Investigations on the effects of dietary insoluble and soluble non-starch polysaccharides (NSP) on host-parasite interactions in laying hen chicks infected with *Heterakis gallinarum* or *Ascaridia galli*

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Dedicated to

the memories of my mother

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LIST OF ABBREVIATIONS

°C	Degree Celsius
µl	Microliter
ADF	Acid detergent fibre
ADG	Average daily weight gain
AIC	Akaike's Information Criterion
BIC	Schwarz's Bayesian Criterion
BW	Body weight
BWG	Body weight gain
CaCo3	Calcium carbonate
CON	Control basal diet
CP	Crude protein
d	Day
DM	Dry matter
EE	Ether extract
EPD	Eggs per day; total number of eggs excreted per worm population of a bird within 24 h
EPD/female	EPD based female worm fecundity; number of eggs excreted per female worm within 24 h
EPG	Eggs per gram of faeces
EPG / female	EPG based female worm fecundity; average number of eggs excreted per female worm through one gram of faeces
FID	Flame ionization detector
FMVO	Futtermittelverordnung (German Feed Regulations)
g	Gram
g	Acceleration of gravity, g-force
GC	Gas chromatography
Hs-Index	Hepato-somatic index
I-NSP	Insoluble non-starch Polysaccharide
$K_2Cr_2O_7$	Potassium dichromate
LSL	Lohmann Selected Leghorn
MCP	Monocalcium Phosphate
ME	Metabolizable energy
N	Total number of observations
n	number of observations per group or treatment
NDF	Neutral detergent fibre
NSP	Non-starch polysaccharide
OM	Organic matter
p. i.	post infection
SCFA	Short chain fatty acids
S-NSP	Soluble non-starch polysaccharide
v/v	volume / volume
VFA	Volatile fatty acids
w/v	Weight / volume

SUMMARY

The objective of this study was to test the hypothesis that low or highly soluble dietary non starch polysaccharides (NSP) differently affect infections with *Heterakis gallinarum* or *Ascaridia galli* in grower layers. Because *H. gallinarum* acts as a vector for transmission of *Histomonas meleagridis*, the agent of 'Blackhead disease', effects of NSP-supplemented diets on interactions between these two parasites were also investigated.

The experiments were conducted between 2007 and 2010 at the Department of Animal Sciences of the Göttingen University. Three experimental diets were used: basal diet (CON), supplying metabolizable energy and nutrients for grower layers according to recommended feeding standards. One hundred gram of pea bran or chicory root meal were added to each kg of CON in diets I-NSP and S-NSP supplying insoluble (I-) or soluble (S-) NSP, respectively. The first study aimed at investigating effects of NSP supplemented diets on interactions between *H. gallinarum* and *H. meleagridis*, including a prophylactic treatment with dimetridazole (0.05%, w/v) via drinking water against *H. meleagridis* for half of the birds. Histomonas free *H. gallinarum* female worms obtained from this study were used for the preparation of infection material applied in the experiment that investigated effects of NSP-supplemented diets on *H. gallinarum* infection. Effects of dietary NSP on either *H. gallinarum*- or *A. gall*-infections were separately investigated in two consecutive experiments, each comprising three identical runs for each nematode species. In each run, three feeding groups of one-day-old female layer chicks were built, each being fed until an age of wk 3 with one of the three experimental diets. At the end of wk 3, the birds were marked with wing tags and weighed. Each feeding group was subdivided into an uninfected control and an infected group of birds, the latter being inoculated with 200 embryonated eggs of *H. gallinarum* or 250 embryonated eggs of *A. galli*, respectively. Daily feed consumption was determined per group throughout the experiment until wk 11. In the last two runs of the *H. gallinarum*-experiment, the infected birds were placed into individual cages and their daily total amounts of faeces, number of eggs per gram of faeces (EPG) and total number of eggs excreted within 24 h (eggs per day, EPD) were determined. In the *A. galli*-experiment, the faeces were collected at the time of slaughter and EPG was determined. The birds were slaughtered 8 wk post infection (p.i.) and their worm burdens were determined. Volatile fatty acids (VFA) and pH were measured in caeca contents.

In the first experiment that dealt with histomonas contaminated *H. gallinarum* (**Chapter-II**), treatment against *H. meleagridis* significantly increased the incidence of *H. gallinarum* infection and the average worm length in all infected groups irrespective of the type of experimental diet consumed by the birds. An interaction between effects of diet and dimetridazole treatment indicated that S-NSP resulted in lowest worm burden in dimetridazole-untreated birds, whereas it caused highest worm burden in treated birds. Within each feeding group, higher worm burdens were determined in treated than in untreated birds. Infection with *H. gallinarum* reduced the body weight (BW) of the chicks, and *H. meleagridis* aggravated this effect. Dimetridazole-treated and untreated uninfected birds developed similar BW. Both NSP-supplemented diets, with S-NSP being inferior to I-NSP, led to lower BW of the birds.

In the histomonas free *H. gallinarum*-experiment (**Chapter-III**), the NSP-supplemented diets elevated the incidence of infection, the average number of larvae and the total worm burden compared to CON. The worm length was not influenced by the type of diet. The daily amount of faeces increased in NSP-fed birds. The EPG, EPD and female worm fecundity (EPD/female worm) were elevated after feeding S-NSP, whereas I-NSP led to lower EPG/female worm. The EPD increased in the sequence of CON < I-NSP < S-NSP. Both, the NSP-supplemented diets and infection led to reduced BW of birds and infection additionally impaired the feed conversion rate. The NSP-supplemented diets increased average length of caecum with S-NSP exerting a stronger effect than I-NSP. Filled caeca weight was increased by S-NSP. The infection increased the weight of filled and emptied (washed) caeca. Feeding S-NSP lowered intracaecal pH and molar proportion of acetate and increased that of butyrate compared to CON and I-NSP. Caecal pool of VFA was increased with S-NSP. Infection increased intracaecal pH, accompanied by lower molar proportion of butyrate and reduced caecal pools of VFA.

In the *A. galli*-experiment (**Chapter-IV**) both NSP-diets, particularly S-NSP, increased incidence of infection and worm burden of the birds, but the development (length) and fecundity of the nematode remained unaffected. *A. galli*-infection caused a less efficient feed utilization for body weight gain (BWG) resulting in lower BW irrespective of type of diet consumed. NSP-fed birds, particularly those on S-NSP, showed retarded BW development compared to birds receiving CON. Intracaecal pH was lowered by feeding S-NSP but was unaffected by *A. galli*-infection. Both NSP-diets increased caecal VFA pool size, S-NSP exerting a greater effect than I-NSP. Infected birds had smaller caecal VFA pool size than their uninfected counterparts consuming the

corresponding diets. S-NSP also led to higher weights of splanchnic tissues and intestinal tract. These effects were less pronounced in I-NSP fed chicken.

The results show that *H. meleagridis* does not only harm the definitive host, but also its vector, *H. gallinarum*. Both, insoluble and soluble dietary NSP favor *H. gallinarum* infection while S-NSP additionally intensifies histomonas-infection, which then impairs establishment and development of *H. gallinarum*. The pea bran and chicory root meal used as sources of insoluble and soluble dietary NSP, respectively, favored the establishment of histomonas-free *H. gallinarum* in grower layers. Inulin rich chicory root meal additionally enhanced fecundity of this nematode. Insoluble and soluble dietary NSP retard growth performance, alter gastrointestinal environment and lead to higher weights of splanchnic tissues associated with an elevated establishment of *A. galli* in grower layers. The NSP supplemented diets, and S-NSP in particular, may have altered the gastrointestinal environment, which in turn enhanced nematode infections. It is concluded that the two natural sources of insoluble and soluble NSP offer no potential as protecting agents against the parasitic infections in chicken. Therefore, suitable measures of precaution should be applied to production systems particularly prone to gastrointestinal parasitic infections and where diets with relatively high NSP-contents are fed.

CHAPTER - I

BACKGROUND

1.1. Foreword

As a consequence of recent changes in consumer demands toward chemical-residues free animal products and increasing public concern about animal welfare, conventional cage production systems for laying hens are being replaced by outdoor/free-range systems in the EU (Gauly et al., 2001; Permin and Ranvig, 2001). The proportion of laying hen farms in the form of non-cage production systems increased from 6.7 % in 2000 to 33.8% in 2007, and will be 100% by 2010 in Germany (ZMP, 2008). In some countries, e.g. in Switzerland the battery cages have completely been banned (Kaufmann-Bart and Hoop, 2009), and in Austria, almost 70% of the laying hens are kept in the non-cage production systems (ZMP, 2008). These changes reflect a transition period that is determined by an EU-wide ban on the use of battery cages (un-enriched cages) for laying hens, and will enter into force in January 2012 (Anonymous, 1999). Changes that resulted from increased numbers of chickens kept in outdoor-floor husbandry systems have caused re-emerging parasitic infections (Permin et al., 1999; Thamsborg et al., 1999; Fossum et al., 2009; Kaufmann and Gauly, 2009). The prevalence of nematode infections in battery cage systems was low (< 5%; Permin et al., 1999). However, because of the faeces management that allows nematodes to complete their life cycles, and the frequent contact of animals with faeces, there is an increased risk for ingestion of parasitic stages in floor-outdoor access husbandry systems. Among the parasitic infections, the most prevalent infections are with *Heterakis gallinarum* and *Ascaridia galli* (Permin et al., 1999; Kaufmann and Gauly, 2009). These two parasites are probably the most important nematode species of economical importance in chickens.

Fibre rich diets for poultry are expected to increase in the future, particularly in organic poultry production (Sundrum et al., 2005; Van de Weerd et al., 2009). In addition, poultry housed in floor systems can ingest fibre rich litter material from the floor. Similarly, layers in modified cages can also pick up fibrous material from the litter bath (Hetland et al., 2004). In free-range systems, birds can directly consume plants available in the outdoor area. Non starch polysaccharides (NSP) are the major part of dietary fibre. Monogastric animals do not possess own digestive enzymes for NSP degradation. Depending on its fermentability, this class of carbohydrates can either less or highly be utilized by the microorganisms in the distal intestinal tract (Englyst, 1989; Bach Knudsen, 2001). There is evidence that dietary NSP may interact with parasites of the host animals. Feeding grower layers with NSP that form viscous digesta, favored the development of *A.*

galli (Daenicke et al., 2009). In pigs, the type of dietary NSP has been shown to affect the establishment, development and fecundity of pig-specific common nematodes differently (Petkevičius et al., 1997; 2001; 2003). The main objective of the present study was to investigate the effects of less or highly fermentable dietary NSP on *H. gallinarum* and *A. galli* infections in growing layer hens. Because of the vector role of *H. gallinarum* in the transmission of *Histomonas meleagridis*, the effects of NSP supplemented diets on interactions between these two parasites were also investigated.

1.2. General introduction

1.2.1. Nutrition of host animal and parasitic infections

Feed is probably the most important entity in poultry production that can expose the birds to a wide variety of factors through the gastrointestinal tract (Yegani and Korver, 2008). Dietary characteristics can modulate a bird's susceptibility to infectious challenges and subtle influences due to the level of nutrients, or the types of ingredients may at times be of critical importance (Klasing, 1998). There exists a large body of evidence that the host animal nutrition can alter the interactions between the host animals and their parasites (Coop and Holmes, 1996; Coop and Kyriazakis, 1999; Stear et al., 2007). Nutrition can affect resistance and/or resilience status of host animals through specific adaptive physiological responses and/or certain immune regulations (Figure 1).

Interactions between the host and nutrition can be considered from two interrelated perspectives. Firstly, the effects of nutrition on the metabolic disturbances and pathophysiology induced by parasitism, and secondly the influence of nutrient availability on the ability of the host to mount an effective response against parasite establishment and/or development and to induce parasite rejection. The level of nutrition can thus influence the 'resilience' and resistance' of the host to parasitic infections (Coop and Kyriazakis, 1999). Nutrition can affect gastrointestinal nematodes through its influence on resistance, i.e. the ability to regulate gastrointestinal nematode establishment, fecundity and survival. This is mainly mediated through acquired immunity, and thus nutrition has the potential to affect the rate of acquisition and/or the degree of expressing of immunity (Kyriazakis and Houjdijk, 2006).

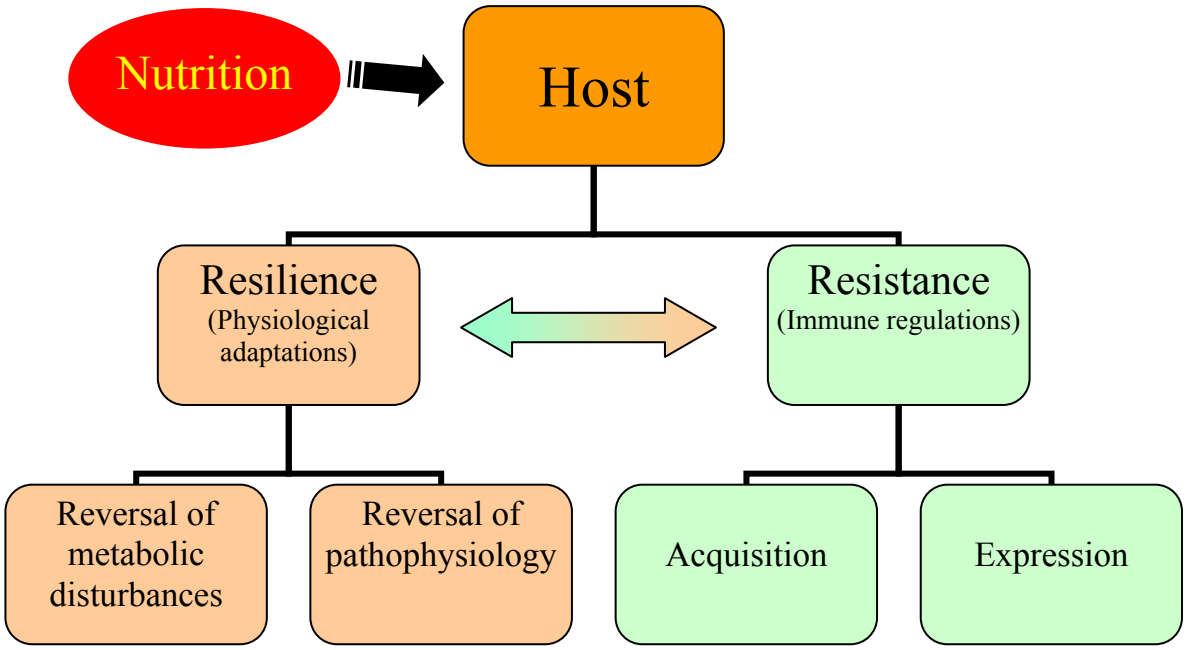

Figure 1. Host-nutrition and parasite interaction concepts (modified after Coop and Kyriazakis, 1999; Hoste, 2001; Kyriazakis and Houjdijk, 2006).

Resilience can be considered as the host's ability to maintain a reasonable level of productivity in the face of a parasitic challenge (Coop and Kyriazakis, 1999). In other words, resilience is the ability of the host to maintain its physiological functions and to tolerate the detrimental effects due to parasites (Hoste, 2001). Further common terms describing interactions between host and parasitic infections are susceptibility and tolerance. Susceptibility is the opposite of resistance. Tolerance is similar to resilience, and refers to the ability of a host to perform despite the presence of infection. Resilience is preferred over tolerance, because it is used to avoid the confusion with immunological tolerance (Abdelqader, 2007). Effects of protein supplementation on resistance and/resilience of gastrointestinal nematodes of ruminants are well known (Stear et al., 2007). Wallace et al. (1995) showed that protein supplementation did not influence worm burden of lambs infected with a blood sucking nematode, *Haemonchus contortus*, but lowered faecal egg counts and increased packed red cell volume. Dietary supplementation with urea also enhanced resistance and resilience to *Trichostrongylus colubriformis* (Knox and Steel, 1999).

Certain dietary components can directly influence gastrointestinal parasites through their antiparasitic compounds. Various secondary plant metabolites, e.g. phenolic metabolites, nitrogen containing metabolites and terpenoids, are thought to have

antiparasitic properties (Coop and Kyriazakis, 2001). Plants rich in tannins, a class of phenolic secondary metabolites, are known to have detrimental effects on gastrointestinal parasitic infections of ruminants (Hoste et al., 2006).

It appears that effects of nutrition on gastrointestinal nematodes have extensively been examined in ruminants. However, there is evidence that nutrition can influence poultry parasites too. Among the parasitic infections, the most prevalent infections are with *Heterakis gallinarum* and *Ascaridia galli* (Permin et al., 1999; Kaufmann and Gauly, 2009). These two parasites are probably the most important nematode species of economical importance in chickens. It has been reported that vitamins (Idi et al., 2007), minerals (Gabrashanska et al, 2004) protein or amino acids (Riedel and Ackert, 1951; Daş et al., 2010) alter infections of poultry with *A. galli*. Compared to *A. galli*, less is known about dietary effects on the caecal worm, *Heterakis gallinarum*. Basic features of the two nematodes are summarized in the following table.

Table. Basic features of *Ascaridia galli* and *Heterakis gallinarum.*

	A. galli	***H. gallinarum***
Life cycle	Direct	Direct
Infective stage	L3	L3
Histotrophic phase	+	+ (?)
Prepatent period	4-8 wk	24 d
Adult length, cm		
Female	6.0 - 12.0	1.0 -1.5
Male	5.0 - 7.8	0.7-1.3
Predilection site	small intestine	ceca
Feeding on	Digesta	Digesta / bacteria

Both nematodes have direct life cycles, i.e. require no intermediate host to transmit to their definitive hosts (Herd and McNaught, 1975; McDougald, 2005). Infection starts with ingestion of infective larval stages (L3) in form of embryonated eggs by the host animal. Embryonated eggs of *A. galli* containing L3 larva hatch in the proventriculus or duodenum within 24 h after ingestion (Idi, 2004). The larva invades the mucosal layer of the intestine, where a histotropic phase takes place. The histotropic phase is a normal part of the life cycle of *A. galli* and it lasts approximately 7 to 50 days, depending on infection dose (Herd and McNaught, 1975). The pathogenicity of *A. galli* is considered to be stronger during histotropic, larval development, resulting in inflammation and injury to the

intestinal wall and to the host's absorption of metabolic waste from the nematode (Ramadan and Abou Znada, 1991). It is not clear whether life cycle of *H. gallinarum* involves a histotropic phase. However, according to Van Grembergen (1954), Hsü et al. (1940) have shown the phenomena for *H. gallinarum*. Prepatent period, the time required from ingestion of L3 larvae until mature adult parasites are producing eggs, for *A. galli* is between 4-8 wk (Idi, 2004; Ramadan and Abou Znada, 1992). *H. gallinarum* has an average prepatent period of 24 d, however, it was shown that the females can produce eggs as early as 21 d after infection (Fine, 1975).

A. galli is the largest nematode parasite of poultry. The length of adults varies between 5 to 7.76 cm in males and 6 to 11.6 cm in females, respectively (Idi, 2004; Ramadan and Abou Znada, 1992). *A. galli* resides mainly in the upper part of the small intestine, but also can be found in the distal parts, i.e. ileum. *A. galli* infection can influence digestion and absorption of nutrients (Hurwitz et al., 1972ab; Walker and Farrell, 1976). *A. galli* not only retards performance but can also threaten the general intestinal health of the birds. Dahl et al. (2002) reported that chickens infected with *A. galli* are at higher risk of being subjected to outbreaks of fowl cholera with *P. multocida*.

H. gallinarum has a narrow predilection site, i.e. the caeca, and is regarded as a relatively less pathogenic nematode (Taylor et al., 2007). However, the importance of this nematode lies in its role as a main vector for the transmission of *Histomonas meleagridis*, the causative agent of 'blackhead' disease (McDougald, 2005). Susceptibility of turkeys to histomonas infection is higher than that of chickens. However, histomonioasis outbreaks can increase flock mortality and decrease egg production in laying hens (Esquenet et al., 2003).

Gastrointestinal bacterial flora seems to play important roles in establishment of both *A. galli* and *H. gallinarum.* Johnson and Reid (1973) showed that lower number of *A. galli* larvae established themselves in germ-free chickens than in chickens with conventional flora. Chickens inoculated with single species of bacteria harbored higher number of larvae than germ-free birds, but had lower number of larvae than those with a conventional flora. Although establishment of *A. galli* is enhanced by the presence of bacteria, it was shown that germ-free birds harbor established larvae (Johnson and Reid, 1973). For *H. gallinarum*, the role of bacteria seems to be more vital (McDougald, 2005). Springer et al. (1970) showed that *Heterakis* larvae were not able to survive when injected into caeca of gnotobiotic birds. Moreover, *H. gallinarum* is regarded as a bacteria feeder (Bilgrami and Gaugler, 2004). *H. meleagridis* attains full virulence in the caeca of chicken

only in combination with the presence of several bacteria species (Springer et al., 1970; McDougald, 2005). Because both *A. galli* and *H. gallinarum* reside in the gastrointestinal tract, it is likely that an altered gastrointestinal environment due to dietary characteristics may influence their establishment and fecundity. Moreover, dietary characteristics may aslo alter interactions between bacteria dependent *H. gallinarum* and *H. meleagridis*.

