of sunflower cake mediated a linear reduction in villus height and the muscularis layers of jejunum (Study I). This was hypothetically due to a stretching of the intestinal wall, following increased contents of digesta in the small intestine. The current finding contradicts the sometimes proposed association between villus height and digestive capacity (Baurhoo *et al.*, 2007) as the reductions in villus height in Study I were accompanied by increased digestibilities of protein and fat. In fact, decreased fat digestibility has previously been reported in conjunction with increased villus heights (Smits *et al.*, 2000). The hypothesis that villus height may be influenced by the fill of the intestinal tract deserves attention in future evaluations of effects of fibrous raw materials on intestinal morphology.

The two exogenous enzymes did not significantly affect the jejunal mucosa and underlying intestinal tissues in Study II, but the combination of xylanase and protease reduced the length of ileum. In contrast, Wu *et al.* (2004a) found that another xylanase preparation increased villus height in ileum. Theoretically, it may be more probable to detect eventual morphological effects from viscosity reducing enzymes posterior of mid-jejunum since Choct *et al.* (1999) demonstrated increasing viscosities along the small intestine of broiler chickens.

The fact that the protease reduced the empty weight of jejunum, compared to the combination of the protease and xylanase, may indicate that the individual action of the enzymes actually may be compromised in the presence of each other. Intriguingly, Naveed (1998) observed better performance results in broiler chickens from adding a xylanase or a cellulase, compared to when the two enzymes were mixed together with a protease in the diet. These authors speculated that the observed effect might be explained by "degradation of the carbohydrases by the protease".

In Study II the coccidiostat reduced villus height by 12%, although the difference failed to reach statistical significance. Moreover, the coccidiostat significantly reduced the length of the small intestine. Earlier studies have shown that the use of antibiotics in broiler chicken diets improves performance and mediates reductions in villus height (Miles *et al.*, 2006). Similarly, coccidiostats are believed to increase performance via their effects on the intestinal microflora (Elwinger *et al.*, 1998).

From Study II it was found that the proportional length of jejunal villi stained with an antibody to PCNA averaged 87%, but no significant differences between treatments were seen. Uni *et al.* (1998) showed that intestinal cell proliferation in broiler chickens, unlike mammals, occurs not only in the crypts but also along the villus axis. Compared to present data, others have reported lower degrees of villus staining in young chicks (Zekarias *et al.*, 2005; Geyra *et al.*, 2001) and turkey poults (Girish *et al.*, 2010) but it should be emphasized that laboratory procedures and evaluation techniques in these experiments were not uniform to Study II.

Bacterial flora of the digestive tract

Inclusion of 20% sunflower cake in the diet reduced *Clostridium* spp. counts in jejunal digesta (Study I). The underlying reason for this reduction may be ascribed the higher fat and CP digestibility seen in birds fed 20% sunflower cake, as *e.g. C. perfringens*, benefit from impaired nutrient utilization by the host (Choct *et al.*, 2006; Knarreborg *et al.*, 2002b). Because *C. perfringens* is known to exert bile salt hydrolase activity (Knarreborg *et al.*, 2002a) their impact on fat digestion is probably negative. This notion may support the current association between the improved fat digestibility and reduced *Clostridium* spp. counts.

In Study I, lactic acid was the predominant fermentation metabolite screened in jejunal digesta, but concentrations were highly variable within treatments and there was no obvious correlation with *Lactobacillus* spp. counts. Acetic and propionic acid concentrations decreased linearly due to the inclusion of sunflower cake. This finding may be unexpected as the sunflower cake contained large amounts of fibre, which intuitively would increase bacterial fermentation. It should be emphasized though, that in Study I the fibres present in the sunflower cake were primarily of insoluble nature, and that the s-NSP contents did not differ markedly between diets. Others have found that the concentration of short chain fatty acids in digesta is typically higher in diets characterized by s-NSP compared to i-NSP (Jørgensen *et al.*, 1996) which may explain the observed lack of increase in acetic and propionic acid concentration in the present trial.

In Study II, a pilot test allowed for a small number of bacterial groups to be identified from jejunal and caecal samples using molecular techniques. When matched with an in-house database, the generated DNA fragments (TRFs) corresponded to the groups of *Lactobacillus, Streptococcus* and *Clostridiales*. In broiler chickens, it was previously demonstrated that *Lactobacilli* and *Clostridia* are the two most predominating bacterial groups occupying the jejunum and caeca, respectively (Gong *et al.*, 2007).