1.2.2. Non starch polysaccharides (NSP)

Today's poultry diets consist of highly concentrated feedstuffs providing efficient digestion and utilization. The diets are mainly based on cereals and protein rich ingredients. Fibre is rather regarded as nutrient diluent or anti-nutrient, depending on its solubility. Cereals and legumes, the bulk of commercial poultry diets, contain a significant amount of fibre (Hetland et al., 2004). Plant polysaccharides can be separated broadly into two distinct and chemically well-defined types; the storage polysaccharide starch (α-glucan) and the cell-wall polysaccharides (non- α-glucan), which may conventionally be called non starch polysaccharides (NSP). The term dietary fibre is used for the sum of NSP and lignin (Bach Knudsen, 2001). A typical arrangement of cereal polysaccharides is illustrated in the following figure.

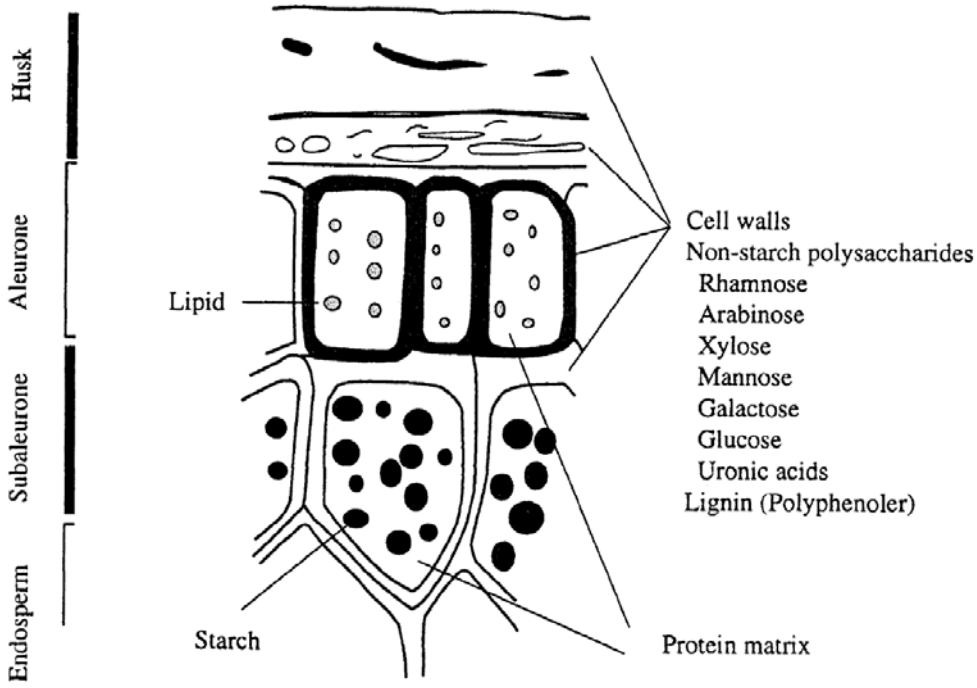

Figure 2. Example of cell wall materials from oats (Bach Knudsen, 2001).

As shown in the figure, plant polysaccharides consist of the storage polysaccharide starch and the cell wall polysaccharides. Starch is composed of amylose and amylopectin, which contain α-(1-4) and α-(1-6)-glucosidic linkages, respectively (Montagne et al., 2003). The building blocks of the cell wall polysaccharides are the pentoses arabinose and xylose, the hexoses glucose, galactose and mannose, the 6-deoxyhexoses rhamnose and fucose, and the uronic acids glucuronic and galacturonic acid (Bach Knudsen, 2001). Another major component of the cell wall is lignin which can be described as very branched networks built up by phenylpropane units. Lignin cements and anchors the cellulose microfibrils and other matrix polysaccharides (Bach Knudsen, 2001). The main polysaccharides of plant cell walls are cellulose, pectins, β-glucans [mixed linked β (1→3) (1→4)-D-glucan (β-glucan)], pentosans, xylans (Montagne et al., 2003; Bach Knudsen, 2001).

Dietary starch can be hydrolyzed by pancreatic α-amylase and may therefore be digested in the small intestine and be absorbed as glucose (Englyst, 1989). It is generally accepted that starch is well digested in the gastrointestinal tract (Classen, 1996; Józefiak et al., 2004). In contrast to starch, NSP are not susceptible to the endogenous enzymes and, depending on their fermentability can either less or highly be utilized by the microorganisms in the distal parts of the gastrointestinal tract (Englyst, 1989; Schneeman, 1999; Montagne et al., 2003). Cellulose and xylans belong to insoluble NSP, whereas pectins, β-glucans and arabinoxylans are considered as soluble NSP (Hetland et al., 2004). Plants generally contain a mixture of soluble and insoluble NSP in a ratio that varies between plants, plant parts, and stage of maturity (Montagne et al., 2003; Hetland et al., 2004).

Inulin and oligofructose are comparable to dietary fibre in that they are composed of multiple saccharide units, which are soluble in water and are not digested by the endogenous enzymes found in the intestines (Schneeman, 1999). Chemically, inulin fructo-oligosaccharides (FOS) are composed of linear chains of fructose units, linked by β-(2→1) fructosyl-fructose bonds, often terminated by a glucose unit (Ten Bruggencate et al., 2004; Roberfroid, 2005). The number of fructosyl moieties ranges from 2 to 60 for inulin and from 2 to 7 for FOS. In vitro, fermentation experiments revealed that molecules with a degree of polymerization (DP) > 10 are fermented, on average, half as quickly as molecules with a DP of < 10 (Ten Bruggencate et al., 2004; Rehman et al., 2008). The only plant that has so far been used industrially for the extraction of inulin-type fructans belongs to the *Compositae* family, i.e. chicory (Roberfroid, 2005).

1.2.3. Physiological effects of NSP

The main physico-chemical properties of dietary fibre with nutritional significance are the cation exchange capacity, hydration properties, viscosity and organic compound absorptive properties (Bach Knudsen, 2001). However, hydration and viscosity associated properties of dietary fibre appear to have been studied most extensively in poultry nutrition (Hetland et al., 2004). The hydration properties are characterized by the swelling capacity, solubility and water holding capacity, and are linked to the type of polymers that make up the cell wall and their intermolecular association. Water holding capacity is also used to describe hydration properties and reflects the ability of a fibre source to incorporate water within its matrix. In general cereal fibre tends to have lower water holding capacity than fibre sources high in pectin containing materials. The majority of polysaccharides give viscous solutions when dissolved in water. The viscosity is primarily dependent on the molecular weight of the polymer and the concentration. Large molecules increase viscosity of diluted solutions and their ability to do this mainly depends on the volume they occupy. The volume of the polymers is much greater than that of monomers and the volume occupied by one polymer coil will be greater than the combined volume of two coils each half its length (Bach Knudsen, 2001). Because of the importance of the complex interactions between different chemical components of plant tissues, it makes little sense from a nutritional point of view, to describe dietary fibre solely in chemical terms. Rather, it may be better to describe the cell wall polysaccharide components of feedstuffs in terms of their physicochemical properties, which are likely to be related to their physiological effects (Smits and Annison, 1996).

1.2.3.1. Insoluble NSP

The insoluble fibre fraction has traditionally been regarded as a nutrient diluent in monogastric animal diets (Hetland et al., 2004). Insoluble polysaccharides such as cellulose and xylans can hold water as they behave like sponges but their viscosity properties are relatively unimportant (Smits and Annison, 1996). In contrast to soluble fibre, insoluble fibre is not extensively degraded by bacterial fermentation in poultry, which makes its influence on the composition and quantity of the microflora relatively insignificant (Hetland et al., 2004). Therefore, unlike soluble fibre, insoluble fibre does merely influence the composition and population size of the gastrointestinal microflora

(Shakouri et al., 2006). The most obvious effect of insoluble NSP is the increased bulk of digesta in the intestinal tract. This may be handled either by a larger capacity of the digestive system or a faster passage rate through the tract (Hetland et al., 2004). Inclusions of insoluble NSP in poultry diets have certain positive effects on animal welfare. Van Krimpen et al. (2007; 2008) reported that hens fed diets high in insoluble NSP increased time spent for eating and reduced aggressive pecking behaviours. Diets supplemented with NSP may also stimulate the development of the gizzard suggesting improved digestive functioning (Van Krimpen et al., 2009).

1.2.3.2. Soluble NSP

It is well known that soluble NSP exert anti-nutritive effects in growing poultry through viscosity associated effects (Choct and Annison, 1992; Daenicke et al., 1999; Francesch and Brufau, 2004; Daenicke et al., 2009). Soluble fibres can produce high viscosity in the small intestine and thereby inhibit digestion and absorption. High viscosity can affect feed intake due to slower digesta passage rate, which in turn causes microbial proliferation in the intestine (Van der Klis et al., 1993; Hetland et al., 2004; Yegani and Korver, 2008). The water holding capacity of soluble fibre is associated with sticky droppings (Hetland et al., 2004). Due to their NSP contents, barley, wheat, rye and oats can increase viscosity, decrease digesta passage rate, digestive enzymatic activities and nutrient digestibility, which may consequently cause depressed feed conversion efficiency and growth rate of birds (Yegani and Korver, 2008). The viscous properties of NSP can impair the diffusion and convective transport of lipase, oils and bile salt micelles within the gastrointestinal tract. NSP induced increased viscosity may stimulate mucus secretion by the Goblet cells. Morever, increased viscosity may reduce the contact between potential nutrients (e.g. fats) and the digestive secretions (e.g. lipases, bile salts), and impair the transport to the epithelial surface (Smits and Annison, 1996). NSP-caused high digesta viscosity is often associated with increased gastrointestinal capacity. Iji et al. (2001) reported that the gross weight of the intestines as well as the mucosal morphometry of the small intestine was increased by NSP of highly viscous properties. Viscosity associated effects of soluble NSP are not limited to impaired digestion and absorption of nutrients, but are also closely related to the microbial proliferation in the gastrointestinal tract. Increased average digesta retention time due to higher viscosity, likely provides favorable conditions for bacterial proliferation and activity. Because most of the soluble NSP are fermentable, local or

systemic effects of pathogenic bacteria may threaten general health of birds (Smits and Annison, 1996; Shakouri et al., 2006; Yegani and Korver, 2008). Viscosity associated anti-nutritive effects of soluble NSP can to some extent be eliminated through exogenous NSP degrading enzymes supplemented to the diets (Castanon et al., 1997; Dusel et al., 1998; Mikulski et al., 2006; Józefiak, et al., 2007; Daenicke et al., 2009).

1.2.3.3. Inulin

Unlike NSP with high viscosity properties, inulin does not appear to increase intestinal viscosity (Schneeman, 1999). It can act as a prebiotic, i.e., it may be a selectively fermented ingredient that allows specific changes in the composition or activity of the gastrointestinal microbiota (Rehman et al., 2008). Due to its β-(2→1) linkages, it is resistant to enzymatic hydrolysis in the upper gastrointestinal tract and reaches intact to distal parts of the tract, where it is completely fermented (Juskiewicz and Zdunczyk, 2004). End products of inulin fermentation are short chain fatty acids (SCFA), carbon dioxide, methane and hydrogen (Donalson et al., 2008). Although fermentation of inulin may start already in the ileum, caeca are the main site of microbial fermentation in chickens (Juskiewicz et al., 2005). An inulin-dependent stimulation of metabolic activity of beneficial intestinal bacteria has been reported for turkeys (Juskiewicz et al., 2005) and chickens (Rehman et al., 2007). Although many reports suggest that inulin stimulated bacteria may inhibit colonisation of intestinal pathogens resulting in a fermentation benefical to the health of the animals (Juskiewicz et al., 2005; Rehman et al., 2007; Donalson et al., 2008), there are exceptions. Ten Bruggencate et al. (2004) showed that inulin and fructo-oligosaccharides impaired resistance to salmonella infections in rats. According to these authors this might be due to rapid production of fermentation metabolites and subsequent impairment of the mucosal barrier.

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CHAPTER - II

Non-starch polysaccharides alter interactions between *Heterakis gallinarum* and *Histomonas meleagridis*

Non-starch polysaccharides alter interactions between *Heterakis gallinarum* and *Histomonas meleagridis*

Abstract

Nutrition of the host animal may not only influence interactions between the host and its parasites, but also relations between different parasites species residing on the same host. We investigated effects of insoluble and soluble non-starch polysaccharides (NSP) on establishment and development of *Heterakis gallinarum* in chicken being treated or left untreated against *Histomonas meleagridis*.

Six groups of one-day-old birds were allocated to three diets, two on each diet. The birds were fed *ad libitum* either a basal diet (CON), or CON + insoluble NSP (I-NSP) or CON + soluble NSP (S-NSP) until an age of 11 wk. At an age of 19 d, one of each diet groups was prophylactically treated for 9 d with dimetridazole (0.05%, w/v) via drinking water against histomonas. The remaining three groups were left un-treated. Two days after starting dimetridazole treatment (at 3 wk), each of the 6 groups was divided into two sub-groups. One dimetridazole treated and one dimetridazole un-treated groups of birds on each diet (6 groups) were infected with 200 embryonated eggs of *H. gallinarum* that were previously harvested from histomonas carrying *H. gallinarum* infected chickens. The remaining 6 groups of uninfected birds, either treated or left un-treated against *H. meleagridis,* served as controls. Worm burdens of infected birds were determined 8 wk p.i.

Treatment against *H. meleagridis* significantly increased incidence of *H. gallinarum* infection and average worm length in all infected groups independent of the diet consumed ($P<0.001$). An interaction between effects of diet and dimetridazole treatment on worm burden ($P<0.001$) indicated that the S-NSP diet resulted in lowest worm burden in dimetridazole un-treated birds, whereas it caused the highest worm burden in the treated birds ($p<0.05$). Furthermore, the treatment resulted in higher worm burdens when compared to un-treated birds on the corresponding diets ($p<0.05$). Infection with *H. gallinarum* impaired body weight (BW) of the chicks ($p<0.05$) and *H. meleagridis* aggravated this effect ($p<0.05$). Dimetridazole treated and un-treated uninfected birds developed similar BW ($p>0.05$). Both NSP supplemented diets resulted in lower ($p<0.05$) BW when compared with the CON diet, S-NSP being inferior to I-NSP ($p<0.05$).

It is concluded that *H. meleagridis* harms the definitive host as well as *H. gallinarum*. Both insoluble and soluble NSP supplemented diets favor *H. gallinarum* infection while S-NSP additionally intensifies histomonas infection, which then impairs establishment and development of *H. gallinarum*.

Keywords: *Heterakis gallinarum; Histomonas meleagridis; chicken; vector; host diet;* non-starch polysaccharides.

2.1. Introduction

Because the conventional battery cages will be banned in the European Union by 2012, floor- and outdoor production systems are spreading. However, these systems bear an increased risk of parasitic infections in poultry (Thamsborg et al., 1999; Fossum et al., 2009). *Heterakis gallinarum*, a caecal worm, is one of the most common nematodes in poultry with a prevalence ranging from 68% to 80% (Permin et al., 1999; Maurer et al., 2009) especially in organic/free ranging flocks. In spite of its often neglected *per se* pathogenicity, the importance of the nematode lies in its role as a main vector for the transmission of *Histomonas meleagridis*. The host becomes infected by the ingestion of *H. meleagridis* infected embryonated eggs of the nematode (Levine, 1985; McDougald, 2005). Prevalence of histomonas in layer hens in Europe is slightly increasing (Kaufmann-Bart and Hoop, 2009) and infection outbreaks had not been reported for decades because layers were kept in cages (Esquenet et al., 2003). Transmission of *H. meleagridis* among individuals within or between chicken flocks depends on the presence of *H. gallinarum* (McDougald, 2005). In contrast to turkeys a direct transmission of the histomonads, via the so-called phenomena of cloacal drinking, does not happen in chickens (Hu et al., 2006).

Nutrition of the host animals may not only influence interactions between the host and its parasites but also relations between different parasites species residing on the same host. Non-starch polysaccharides (NSP) constitute an important part of dietary fibre. Dietary NSP are not digested by the endogenous enzymes and, depending on their fermentability can either less or highly be degraded and utilized by the microorganisms in the hind parts of the gastrointestinal tract (Englyst, 1989; Schneeman, 1999). Caeca are the main site of microbial fermentation in chickens (Juskiewicz et al., 2005). Dietary NSP are known to alter microbial composition in the gastrointestinal tract, particularly in caeca, the predilection site of *H. gallinarum* and *H. meleagridis*. As shown by Petkevičius et al.

(1997), NSP varying in intestinal fermentability influence establishment of common nematode infections of pigs differently. Likewise, an altered caecal environment, induced by feeding NSP supplemented diets may affect relations between *H. gallinarum* and *H. meleagridis* and their effects on performance in poultry. Therefore we aimed at investigating the effect of histomonas infection on establishment and development of *H. gallinarum* in chickens fed NSP supplemented diets varying in their fermentability. The objective of the present study was to estimate effects of insoluble and soluble NSP on interactions between *H. gallinarum* and *H. meleagridis* in chicken being treated or not treated against *H. meleagridis*.

2.2. Materials and methods

2.2.1. Birds, diets and experimental infections

A total of 360 one-day-old Lohmann Selected Leghorns chicks, obtained from a commercial hatchery, were used. The one-day-old chicks were weighed together and randomly divided into 6 feeding groups. The groups of birds were allocated to three diets, two groups for each diet. The birds were fed *ad libitum* until an age of 11 weeks (wk) either a basal diet (CON) supplying recommended metabolizable energy (ME) and nutrients for grower layers (NRC, 1994) or the basal diet plus insoluble non-starch polysaccharide (I-NSP) or the basal diet supplemented with soluble non-starch polysaccharide diet (S-NSP). The I-NSP diet contained additional pea bran meal (Exafine 500, Socode, Belgium) and the S-NSP diet additional chicory root meal (Fibrofos, 60, Socode, Belgium) as the natural NSP sources. Pea bran and chicory root meal amounted to 9.1% of the I-NSP and S-NSP feed mixtures, respectively. The diets were mixed on air dry-basis conditions and were pelleted. Each feeding group was kept in a pen scattered with wood shavings.

At an age of 19 d, a prophylactic treatment with dimetridazole (0.05%, w/v) via *ad libitum* offered drinking water was started and continued for 9 d for one group on each diet. The remaining three groups, each on one diet, were left un-treated. Two days after starting the dimetridazole treatment, e.g. at an age of 3 wk, each of the 6 groups was divided into two sub-groups ending up with 12 final experimental groups. One dimetridazole treated and one dimetridazole un-treated groups of birds on each diet (6 groups) were infected with 200 embryonated eggs of *H. gallinarum,* which were

previously harvested from *H. gallinarum* and concomitant histomonas infected chickens. The remaining 6 groups of birds, either treated or left un-treated against *H. meleagridis,* served as uninfected controls. The experimental structure of the final groups and number of birds allocated to each group are shown in Table 1.

Table 1. Number of birds allocated to the experimental groups.

	Dimetridazole un-treated		Dimetridazole treated[1]	
Diet	Un-infected	Infected[2]	Un-infected	Infected[2]
CON, N=118	29	30	23	36
I-NSP, N=123	26	34	24	39
S-NSP, N=119	27	30	23	39

[1] The birds were treated with dimetridazole (Chevi-col© Pulver, Chevita GmbH, Germany). The compound was given via *ad libitum* offered drinking water at a concentration of 0.05% (w/v) from 2 d before inoculating *H. gallinarum* eggs to 7 d post-infection.
[2] Each bird was infected with 200 embryonated eggs of *H. gallinarum* previously harvested from histomonas and concomitant *H. gallinarum* infected chickens.

2.2.2. Infection material

The infection material was produced at the Department of Animal Science, University of Goettingen, Germany. Adult female worms, harvested from intestines of naturally infected chickens collected at different farms, were used as the original material of infection. The eggs had been used in a previous trial and shown to produce Histomonas-typical pathological lesions in chickens. Presence of *H. meleagridis* was macroscopically and microscopically confirmed in the caecal and liver tissues. The worms were harvested and used for the present investigation. For embryonation, intact female worms were incubated at room temperature (20-25 °C) for 3 weeks in a media containing 0.5% (vol/vol) formalin as described by Puellen et al. (2008). After embryonation, the worms were cut into pieces, and the eggs were squeezed out using a pestle placed on a sieve. The residual worm tissues on the sieve were flushed and removed, and the eggs were gathered. The embryonated eggs were stored at + 4°C until the infection day. On the infection day, the number of eggs/ml aqueous suspension was determined using a McMaster egg counting chamber. Only eggs in the vermiform and infective larval stages were classed and counted as embryonated. The counting procedure was repeated five times and the arithmetic mean was calculated. The infection dose was then adjusted to 200 eggs / 0.2 ml

of final suspension. Uninfected control birds were given 0.2 ml of 0.5% formalin as placebo.