In the present study, the addition of a xylanase or a protease, or their combination, to the wheat-based diet did not affect the relative abundance of the bacteria identified. Others have reported similar results when using only the xylanase in question (Engberg *et al.*, 2004).

Adding only a protease to the feed in the present study depressed the relative abundance of TRFs identified as *Lactobacillus* in both jejunal and caecal samples. On the other hand, the protease increased the relative abundance of TRFs matched with *Streptococcus* and *Clostridiales* in jejunum and caeca, respectively. Unfortunately, the data available from the present trial do not provide enough information to satisfactorily explain this finding.

The coccidiostat dramatically reduced the relative abundance of TRFs identified as *Lactobacillus* in jejunum, but the variation between individuals was large. In caecal samples, the coccidiostat failed to significantly reduce the relative abundance of TRFs matched with *Lactobacillus* and *Clostridiales,* even though the latter was depressed to almost zero. It was previously shown that coccidiostats inhibit *Clostridium* spp. in caeca (Elwinger *et al.*, 1998).

It may be suggested that the results reported from this pilot study be interpreted with care. This is partly because what characterizes an intestinal microflora advantageous to the bird is not fully understood. In the present study for example, the relative abundances of selected TRFs differed markedly between the three enzyme treatments, yet they all improved FCR and nutrient digestion analogously. Moreover, even if *Lactobacillus* is considered part of the healthy, natural microflora, some Lactobacilli do have the ability to impair fat digestion of the host (Guban *et al.*, 2006). The same reasoning can be applied to *Clostridiales*, a group which does include the potentially detrimental *C. perfringens,* but can at the same time be found in large numbers in the caeca of healthy animals (Gong *et al.*, 2007).

6.1.2 The functional laying hen

Consumption and utilization of fibrous feeds

Feeding layers supplementary roughage improved the FCR from 2.07 to 1.96 (Study III). Nutrients obtained from the roughage unlikely accounted for this effect as its nutritional value was low and the mean consumption amounted to 2.9 g/hen and day. Furthermore, this figure is probably an overestimation as eventual spillages were not corrected for. Instead, differences in FCR probably reflected the observed variations in plumage conditions, described further below. The condition of the plumage is important to its insulating capacity and consequently to energy expenditure. Thus, birds with damaged plumages eat more to regulate their body temperature (Peguri & Coon, 1993; Tauson & Svensson, 1980) thereby negatively affecting feed efficiency. The significance of plumage condition applies in particular to systems wherein the climate may be more difficult to control.

In Study III there was a strong tendency that also the diet containing 26% sunflower cake reduced the FCR, but this effect could not be attributed to differences in plumage conditions. Instead it seems probable that the high oil content increased the utilization of carbohydrates (Mateos & Sell, 1981). Because starch was the largest defined nutrient fraction in the experimental feeds, it may have been that carbohydrate utilization was a major determinant of feed efficiency. In contrast to the findings of Study II with broiler chickens, the higher level of s-NSP in the layer diet containing 26% sunflower cake (Study III) did not reduce feed utilization. This difference may be ascribed the fact that the negative impact of s-NSP declines with age (Petersen *et al.*, 1999).

The experimental lines divergently selected on feather pecking (Study IV) consumed on average very small amounts of feed, which partly reflected their low rate of lay. The latter feature was an effect of the selection programme which did not account for performance measures. HFP weighed more and consumed more feed than LFP hens, and it was found that the larger feed intake in HFP hens correlated with their inferior plumage condition, discussed further below.

In general, both lines avoided the feed supplemented with 8% spelt hulls, but despite large individual variation, there was a significantly larger preference for this feed in HFP compared to LFP hens. This finding, which is the first of its kind, poses some interesting hypotheses. If there is an actual demand for fibrous components in laying hens, the comparatively larger preference of HFP hens for the feed supplemented with spelt hulls would indicate that this eventual demand co-varies with abnormal pecking behaviour. Such an explanation would shed new light on observations indicating that fibrous feeds may reduce feather pecking and cannibalism (van Krimpen *et al.*, 2009; Hartini *et al.*, 2002; Wahlström *et al.*, 1998; Bearse *et al.*, 1940).

However, it cannot be ruled out that feather pecking represents a redirected foraging behaviour, as proposed by *e.g.* Blokhuis (1986). As such, hens prone to feather pecking may have a stronger incentive to investigate, by means of pecking and consuming edible particles in their environment. If so, this would explain why HFP hens consumed more of the feed supplemented with spelt hulls, even if there was a larger preference for the control feed. Why LFP hens steadily increased their proportional intake of feed supplemented with spelt hulls from day 4 and onwards is unclear and deserves further attention in future studies with these experimental lines.

The role of fibres in the digestive tract

The sunflower cake feed (Study III) contained more s-NSP than the control, and this may explain the increased moisture content of excreta. It is not fully understood by what means s-NSP influences water consumption and retention, but in the study of Langhout *et al.* (2000) the presence of bacteria was responsible for the higher water intake and increased viscosity seen in broiler chickens fed s-NSP. In Study III, it is not known whether the sunflower cake feed mediated an increase in water consumption or a decrease in water retention, or both. In general, however, it seems that easily fermentable fibres may damage the intestinal mucosa due to increased bacterial growth, thereby causing a loss of intracellular electrolytes and water to the intestinal lumen.

In Study III the elevated faecal moisture implied that the proportion of eggs contaminated with faeces increased and the foot-pad cleanliness was reduced. The former finding bears particular relevance in practice as the number of dirty eggs influences the payment to the egg producer.

Feeding the sunflower cake diet to LB housed in furnished cages dramatically influenced hepatic characteristics. The reduced fat content of the livers fits well with earlier findings that certain fibres may reduce hepatic lipids (Akiba & Matsumoto, 1980). This phenomenon can probably be explained by certain fibres' capacity to bind to bile salts (Kritchevsky & Story, 1974) which are synthesized from cholesterol in the liver (Pettersson & Åman, 1988). It may also be that the diet in Study III shifted the bacterial flora towards a profile of higher bile salt conjugation activity (Knarreborg *et al.*, 2002a). However, the higher fat content of the sunflower cake diet presumably influenced liver lipids contents too, via inhibition of hepatic lipogenesis (Hillard *et al.*, 1980). In practical nutrition, the results from the present trial may be implicated in diet formulations to hens prone to fatty liver disease.

Foraging and abnormal pecking behaviours

The hybrids in Study III demonstrated notable differences in their use of the outdoor hen-runs. Whereas LB utilized their hen-runs extensively and soon had depleted the vegetation, very few LSL hens were registered in the hen-runs. It has been shown that proactive behaviour has a genetic component and that it correlates with *e.g.* feather pecking (Jensen *et al.*, 2005). In Study III, however, more wood shavings were added to LSL groups indoors as they more rapidly depleted their litter compared to LB hens. Earlier work has demonstrated differences among genotypes in the propensity to peck litter substrates (Savory & Mann, 1997) and maintaining a loose and dry litter throughout the laying period is important to control abnormal pecking behaviours (Green *et al.*, 2000). Even if the provision of *e.g.* wood shavings does not necessarily stop feather pecking (Huber-Eicher & Wechsler, 1998) continuous provisions of fibrous litter substrates constitutes a practically feasible means for egg producers to assure that the hens are exposed to foraging material and i-NSP.

Interestingly, LB hens assigned to the control feed in Study III were less frequently seen in the hen-runs, compared to when fed the sunflower cake feed or supplementary roughage. Hypothetically, this finding may reflect an increased fearfulness in these birds as they displayed a relatively high level of abnormal pecking behaviours, accounting for approximately two-thirds of total mortality (10.2%). It has been shown that fearfulness in laying hens is associated with unwillingness to visit outdoor areas (Mahboub *et al.*, 2004).

Damage to the vent area following abnormal pecking behaviour was reduced with supplementary roughage. It may be rash to stress that this was due to the fibres present in the roughage because the mere presence of a coarse substrate, which invited manipulation, possibly played an important role. As such, the roughage might have had an alleviating effect on the propensity to perform misdirected foraging pecks towards conspecifics. In fact, Huber-Eicher and Wechsler (1998) showed that the positive effect of providing long-cut straw on foraging behaviour and reduction of feather pecking could also be achieved with the provision of polystyrene blocks.

It is worth considering that beak-inflicted damage was reduced in groups provided roughage, despite the very low amounts consumed (2.9 g/d). In the experiment of Steenfeldt *et al.* (2007) abnormal pecking behaviours were dramatically reduced following roughage supplementation, but supplement intakes were very high (58-108 g/hen and day) which makes implementation to large-scale egg producers somewhat more difficult.