2.2.3. Management of the birds

The litter was replaced once (wk 1-3) or twice (wk 4-11) a week. Room temperature was gradually decreased from 34 °C on the first day (d) to 26 °C in wk 3 and thereafter decreased by 2-3°C per wk, ending at 18-20 °C from wk 6 onwards. A 24 h lighting period was maintained for the first 2 days and was then reduced to 16 h/d at the end of the first week. By wk 8, it was reduced to 12h/d and subsequently maintained until the end of the experiment. At the end of wk 3, the birds were marked with wing tags and individual body weights (BW) were recorded for the first time and thereafter at weekly intervals for 5 wk post-infection. Group feed consumption was determined daily. Drinking water was offered *ad libitum*. The birds did not get any vaccination or anthelmintic treatment throughout the experimental weeks. The experimental stable was thoroughly disinfected 10 d before introducing the birds.

2.2.4. Necropsy

Sentinel birds were subjected to necropsies to determine infection induced macro- and microscopical lesions and to detect *H. meleagridis* in caecal sections. For this purpose, 4-5 birds from each of 12 experimental groups were examined in wk 2, 3, and 5 p.i., respectively. The caecal samples were fixed for 24 hours in 4% phosphate-buffered formalin, embedded in paraffin and processed for Hematoxylin and Eosin (H&E) staining according to standard methods. Tissue sections were examined microscopically for infection induced lesions such as epithelial erosion and ulceration, lymphocyte and heterophil infiltrations as well as for the presence of the histomonads in the tissue. The sentinel birds are not included in the animal numbers shown in Table 1.

2.2.5. Worm harvest

All the birds were slaughtered after electrical stunning 8 wk p.i. After slaughtering, the gastrointestinal tract was removed, caeca were separated and the worm burdens were quantified by the procedure described by Gauly et al. (2008). Briefly, caeca were opened,

the content was removed, and the caecal walls were flushed to remove the worms. The caecal content was flushed with low-pressure tap water through a sieve with a mesh aperture of 100 μm, and the residues were transferred into Petri dishes to be examined by using a stereomicroscope. Average intact worm length was estimated by measuring 10 female and 10 male randomly selected worms per each bird. In cases of lower than 10 worms per sex were available, all the intact worms were measured. Caeca from uninfected control birds (20% of each group) were also processed to confirm infection free status of the controls.

2.2.6. Chemical analyses of the diets

The composition, nutrient and energy contents of the experimental diets are given in Table 2. At weeks 1, 6 and 11, representative feed samples were taken and analyzed for dry matter (DM), crude ash (CA), crude protein (CP), sugar, starch, and ether extract (EE) using standard methods (Naumann and Bassler, 1997). Neutral and acid detergent fibre (NDF and ADF, respectively) were analyzed according to Van Soest et al. (1991) and results are given exclusive of ash. The metabolizable energy of the diets (MJ ME/kg DM) was calculated (FMVO, 2008). Insoluble and soluble non-starch polysaccharides were measured using an enzymatic test (Megazyme, 2007). Inulin was determined according to Naumann and Bassler (1997).

Table 2. Composition and analysis of the experimental diets.

Item	CON[1]	I-NSP[2]	S-NSP[3]
Components, g/kg (as fed-basis)			
Barley	290	264	264
Wheat	540	491	491
Fishmeal[4]	80	73	73
Casein	45	41	41
Soybean oil	20	18	18
Premix[5]	10	9	9
MCP	9	8	8
CaCO3	6	5	5
Pea bran[6]	-	91	-
Chicory root meal[7]	-	-	91
Analyzed composition			
DM, g/kg	896	901	894
Nutrient, g/kg DM			
Ash	54	52	54
CP	222	207	207
NDF	115	164	110
ADF	31	86	41
Ether extract	38	35	36
Starch	491	439	411
Insoluble NSP	102	170	104
Soluble NSP	18	22	24
Inulin	-	-	70
ME, MJ/kg DM[8]	13.28	12.05	11.85

[1] Basal diet.
[2] Insoluble non-starch polysaccharide supplemented diet = 1000 g CON plus 100 g pea bran.
[3] Soluble non-starch polysaccharide supplemented diet = 1000 g CON plus 100 g chicory root meal.
[4] Fishmeal; 64% CP and 8% CL.
[5] Supplied per kg of premix: 1.200.000 IU vitamin A, 350.000 IU vitamin D3, 4.000 mg vitamin B1, 800 mg vitamin B2, 600 mg vitamin B6, 3.200 mg vitamin B12, 450 mg vitamin K3, 4.500 mg nicotinic acid, 1.500 mg Ca-pantothenate, 120 mg folic acid, 5.000 mg biotin, 55.000 mg choline chloride, 3.200 mg Fe, 3.200 mg Fe-(II)-Sulphate, 1.200 mg Cu-(II)-sulfate pentahydrate, 10.000 mg Mn-(II)-oxide, 8.000 mg Zn-Oxide, 160 mg iodine, 160 mg Ca-iodine-hexahydrate, 40 mg Na-Selenite, 64 mg Cobalt, 64 mg basic Co-(II)-Carbonate-monohydrate, 10.000 mg BHT (Product code: 77046, Vilomix, Germany).
[6] Pea bran: contained 86.9% crude fibre (Exafine 500, Socode, Belgium).
[7] Chicory root meal: average polymerization degree (DP) of inulin = 9. (Fibrofos 60, Socode, Belgium).
[8] ME = metabolizable energy, MJ/kg DM= [(g CP/kg DM x 0.01551) + (g CL/kg DM x 0.03431) + (g starch/kg DM x 0.01669) + (g sugar/kg DM x 0.01301)]. Sugar contents of the diets were estimated based on sugar contents of the components.

2.2.7. Statistics

2.2.7.1. Incidence of H. gallinarum infection

Effects of the diets on the incidence of *H. gallinarum* infection (proportion of worm-harboring birds to the experimentally infected birds) were analyzed using GENMOD procedure of SAS (2010) with a logit link function. The GENMOD procedure fits the generalised linear models and suited for responses with binary outcomes (Kaps and Lamberson, 2004). Because all the dimetridazole treated birds of I-NSP and S-NSP fed birds harbored worm(s), the infected groups were not comparable for the incidence of infection within dimetridazole treated groups. Therefore effect of diet on the incidence of infection was estimated within dimetridazole untreated groups. To find out the effect of dimetridazole treatment on the incidence of *H. gallinarum* infection, each dimetridazole treated infected group was compared with its un-treated corresponding group on the same diet.

2.2.7.2. H. gallinarum worm burden and worm length

Transformation with a natural logarithm (ln) function [ln(y) = ln(y+1)] was applied to worm burden data that were positively skewed (Skewness > 0) and showed non-normal distribution (Kolmogorow-Smirnow, $p<0.05$) to correct for heterogeneity of variance and to produce approximately normally distributed data. After transformation the variances were still not equal among the groups. Therefore the transformed data were analyzed with a mixed model (Proc Mixed), by which unequal variances were taken into account. This approach improved fit statistics of the model as indicated with smaller BIC and AIC values. The statistical model for the worm burden, worm length and sex ratio included fixed effects of diet (1-3), Dimetridazole treatment (0, 1), interaction effect between diet and dimetridazole treatment, and the residual error term.

2.2.7.3. Growth and feed utilization data

Body weight (BW) and feed:gain ratio of the birds were evaluated for a period of five weeks (*p.i.)*, covering the pre-patent period of the nematode. The data were analyzed with a 3-way ANOVA that included fixed effects of diets (CON, I-NSP, S-NSP), *H.*

gallinarum infection (infected, not infected), the dimetridazole treatment (untreated, treated), all possible interactions between these factors and the residual error term. Because effects of infection(s) and dimetridazole treatment could only be estimated for the post-infectional period, BW and feed:gain of the birds in the pre-infectional period was analyzed with another model. This model included fixed effect of diet and block effect of double feeding system for each diet (two separate groups were on each diet) in this period.

2.2.7.4. Presentation of the results and multiple comparison tests

After infection at the end of wk 3, the birds were kept according to a 3 x 2 x 2 factorial arrangement of treatments with diet, infection and the dimetridazole treatment as the main factors. Therefore, unless no significant interactions between the effects of the main experimental factors were encountered, the data are presented as the main effects of diet, infection and the dimetridazole treatment. In case of significant interactions between the main factors were encountered, the results are presented for the corresponding single groups under influence of the interacting factors.

Tukey-Kramer test was used to partition differences among feeding groups when a significant non-interactive main effect was encountered. In cases of significant interactions, sub-groups were separated using the Tukey-Kramer post-hoc test (alpha=0.05). All the statistical analyses were performed with SAS (2010).

2.2.8. Ethical consideration

The experimental procedures followed the animal welfare rules. The infection dose (200 eggs) given to each bird was within the range of the worm burdens that can be observed in natural sub-clinical infections. The procedures for experimental infections followed the guidelines suggested by the World Association for the Advancement of Veterinary Parasitology for evaluating the effectiveness of anthelmintics in chickens and turkeys (Yazwinski et al., 2003).

2.3. Results

2.3.1. Incidence of H. gallinarum infection

As shown in Figure 1, dimetridazole treatment increased incidence of *H. gallinarum* infection in all the feeding groups (P<0.001). Without dimetridazole treatment, there was an effect of diet on the incidence of infection (P=0.0014). The I-NSP diet led to higher incidence of *H. gallinarum* infection in comparison to S-NSP (p<0.05). Further, the incidence of infection in the CON group tended to be lower than in the I-NSP group (p=0.059) and higher than in the S-NSP group (p=0.089). Dimetridazole treatment resulted in a high incidence of *H. gallinarum* infection (>90%) in all three feeding groups with insufficient variation for comparisons within dimetridazole treated groups.

2.3.2. H. gallinarum establishment rate

Establishment rates of the *H. gallinarum* eggs were lower than 7% in all the dimetridazole un-treated feeding groups, whereas dimetridazole treatment resulted in 12.1%, 17.7% and 27.4% establishment rates after feeding CON, I-NSP and S-NSP, respectively (Figure 2).

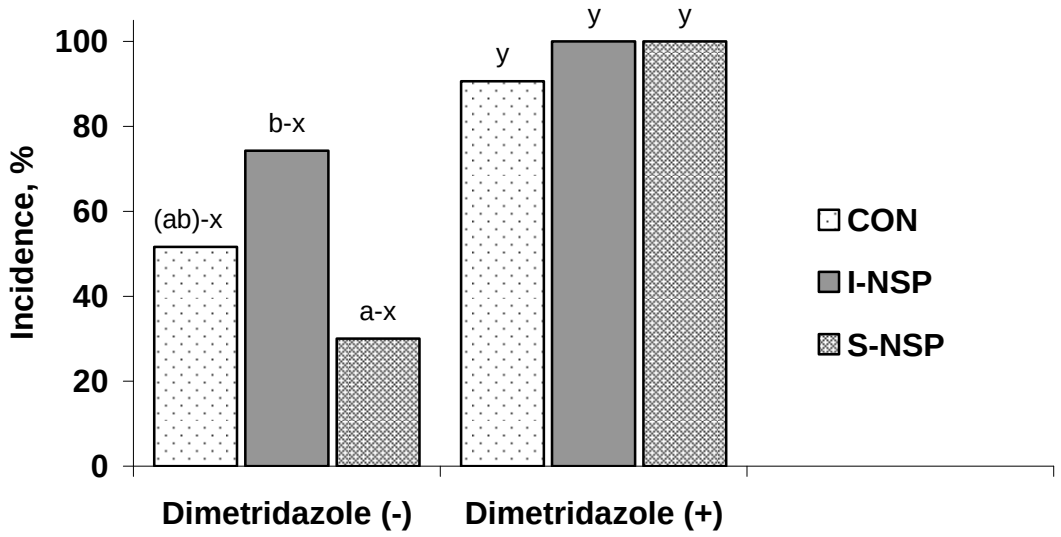

Figure 1. Incidence of *H. gallinarum* infection without (-) and with (+) dimetridazole treatment of the birds on different diets (n= 30-35 per group).

ab : Dimetridazole un-treated groups sharing no common letters differ (p<0.05).
(ab) : Groups sharing the same letters in brackets tend to differ (a: p=0.059; b: p=0.089).
xy : Dimetridazole treated or un-treated groups on the same diet with no common letter differ (P<0.001).

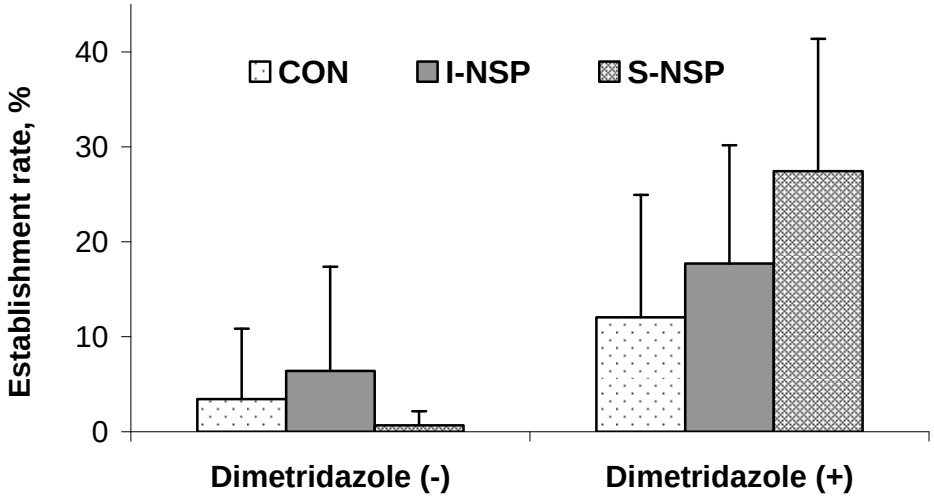

Figure 2. Establishment rate (%) of *Heterakis gallinarum* after a single dose (200 eggs/bird) inoculation of *Histomonas meleagridis* positive eggs in chickens, left untreated (-) or treated (+) with dimetridazole (n= 30-35 per group, means and standard deviations on the error bars).

2.3.3. Average worm burden

Uninfected control birds were free of *H. gallinarum* as confirmed by examination of the cecal contents. As shown in Table 3, there was a significant interaction between diet and the dimetridazole treatment on worm burdens of the birds ($P<0.001$). Dimetridazole treated S-NSP fed birds harboured higher number of worms than all dimetridazole treated and un-treated groups of birds ($p<0.05$). All the dimetridazole treated groups had higher worm burdens in comparison to the corresponding un-treated feeding groups ($p<0.05$). Without dimetridazole treatment, I-NSP-fed birds had higher worm burdens than S-NSP-fed birds ($p<0.05$). With dimetridazole treatment, S-NSP led to higher worm burdens than CON and I-NSP ($p<0.05$). Dimetridazole treated I-NSP-fed birds tended to harbour higher numbers of worms than the CON-fed birds ($p=0.093$). Worm burdens of dimetridazole un-treated I-NSP fed birds and dimetridazole treated CON fed birds did not differ ($p>0.05$), whereas dimetridazole treated I-NSP fed birds had higher worm burdens than all the dimetridazole un-treated groups ($p<0.05$). Without dimetridazole treatment, no larval stages were found in the S-NSP-fed birds and only a few in the CON and I-NSP fed birds (3-4 birds). Dimetridazole application resulted in 0.1 larvae per bird after feeding CON, whereas I-NSP and S-NSP fed birds harboured 3.5 and 2.1 larvae, respectively.

2.3.4. Sex ratio and worm length

Proportion of numbers of female to male worms (sex ratio) was, in general, in favor of the male worms (Table 4). No significant effects of diet, dimetridazole treatment or interaction between these two factors on the sex ratio were observed ($P>0.05$). Male and female worm length remained unaffected by the type of diet ($P>0.05$), however, dimetridazole treatment led to an increase in length of the male and female worms irrespective of the type of diet consumed by the birds ($P<0.001$). There was no interaction effect of diet and dimetridazole treatment on worm length ($P>0.05$).

Table 3. Interaction of the diets and dimetridazole treatment on average *H. gallinarum* worm burdens in dimetridazole treated and untreated birds[1].

	Without dimetridazole			**With dimetridazole**			***P-values,* ≤**		
Log-worm burden	**CON**	**I-NSP**	**S-NSP**	**CON**	**I-NSP**	**S-NSP**	***Diet***	***Dimetridazole***	***Interaction***
LS-MEANS	0.94[ab]	1.62[bc]	0.45[a]	2.50[cd*]	3.28[d*]	3.85[e]	*0.006*	*0.001*	*0.001*
SE	*0.236*	*0.240*	*0.144*	*0.254*	*0.156*	*0.115*			

[1]: n = 30-35 per each infected group.
(abcde): Groups with no common superscript differ (Tukey, p<0.05).

(*): Groups sharing the sign tend to differ (Tukey, p = 0.093).

Table 4. Effects of the diets and the dimetridazole treatment on sex ratio and average worm length.

	***Diets*[1]**					***Dimetridazole treatment*[2]**				***Interaction P,* ≤**
Item	**CON**	**I-NSP**	**S-NSP**	***PSE*[3]**	***P,* ≤**	**(-)**	**(+)**	***PSE*[2]**	***P,* ≤**	
Sex ratio (N=133)	0.84	0.95	0.87	*0.134*	*0.750*	0.87	0.90	*0.121*	*0.773*	*0.818*
Average male length, mm (N=117)	9.12	9.38	8.96	*0.379*	*0.368*	8.45[a]	9.86[b]	*0.282*	*0.001*	*0.4916*
Average female length, mm (N=115)	10.60	10.81	10.29	*0.280*	*0.111*	9.44[a]	11.70[b]	*0.224*	*0.001*	*0.2244*

(ab): Groups with no common superscript in a row within a factor differ (Tukey, p<0.05).
[1] CON = basal diet; I-NSP = 1,000 g CON + 100 g pea bran; S-NSP = 1,000 g CON + 100 g chicory root meal.
[2] The birds were treated with dimetridazole (Chevi-col© Pulver, Chevita GmbH, Germany). The compound was given via *ad libitum* offered drinking water at a concentration of 0.05% (w/v) from 2 d before inoculating *H. gallinarum* eggs to 7 d post-infection.
[3] Pooled SE.

2.3.5. Mortality, clinical observations and pathology

The overall mortality (up to wk 11) was less than 3% and most of the deaths occurred in the first week of the pre-infectional period (2.5%). Following the experimental infection, there was a decrease in feed intake of the infected birds that were left un-treated against histomonas, whereas only a slight decrease was observed in association with dimetridazole application (Figure 3).

Due to the limited number of sentinel birds at each of the examination weeks, the intensity of pathological and histo-pathological lesions at the caecal level could not be quantified for each feeding group. Dimetridazol untreated infected birds showed macroscopic lesions such as thickening of the caecal wall and fibrinous to fibrino-hemorrhagic exudates in the caecal lumen. Histologically, severe hyperplasy of tunica muscularis, numerous histomonads, massive lymphocyte, heterophil and macrophage infiltrations, as well as coagulation necrosis have been observed in 2 and 3 wk p.i.. At 5 wk p.i., the severity of the lesions declined. After dimetridazole treatment, macroscopical lesions were not observed in either of the feeding groups. Microscopicaly, dimetridazole treated infected birds from all feeding groups exhibited mild to moderate lesions of the caecal tissue i.e., lymphocytes infiltration in the lamina propria and formation of lymphoid aggregations. Histomonads were absent. Uninfected birds, either left untreated or treated with dimetridazole, were negative for histomonads and the pathological lesions.