The absence of effects from the sunflower cake diet on abnormal pecking behaviours was likely related to its fibre quality. Including sunflower cake in the diet mediated a larger increase of s-NSP (+36%) than of i-NSP (+18%) and in experiments where fibrous feeds reduced abnormal pecking behaviours the fibre sources have primarily been insoluble in nature (van Krimpen *et al.*, 2009; Hartini *et al.*, 2002; Bearse *et al.*, 1940).

In agreement with Su *et al.* (2006) plumage conditions differed significantly between the experimental lines divergently selected on feather pecking (Study IV). In the present study it should be borne in mind that damage to the plumage originated from pecks delivered in the group housing system where HFP and LFP hens were kept mixed together. Thus, the inferior plumage condition of HFP indicates that these birds not only pecked their conspecifics to a greater extent, but also received more pecks than LFP hens. This finding contrasts that of Savory and Mann (1997) and Kjaer and Sørensen (1997). There seems to exist no heritable component in being the recipient of pecks (Kjaer & Sørensen, 1997) which is why it is unlikely that the selection on feather pecking activity also has resulted in hens receiving pecks. Instead, the observed association between damaged plumage condition and the propensity to severe feather pecking in Study IV may derive from self-pecking.

In Study IV the higher feather pecking activity in HFP hens was mirrored in larger numbers of feathers plucked from the plastic dish mounted to the cage front. Some of the hens had plucked all feathers presented within a few minutes, indicating that the incentive to pluck feathers was very strong. However, the number of feathers plucked did not correlate with the number of pecks delivered in the group housing system. Further, no correlation was found between the number of feathers plucked and the previous consumption of feed supplemented with spelt hulls. Lack of correlations between actual feather pecking and inanimate models has been reported earlier (Rodenburg & Koene, 2003) and in the present trial the sample size was most probably restricting.

Feathers resemble i-NSP in their action in the gastrointestinal tract (Harlander-Matauschek *et al.*, 2006) and in the experimental lines used in Study IV it was previously demonstrated that HFP ingested more feathers than LFP hens (Harlander-Matauschek *et al.*, 2007). In Study IV, however, it was unfortunately not possible to quantify feather ingestion as the manure belts contained a mix of feathers shed from the plumage and feathers plucked from the plastic dish.

6.2 On the interpretation and applicability of trials with fibrous feeds

There are a number of factors which affect the interpretation and application of the present results in practice. These factors include analytical shortcomings with respect to fibres in commercial feed production and inherent ambiguities in experiments addressing the nutritional impact of fibres.

6.2.1 Analytical procedures

Historically, the lack of appropriate analytical procedures for a meaningful characterization of fibres has likely contributed to the confusion on whether the fibre fraction of feeds should be regarded inert, anti-nutritive, or on the contrary, beneficial to fowls. The notion that fibres may be nutritionally detrimental is reflected in *e.g.* simple prediction equations of the energy value of wheat:

$$AME_n = 3626 - 99CF + 23CP - 89ASH \qquad \text{(Larbier \& Leclerq, 1994)}$$

(where CF, CP and ash are expressed in % DM and energy in kcal/kg DM). However, the use of ‘crude fibre’ as a negative factor in such equations may seem contradictory to results from experiments where fibres have exerted a distinguished positive effect, see *e.g.* Davis and Briggs (1947) and Rogel *et al.* (1987) or Jiménez-Moreno *et al.* (2009b).

Today we know that the information obtained from the ‘crude fibre’ analysis is of limited relevance in poultry nutrition. Still, there are currently no demands on the declaration of fibres other than the ‘crude fibre’ analysis of feedstuffs or compound feeds marketed in the European Union (EC, 2009). Thus, if the growing knowledge on the significance of fibre quality is to be applied in the field, actors on the feed market need to find the most practically and economically feasible, yet biologically relevant, methods to characterize fibres. Since there exists a large spectrum of more or less time consuming and technically challenging procedures for the determination and characterization of NSP, as reviewed by Lunn and Buttriss (2007), such a task would probably gain from co-ordination between poultry nutritionists and experts in carbohydrate chemistry. It seems probable that advancements in analytical specificity, in conjunction with an increasing awareness of the impact of fibre quality, will benefit the development of commercially available analytical kits and procedures.

6.2.2 The energy content of fibrous feeds

The energy content of feeds is an entity of great significance to researchers in poultry nutrition. In essence, this is because feed intake is often negatively correlated with the energy value of the feed. It may therefore be argued that all diets in an experiment should be iso-energetic since low-energy diets otherwise will increase feed consumption and their appraisal be penalized. This applies in particular to experiments with fibrous feeds as the fibre fraction of feeds is often associated with low AME_n values. The typically low energy value of fibrous feeds mainly derives from three properties of fibres. First, a high content of fibres generally means that there is less room for more energy-dense fractions such as fat, starch and protein in the material. Second, the fibre fraction of cereals, oil seeds and legumes is normally composed of variable amounts of s-NSP (Knudsen, 1997) meaning that high fibre contents may imply limitations in digestive capacity. Such limitations compromise the utilization of the potentially metabolizable energy present in the feed. Third, even if fibres escaping digestion by endogenous enzymes can be fermented by bacteria into end products metabolizable to the host, their contribution to the energy requirement of the fowl is marginal (Jørgensen *et al.*, 1996).

When formulating poultry feeds, low energetic values of fibrous materials can be compensated for by the addition of fat. However, this approach leads to the question whether eventual effects derive from the fibres or the added fat. This issue is further complicated by the fact that both fibres and fat influence *e.g.* lipid metabolism (Akiba & Matsumoto, 1980; Butler, 1976; Kritchevsky & Story, 1974) and retention time of digesta (Ferrando *et al.*, 1987; Mateos *et al.*, 1982) and nutrient utilization (Jiménez-Moreno *et al.*, 2010; Mateos *et al.*, 1980).

In the present thesis, different approaches were used to address the issue of low energetic values in some of the fibrous materials tested. In Study I, no attempt was made to compensate the low energy value of the test feeds as the highest inclusion of the fibrous sunflower cake (30%) resulted in a total amount of 7.6% fat in the diet which made further fat additions virtually impossible with respect to pelleting. No energy compensation was required in Study II as it addressed the effects of two enzymes and a coccidiostat added on top of a basal diet. In Study III, soya oil was added to achieve a similar level of AME_n in the experimental feed containing 26% sunflower cake (11.4 MJ/kg) compared to the control (11.3 MJ/kg) calculated according to the European prediction equation (Rose, 1997). In Study IV, spelt hulls were included in the diet on the expense of wheat and no adjustments for differences in nutritional value were made. Here, it was reasoned that since the objective was to determine the preference for fibres, rather than egg production and feed efficiency, this was an appropriate way of formulating the experimental diets.

In summary, independent of which approach is used the typically low energy value of fibrous materials restrict the possibility to generalize from trials wherein large amounts of fibrous materials are fed.

6.2.3 Fibre intake from other sources than the feed

Broiler chickens

Although commonly overlooked, voluntary consumption of fibrous litter constitutes a confounding factor in experiments evaluating the effects of fibres present in the feed. During experimental conditions broiler chickens ingest significant amounts of litter (Malone & Chaloupka, 1983) and when added to concrete floors the ingestion of wood shavings may alleviate the occurrence of maldeveloped proventriculi and gizzards (Riddell, 1976). Recently, Kaldhusdal *et al.* (2012) showed that access to wood shavings improved feed efficiency, reduced ileal starch contents and caecal *Clostridium perfringens* counts.

In nutrition experiments addressing fibres it is important that the ingestion of i-NSP rich litter is controlled, or accounted for. If not, it clearly may confound, or limit, the effects of adding i-NSP sources to the diet. For example, Swick *et al.* (2012) recently demonstrated that broiler chickens reared on hardwood shavings had larger gizzards and better feed conversion than when reared on paper. Furthermore, it was shown that the addition of 7% oat hulls to a wheat and soybean meal-based diet improved feed efficiency, but only when the birds were reared on paper and not on hard wood shavings.

It should also be noted that feeding regimens including the use of whole wheat has been found to increase gizzard size and improve feed efficiency, even when broiler chickens are reared on wood shavings (Wu & Ravindran, 2004). Consequently, in areas such as Sweden, where whole wheat feeding concepts are employed and the majority of broiler chickens are reared on wood shavings, the benefits from adding raw materials rich in i-NSP can be expected to be much lower than in areas where these regimens are not used.

Laying hens

A much similar argumentation can be applied to laying hens housed in modern housing systems, *i.e.* furnished cages, single-tier floor systems and aviaries. Previous estimations of fibrous litter consumption in furnished caged layers (Hetland & Svihus, 2007) were recently supported by Kalmendal *et al.* (2012a). In this trial it was also found that the amount of litter consumed varied between genotypes, as illustrated in Table 6.