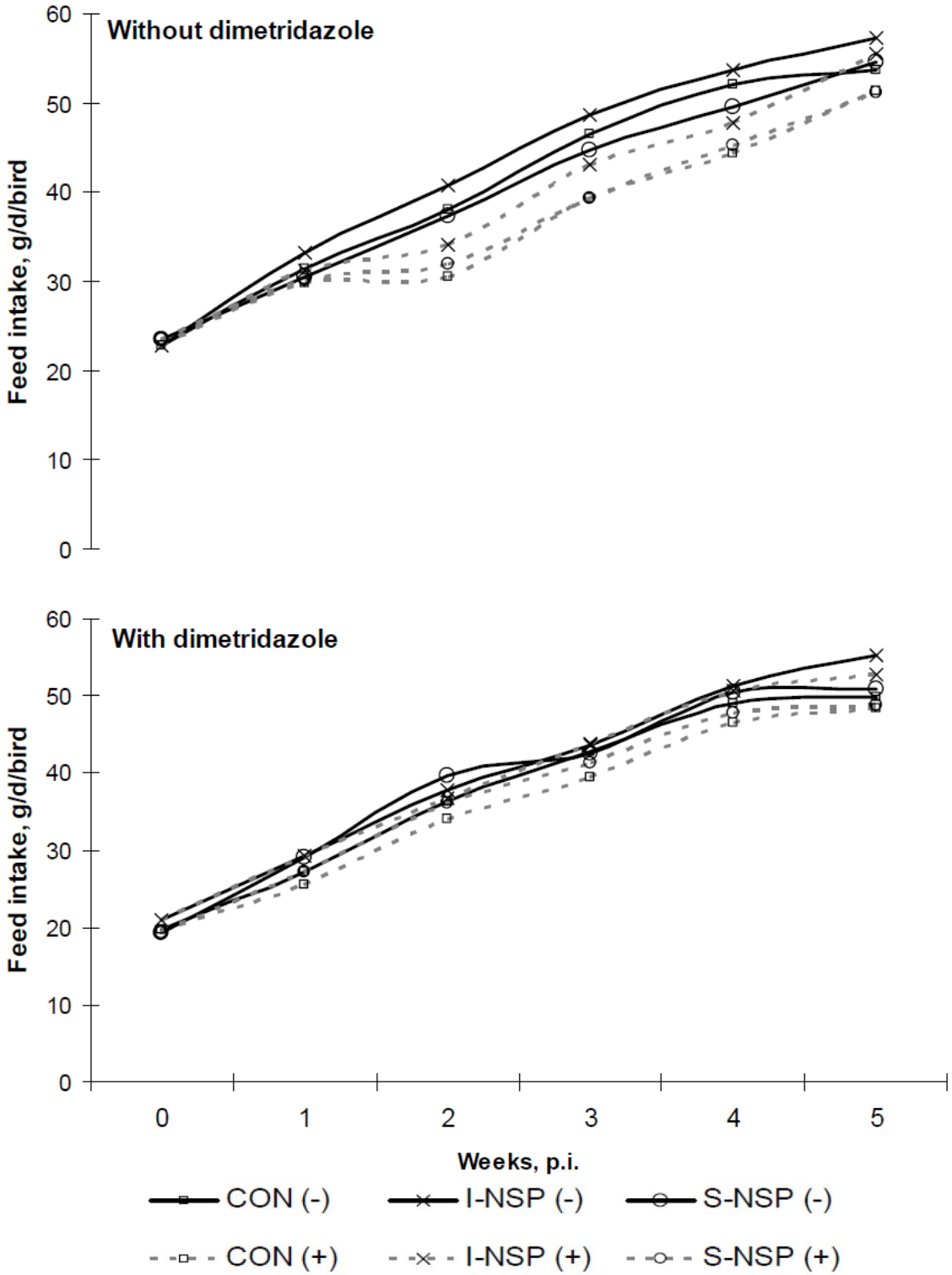

Figure 3. Average daily feed intake of *H. gallinarum* infected (+) and uninfected control (-) groups on different diets, without and with the dimetridazole treatment.

2.3.6. Host animal growth performance and feed utilization in the pre-infectional period

During the pre-infection period (1-3 wk), birds being supplied with I-NSP consumed 2.8 % more feed compared to CON (Table 5). No significant effect of double feeding system for each diet was found for BW and feed:gain ratio in the pre-infectional period (P>0.05). CON-fed birds had higher body weights than S-NSP fed birds (p<0.05), whereas I-NSP fed birds did not differ from birds of CON- or S-NSP-groups (p>0.05). Feed:gain ratio was smaller in CON than in NSP fed birds (p<0.05).

Table 5. Effects of diet on feed intake, body weight (BW), and feed:gain ratio in the pre-infectional period (1-3 wk)**.**

	Diet[1]				
Item	**CON**	**I-NSP**	**S-NSP**	***PSE*** [2]	***P-value***
Feed consumption[3], g/bird	351	361	351	-	-
BW[4], g	212[a]	207[ab]	203[b]	1.533	0.001
Feed:gain, g/g	2.03[a]	2.14[b]	2.14[b]	0.020	0.001

[1] CON = basal diet; I-NSP = 1,000 g CON + 100 g pea bran; S-NSP = 1,000 g CON + 100 g chicory root meal.
[2] Pooled SE.
[3] Calculated from daily group consumptions.
[4] Body weight at the end of wk 3 of life. The average one-day-old weight was 37 g.

2.3.7. Host animal growth performance and feed utilization up to 5 wk p.i.

As shown in Table 6, the effects of diet on BW and feed:gain were significant (P<0.001). Feeding CON resulted in higher BW and lower feed:gain ratios in comparison to feeding the NSP diets. Feeding I-NSP resulted in higher BW development but also higher feed:gain ratio in comparison to feeding S-NSP (p<0.05). There were significant interactions between effects of dimetridazole treatment and infection for BW and feed gain ratio (P<0.05). Uninfected birds with and without dimetridazole treatment had almost the same BW (p>0.05) whereas infected birds were lighter (p<0.05). Dimetridazole un-treated infected birds had lower BW than the dimetridazole treated infected birds (p<0.05). The application of dimetridazole decreased the feed:gain ratio of infected and uninfected birds compared to the un-treated birds (p<0.05). Infected un-treated birds had higher feed:gain ratio than the uninfected un-treated birds (p<0.05).

Table 6. Effects of the investigated factors on feed consumption, body weight (BW), and feed:gain ratio (as LSMEANS and SE)**.**

	Feed intake[1]	**BW**[2]		**Feed:gain**	
Item	g/bird	g/bird	*SE*	g/g	*SE*
Diet					
CON	1775	706[a]	*4.851*	2.67[a]	*0.023*
I-NSP	1886	683[b]	*4.783*	2.95[b]	*0.023*
S-NSP	1793	665[c]	*4.856*	2.88[c]	*0.023*
Infection[3] **x dimetridazole**[4]					
Un-infected / dimetridazole (-)	1931	708[a]	*5.752*	2.86[a]	*0.028*
Infected / dimetridazole (-)	1778	633[c]	*5.377*	2.98[b]	*0.026*
Un-infected / dimetridazole (+)	1827	709[a]	*6.221*	2.74[c]	*0.030*
Infected / dimetridazole (+)	1767	688[b]	*4.877*	2.74[c]	*0.023*
P-values[5]					
Diet	-	*0.001*		*0.001*	
Infection	-	*0.001*		*0.038*	
Dimetridazole treatment	-	*0.001*		*0.001*	
Diet x infection	-	*0.563*		*0.687*	
Diet x dimetridazole	-	*0.950*		*0.721*	
Infection x dimetridazole	-	*0.001*		*0.019*	
Diet x infection x dimetridazole	-	*0.951*		*0.412*	

(abc): Values with no common superscripts within a factor indicate differences (Tukey, $p<0.05$).
[1] Calculated from daily group consumptions.
[2] Body weight at the end of wk 8 of life, i.e., at 5 wk p.i. .
[3] The birds were treated with dimetridazole (Chevi-col© Pulver, Chevita GmbH, Germany). The compound was given via *ad libitum* offered drinking water at a concentration of 0.05% (w/v) from 2 d before inoculating *H. gallinarum* eggs to 7 d post-infection.
[4] *H. gallinarum* infection with 200 embryonated eggs of *H. gallinarum* previously harvested from histomonas and concomitant *H. gallinarum* infected chickens.
[5] P-values derived from the 3-way ANOVA analysis. LSMEANS and SE are presented either for significant non-interactive main effects (Diet) or for significant interactions between any of the main factors (in this case, interaction between infection x dimetridazole treatment).

2.4. Discussion

Under natural conditions, the vast majority of Heterakis eggs from chicken are *H. meleagridis* positive (McDougald, 2005). The minimum possible infection rate, i.e., the proportion of Histomonas-carrying *H. gallinarum* eggs that are obtained from sub-clinically infected birds and those are able to produce clinical histomonosis, has been estimated to be 1:139 (Lund, 1958). In any case, it can be assumed that the histomonas proven positive infection dose used led to a double infection in all infected birds. This is confirmed by the presence of the protozoon and histomonas-associated lesions on caecal

tissues of the sentinel birds examined by necropsy. Dimetridazole is known to be highly effective against *H. meleagridis* in chicken (Hu and McDougald, 2004). Histomonads are released from the larva during molting rather than at death of larvae (McDougald, 2005). Lund (1968) showed that the histomonads are liberated from the larva starting as early as 4.5 d after inoculation of the eggs. Therefore, the dimetridazole treatment in the present study starting from 2 d before infection to 7 d p.i. can be regarded as effective to eliminate histomonas infection in the treated birds.

The results of the present study indicate that *H. meleagridis* decreased incidence, establishment rate and development of *H. gallinarum* in all feeding groups when compared with the corresponding histomonas free feeding groups. This is in agreement with observations of Lund (1958) who reported that caeca with clinical evidence of blackhead disease (histomonosis) contain fewer or no worms at all when compared to unaffected caeca in chickens and turkeys. We measured a decrease by almost 20% in length of histomonas infected heterakis females. Lund (1958) reported a decline by 5.1% in worm length of histomonas infected female *H. gallinarum*. Female worm length is the best predictor of fecundity in *H. gallinarum* (Tompkins and Hudson, 1999). Though quantification of female worm fecundity was not in the scope of this study, histomonas infection might have impaired female worm fecundity as well. Detrimental effects of parasites on their vectors are known for a wide range of parasite-vector systems (Elliot et al., 2003). For instance, malaria-infected mosquitoes are less fit as to longevity and reproductive success than unaffected ones (Hogg and Hurd, 1997; Hurd et al., 2005). Although the evolutionary mechanisms behind detrimental effects of parasites on their vectors are not fully understood, it might be hypothesized that *H. meleagridis* exerts a selection pressure on *H. gallinarum* resulting in a more suitable worm population for the transmission of histomonads.

The type of diet played an important role in the interaction between the two parasites, since feeding S-NSP resulted in lower incidence and total worm burden than I-NSP in case of dual infection, but this diet resulted in highest worm burden when histomonas was eliminated. The effect of S-NSP high probably indicates the involvement of the caecal bacteria in the relations of the two parasites. Bacteria play critical roles in the life cycle of the two parasites (McDougald, 2005). *H. meleagridis* attains full virulence in the caeca of chicken only in combination with the presence of several bacteria species (Springer et al., 1970; McDougald, 2005). Springer et al. (1970) showed that *Heterakis* larvae were not even able to survive when injected into caeca of gnotobiotic birds, indicating that the role

of bacteria is more vital for the survival of *Heterakis* than of histomonads. Moreover, *H. gallinarum* is considered as a bacteria feeder (Bilgrami and Gaugler, 2004). The S-NSP diet contained inulin, a highly fermentable substance which acts as a prebiotic and has been shown to increase intestinal bacteria counts and their metabolic activity in turkeys (Juskiewicz et al., 2005) and in chickens (Rehman et al., 2008a). Fermentation of inulin by the microorganisms markedly changes caecal environment in terms of a lowered pH and altered profile of short chain fatty acids that consequently increase caecal size in chicken (Juskiewicz et al., 2005; Rehman et al., 2008a, b). It can be assumed that abundance of caecal bacteria induced by feeding S-NSP may have encouraged the development of histomonas infection. Lower incidence and worm burdens as well as retarded worm growth in histomonas infected birds may be explained by the heavy pathological lesions which can be considered as a harsh environment for *H. gallinarum* to establish and to further develop. On the other hand, S-NSP favored the establishment of *H. gallinarum* in the absence of histomonads as indicated by the highest worm burden in the birds treated against *H. meleagridis*.

Both, dual and single infections resulted in lower body weight development of the birds. This might have been caused by partial diversion of nutrients from growth to development of immunity (Kyriazakis and Houdijk, 2006), and to support repair of damaged mucosal structures of the intestine in infected birds (Hoste, 2001). Moreover, the infection induced a decrease in feed intake particularly in the dimetridazole un-treated birds, being associated with a reduced intake of ME and essential nutrients such as essential amino acids, minerals, trace elements and vitamins, may have contributed to the retarded body weight development of the birds in all feeding groups.

Although a single *H. gallinarum* infection impaired body weight development of the birds, *H. meleagridis* aggravated this effect. Though in dimetridazole un-treated birds the S-NSP diet resulted in lower infection rate and worm burden than I-SNP, I-NSP led to higher body weight development than S-NSP. This may also suggest that S-NSP intensified histomonas infection which then impaired establishment and development of *H. gallinarum*, exerting an additional negative effect on host growth performance.

2.5. Conclusion

Histomonas meleagridis harmed the definitive host as well as *H. gallinarum*. Insoluble and soluble NSP supplemented diets favored *H. gallinarum* infection while S-

NSP additionally intensified histomonas infection, which then impaired establishment and development of *H. gallinarum*. Therefore, dietary NSP appear not to be suited to protect chicken against infections with *H. gallinarum* and *H. meleagridis*.

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CHAPTER - III

Effects of dietary non-starch polysaccharides on establishment and fecundity of *Heterakis gallinarum* in grower layers

Effects of dietary non-starch polysaccharides on establishment and fecundity of *Heterakis gallinarum* in grower layers

Abstract

It was hypothesized that the establishment and fecundity of *Heterakis gallinarum* in chicken may be affected by dietary non-starch polysaccharides (NSP), which are known to alter the intracaecal environment. Therefore, a total of 670 one-day-old female layer chicks were fed *ad libitum* for 11 wk one of the following experimental diets. The birds were fed either a basal diet (CON) or a basal diet plus pea bran rich in insoluble NSP (I-NSP), or a basal diet plus chicry root meal as a source of inulin rich soluble NSP (S-NSP) in a three-times repeated experiment. At the end of wk three, each feeding group was subdivided into an uninfected and an infected group of birds each inoculated with 200 embryonated eggs of *H. gallinarum*. All the birds were slaughtered 8 wk post infection, and their worm burdens, as well as the nematode egg excretion were determined.

The NSP supplemented diets and also infection led to reduced body weights (BW) of birds and impaired the feed conversion rate ($P<0.001$). The NSP supplemented diets increased average length of caecum ($P<0.001$), with S-NSP exerting a stronger effect than I-NSP ($P<0.05$). Full caeca weight was increased by S-NSP ($P<0.001$). The infection increased the weight of full and empty (washed) caeca ($P\leq0.027$). Feeding S-NSP lowered intracaecal pH and molar proportion of acetate and increased that of butyrate compared to CON and I-NSP ($P<0.001$). Caecal pool of volatile fatty acids (VFA) was increased with S-NSP ($P<0.001$). Infection increased intracaecal pH ($P=0.002$) accompanied by lower molar proportion of butyrate ($P<0.001$) and reduced caecal pools of VFA ($P<0.001$).

The NSP-diets elevated incidence of infection ($P<0.01$), average number of larvae ($P<0.009$) and total worm burden ($P<0.001$) compared to CON. The worm length was not influenced by the diet ($P>0.05$). The daily amount of faeces increased in NSP-fed birds ($P<0.001$). Number of eggs per gram of faeces (EPG), number of eggs excreted per worm population of a bird within 24 h (EPD) and female worm fecundity (EPD/female worm) were elevated after feeding S-NSP ($P\leq0.002$), whereas I-NSP led to lower EPG/female worm ($P<0.05$). The EPD increased in the sequence of CON < I-NSP < S-NSP ($P<0.001$).

It is concluded that the pea bran and chicory root meal used as sources of insoluble and soluble dietary NSP, respectively, provided favorable conditions for the establishment

of *H. gallinarum* in grower layers. Chicory root meal additionally enhanced fecundity of the nematode. Therefore, the two natural sources of insoluble and soluble NSP offer no potential as protecting agents against *H. gallinarum* infections in chicken.

Keywords: *Non-starch polysaccharides; Heterakis gallinarum; worm fecundity; pea bran; chicory root; inulin; chicken; caeca.*

3.1. Introduction

The diet can alter the interactions between the host animals and their parasites (Coop and Holmes, 1996; Coop and Kyriazakis, 1999; Stear et al., 2007). Dietary vitamins (Idi et al., 2007), minerals (Gabrashanska et al, 2004), protein or amino acids (Riedel and Ackert, 1951; Daş et al., 2010) have been shown to influence infections of poultry with the common fowl parasite *Ascaridia galli*. Dietary non-starch polysaccharides (NSP) influenced infections of pigs with *Oesophagostomum dentatum* (Petkevičious et al., 1997; 2001; 2003) and of chickens with *Ascaridia galli* (Daenicke et al., 2009). As NSP are only degradable by the intestinal microbiota (Englyst, 1989), their effects on nematode infections should mainly be ascribable to alterations of digesta characteristics and intestinal microbial fermentation.

In recent years, *Heterakis gallinarum* has become more important with the increasing number of poultry kept in floor husbandry systems, where the prevalence of this parasite may reach 80% (Permin et al., 1999; Maurer et al., 2009). The nematode is known as the main vector for the transmission of *Histomonas meleagridis*, which is brought about by the ingestion of embryonated eggs of the nematode by the host animal (McDougald, 2005). Dietary NSP have been shown to interfere with the interrelation between *H. gallinarum* and *Histomonas meleagridis* (Daş et al., 2009). Therefore, it must be ensured that the nematode is free of *H. meleagridis* if dietary effects on infection parameters for the worm are investigated.

The caeca are the main sites of microbial fermentation in poultry (Józefiak et al., 2004) and are the predilection sites of *H. gallinarum*. We hypothesized that the establishment and fecundity of *H. gallinarum* may be affected and regulated by dietary NSP, which are known to alter the intracaecal environment. Therefore, the objective of the present investigation was to examine the effects of low or highly fermentable NSP on the establishment and fecundity of the nematode as well as on parameters of caecal

fermentation and performance of grower layers experimentally infected with *H. gallinarum*.

2. Material and methods

3.2.1. Experimental design, diets and management of the birds

In a three times repeated experiment, conducted in the years 2008 - 2009, a total of 670 one-day-old female Lohmann Selected Leghorn (LSL) chickens were used. The chicks were weighed together and divided within each repetition into 3 feeding groups. Each feeding group was fed *ad libitum* one of the following pelleted experimental diets (Table 1): basal diet (CON), basal diet plus insoluble NSP (I-NSP), and basal diet plus soluble NSP (S-NSP) from hatch until wk 11 of life. Insoluble NSP were supplied by mixing on air dry-basis one kg CON with 100 g pea bran (Exafine 500, Socode, Belgium). For S-NSP, one kg CON was mixed with 100 g chicory root meal (Fibrofos 60, Socode, Belgium). Daily feed consumption was monitored per group. Drinking water was offered *ad libitum.*

Until wk three, each feeding group was kept in a pen scattered with wood shavings. The litter was replaced once (wk 1-3) or twice (wk 4-11) a week. Room temperature was gradually decreased from 34 °C on the first day (d) to 26 °C in wk three and thereafter decreased by 2-3°C per wk, ending at 18-20 °C from wk six onwards. A 24 h lighting period was maintained for the first two days and was then reduced to 16 h/d at the end of the first week. By wk eight, it was reduced to 12h/d and subsequently maintained until the end of the experiment. At the end of wk three, the birds were marked with wing tags and individual body weights (BW) were determined for the first time and thereafter at weekly intervals.

3.2.2. Experimental infection

The inocula were prepared at the Department of Animal Science, University of Goettingen, Germany. Adult female worms, harvested from intestines of naturally infected chickens from different farms, were used as the original source of infection material. The infection material was passed for one generation in a preliminary animal trial in which a dimetridazole treatment was applied to chickens to eliminate possible contamination of *Histomonas meleagridis*. Controls confirmed that the new batch of infection material was obtained from *H. meleagridis* free Heterakis-infected birds. For embryonation, the second generation female worms were incubated at room temperature (20-25 °C) for three weeks in media containing 0.1% (wt/vol) potassium dichromate ($K_2Cr_2O_7$) as described by Puellen et al. (2008). After embryonation, the worms were cut into pieces, and the eggs

were squeezed out using a pestle placed on a sieve. The residual worm tissues on the sieve were flushed away, and the eggs were collected. The embryonated eggs were stored at 4 °C until the infection day. On the infection day, the number of eggs/ml aqueous suspension was determined using a McMaster egg counting chamber. Only eggs in the vermiform and infective larval stages were classed and counted as embryonated. The counting procedure was repeated five times and the arithmetic mean was calculated. The infection dose was then adjusted to 200 eggs/0.2 ml of final suspension. In the second and third repetitions, the eggs of female worms harvested in the previous experimental run were used as infection material and prepared in the same way. Therefore, age of the eggs at infection was around 8 mo, 3 mo and 1 mo in the first, second and third repetition, respectively.