It was found that litter intake in LB was higher when crumbled straw pellets were used as litter bath substrate compared to saw dust. Overall, differences in daily fibre intakes tended to affect egg shell quality, hypothetically due to the capacity of certain fibres to bind minerals (van der Aar *et al.*, 1983). As seen in Table 6, the large coefficient of variation (CV) indicates that litter intake varied extensively even within treatments.

Table 6. *Litter intake in furnished cages. Lohmann Brown (LB) and Lohmann Selected Leghorn (LSL) were fed a wheat and soybean meal diet (Control) or a diet supplemented with 3% straw pellets (Fibre). In the litter baths, hens were provided with saw dust or crumbled straw pellets. Figures are presented in g per hen and day, with the coefficient of variation (CV). Within hybrids (H) or treatments (T) values with different superscripts differ significantly (P<0.05).*

	Hybrids (H)		Treatments (T)				P-values		
	LB	LSL	Control /Saw dust	Control /Straw pellets	Fibre /Saw dust	CV (%)	H	T	H x T
Litter intake	3.3^a	1.3^b	2.1^b	2.6^a	2.0^b	47.7	<0.01	0.02	<0.01

Quantifying litter consumption in floor or aviary systems is more difficult as the material becomes mixed with the manure. However, indirect evidence of litter consumption in layers originate from *e.g.* Hetland *et al.* (2005). As referred to earlier in the text, these authors conducted an experiment with hens housed in a floor system and found that access to litter (wood shavings) resulted in larger gizzards compared to hens housed in barren cages. Additional support of this notion derive from a recent study by Kalmendal *et al.* (2012b). Here, feeds and hybrids were identical to the trial referred to in Table 6, but this time performed in a one-tier floor system wherein wood shavings were used as litter substrate. Due to a series of unfortunate events, outbreaks of cannibalism occurred early in egg production. All dead birds were necropsied (n=188) and the gastrointestinal tract examined. Litter material and feathers in the gastrointestinal tract were found in 31% and 38% of the birds, respectively. Because the effects of fibrous feed components are similar to the effects of ingested wood shavings (Hetland *et al.*, 2003) and feathers (Harlander-Matauschek *et al.*, 2006) in the gut, these findings strongly suggest that consumption of structural components from other sources than the feed need consideration in nutritional experiments.

7 Conclusions

This thesis demonstrates that the significance of fibres to the functionality of broiler chickens and laying hens depends on both quantity and quality of the fibres ingested. In summary, the following conclusions were drawn:

- Increasing the dietary levels of a feed ingredient distinguished by insoluble fibres improved fat and protein digestion in broiler chickens, but not in a dose-dependent manner. The effects were reflected in altered characteristics of the small intestinal tissue and microflora of the gut lumen.

- Adding a xylanase to a diet based on wheat increased feed utilization in broiler chickens. Corresponding effects were seen with the inclusion of an exogenous protease, but not a coccidiostat, in the diet. Treatment differences in feed utilization were generally not mirrored by macroscopic or microscopic changes of the small intestine. The protease and coccidiostat affected the relative abundance of selected bacterial groups in jejunum and caeca.

- Supplemental roughage reduced beak-inflicted injuries in laying hens. This was accompanied by improved feed efficiency, likely reflecting reduced energy expenditure due to better plumage conditions. The two hybrids studied differed in their foraging behaviours.

- Formulating a layer diet with large amounts of cold-pressed sunflower cake mediated an increase in the contents of soluble fibres and fat. This implied impaired hygienic conditions and reduced fat in the liver. The diet tended to improve feed efficiency, probably due to beneficial effects of a higher dietary fat level on digestion.

- In support of the hypothesized correlation between injurious pecking and the fibre content of feeds, laying hens predisposed to perform severe feather pecking preferred a diet supplemented with spelt hulls to a greater extent than hens not predisposed to severe feather pecking.

- Inherent ambiguities in fibre nutrition trials make it difficult to separate the effects of increasing amounts of fibrous feedstuffs from those of *e.g.* increased level of dietary fat or nutrient dilution.

- Nutritional and physiological effects associated with fibre intake do not merely mirror the fibre content of the feed, but may depend on the ingestion of *e.g.* litter and feathers.

8 Prospects for research and applications

Interpretations of the impact and applicability of the results must take into account differences in housing systems, feeding regimens and biological characteristics between different animal categories and genotypes. The following issues deserve attention in future research:

- Different exogenous enzymes' mode of action, their interactions and impact in relation to diet characteristics.

- Determination of tolerance levels of soluble fibres, particularly in the young chick.

- Interactions between mineral retention and fibres ingested from both feed and litter.

- Dietary preferences and nutritional demands of laying hens predisposed to perform abnormal pecking behaviour.

Actors in the fields of commercial feed and poultry production may act on the present results by:

- Finding the practically, economically and biologically most relevant ways of characterizing fibres in raw materials and compound feeds.

- Increasing the egg production sector's awareness on the significance of litter provision and quality, as litter may serve both as a foraging material and a dietary source of insoluble fibres.

- Considering the dietary levels of fat and fibres in feeds for layers prone to fatty liver disease.

- Determining the impact of exogenous enzymes, in relation to coccidiostats, in practical conditions.

9 Svensk sammanfattning

Fibrer återfinns i de flesta vegetabiliska foderråvaror, men fjäderfä saknar förmågan att bryta ned dem i tarmkanalen. Trots detta kan fiberfraktionen i fodret ha en betydande inverkan på magtarmkanalens utseende och funktion, samt på upptaget och omsättningen av näringsämnen. Detta beror bl.a. på vissa fibrers förmåga att stimulera magtarmkanalens rörelser, samt att fibrer utgör substrat för tillväxt av olika grupper av bakterier i tarmen.

Hur fågeln påverkas av fodrets fiberfraktion speglar i hög grad hur fibrerna interagerar med vatten. Fibrer som är lättlösliga i vätskor kan binda stora mängder vatten, vilket ökar viskositeten i tarmen. Ökad viskositet medför att de enzymer som fågeln utsöndrar i tarmen hindras från att komma i kontakt med fodrets näringsämnen. Därmed kan osmälta fraktioner av t.ex. protein och fett istället utnyttjas av tarmens bakterier, varav vissa är patogena och kan skada tarmslemhinnan. Skador på tarmslemhinnan medför i sin tur sämre näringsupptag och risk för nedsatt hälsa hos djuret.

Fiberfraktioner som är svårlösliga i vätskor ökar inte viskositeten i tarmen på motsvarande sätt. De har därför inte heller den negativa inverkan på näringsupptaget som lättlösliga fibrer. Svårlösliga fibrer stimulerar istället magtarmkanalens rörelser. Detta är särskilt viktigt för hönsfåglar vars mekaniska nedbrytning av födan sker i den övre delen av magtarmkanalen, kallad muskelmagen.

Idag vet vi att fibrernas löslighet till stor del förklarar deras inverkan på fågeln, men historiskt har det saknats lämpliga analysmetoder för att mäta fibrernas egenskaper. I det här doktorsarbetet har moderna tekniker för att karaktärisera fiberfraktionen i fodret utnyttjats. Olika fodermedels effekt på näringsupptag, produktion, hälsa och beteende har satts i relation till kvalitén på fibrerna i dieten.

I två experiment vid SLU undersöktes effekten av svårlösliga, respektive lättlösliga fibrers inverkan på slaktkycklingar. Den första studien visade att ökande inblandning av svårlösliga fibrer stimulerade upptaget av fett och protein från fodret. Troligtvis kan resultatet delvis förklaras med de svårlösliga fibrernas stimulerande inverkan på magtarmkanalens rörelser. Det förbättrade upptaget av fett och protein korrelerade med sjunkande nivåer av potentiellt skadliga bakterier (*Clostridium* spp.) samt en förtunning av tarmväggen. Effekten på tarmväggen var sannolikt ett resultat av att mängden digesta i tarmen ökade med stigande inblandning av svårlösliga fibrer, som sannolikt har en uttänjande effekt på tarmen.