At the end of wk three, each feeding group was subdivided into an uninfected control group (40% of birds) and an infected group (60%). The infected groups were inoculated with 200 embryonated eggs of *H. gallinarum* per bird, which were administered orally by a 5 cm esophageal cannula. Uninfected control birds were given 0.2 ml of an aqueous placebo. Uninfected birds were left in their previous pens, whereas birds of each infected group were placed in new pens within the same experimental stable. The birds did not get any vaccination or anthelmintic treatment throughout the experimental period. The stable was thoroughly disinfected at least 10 d before introducing the birds.

3.2.3. Chemical analyses of the diets

The compositions of the diets are same as given in Chapter 2. Nutrient contents of the experimental diets are given in Table 1. Feed samples were taken regularly (at weeks 1, 6, 11) during each experimental repetition and were analyzed for dry matter (DM), crude ash (CA), crude protein (CP), sugar, starch, and ether extract (EE) using standard methods (Naumann and Bassler, 1997). Neutral and acid detergent fibre (NDF and ADF, respectively) were analyzed according to Van Soest et al. (1991) and results are given exclusive of ash. The metabolizable energy of the diets (MJ ME/kg DM) was calculated (FMVO, 2008). Insoluble and soluble non-starch polysaccharides were measured using an enzymatic test (Megazyme, 2007). Inulin was determined according to Naumann and Bassler (1997).

Table 1. Composition and analysis of the experimental diets.

Item	CON[1]	I-NSP[2]	S-NSP[3]
Analyzed composition			
DM, g/kg	894	896	895
Nutrient, g/kg DM			
Ash	53	51	53
CP	216	199	202
NDF	111	166	110
ADF	33	90	40
Ether extract	40	36	37
Starch	514	476	435
Insoluble NSP	103	172	104
Soluble NSP	20	23	25
Inulin	-	-	70
Calculated energy			
ME, MJ/kg DM[4]	13.64	12.58	12.21

[1] Basal diet.
[2] Insoluble non-starch polysaccharide supplemented diet = 1000 g CON plus 100 g pea bran.
[3] Soluble non-starch polysaccharide supplemented diet = 1000 g CON plus 100 g chicory root meal.
[4] ME, MJ/kg DM= [(g CP/kg DM x 0.01551) + (g CL/kg DM x 0.03431) + (g starch/kg DM x 0.01669) + (g sugar/kg DM x 0.01301)]. Sugar contents of the diets were estimated based on sugar contents of the components.

3.2.4. Faecal sampling and post-mortem examinations

During the last four days of the last two repetitions, birds of the infected groups were placed into individual cages for a 24 h period of faeces collection (12 birds d^{-1} $group^{-1}$). In the cages, the birds had free access to feed and water. Faeces excreted by each bird accumulated in plastic bag-covered boxes underneath the cage. The total amount of faeces per bird/day was weighed, transferred into a plastic cup and stirred thoroughly for at least 3 minutes to get a paste-like consistency that guaranteed a homogeneous distribution of the eggs in the faeces. The number of eggs per gram of faeces (EPG) was quantified using a modified McMaster counting technique (MAFF, 1986) and saturated NaCl as the flotation liquid (density = 1.2 g/ml). The minimum detection level was 50 eggs / g faeces.

The birds were slaughtered after electrical stunning eight wk post-infection (p.i.) at an age of 11 wk. Immediately after slaughter, the gastrointestinal tracts were removed and the visceral organs were separated. Weights of liver (+gall bladder), pancreas, full caeca as well as length of small intestine and each caecum were measured. Intact caeca from 10 birds per group (60 per repetition) were weighed, frozen and stored at -18 °C until analyzed for volatile fatty acids (VFA).

The caeca of the infected birds were further processed for parasitological examinations to determine incidence and number of adult worms, as well as number of larvae. The caeca were opened with scissors, the content was removed, and the caecal walls were flushed to remove the worms. The surface moisture of the empty caeca was removed using paper towel, and the empty caeca weight was determined. The caecal content was flushed with low-pressure tap water through a sieve with a mesh aperture of 100 μm, and then transferred into Petri dishes to be examined by a stereomicroscope (Gauly et al., 2008). The adult and immature worms were counted and the adults were sexed. Average intact worm length was estimated by measuring 20 female and male worms per bird. In cases of lower than 20 worms per sex were available, all the intact worms were measured. Caeca samples of uninfected control birds (15-20% of each group) were also processed to confirm infection free status of these groups. The caeca of the residual birds of each uninfected group were pooled and checked for the presence of the nematode. A total number of 475 birds and 287 faecal samples were examined for determination of worm burdens and EPG, respectively.

3.2.5. pH and volatile fatty acids (VFA)

The frozen intact caeca were thawed at room temperature. The caecal content was removed from the caeca, and 2 g were weighed and immediately afterwards suspended in 10 ml of distilled water. The sample was mixed using a vortex for around 5 seconds. The pH was directly measured in this suspension using a pH electrode (InLab®Easy BNC, Fa. Mettler Toledo) connected to a pH meter (GC 811, Fa, Schott). Thereafter, the suspension was centrifuged at 2000 x *g* at room temperature for 20 min. Five ml of supernatant was transferred to a glass tube, which contained 250µl international standard (4% methyl-valeric acid in formic acid). The mixture was vortexed and two parallel sub-samples of 1.5 ml each were transferred to Eppendorf tubes. The parallels were centrifuged at 10000 x *g* at room temperature for 10 min. After centrifugation, the samples were stored in a refrigerator (+4°C) until gas chromatography.

For gas chromatography, a combined internal/external standard procedure was applied using a packed column (10% Carbowax 20 MTPA SP1000 with 1% H_3PO_4 on Chromosorb WAW, 80/100). Temperature for injection port was 170 °C, for detector 200 °C and for column 120 °C (isothermal). The gas chromatograph (Shimadzu GC 14B) was equipped with a flame ionization detector (FID) and hydrogen was used as the carrier

gas (Da Costa Gomez, 1999; Abel et al., 2002). The average of the parallels was used for calculations.

The remaining caecal contents after sampling for VFA were used to determine dry matter, crude ash and organic matter of the caecal contents.

3.2.6. Data management and statistical analyses

3.2.6.1. Parameter definitions, transformations and restrictions

Because the data of the infection variables positively skewed (Skewness > 0) and showed non-normal (Kolmogorow-Smirnow, $p<0.05$) distributions, log-transformations were employed. For this, individual infection parameters that described worm counts (establishment rate, number of males, females, larvae, and total worm burden), EPG, total number of eggs excreted per worm population of a bird within 24 h (eggs per day; EPD) and female worm fecundity parameters were transformed by using the natural logarithm (ln) function [ln(y)=Log(y)] to correct for heterogeneity of variance and to produce an approximately normally distributed data set. Establishment rate was defined as the number of worms per bird in relation to infection dose. Adult female worm fecundity was defined based on both EPG (EPG per female worm) and EPD (EPD per female worm). Lengths of the male and female worms, sex ratio (numbers of females / males) and the amount of daily faeces of the infected birds were left untransformed.

In preliminary analyses (with fixed effect of repetition), no significant ($P>0.05$) interaction effects of diet x infection x experimental repetitions on any of the performance parameters (e.g., BW, ADG) were observed. This partly indicated reproducible effects of diet and infections on the performance parameters over the repetitions. However, because the experimental repetitions were performed in different periods of time, the effect of repetition was included in the models as a random factor to ensure safe generalization of the effects of the main factors (diet and infection) and to avoid any possible confounding effect of time, in which the repetitions were performed, with any of the main factors.

3.2.6.2. Statistics

Statistical analyses were performed with SAS V9.1.3 (2010). Mortality data of the 3 repetitions were pooled, because the overall level was below 5 %. The effect of diet on mortality in the pre-infection period (wk 1-3) was analyzed with logistic regression method using the GENMOD procedure with the logit link function. The GENMOD procedure fits the generalised linear models and suited for responses with binary outcomes (Kaps and

Lamberson, 2004). For the infected period (wk 4-11) the mortality model was extended to the effects of diet, infection and the interaction effect between diet and infection.

The effect of diet on worm-harboring birds as a proportion of experimentally infected birds (incidence of infection) was separately analyzed for each repetition. The differences between incidences of infection among the infected groups were analyzed with Fisher's exact test, performed for all possible pair-wise combinations of the three infected groups.

For establishment rate, worm counts and nematode egg excretion variables, the model included fixed effect of the diets and random effect of experimental repetitions using the Proc MIXED. Data of VFA and visceral organ measurements were analyzed with another mixed model that included fixed effects of diets, infection as well as interaction effect of diet and infection. Effect of experimental repetition was included in the model as random.

The model for the repeatedly measured performance variables (e.g. BW, feed:gain) included fixed effects of diet, infection, experimental weeks (as age of birds) as well as all possible interactions among these factors. The effect of experimental repetitions was included in the model as a random factor. Furthermore, individual random effect of the birds as the repeated subject within a repetition over the experimental weeks, was also included in the model presented below.

$$Y_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + a_l + b_{k(l)} + \varepsilon_{ijklm}$$

Where**;**

Y_{ijklm}= observation.

μ = the overall mean.

α_i = the effect of diet (i = 1,2,3).

β_j = the effect of infection (j = 0,1).

γ_k = the effect of experimental weeks (k= 3-11 wk).

$(\alpha\beta)_{ij}$ = the interaction effect between diet and infection.

$(\alpha\gamma)_{ik}$ = the interaction effect between diet and experimental weeks.

$(\beta\gamma)_{jk}$ = the interaction effect between infection and experimental weeks.

$(\alpha\beta\gamma)_{ijk}$ = the interaction effect among diet, infection and experimental weeks.

a_l = random effect of repetitions (l=1,2,3).

$b_{k(l)}$ = random effect of individual bird within repetition over the experimental weeks, the variance between repeated measurements of the birds (subject) within a repetition.

ε_{ijklm} = residual random error.

The repeatedly (weekly) measured variables were assumed to be correlated from one measurement date to the next, and thus the covariance structure was set to be compound symmetry.

3.2.6.3. Presentation of the results

After infection at the end of wk three, groups of uninfected and infected chickens were kept according to a 3 x 2 factorial arrangement of treatments with diet and infection as the main factors. Therefore, unless no significant interactions between the effects of diet and infection were encountered, the data are presented as the main effects of diet and infection. In case of significant interactions between diet and infection, the results are either presented for the 6 single treatments or are mentioned correspondingly in the text. Tukey adjusted post-hoc comparisons (Alpha= 0.05) were performed to either partition effects of the main factors or to determine single group differences when a non interactive significant main effect or a when significant interaction effect of the main factors was encountered, respectively.

For the effects of the main experimental factors, the results are presented as least square means (LSMEANS) with common pooled standard error (PSE). The PSE, calculated from the output of mixed models for balanced data, was confirmed to be the same as for a balanced data set that could be calculated from the output of GLM procedure as Root Mean Square Error divided by the square root of the number of observations per treatment mean as described by Pesti (1997). Because the numbers of observations in the groups were not always balanced for certain data, the most conservative (the largest) standard error of LSMEANS was represented as the pooled SE.

3.2.7. Ethical consideration

The experimental procedures followed the animal welfare rules. The infection dose (200 eggs) given to each bird was within the range of the worm burdens that can be observed in natural sub-clinical infections. The procedures for experimental infections followed the guidelines suggested by the World Association for the Advancement of Veterinary Parasitology for evaluating the effectiveness of anthelmintics in chickens and turkeys (Yazwinski et al., 2003).

3.3. Results

3.3.1. Mortality, feed consumption and performance

Birds consuming S-NSP had a higher mortality rate (4.2%) than those on the I-NSP diet (0.3%) during the pre-infection period from wk 1-3 (P=0.016). Most of the deaths occurred in the first week. After infection (wk 4-11), infected birds had a mortality of 2.5% compared to 0.4 % in uninfected birds (P=0.032). Most of the mortality cases were due to cannibalism, which developed within a few weeks after infection mainly after introducing birds to the new pens. During the pre-infection period (wk 1-3), birds receiving I-NSP and S-NSP consumed 6 % more and roughly 1 % less feed, respectively, compared to CON (Table 2). The coefficient of variation (CV) of feed consumption, calculated for repetitions within feeding groups, was less than 3 %. Regarding the entire experimental period (wk 1-11), birds on the I-NSP and S-NSP diets consumed 8 % and almost 2 % more than the CON fed group and the CV within feeding groups was smaller than 5 %. Compared to CON, the NSP-diets reduced the BW gain of birds and impaired the feed conversion rate with I-NSP entailing a greater amount of feed per unit BW gain than S-NSP (P<0.001). Infected birds consumed 1.6 % less feed and had a lower BW (P<0.001) accompanied by a higher feed:gain ratio (P<0.001) than their uninfected counterparts.

Table 2. Effects of diet and *H. gallinarum* infection on feed consumption, body weight (BW), and feed:gain ratio.

	Diet[1]					***H. gallinarum* infection**[2]				***Interaction P-value***
Item	**CON**	**I-NSP**	**S-NSP**	***PSE*** [3]	***P-value***	**Uninfected**	**Infected**	***PSE*** [3]	***P-value***	
Pre-infection period (wk 1-3)										
Feed consumption[4], g/bird	336	356	332	-	-	no	no	no	no	no
BW[5], g	201[a]	192[b]	192[b]	8.778	0.001	no	no	no	no	no
Feed:gain, g/g	2.10[a]	2.38[b]	2.20[c]	0.094	0.001	no	no	no	no	no
Entire period (wk 1-11)						no	no	no	no	no
Feed consumption[4], g/bird	2910	3146	2964	-	-	3030	2983	-	-	-
BW[6], g	989[a]	960[b]	954[b]	16.100	0.001	978[A]	957[B]	16.050	0.001	0.582
Feed:gain, g/g	3.30[a]	3.64[b]	3.50[c]	0.067	0.001	3.43[A]	3.53[B]	0.066	0.001	0.430

[(abc) or (AB)]: Different letters within each factor on the same line indicate differences (Tukey, $p<0.05$).
no: no infection effect in the pre-infection period.
[1] CON = basal diet; I-NSP = 1,000 g CON + 100 g pea bran; S-NSP = 1,000 g CON + 100 g chicory root meal.
[2] Uninfected controls or infected with 200 eggs of *Heterakis gallinarum*.
[3] Pooled SE.
[4] Calculated from daily group consumptions, therefore no statistical comparison could be performed (-).
[5] Body weight at the end of wk 3 of life.
[6] Estimated from body weight of birds from wk 3-11 of life, presented as BW at the age of 11 wk.

3.3.2. Incidence of infection, worm burdens and worm fecundity

In repetitions 1 and 3, feeding NSP led to higher incidences of infection than CON (P<0.01), but no difference was observed in the second repetition. Almost all NSP fed birds had at least one worm in all three repetitions. In CON fed birds, 88 %, 100% and 81 % were harboring worms in the first, second and third repetition, respectively. Birds on the NSP-diets had higher establishment rate (P<0.001), number of larvae (P<0.009) and total worm burden (P<0.001) than those receiving CON (Table 3). There was a trend for a lower ratio of female to male worms in birds being fed S-NSP instead of CON (P=0.094). The worm length was not influenced by diet (P>0.05).

Table 3. Effect of diet on establishment rate, average number of worms per bird, sex ratio and length of worms in birds infected with *Heterakis gallinarum* (200 eggs/bird).

	Diet[1]				
Item	**CON**	**I-NSP**	**S-NSP**	***PSE***[2]	***P-value,* ≤**
Establishment rate[3], %	26.7[a]	46.9[b]	49.2[b]	11.959	0.001
Number of female worms[3]	26.6[a]	46.2[a]	47.6[b]	12.811	0.001
Number of male worms[3]	26.6[a]	45.9[a]	49.7[b]	11.481	0.001
Number of larvae[3]	0.15[a]	1.62[b]	1.03[b]	0.470	0.009
Total worm burden[3]	53.4[a]	93.7[b]	98.4[b]	23.917	0.001
Sex ratio, F/M	1.07	1.03	0.95	0.072	0.094
Female worm length, mm	11.28	11.47	11.37	0.302	0.111
Male worm length, mm	9.59	9.66	9.65	0.217	0.578

([ab]): Values with no common letters within rows differ (Tukey, p<0.05).
[1] CON = basal diet; I-NSP = 1,000 g CON + 100 g pea bran; S-NSP = 1,000 g CON + 100 g chicory root meal
[2] Pooled SE.
[3] LSMEANS and PSE represent untransformed data, P-values are based on the transformed data.

The amount of faeces increased in the NSP-fed birds (P<0.001) irrespective of the type of NSP (Table 4). The EPG, the EPD and EPD/female worm were elevated after feeding S-NSP (P≤0.002), whereas I-NSP led to lower EPG/female worm (P<0.05). The EPD increased in the sequence of CON < I-NSP < S-NSP (P<0.001).

Table 4. Effect of diet on the amount of faeces, the excretion of nematode eggs and the fecundity estimates of worms in birds infected with *Heterakis gallinarum* (200 eggs / bird).*

	Diet [1]				
Item	**CON**	**I-NSP**	**S-NSP**	***PSE***	***P-value,* ≤**
Faeces, g bird^{-1} d^{-1}	28.26^{a}	37.68^{b}	36.43^{b}	4.70	0.001
EPG[2]	449^{a}	581^{a}	780^{b}	180.91	0.002
EPG / female worm[3]	13.2^{a}	10.4^{b}	14.9^{a}	4.85	0.001
EPD[4]	12148^{a}	19138^{b}	26181^{c}	3548.59	0.001
EPD / female worm[5]	321.4^{a}	344.0^{a}	490.8^{b}	96.10	0.002

(*): LSMEANS and pooled standard error (PSE) represent untransformed data, P-values and multiple comparisons are based on transformed data.
(^{abc}): Values with no common letters within rows differ (Tukey, p<0.05).
[1] CON= basal diet; I-NSP = 1,000 g CON + 100 g pea bran; S-NSP = 1,000 g CON + 100 g chicory root meal.
[2] Number of eggs per gram of faeces.
[3] EPG based female worm fecundity: average number of eggs excreted per female worm through one gram of faeces.
[4] Number of eggs per day; total number of eggs excreted per worm population of a bird within 24 h.
[5] EPD based female worm fecundity; number of eggs excreted per female worm within 24 h.

3.3.3. Visceral organ development

Both types of NSP increased the relative pancreas weight, the caecum length and the empty caeca weight (P<0.001; Table 5), and, with the exception of relative pancreas weight, S-NSP exerted a greater effect than I-NSP (P<0.05). The absolute liver weight was lower in S-NSP fed birds than the CON fed birds (P=0.005), however relative weight of liver to BW (hepato-somatic index) was not influenced by the diets (P=0.629). The small intestine length and the full caeca weight were increased by S-NSP (P<0.05). Infection reduced liver weight (P=0.005) and increased the relative pancreas weight (P=0.003) as well as the full caeca weight (P=0.027). Empty caeca weight was also elevated by infection (P<0.001). An interaction between diet and infection (P=0.039) revealed, that within each feeding group infected animals had heavier empty caeca than the uninfected ones, but uninfected S-NSP fed birds exceeded infected ones receiving CON and did not differ from infected I-NSP-fed birds.

Table 5. Effects of diet and *H. gallinarum* infection on the size of certain visceral organs.

	Diet[1]					***H. gallinarum* infection**[2]				***Interaction***
	CON	**I-NSP**	**S-NSP**	***PSE*** [3]	***P,* ≤**	**-**	+	***PSE*** [3]	***P,* ≤**	***P-value***
Liver, g	18.0^{a}	17.7^{ab}	17.3^{b}	0.499	0.005	17.9^{A}	17.5^{B}	0.494	0.005	0.849
HS-Index[4], % (Liver/BW)	1.84	1.84	1.85	0.020	0.629	1.85	1.84	0.020	0.340	0.587
Pancreas, g	2.35	2.42	2.42	0.044	0.097	2.39	2.40	0.042	0.559	0.479
g Pancreas / 100 g BW	2.41^{a}	2.53^{b}	2.60^{b}	0.087	0.001	2.47^{A}	2.56^{B}	0.086	0.003	0.244
Small int. length, cm	108.6^{a}	108.5^{a}	112.6^{b}	1.505	0.001	110.2	109.6	1.482	0.362	0.669
Caecum length, cm	13.7^{a}	14.1^{b}	15.7^{c}	0.083	0.001	14.4	14.5	0.074	0.242	0.075
Full caeca weight, g	6.42^{a}	6.54^{a}	9.52^{b}	0.178	0.001	7.35^{A}	7.64^{B}	0.171	0.027	0.214
Empty caeca weight, g	2.46^{a}	2.61^{b}	3.29^{c}	0.085	0.001	2.59^{A}	2.98^{B}	0.083	0.001	0.039

[(abc) or (AB)]: Different letters within each factor on the same line indicate differences ($p<0.05$).
[1] CON = basal diet; I-NSP = 1,000 g CON + 100 g pea bran; S-NSP = 1,000 g CON + 100 g chicory root meal.
[2] Uninfected controls (-) or infected with 200 eggs of *Heterakis gallinarum* (+).
[3] Pooled SE.
[4] HS-Index: Hepato-somatic index = liver/BW*100.