Det andra försöket visade att de negativa effekterna av de lättlösliga fibrer som återfinns i vete kan reduceras med tillsats av ett fibernedbrytande enzym (xylanas) i fodret. Tillsatsen av enzymet ökade upptaget av stärkelse, fett och energi från fodret. Upptaget av protein och enskilda aminosyror tenderade att öka på motsvarande sätt, men effekten var inte statistiskt säkerställd. Jämförbara effekter sågs vid tillsatsen av ett proteinnedbrytande enzym (proteas) i fodret, men inte vid tillsats av koccidiostatika, d.v.s. en fodertillsats som normalt används i slaktkycklinguppfödning för att hämma tillväxten av särskilda tarmparasiter, s.k. koccidier. Tillsatsen av proteas och koccidiostatika i fodret förändrade även artsammansättningen av bakterier i magtarmkanalen. Genom att tillsätta en kombination av de två enzymerna som användes i studien (xylanas och proteas) erhölls motsvarande resultat som när enzymerna användes var för sig. I det här försöket sammanföll inte de positiva effekterna av enzymtillsatserna på näringsupptaget med några förändringar i tarmväggens utseende.

I ytterligare två försök undersöktes förhållandet mellan fiberintag och produktion, hälsa och välfärd hos värphöns. Särskild vikt lades vid två oönskade och skadliga beteenden; fjäderplockning och hackning. Det ena försöket utfördes vid SLU och kartlagde effekten av att ge frigående höns tillgång till grovfoder, eller ett foder med högre innehåll av fibrer och vegetabilisk olja. Höns som fick grovfoder uppvisade färre hackskador samt ett bättre utnyttjande av fodrets näringsinnehåll. Fjäderdräkten isolerar mot kyla, varför hönsen med mindre skadad fjäderdräkt sannolikt kunde använda mer av fodrets näring till äggbildning, istället för att reglera kroppstemperaturen. Även höns hållna i inredd bur, d.v.s. en bur försedd med sittpinne, ströbad och rede, fick äta fodret med högre innehåll av fibrer och vegetabilisk olja. Detta resulterade i lägre fetthalt i levern; ett resultat som kan vara värdefullt i praktiken då värphöns ibland drabbas av leverförfettning. Samma foder tenderade också att bidra till ett högre näringsutnyttjande. Detta kan bero på fodrets högre innehåll av vegetabilisk olja, som antas stimulera upptaget av kolhydrater hos värphöns. Den hygieniska miljön i stallet försämrades dock i de grupper som utfodrades med det mer fiberrika fodret. Detta sammanföll med ökad vattenhalt i träcken, vilket kan antas hänga samman med att fiberfraktionen i fodret var förhållandevis lättlöslig. Lättlösliga fibrer har i tidigare studier visats öka träckens vattenhalt hos fjäderfä. Försöket visade också på tydliga skillnader mellan vita och bruna höns. Förutom skillnader i produktion vistades bruna höns mer i rastgårdarna, där de också konsumerade merparten av all vegetation, medan vita höns spenderade mer tid inomhus i stallet. Både vita och bruna höns konsumerade det kutterspån som användes som strömaterial i stallet, men konsumtionen var större bland vita än bruna höns.

Det sista försöket utfördes på universitetet i Hohenheim, Tyskland, där man korsat fram två typer av värphöns; en avelslinje som är mycket benägen till en allvarlig form av fjäderplockning, eller hackning, och en avelslinje som inte alls uppvisar motsvarande beteende. Höns från respektive avelslinje tilläts välja fritt mellan ett vanligt hönsfoder och ett mer fiberrikt foder innehållandes skal från speltvete. Hypotesen var att de höns som utförde fjäderplockning skulle äta mer av det fiberrika fodret, eftersom tidigare studier har indikerat att det finns en koppling mellan fodrets innehåll av fibrer och fjäderplockning. Försöket i Tyskland styrkte denna hypotes; höns som uppvisade en mycket allvarlig form av fjäderplockning, eller hackning, konsumerade mer av det fiberrika fodret än höns som inte uppvisade beteendet.

Sammanfattningsvis visar den här doktorsavhandlingen att fodrets innehåll och kvalité av fibrer påverkar slaktkycklingar och värphöns i varierande utsträckning. Två generella slutsatser kan dras; för det första bör fodrets innehåll av lättlösliga fibrer begränsas, samt dess negativa effekter lindras med tillsats av fibernedbrytande enzymer. Detta gäller särskilt unga djur, såsom slaktkycklingar. För det andra kan svårlösliga fibrer, utöver att stimulera magtarmkanalens funktion, ha en lugnande effekt på värphöns benägna till fjäderplockning eller hackning. I synnerhet bör tillämpningen av den senare slutsatsen väga in skillnader mellan olika system för inhysning och utfodring, eftersom effekten av fibrerna i fodret kan sammanfalla med effekterna av utevistelse samt tillgången på grovfoder, fiberrikt strömaterial och hel spannmål.

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