3.3.4. Biochemical characteristics of the caeca

As shown in Table 6, percental DM of caecal contents was not influenced by diet ($P=0.246$) but was decreased by infection ($P<0.001$). Feeding S-NSP decreased the proportion of crude ash and increased organic matter in the DM of caecal contents compared to feeding CON and I-NSP ($P<0.001$). Infection did not influence crude ash and organic matter in the DM of caecal contents ($P>0.05$). Feeding S-NSP reduced intracaecal pH and molar proportion of acetate and increased that of butyrate as well as the caecal pools of individual and total VFA compared to CON and I-NSP ($P<0.001$). Infection increased pH ($P=0.002$) accompanied by lower molar proportion of butyrate ($P<0.001$), pools of acetate ($P=0.003$), butyrate ($P<0.001$) as well as the total VFA pool ($P<0.001$) of the caecal contents. Significant interaction effects of diet and infection were observed for the caecal propionate pool ($P=0.014$), which was smaller ($P<0.05$) in infected CON and I-NSP fed birds and reached a similarly ($P>0.05$) high level in all the other groups (Figure).

Table 6. Effects of diet and *H. gallinarum* infection on biochemical characteristics of the caeca.

	Diet[1]					***H. gallinarum* infection**[2]				***Interaction P-value***
Item	**CON**	**I-NSP**	**S-NSP**	***PSE*** [3]	***P*, ≤**	**Inf. (-)**	**Inf. (+)**	***PSE*** [3]	***P*, ≤**	
Dry matter (DM), %	17.48	17.63	18.19	*0.398*	*0.246*	18.42^{A}	17.11^{B}	*0.350*	*0.001*	*0.589*
Crude ash, (% of DM)	13.86^{a}	13.15^{a}	11.41^{b}	*0.377*	*0.001*	12.65	12.97	*0.339*	*0.317*	*0.373*
Organic matter, (% of DM)	86.14^{a}	86.85^{a}	88.59^{b}	*0.377*	*0.001*	87.35	87.03	*0.339*	*0.317*	*0.373*
pH	6.61^{a}	6.59^{a}	6.00^{b}	*0.153*	*0.001*	6.32^{A}	6.49^{B}	*0.151*	*0.002*	*0.210*
VFA Molar ratios, %										
Acetate	69^{a}	71^{a}	66^{b}	*1.301*	*0.001*	68	69	*1.229*	*0.091*	*0.785*
Propionate	15	15	14	*2.142*	*0.581*	14	15	*2.093*	*0.389*	*0.278*
Butyrate	16^{a}	15^{a}	20^{b}	*1.219*	*0.001*	18^{A}	16^{B}	*1.183*	*0.001*	*0.212*
VFA Pool, µmol[4]										
Acetate	262.6^{a}	247.0^{a}	351.3^{b}	*20.769*	*0.001*	312.8^{A}	261.1^{B}	*18.945*	*0.003*	*0.116*
Propionate	52.5^{a}	47.5^{a}	69.5^{b}	*8.416*	*0.001*	62.0^{A}	51.0^{B}	*8.210*	*0.003*	*0.014*
Butyrate	61.0^{a}	52.6^{a}	108.9^{b}	*4.705*	*0.001*	86.4^{A}	62.0^{B}	*3.799*	*0.001*	*0.812*
Total	376.2^{a}	347.1^{a}	529.7^{b}	*30.091*	*0.001*	461.2^{A}	374.2^{B}	*27.403*	*0.001*	*0.144*

[(abc) or (AB)]: Different letters within each factor on the same line indicate significant differences (Tukey, $p<0.05$).

[1] CON: Basal diet; I-NSP: Insoluble non-starch polysaccharide supplemented diet; S-NSP: Soluble non-starch polysaccharide supplemented diet.

[2] Uninfected controls (-) or infected (+) with 200 eggs of *Heterakis gallinarum*.

[3] Pooled SE.

[4] Calculated as multiplication of VFA concentration by the total amount cecal digesta.

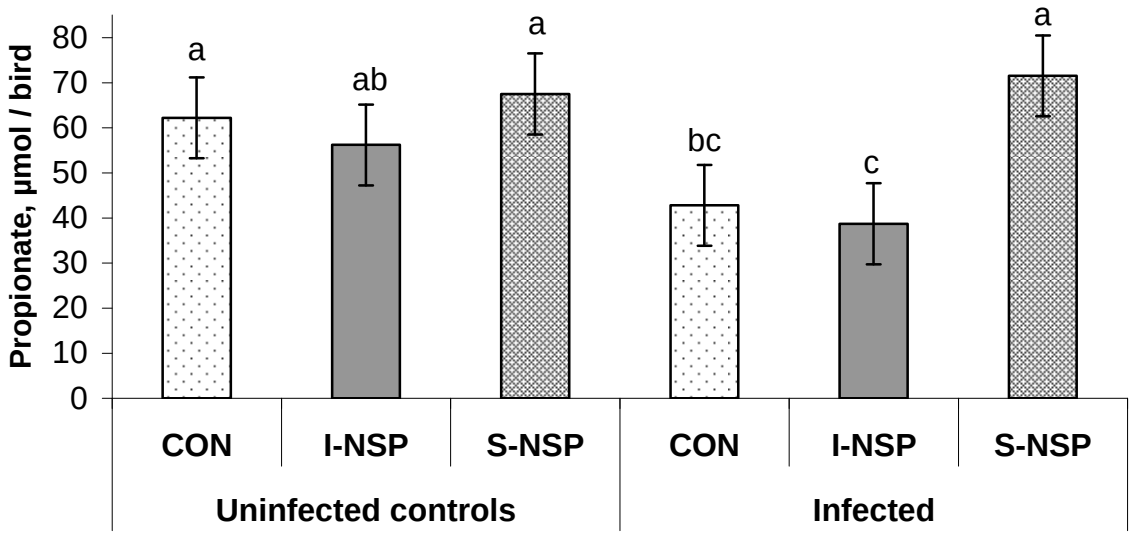

Figure Interaction effect (P=0.014) of diet and infection on the propionate pool.
(^{abc}): Different letters among the groups indicate significant differences (Tukey, $p<0.05$).

3.4. Discussion

The inclusion of pea bran and chicory root meal implied a nutrient dilution in I-NSP and S-NSP diets compared to CON. However, because chickens are able to increase their feed intake when a nutritionally diluted diet is offered (Forbes and Shariatmadari, 1994; Halle, 2002; Van Krimpen et al., 2007; Daş et al., 2010), the NSP fed birds could have consumed similar amounts of basal mixture nutrients as the CON fed birds. In fact, this was the case with I-NSP, whereas with S-NSP the increase in feed intake was not large enough to reach a similar level of basal mixture intake compared to CON. In spite of these differences in feed intake and the larger intestinal capacity of S-NSP fed birds, the NSP fed birds developed lower BW than those on the CON.

The increased sizes and weights of empty caeca, pancreas and small intestine length indicate that NSP feeding, with S-NSP in particular, have caused preferred channeling of nutrients to the development of splanchnic tissues and the intestinal tract. Increased relative pancreas in I-NSP and S-NSP fed birds indicates an adaptive response induced either directly by the diet (Iji et al., 2001) or indirectly by the intestinal microbiota. The longer small intestine in S-NSP fed birds indicates stimulated fermentation in the pre-caecal intestine too. Greater caeca size has repeatedly been observed after feeding chicken with NSP (Redig, 1989; Clench and Mathias, 1995; Jørgensen et al., 1996; Józefiak et al., 2004). Fermentation of inulin in chicken has been reported to selectively support butyrate

producing microorganisms and to lower luminal pH (Marounek et al., 1999; Rehman et al., 2008a,b). Volatile fatty acids and butyrate in particular are known to exert trophic effects on the intestinal mucosa (Montagne et al., 2003). In the present study, the pools of VFA and butyrate were increased and the pH was lowered in the caeca of birds on the chicory root meal supplemented diet in agreement with the known effects of intestinal inulin fermentation in chicken.

Dietary NSP intensified *H. gallinarum* infection in grower layers. Soluble NSP enhanced fecundity of the female worms, which would contribute to a strongly contaminated environment and create a higher risk for new or re-infections of birds under field conditions. Soluble NSP also led to higher mortality of the birds in the pre- but not in the post-infectional period from wk 4 onwards. This is in agreement with finding that the adaptation of NSP-degrading enzyme activity requires time (Iji et al., 2001). Although viscous S-NSP may stimulate proliferation of health impairing microorganisms in the small intestine (Smits and Annison, 1996; Rehman et al., 2007), soluble NSP in the form of inulin do not affect digesta viscosity (Schneeman, 1999). Infected birds had higher mortality rate than their uninfected counterparts consuming the same diets, but most of the deaths were due to cannibalism. Because in most cases, the intestines of victims were partly or completely eaten by the pecking birds, it was not possible to investigate whether cannibalism would relate to the infection status of the birds. Though a tendency toward increased agonistic behavior in *A. galli* infected birds has been reported (Gauly et al., 2007), cannibalism developed shortly after introducing the infected birds to the new pens in the present study and this may also have contributed to the erratic behavior developed during the establishment of a new social hierarchy in the infected groups.

Heterakis gallinarum is commonly regarded as a non-pathogenic nematode (Taylor et al., 2007). However, the present study shows that the efficiency of feed utilization and growth performance were impaired in infected birds independent of the type of diet. This might have been caused by partial diversion of nutrients from growth to development of immunity (Kyriazakis and Houdijk, 2006), and to support repair of damaged mucosal structures of the intestine in infected birds (Hoste, 2001). Infection induced slight decrease in feed intake of the birds might have also contributed to the retarded growth of the infected birds.

The average establishment rate for the CON fed birds were similar to those reported for chickens infected either with 100 eggs (Gauly et al. 2008) or with 3-9 eggs/bird (Fine, 1975). Insoluble and soluble NSP supplemented diets almost doubled the

establishment rate. Establishment rate of *H. gallinarum* in a pheasant host system was shown to be density dependent, i.e., the success rate of larvae developing to adult stage decreases as the infection dose increases (Thompkins and Hudson, 1999). The same pattern has also clearly been observed for *A. galli* in experimentally infected birds with different infection doses (Permin et al., 1997). With regard to the density dependent characteristic of the establishment rate, the great difference observed in the present study between CON and NSP fed birds indicates that manifold favorable conditions were provided to the nematode by feeding NSP. Although no difference between worm burdens of I-NSP and S-NSP fed birds was observed, these two diets differed in their effects on the fecundity of the nematode.

Because the nematode's eggs are passed to the external environment throughout caecal droppings (Fine, 1975), which are periodically excreted within a day (Clarke, 1979), 24 hour of collection of the final faeces, originating both from intestines and caeca seems to be crucial for reliable egg quantification of this nematode. The EPG is widely used for estimating the intensity of nematode infections in living host animals, it may however be influenced by the amount of faeces, which was elevated in the NSP fed birds confirming earlier results of others (Van der Klis et al., 1993; Jørgensen et al., 1996). Feeding I-NSP led to higher worm burden, but did not increase EPG, and a lower EPG based fecundity (EPG/female worm) was calculated in comparison to CON because of the greater amount of faeces. Feeding S-NSP also led to greater worm burden and faeces amount, and EPG was in addition increased without an effect on EPG based worm fecundity. Thus, the EPG rendered unsatisfactory information about the actual infection intensity in the birds. Based on EPD, I-NSP and particularly S-NSP increased worm egg excretion compared to CON and the fecundity (EPD/female worm) was also elevated after feeding S-NSP. As shown in the present study, the inclusion of the total daily amount of faeces for the calculation of EPD eliminates dilution effect of faeces and provides more accurate information about the actual infection status of the host animal as well as for the actual worm fecundity estimate than EPG alone.

Infection increased caeca size possibly by a histotropic phase, in which the larvae embed themselves into caecal tissue during the larval development as observed in *A. galli* infection (Herd and McNaught, 1975). It has been shown that bacteria play an important role for the establishment of *H. gallinarum* (Springer et al., 1970). The inulin supplied with S-NSP in the present study can be regarded as prebiotic similar to pure inulin which has been shown to increase bacteria counts and metabolic activity in turkeys (Juskiewicz et al.,

2005) and in chickens (Rehman et al., 2008b). It can be assumed that the establishment and fecundity of the bacteria feeder *H. gallinarum* (Bilgrami and Gaugler, 2004) was stimulated by NSP fermenting caecal bacteria. An explanation for the reduced caecal VFA pool observed in infected birds can only be found if the rates of VFA production and utilization respectively are investigated in more detail. Undoubtedly, the nematode benefited from the altered NSP dependent caecal environment resulting in higher establishment rate and enhanced fecundity. However, when compared to the effects of S-NSP on the gastrointestinal organ development and the parameters describing the intracaecal environment (organic matter, pH, VFA), the effects of I-NSP were less prominent than that of S-NSP. Enlarged caecal size (length and empty weight) induced by feeding both NSP diets can be interpreted as an indication of a higher possibility (larger space, less competition etc.) for establishment of larvae, while further altered caecal environment due to feeding S-NSP corresponds well to the enhanced fecundity of the nematode in the S-NSP fed birds.

Previous studies showed that insoluble NSP provide favorable conditions to *Oesophagostomum dentatum*, the nodule worm of pigs, whereas S-NSP (inulin) was shown to have adverse effects on this parasite (Petkevičious et al., 1997; 2001; 2003). Based on these results, the authors have proposed inulin as a potential dewormer (Petkevičious et al., 2003). The results reported from the pig studies are in agreement with the favorable effects of I-NSP to *H. gallinarum.* However, in contrast to O. *dentatum,* the inulin rich S-NSP fed in the present study also provided most favorable conditions to *H. gallinarum*. The differences between responses of different worm species to the same substance is of interest, and should further be investigated.

3.5. Conclusion

It is concluded that the pea bran and chicory root meal used as sources of insoluble and soluble dietary NSP, respectively, provided favorable conditions for the establishment of *H. gallinarum* in grower layers. Inulin rich chicory root meal additionally enhanced fecundity of the nematode. Therefore, the two natural sources of insoluble and soluble NSP offer no potential as protecting agents against *H. gallinarum* infections in chicken.

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CHAPTER -IV

Effects of dietary non-starch polysaccharides in *Ascaridia galli*-infected grower layers

Effects of dietary non-starch polysaccharides in *Ascaridia galli*-infected grower layers

Abstract

This study examined if the establishment and fecundity of *Ascaridia galli* in chicken can be controlled by dietary non-starch polysaccharides (NSP). One-day-old female layer chicks (N=604) were fed *ad libitum* either a basal diet (CON) or CON plus pea bran rich in insoluble NSP (I-NSP), or CON plus chicory root meal as a source of inulin rich soluble NSP (S-NSP) for 11 wk in a three times repeated experiment. At the end of wk 3, each feeding group was subdivided into an uninfected and an infected group of birds, the latter being inoculated with 250 embryonated eggs of *A. galli*, respectively. The birds were slaughtered 8 wk post infection and their worm burdens and the faecal nematode egg excretion were determined. Volatile fatty acids (VFA) and pH were determined in caeca contents.

Both NSP diets, particularly S-NSP, increased incidence of infection ($P<0.05$) and worm burden of birds ($P<0.001$), but the development (length) and fecundity of the nematode remained unaffected ($P>0.05$). *A. galli* infection caused a less efficient feed utilization for body weight gain (BWG, $P=0.013$) resulting in lower body weights ($P<0.001$) irrespective of type of diet consumed. NSP-fed birds, particularly those on I-NSP, consumed more feed per unit BWG and showed retarded body weight development ($P<0.001$) compared to birds receiving CON. Birds receiving I-NSP had higher body weights than those consuming S-NSP ($P<0.05$). Intracaecal pH was lowered by feeding S-NSP ($P<0.05$) independent of *A. galli* infection ($P=0.223$). Both NSP diets increased total VFA pool size ($P<0.001$), S-NSP exerting a greater effect than I-NSP ($P<0.05$). Infected birds had smaller total VFA pool size than their uninfected counterparts consuming the corresponding diets ($P=0.028$). S-NSP also led to higher weights of splanchnic tissues and intestinal tract ($P<0.05$). These effects were less pronounced in I-NSP fed birds.

The results suggest that insoluble and soluble dietary NSP retard growth performance, alter gastrointestinal environment and lead to higher weights of splanchnic tissues associated with an elevated establishment of *A. galli* in grower laying hens. It is concluded that the NSP used in this study had no potential for controlling *A. galli* infection in the birds.

Keywords: *Non-starch polysaccharides;* Ascaridia galli*; pea bran; chicory root; inulin; chicken.*

4.1. Introduction

Dietary non-starch polysaccharides (NSP) are fermentatively degraded in the distal sections of the gastrointestinal tract (Englyst, 1989; Schneeman, 1999). Apart from certain anti-nutritive effects of NSP mainly associated with increased digesta viscosity (Daenicke et al., 1999; Francesch and Brufau, 2004; Daenicke et al., 2009), dietary NSP may support animal welfare and health. Van Krimpen et al. (2008) reported that hens fed diets high in insoluble NSP increased time spent for eating and reduced aggressive pecking behaviours. Coarsely ground NSP supplemented diets may also stimulate the development of the gizzard suggesting improved digestive functioning (Van Krimpen et al., 2009). The soluble NSP inulin is known for its prebiotic effect (Schneeman, 1999) and inulin-dependent stimulation of metabolic activity of beneficial intestinal bacteria has been reported for turkeys (Juskiewicz et al., 2005) and chickens (Rehman et al., 2007; Rehman et al., 2008a).

Similar to some other EU countries the number of chickens kept in floor husbandry is increasing in Germany (ZMP, 2008), but in these systems the birds are in close contact with their faeces, allowing nematodes to complete their life cycles. The prevalence of common poultry nematodes has been reported to be higher in outdoor/floor husbandry systems than in conventional ones (Permin et al., 1999; Kaufmann and Gauly, 2009). The common fowl parasite, *Ascaridia galli* is a widespread nematode of chickens that resides in the small intestine. There is evidence that dietary NSP may interact with parasites of the host animals. Daenicke et al. (2009) showed that feeding viscous NSP favored the development of *A. galli*. In pigs, the type of dietary NSP has been shown to affect the establishment, development and fecundity of common nematodes (Petkevičius et al., 1997; 2001; 2003). The effects of NSP on nematode infections are assumed to be mainly attributable to alterations of digesta characteristics and intestinal microbial fermentation. We hypothesized that the establishment and fecundity of *A. galli* in chicken is affected by dietary NSP. The objective of the present study was to investigate the effects of low or highly fermentable NSP on the establishment and fecundity of the nematode as well as on the performance of grower layers experimentally infected with *A. galli* in order to examine if the nematode infection can be controlled by dietary NSP.

4.2. Material and methods

In three repetitions, 604 female Lohmann Selected Leghorn (LSL) chicks were used in the time interval from July 2007 to July 2008. The chicks were purchased from a commercial hatchery as one-day-old bird. Within each repetition, the chicks were weighed together and randomly divided into three feeding groups. Each feeding group was fed *ad libitum* until wk 11 of life one of the following pelleted diets: basal diet (CON), CON plus insoluble NSP (I-NSP), and CON plus soluble NSP (S-NSP) (Table 1). Insoluble NSP were supplied by mixing on air dry-basis one kg CON with 100 g pea bran (Exafine 500, Socode, Belgium). For S-NSP, one kg CON was mixed with 100 g chicory root meal (Fibrofos 60, Socode, Belgium). Daily feed consumption was determined per group throughout the experimental weeks. Drinking water was offered *ad libitum.*

Until wk three, each feeding group was kept in a pen scattered with wood shavings. The litter was replaced once (wk 1-3) or twice (wk 4-11) a week. Room temperature was gradually decreased from 34 °C on the first day (d) to 26 °C in wk 3 and thereafter decreased by 2-3°C per wk, ending at 18-20 °C from wk six onwards. A 24 h lighting period was maintained for the first two days and was then reduced to 16 h/d at the end of the first week. By wk eight, it was reduced to 12h/d and subsequently maintained until the end of the experiment. At the end of wk three, the birds were marked with wing tags and individual body weights (BW) were taken for the first time and thereafter at weekly intervals.

2.2. Infection material and experimental A. galli *infections*

The infection material was prepared at the Department of Animal Sciences, University of Göttingen, Germany. Adult female *A. galli* worms harvested from the intestines of naturally infected chickens were used as the source of eggs. Preparation techniques for the infection material (egg harvest, embryonation procedures etc.) were performed as described in a previous study (Daş et al., 2010a) in details. On the infection day, number of eggs/ml suspension was determined and the infection dose was adjusted to 250 eggs/0.2 ml of final suspension. Eggs only in the vermiform and infective larval stages were classed and counted as embryonated. Three sub-groups of birds, each for one feeding group, were infected at an age of three wk using a five cm oesophageal cannula. The remaining three groups of uninfected control birds were given 0.2 ml of an aqueous 0.1%

solution (w/v) of potassium dichromate as placebo. Average number of birds inoculated with eggs per each feeding group in each repetition ranged from 30 to 35.

In the second and third repetitions, mixed batches of eggs from female worms harvested in the preceding repetition as well as eggs from worms of naturally infected field chickens were used as the infection material and prepared in the same way. Average age of the eggs (after embryonation) on infection days was around three, five and one mo in the first, second and third repetition, respectively.

After inoculation of the eggs, birds of three uninfected control groups were left in their previous pens, whereas birds of each infected group were placed in new pens within the same experimental stable equipped with six pens. The birds did not get any vaccination or anthelmintic treatment throughout the experimental period. The stable was thoroughly cleaned and disinfected at least two wk before introducing the birds.

2.3. Slaughter process, faecal samples and post-mortem examinations

All the birds were slaughtered after electrical stunning 8 wk post-inoculation (p.i.) at an age of 11 wk. The slaughtering was accomplished within three h on the day of slaughter. Individual faecal samples were collected during the slaughter process either as freshly dropped faeces or - if available - directly from the colon. The individual faecal samples were examined for estimating number of eggs per gram of faeces (EPG) using a modified McMaster counting technique with a sensitivity of 50 eggs/g faeces (MAFF, 1986).

Immediately after slaughtering, the gastrointestinal tracts were removed and the visceral organs were separated. The small intestines of the infected birds were opened longitudinally with scissors. The contents were flushed with tap water through a sieve with a mesh aperture of 100 μm, and then transferred into one or more Petri dishes, depending on the amount of content. Thereafter, the incidence and number of adult worms and larvae were determined using a stereomicroscope. The adults were sexed and a maximum number of 10 (range 1-10) intact adult female and male worms per bird were measured for length (Gauly et al., 2002). Small intestines from uninfected control birds (15-20% of each group) were also processed to verify infection-free status of these groups. The remaining small intestines from the uninfected control birds were opened and macroscopically checked for the presence of adult worms.

Weights of liver (+gall bladder), pancreas, full caeca as well as length of small intestine and each caecum were measured. In the last two repetitions, caeca from 10 birds

per group (60 per repetition.) were weighed and frozen stored at -18 °C until analyzed for volatile fatty acids (VFA).

2.4. pH and volatile fatty acids (VFA)

The frozen caeca were thawed at room temperature for pH measurement and VFA analyses. The caecal content was removed from the caeca and the amount was quantified. A two g sample of caecal content was weighed and suspended in 10 ml of distilled water. The sample was mixed using a vortex for around five seconds. The pH was directly measured in this suspension using a pH electrode (InLab®Easy BNC, Fa. Mettler Toledo) connected to a pH meter (GC 811, Fa, Schott). Thereafter, the suspension was centrifuged at 2000 x *g* at room temperature for 20 min. Five ml of supernatant was transferred to a glass tube, which contained 250µl international standard (4% methyl-valeric acid in formic acid). The mixture was vortexed and two parallel sub-samples of 1.5 ml each were transferred to sample tubes. The tubes were centrifuged at 10.000 x *g* at room temperature for 10 min. After centrifugation, the samples were stored in a refrigerator (+4°C) until gas chromatography.

For gas chromatography, a combined internal/external standard procedure was applied using a packed column (10% Carbowax 20 MTPA SP1000 with 1% H_3PO_4 on Chromosorb WAW, 80/100). Injection port temperature was 170 °C, for detector 200 °C and for column 120 °C (isothermal). The gas chromatograph (Shimadzu GC 14B) was equipped with a flame ionization detector (FID) and hydrogen was used as the carrier gas (Da Costa Gomez, 1999; Abel et al., 2002). The average of two parallel analyses for each sample was used for calculations.

In the last repetition, the remaining caecal contents after sampling for VFA were used to determine dry matter and crude ash contents in order to calculate the organic substance in the caeca samples.

2.5. Chemical analyses of the diets

Feed samples were taken regularly during each experimental repetition and were analyzed for dry matter (DM), crude ash (CA), crude protein (CP), sugar, starch, and ether extract (EE) using standard methods (Naumann and Bassler, 1997). Neutral and acid detergent fibre (NDF and ADF, respectively) were analyzed according to Van Soest et al. (1991). The metabolizable energy of the diets (MJ ME/kg DM) was calculated (FMVO, 2008). Insoluble and soluble NSP were measured using an enzymatic test (Megazyme,

2007). Inulin content of S-NSP diet was determined according to Naumann and Bassler (1997). The composition and analyzed nutrient contents of the experimental diets are given in Table 1.

Table 1. Composition and analysis of the experimental diets.

Item	CON	I-NSP	S-NSP
Analyzed composition			
DM, g/kg	898	900	900
Nutrients, g/kg DM			
Ash	55	52	55
CP	218	198	203
NDF	113	173	122
ADF	34	92	36
Ether extract	40	36	37
Starch	486	446	423
Insoluble NSP	103	177	102
Soluble NSP	20	24	26
Inulin	-	-	77
ME, MJ/kg DM[1]	**13.21**	**12.06**	**12.02**

[1] The metabolizable energy of the diets: calculated according to the formula given by the German regulations for complete poultry feed mixtures (FMVO, 2008). ME, MJ/kg DM= [(g CP x 0.01551) + (g CL x 0.03431) + (g starch x 0.01669) + (g sugar x 0.01301)]. Sugar contents of the diets were estimated based on sugar contents of the components.

4.2.6. Data management and statistical analyses

4.2.6.1. Parameter definitions, transformations and restrictions

Because the data of the infection variables positively skewed (Skewness > 0) and showed non-normal (Kolmogorow-Smirnow, $p<0.05$) distributions, log-transformations were employed. For this, individual infection parameters that described worm counts (establishment rate, number of males, females, larvae, and total worm burden), number of eggs per gram of faeces (EPG) and female worm fecundity parameters were transformed by using the natural logarithm (ln) function [ln(y)=Log(y+1)] to correct for heterogeneity of variance and to produce an approximately normally distributed data set. Establishment rate was defined as the number of worms per bird in relation to infection dose. Worm egg excretion was quantified as the number of eggs per gram of faeces (EPG) in birds that had had a faecal sample. Fecundity of adult female worms was defined as EPG per female

worm. Lengths of the male and female worms and sex ratio (numbers of females / males) were left untransformed.

Because the experimental repetitions were performed at different periods of time and we used different batches of worm eggs, the effect of repetition was included in the models as a random factor to ensure safe generalization (with a cost of elevated standard errors) for the effects of the main experimental factors and to avoid any possible confounding effect of time, in which the repetitions were performed, with any of the main factors.

2.6.2. Statistics

Effects of the diets on the incidence of *A. galli* infection (proportion of worm-harbouring birds to the experimentally infected birds) were analyzed using GENMOD procedure of SAS (2010) with a logit link function. The GENMOD procedure fits the generalised linear models and suited for responses with binary outcomes (Kaps and Lamberson, 2004). The model included effects of diet and effect of repetition. Because there was no significant interaction effect between diets and repetitions the following reduced model was used (I).

(I) $\eta_{ij} = log\,[p_{ij} / (1 - p_{ij})] = m + \tau_i + r_j$

i= diets; CON, I-NSP, S-NSP

j= repetitions; 1, 2, 3

where;

p_{ij}= the proportion of infected birds on diet i and repetition j

m= the overall mean of the proportion on the logarithmic scale

τ_i = the effect of diet i

r_j= the effect of repetition j

Body weight (BW) and feed utilization (feed:gain) data were analyzed both for pre-infectional (1-3 wk) and for the entire period (1-11 wk). The model for the performance parameters in pre-infectional period included fixed effect of diets, random effect of repetitions and the residual error (II). This model was also used to analyze establishment rate, worm counts, worm length and egg excretion data.

(II) $Y_{ijk} = \mu + \alpha_i + a_j + \varepsilon_{ijk}$

where**;**

Y_{ijk}= observation.

μ = the overall mean.

α_i = the effect of diet (i = 1,2,3).

a_j = random effect of repetition (j =1,2,3).

ε_{ijk} = residual random error.

Organ measurements and VFA data were analyzed with the following mixed model (III).

(III) $\mathbf{Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + a_k + \varepsilon_{ijkl}}$

where**;**

Y_{ijkl}= observation.

μ = the overall mean.

α_i = the effect of diet (i = 1,2,3).

β_j = the effect of infection (j = 0,1).

$(\alpha\beta)_{ij}$ = the interaction effect between diet and infection (ij = 1-6).

a_k = random effect of repetition (k* =1,2,3).

ε_{ijkl} = residual random error.

*: (k=1, 2) for the VFA data.

The model for the repeatedly measured performance variables (BW, feed:gain) in the entire period included fixed effects of diet, infection, experimental weeks (as age of birds) as well as all possible interactions among these factors. The effect of experimental repetitions was included in the model as a random factor. Furthermore, individual random effect of the birds as the repeated subject within a repetition over the experimental weeks, was also included in the model presented below (IV).

(IV) $\mathbf{Y_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + a_l + b_{k(l)} + \varepsilon_{ijklm}}$

Where**;**

Y_{ijklm}= observation.

μ = the overall mean.

α_i = the effect of diet (i = 1,2,3).

β_j = the effect of infection (j = 0,1).

γ_k = the effect of experimental weeks (k= 3-11 wk).

$(\alpha\beta)_{ij}$ = the interaction effect between diet and infection.

$(\alpha\gamma)_{ik}$ = the interaction effect between diet and experimental weeks.

$(\beta\gamma)_{jk}$ = the interaction effect between infection and experimental weeks.

$(\alpha\beta\gamma)_{ijk}$ = the interaction effect among diet, infection and experimental weeks.

a_l = random effect of repetition (l=1,2,3).

$b_{k(l)}$ = random effect of individual bird within repetition over the experimental weeks, the variance between repeated measurements of the birds (subject) within a repetition.

ε_{ijklm} = residual random error.

2.6.3. Presentation of the results

After infection at the end of wk 3, groups of uninfected and infected chickens were kept according to a 3 x 2 factorial arrangement of treatments with diet and infection as the main factors. Therefore, unless no significant interactions between the effects of diet and infection were encountered, the data are presented as the main effects of diet and infection. In case of significant interactions between diet and infection, the results are either presented for the 6 single treatments or are mentioned correspondingly in the text. Tukey adjusted post-hoc comparisons (Alpha< 0.05) were performed to either partition effects of the main factors or to determine single group differences when a non interactive significant main effect or when a significant interaction effect of the main factors was encountered, respectively.

For the effects of the main experimental factors, the results are presented as least square means (LSMEANS) with common pooled standard error (PSE). The PSE, calculated from the output of mixed models for balanced data, was confirmed to be the same as for a balanced data set that could be calculated from the output of GLM procedure as Root Mean Square Error divided by the square root of the number of observations per treatment mean as described by Pesti (1997). Because the numbers of observations in the groups were not always balanced for certain data, the most conservative (the largest) standard error of LSMEANS is represented as the pooled SE.

2.7. Ethical consideration

The experimental procedures followed the animal welfare rules. The infection dose given to each bird (250 eggs) was within the range of the worm burdens that can be observed in natural sub-clinical infections. The procedures for experimental infections followed the guidelines suggested by the World Association for the Advancement of Veterinary Parasitology for evaluating the effectiveness of anthelmintics in chickens and turkeys (Yazwinski et al., 2003).

4.3. Results

4.3.1. Mortality, feed consumption and growth performance

Infected birds did not show clinical signs of infection and the overall mortality rate was low. Uninfected control birds were free of infection as confirmed by microscopic and macroscopic examination of the small intestines. Because the mortality rate in the pre-infectional period was low (1.2 %) no statistical comparison among the feeding groups was done. Birds consuming S-NSP had a slightly higher mortality (2.1 %) than those on the CON (0.9 %) or the I-NSP diet (0.6 %). In the post-inoculation period (wk 4-11), infected and uninfected birds had similar low mortality rates (3 %).

During the pre-infection period, birds receiving I-NSP and S-NSP consumed roughly 4.5 % and 1.7 % more feed, respectively, than those being fed CON (Table 2). The coefficients of variation (CV) for feed consumption calculated within feeding group over 3 repetitions in the pre-infectional period, were less than 6 %. Birds consuming I-NSP had a higher feed:gain ratio in comparison to CON and S-SNP fed birds ($P<0.05$). Both NSP-diets reduced the BW of birds in comparison to CON ($P<0.001$).

In the entire experimental period, birds on the I-NSP and S-NSP diets consumed 8 % and 2 % more than the CON fed birds. The CVs within each feeding groups were lower than 5 %. Compared to CON, birds on the NSP-diets consumed more feed per unit body weight gain (BWG, $P<0.001$) and I-NSP caused higher feed intake than S-NSP ($P<0.05$). Both NSP diets led to retarded body weight development ($P<0.001$) with S-NSP entailing a stronger negative effect than I-NSP ($P<0.05$). Within feeding groups, infected birds consumed almost the same amount of feed as the uninfected birds whereas body weight of the birds was impaired irrespective of type diet ($P<0.001$) resulting in lowered efficiency of feed utilization ($P=0.013$).

Table 2. Effects of diet and *A. galli* infection on feed consumption, body weight development (BW), and feed:gain ratio (N=581).

	Diet[1]					***A. galli* infection**[2]				***Interaction***
Period/Item	**CON**	**I-NSP**	**S-NSP**	***PSE*** [3]	***P*, ≤**	**Uninfected**	**Infected**	***PSE*** [3]	***P-value***	***P-value***
Pre-infection (wk 1-3)										
Feed consumption[4], g/bird	336	351	330	-	-	no	no	no	no	no
BW[5], g	202[a]	197[b]	196[b]	*6.833*	*0.001*	no	no	no	no	no
Feed:gain, g/g	2.08[a]	2.24[b]	2.12[a]	*0.116*	*0.001*	no	no	no	no	no
Entire-period(wk 1-11)										
Feed consumption[4], g/bird	2899	3129	2963	-	-	3006	2990	-	-	
BW[6], g	981[a]	953[b]	939[c]	*11.048*	*0.001*	972[A]	942[B]	*10.936*	*0.001*	*0.296*
Feed:gain, g/g	3.09[a]	3.43[c]	3.30[b]	*0.078*	*0.001*	3.22[A]	3.33[B]	*0.070*	*0.013*	*0.084*

[(abc) or (AB)]: Different letters within each factor on the same line indicate differences ($p<0.05$).
no: no infection effect in the pre-infection period.
[1] CON = basal diet; I-NSP = 1,000 g CON + 100 g pea bran; S-NSP = 1,000 g CON + 100 g chicory root meal.
[2] Uninfected controls or infected with 250 eggs of *A. galli*.
[3] Pooled SE.
[4] Estimated from daily group consumptions.
[5] Body weight at the end of wk 3 of life.
[6] Body weight at the end of wk 11 wk (i.e., 8 wk p.i).

4.3.2. Incidence of infection, worm burdens and worm fecundity

There was a significant effect of diet on the incidence of infection (P<0.001). As shown in Table 3, it was higher with S-SNP than with CON and I-NSP (P<0.05). Feeding I-NSP also tended to increase incidence of infection when compared with CON (P=0.078). Birds on the NSP-diets had higher establishment rates (P<0.001), higher numbers of female (P=0.003) and male worms (P=0.021) as well as higher total worm burden (P<0.001) than those receiving CON. The number of larvae tended (P=0.060) to be higher in S-NSP than in CON fed birds. Sex ratio, worm length, EPG and female worm fecundity remained unaffected by type of diet (P>0.05).

Table 3. Effect of diet on establishment rate, average number of worms per bird, sex ratio, length and egg excretion parameters of worms in birds infected with *Ascaridia galli* (250 eggs / bird).

	Diet[1]				
Item	**CON**	**I-NSP**	**S-NSP**	***PSE***[2]	***P-value,* ≤**
Incidence, % (N=286)	57.0[a]*	68.4[a]*	89.0[b]	-	0.001
Establishment rate[3], %	0.91[a]	1.34[b]	1.36[b]	0.382	0.001
Number of female worms[3]	0.74[a]	1.21[b]	1.19[b]	0.403	0.003
Number of male worms[3]	1.12[a]	1.55[b]	1.50[b]	0.624	0.021
Number of larvae[3]	0.38	0.56	0.70	0.124	0.060
Total worm burden[3]	2.27[a]	3.34[b]	3.40[b]	0.955	0.001
Sex ratio, F/M	0.69	0.67	0.80	0.138	0.687
Female worm length, cm	7.13	7.35	7.46	0.377	0.572
Male worm length, cm	5.15	5.45	5.59	0.120	0.122
Eggs per gram of faeces (EPG)[3]	359	319	223	177	0.363
Fecundity (EPG / female worm)[3]	210	91	125	60	0.576

ab: Values with no common letters within rows differ (Tukey, p<0.05).
*: Values sharing the sign tend to differ (P=0.078).
[1] CON = basal diet; I-NSP = 1,000 g CON + 100 g pea bran; S-NSP = 1,000 g CON + 100 g chicory root meal
[2] PSE: Pooled standard error.
[3] LSMEANS and PSE represent untransformed data, P-values are based on the transformed data.

4.3.3. Visceral organ development

No significant interaction effect was observed between diet and infection on any of the organ measurements (P>0.05). The absolute weights of liver and pancreas were not affected by the type of diet (P>0.05; Table 4), but the NSP diets increased the relative

weights of both organs (P<0.05). The small intestine length, the caecum length and the full caeca weight were increased by S-NSP (P<0.05).

A. galli infection increased, the absolute pancreas weight and the length and weight of caeca as well as the relative liver and pancreas weights (P<0.05). The liver weight tended to be higher in the infected birds (P=0.058). Infected birds had shorter small intestines than their uninfected counterparts receiving the same diets (P=0.007).

4.3.4. Biochemical parameters of the caecal content

As shown in Table 5, both NSP diets increased the amount of caeca content (P<0.001) with S-NSP exerting a greater effect than I-NSP (P<0.05). Feeding S-NSP increased the proportions of dry matter in the caecal contents (P<0.05). The proportion of organic matter was increased (P<0.05) at the expense of crude ash (P<0.05) in the caecal content of S-NSP fed birds. Infection did not influence the proportions of organic matter and ash (P>0.05). The intracaecal pH was lower with feeding S-NSP (P<0.05) and remained unaffected by the infection (P>0.05).

Table 4. Effects of diet and *A. galli* infection on the size of visceral organs (N=567).

	Diet[1]					***A. galli* infection**[2]				***Interaction***
	CON	**I-NSP**	**S-NSP**	***PSE*** [3]	***P, ≤***	**-**	+	***PSE*** [3]	***P, ≤***	***P-value***
Liver, g	17.8	18.0	17.7	*0.363*	*0.472*	17.7	18.0	*0.356*	*0.058*	*0.208*
HS-Index[4], % (Liver/BW)	1.82[a]	1.89[b]	1.90[b]	*0.065*	*0.001*	1.83[A]	1.91[B]	*0.065*	*0.001*	*0.221*
Pancreas, g	2.36	2.38	2.38	*0.076*	*0.726*	2.33[A]	2.41[B]	*0.075*	*0.001*	*0.957*
g Pancreas / 100 g BW	2.42[a]	2.51[b]	2.55[b]	*0.117*	*0.001*	2.42[A]	2.57[B]	*0.116*	*0.001*	*0.902*
Small int. length, cm	105.0[a]	104.9[a]	108.9[b]	*1.148*	*0.001*	107.0[A]	105.5[B]	*1.121*	*0.007*	*0.379*
Caecum length, cm	13.56[a]	13.81[a]	15.12[b]	*0.267*	*0.001*	13.89[A]	14.43[B]	*0.264*	*0.001*	*0.239*
Full caeca weight, g	6.33[a]	6.59[a]	8.89[b]	*0.168*	*0.001*	7.16	7.38	*0.158*	*0.075*	*0.085*

[(abc) or (AB)]: Different letters within each factor on the same line indicate differences ($p<0.05$).
[1] CON = basal diet; I-NSP = 1,000 g CON + 100 g pea bran; S-NSP = 1,000 g CON + 100 g chicory root meal.
[2] Uninfected controls (-) or infected with 250 eggs of *A. galli* (+).
[3] Pooled SE.
[4] HS-Index: Hepato-somatic index = liver/BW*100.

Table 5 Effects of diet and *A. galli* infection on biochemical parameters of caecal content*.

	Diet[1]					*A. galli* infection[2]				*Interaction P-value*
Item	**CON**	**I-NSP**	**S-NSP**	***PSE*** [3]	***P,*** ≤	**Inf. (-)**	**Inf. (+)**	***PSE*** [3]	***P,*** ≤	
Caecal content, g	3.49^{a}	4.13^{b}	5.12^{c}	*0.182*	*0.001*	4.44	4.04	*0.148*	*0.060*	*0.052*
Dry matter (DM), %	16.6^{a}	17.1^{a}	21.0^{b}	*0.484*	*0.001*	19.0^{A}	17.5^{B}	*0.392*	*0.006*	*0.127*
Ash, % (of DM)	14.0^{a}	13.4^{a}	9.9^{b}	*0.390*	*0.001*	12.7	12.1	*0.308*	*0.181*	*0.074*
Organic matter, % (of DM)	85.8^{a}	86.6^{a}	90.1^{b}	*0.307*	*0.001*	87.3	87.9	*0.308*	*0.181*	*0.074*
pH	5.97^{a}	6.03^{a}	5.35^{b}	*0.053*	*0.001*	5.82	5.75	*0.042*	*0.223*	*0.079*
VFA pool, µmol[4]										
Acetate	223^{a}	275^{b}	296^{b}	*15.272*	*0.001*	282^{A}	248^{B}	*12.961*	*0.035*	*0.040*
Propionate	18^{a}	29^{b}	34^{b}	*1.966*	*0.001*	27	27	*1.619*	*0.763*	*0.630*
Butyrate	64^{a}	73^{a}	114^{b}	*9.342*	*0.001*	92^{A}	76^{B}	*8.863*	*0.011*	*0.713*
Total	305^{a}	377^{b}	445^{c}	*25.016*	*0.001*	401^{A}	350^{B}	*22.233*	*0.028*	*0.140*

*: N=120 for ph and VFA data, for the remaining variables N=60.

[(abc) or (AB)]: Different letters within each factor on the same line indicate significant differences (Tukey, $p<0.05$).

[1] CON: Basal diet; I-NSP: Insoluble non-starch polysaccharide supplemented diet; S-NSP: Soluble non-starch polysaccharide supplemented diet.

[2] Uninfected controls (-) or infected (+) with 250 eggs of *A. galli.*

[3] Pooled SE.

[4] Calculated as multiplication of VFA concentration by the total amount cecal digesta.

Pool size of acetate was influenced by interaction between diet and infection (Figure; P=0.040). Infected birds on CON had lower pool size of acetate than all the other infected or uninfected birds on any diet (P<0.05). Pool size of propionate was increased by NSP diets (P<0.05; Table 5) and remained unaffected by infection (P=0.763). Butyrate pool size was increased with S-NSP (P<0.05) and decreased by *A. galli* infection (P=0.011). Both NSP supplemented diets increased the total VFA pool size (P<0.001), S-NSP exerting a greater effect than I-NSP (P<0.05). Infected birds had smaller total VFA pool size than their uninfected birds consuming the corresponding diets (P=0.028).

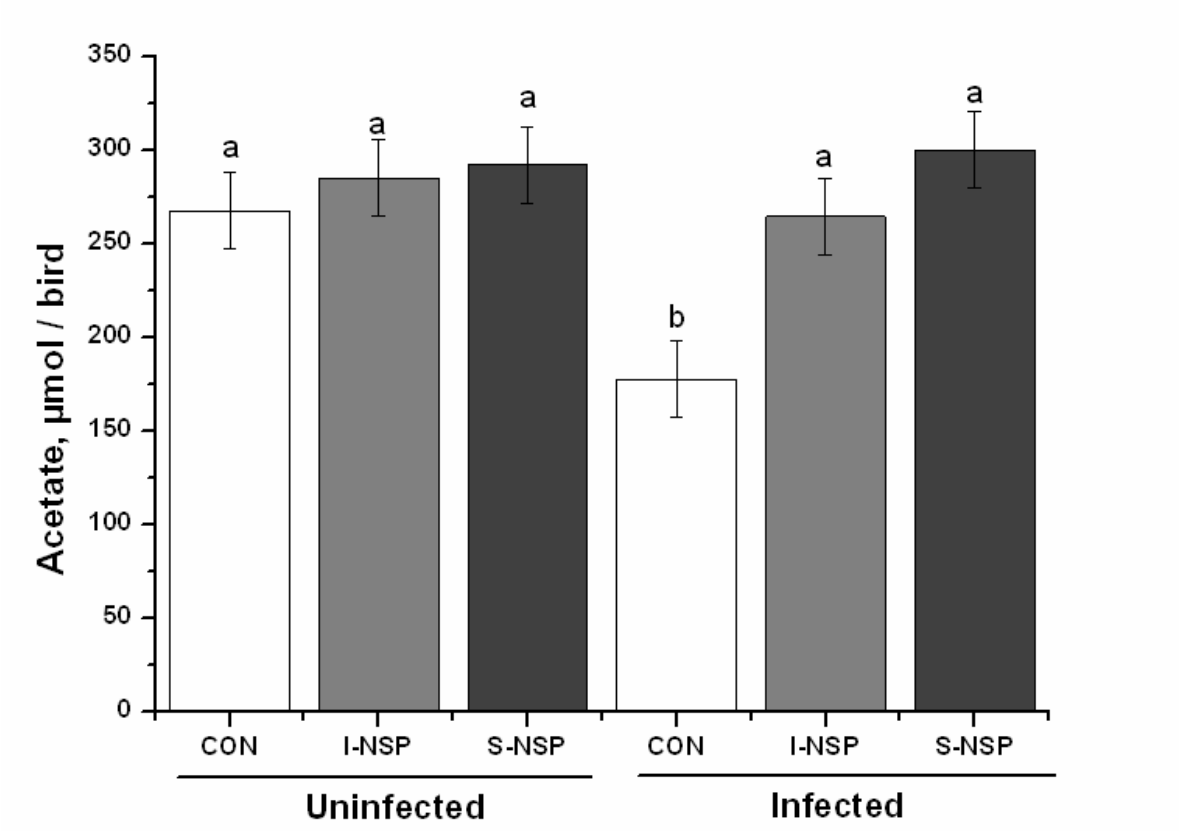

Figure Pool size of acetate in the caeca as influenced by interaction between diet and infection (P=0.040; N=120).

4.4. Discussion

As the study was designed, the authors assumed that the three *ad libitum* offered experimental diets would supply chickens with similar amounts of essential nutrients and energy either via CON alone or with the CON proportions of the I-NSP and S-NSP diets. This assumption was based on expected compensatory feed consumption of birds receiving the nutritionally diluted NSP diets (Forbes and Shariatmadari, 1994; Halle, 2002; Van Krimpen et al., 2007). According to this concept, infection with *A. galli* should clearly be

testable as affected by insoluble and soluble NSP. In fact, birds receiving I-NSP instead of CON increased feed intake by 8 % thereby almost balancing feed dilution brought about by 9% pea bran. In S-NSP fed birds, the increase in feed intake was not large enough to compensate feed dilution and their proportionate CON intake was approximately 7 % lower than that of birds on the pure CON diet. Therefore, differences between birds in their proportionate CON consumption may have additionally influenced the results.

In spite of almost compensatory feed intake, I-NSP fed birds had lower final BW than those on the pure CON diet, indicating that consumption of I-NSP may have been associated with additional metabolic costs. In S-NSP fed birds, the reduced final BW may have at least partially resulted from lower proportionate intake of CON. Inulin, which was fed as a source of soluble NSP, is known to increase digesta bulk (Schneeman, 1999) and feed intake of S-NSP fed birds might have been limited by the capacity of the gastrointestinal tract.

Dietary insoluble and soluble NSP favored *A. galli* infection in terms of higher incidence of infection and worm burdens. The absence of clinical signs of Ascaridiosis and low mortality may be explained by the low worm burdens. The average worm burden of the infected birds was, however, in the range observed by others applying similar or the same infection doses (Riedel and Ackert, 1951; Gauly et al., 2001; Marcos-Atxutegi et al., 2009; Daenicke et al., 2009; Daş et al., 2010a). *A. galli* infection did not influence feed intake of the birds, but caused a less efficient utilization of feed for BWG. This indicates an altered allocation of nutrients from growth to defense reactions against the parasite infection (Kyriazakis and Houdijk, 2006). Diverted allocation of nutrients may include repair of damaged mucosal tissues during the histotropic phase of the nematode (Herd and Mcnaught, 1975) and the acquisition as well as the development of immunity (Marcos-Atxutegi et al., 2009), which may require high metabolic inputs (Colditz, 2008). *A. galli* infection may also influence digestion and absorption of nutrients by reducing proteolytic enzyme activity in the jejunum (Hurwitz et al. (1972a) being associated with an increased recycling of nitrogen (Hurwitz et al., 1972b). Reduced metabolizability of energy and lowered nitrogen retention has been reported for *A. galli* infected chickens (Walker and Farrell, 1976).

Both NSP supplemented diets increased incidence of infection and worm burdens of the birds but the development (length) and fecundity of the nematode remained unaffected. Thus, dietary NSP appear to support larvae establishment. The average female worm lengths measured in the present study are in agreement with earlier reports

(Abdelqader et al., 2007; Daenicke et al., 2009; Marcos-Atxutegi et al., 2009). The average worm fecundity (EPG/female) was slightly lower for the worms of the NSP fed birds and this may have resulted from increased faeces amounts as has been reported for chicken submitted to NSP feeding (Van der Klis et al., 1993; Jørgensen et al., 1996). In a parallel experiment with *Heterakis gallinarum*, NSP fed birds produced roughly 33% more faeces than birds on a NSP-unsupplemented diet. Such increase would lead to lower EPG levels and the EPG based fecundity estimates may be underestimated even at significantly elevated worm burden (Daş et al., 2010b), In the present investigation, no difference in EPG between CON and NSP fed birds was observed because the latter presumably excreted larger daily amounts of faeces. A more precise evaluation of female worm fecundity could be achieved by referring worm egg excretion on total daily faeces excretion of the birds.

Soluble NSP present in certain cereals like e.g., rye, barley, triticale and wheat may exert anti-nutritive effects in poultry through increased intestinal viscosity (Francesch and Brufau, 2004; Józefiak et al., 2004; Daenicke et al., 2009). Daenicke et al. (2009) showed that soluble NSP-caused increased viscosity favored the development of *A. galli* and elevated nematode egg excretion. Soluble NSP in the form of inulin do not affect digesta viscosity (Schneeman, 1999), but, as observed in the present study and in agreement with others (Montagne et al., 2003), led to elevated weights of splanchnic tissues as well as increased sizes of gastrointestinal tract sections. The enlarged caeca can be related to increased microbial activity. The praecaecal section was obviously also exposed to stimulated fermentation, as indicated by increased small intestine size in S-NSP fed birds. The lowered pH and the greater pools of VFA in caeca contents are further indications for an inulin-induced stimulation of intestinal fermentative activity. Consistent with previous observations (Marounek et al., 1999; Rehman et al., 2008a, b), fermentation of inulin was associated with high proportions of butyrate, which in turn can be used by colonocytes as a source of energy for epithelial cell proliferation, thus supporting the enlargement of intestinal tract sections (Montagne et al., 2003).

Increased worm burdens with NSP feeding can be related to an altered gastrointestinal environment in the birds. However, apart from greater effects of S-NSP than I-NSP on enlargement of gastrointestinal organs and caecal VFA pool size, *A. galli* infection also increased caeca size and the relative proportions of liver and pancreas. Increased proportion of liver has been reported in *A. galli* infected birds and may indicate

liver dysfunction and disturbance in carbohydrate metabolism in the infected birds (Ramadan and Abou Znada, 1991).

Energy diluted diets have been suggested for laying hens in order to benefit from compensatorily increased feed intake, thereby balancing nutrient intake of the animals under the condition of organic farming (Sundrum et al., 2005; Van de Weerd et al., 2009). Considering the availability of organic feedstuffs, such energy dilutions will primarily be achieved by fibre, i.e. NSP rich feed components. The results of the present investigation, showing infection supporting effects of dietary NSP, and the inherently increased risk of obligatory outdoor sytems for parasitic infections (Permin et al., 1999; Thamsborg et al., 1999; Fossum et al., 2009) suggest particular measures of precaution for organic poultry husbrandy in order to protect animals against nematode-related health risks.

4.5. Conclusion

Insoluble and soluble dietary NSP retard growth performance, alter gastrointestinal environment and lead to higher weights of splanchnic tissues being associated with an elevated establishment of *A. galli* in grower laying hens. These observations may be particularly relevant for poultry husbandry in organic farming, where relatively fibre, i.e. NSP rich feeding is recommended and where animals with obligatory outdoor access are inherently exposed to high nematode infection risks.

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CHAPTER - V

General Discussions

5. General discussions

5.1. Body weight development and feed intake

As observed in all three experiments, both, the NSP-supplemented diets and the parasitic infections caused a retarded body weight development of the birds. Due to high content of insoluble and presumably undegradable fibre, which is additionally known to accelerate digesta passage rate (Hetland et al., 2004), total organic matter digestibility of I-NSP was presumably lower than that of CON and S-NSP. In spite of compensatory feed intake, I-NSP fed birds had lower final BW than those on the pure CON-diet, indicating that consumption of I-NSP may have been associated with additional metabolic costs. In S-NSP fed birds, the reduced final body weights may have resulted at least partially from lower proportionate intake of basal diet (CON).

The NSP-supplemented diets used in this study were diluted as to the contents of energy and nutrients. When the study was designed, it was assumed that the three ad lib.-offered experimental diets would supply chickens with similar amounts of energy and nutrients either via CON alone or with the CON proportions of I-NSP and S-NSP. This assumption was based on expected compensatory feed consumption of birds receiving the nutritionally diluted NSP-diets (Forbes and Shariatmadari, 1994; Halle, 2002; Van Krimpen et al., 2007). According to this concept, birds on the NSP-supplemented diets would increase their feed consumption to reach a similar level of energy and nutrient intake as the CON-fed birds. In fact, birds receiving I-NSP instead of CON increased feed intake by 8 % thereby almost balancing feed dilution brought about by 9% pea bran. In S-NSP-fed birds, the increase in feed intake was not large enough (2%) to compensate feed dilution and their proportionate CON-intake was approximately 7 % lower than that of birds on the pure CON-diet. These results have repeatedly been observed in the *H. gallinarum*- (Chapter 3) and the *A. galli*- experiments (Chapter 4). In the histomonas-contaminated *H. gallinarum*-experiment (Chapter 2) the increases were slightly lower (6.2% and 1% for I-NSP- and S-NSP-fed birds, respectively) than in the prementioned two experiments. In spite of the larger intestinal capacity of the S-NSP-fed birds, these birds were not able to increase their feed intake similarly to those of the I-NSP-fed birds. The lower nutrient intake in the S-NSP-fed birds may therefore have additionally influenced all the results reported in this study. Although effects of feeding additional insoluble fibre on infections and performance of the birds have clearly been tested by comparing the I-NSP- and CON-feeding groups, the lower nutrient intake in the S-NSP-fed birds might, to some

extent, have interfered with the effects of soluble NSP supplied with chicory root meal. However, these effects can be considered as total diet effects that include the effects of soluble NSP-supplementation and lower nutrient intake.

What may have been the reason for the differences between feed consumption of I-NSP- und S-NSP-fed birds, and why were the latter obviously unable to reach a similar level of intake as the former? Increased intestinal viscosity due to dietary viscous NSP has been reported to reduce feed intake of chickens (Van der Klis et al., 1993). However, soluble NSP in the form of inulin do not affect digesta viscosity (Schneeman, 1999). These results were also confirmed by parallel studies focusing on digestibility of the NSP diets conducted within the same frame of this study. Humburg (2010) observed that neither I-NSP nor S-NSP was associated with increased intestinal viscosity. In fact, this can be expected because the main feedstuffs used in the experimental diets that may increase intestinal viscosity were barley and wheat. Their proportions in the NSP-supplemented diets, after dilution with either pea bran or chicory root, are reduced so that even a lower intestinal viscosity associated with viscous-NSP supplementation could result. Therefore, it can be assumed that lower feed intake in the S-NSP-fed birds must have been associated with factors other than viscosity. Inulin, which was fed as a source of soluble NSP, is known to increase digesta bulk (Schneeman, 1999) and feed intake of S-NSP-fed birds might have been limited by the capacity of the gastrointestinal tract. On the other hand, fermentation of NSP may increase heat production which may negatively influence feed intake of the birds. Jørgensen et al. (1996) showed that fermentation of pea fibre supplemented diets increased heat production of broilers relative to ME by 3-6%. As shown by lowered intracaecal pH and elevated pool size of VFA in the present investigation, the inulin supplemented S-NSP-diet intensified intestinal fermentation more than the I-NSP-diet. Therefore, it may be assumed that intestinal fermentation increased heat production, which contributed to the lower feed intake of the S-NSP-fed birds.

In the *H. gallinarum*-experiments, infection associated reductions in feed intake of the birds were observed. The infection-induced reduction in voluntary feed intake was stronger with histomonas-contamintated *H. gallinarum*-infection than in the pure *H. gallinarum*-infection. In the *A. galli*-experiments there was no difference in feed intake between infected and uninfected birds. This is in agreement with a previous study (Daş et al., 2010) showing that *A. galli*-infection does not influence feed intake of the birds on a nutritionally balanced diet. Anorexia, as observed in case of many parasitic infections, is known as one of the main factors affecting performance of infected animals (Kyriazakis et

al. 1998; Crop and Kyriazakis, 1999; Walkden-Brown and Kahn, 2002). The degree of anorexia may be affected by the species of parasite and its site of infection as well as level of infection and immune responses of the host animal (Knox et al., 2006). A direct comparison between intensity of *H. gallinarum*- and *A. galli*-infections based on number of worms may not be a valid approach. However, number of worms may indicate potential parasitic stimulants that interact with the immune system. Lymphoid structures play an important role in the avian immune system. Yegani and Korver (2008) reported that the chicken's foregut is relatively poor in lymphoid follicles, but they are numerously present in the hindguts, particularly in the caeca. An intense immune stimulation induced by *H. meleagridis and H. gallinarum*, might therefore, have contributed to the decreased feed intake of the infected birds.

Adverse effects of parasitic infections on performance of the birds are not limited to reduction in feed intake, but also to a less efficient utilization of feed for BWG. Immune responses to gastrointestinal antigenic stimulations can negatively affect feed utilization efficiency, are energetically expensive, and divert nutrients away from production (Yegani and Korver, 2008). Parasitic infection-induced less efficient feed utilization often indicates a diverted allocation of nutrients from growth to defense reactions against the parasite infection (Kyriazakis and Houdijk, 2006) including repair of damaged mucosal tissues during the histotropic phase of the nematodes (Herd and Mcnaught, 1975) and the acquisition as well as the development of immunity (Marcos-Atxutegi et al., 2009). These reactions may require high metabolic inputs (Colditz, 2008). *A. galli* infection may also influence digestion and absorption of nutrients by reducing proteolytic enzyme activity in the jejunum (Hurwitz et al., 1972a) being associated with an increased recycling of nitrogen (Hurwitz et al., 1972b). Reduced metabolizability of energy and lowered nitrogen retention has been reported for *A. galli* infected chickens (Walker and Farrell, 1976). *Heterakis gallinarum* is commonly regarded as a non-pathogenic nematode (Taylor et al., 2007). Although infection with this parasite is very common, effects of the infection on the digestion processes are not known. However, the present study shows that the efficiency of feed utilization and growth performance were impaired in *H. meleagridis* and/or *H. gallinarum* infected birds irrespective of the type of diet, and thus indicating negative effects of the caecal parasitic infections on the overall digestion and absorption processes.

5.2. Parasitic infections intensified by the dietary NSP

Feeding NSP-supplemented diets did not only influence mono-infections with *H. gallinarum* or *A. galli*, but also interactions between *H. meleagridis* and *H. gallinarum*. In all parasitic infections, dietary NSP intensified infections as indicated by higher worm burdens of the birds. It was shown that insoluble and soluble NSP differ in their effects on the parasitic infections. The differences were more obvious with *H. gallinarum*- than with *A. galli*-infections. Although both NSP supplemented diets favored the establishment of *H. gallinarum*, S-NSP additionally enhanced female worm fecundity. Thus, it can be expected that feeding S-NSP will aggravate environmental contamination with the excreted *H. gallinarum* eggs and create an increased risk for new infections and re-infections of the birds, if kept in the field. The type of dietary NSP also played an important role in the interaction between *H. meleagridis* and *H. gallinarum*. Feeding S-NSP resulted in lower incidence and total worm burden than I-NSP in case of histomonas contaminated *H gallinarum* infection, whereas this diet led to highest worm burden when histomonas was eliminated.

To our knowledge, effects of dietary NSP on parasitic infections in poultry have only scarcely been investigated. Daenicke et al. (2009) showed that feeding viscous NSP favored the development of *A. galli* and caused a higher level of egg excretion but did not influence worm burden of growing chickens. In contrast to chickens, NSP-feeding has extensively been investigated in pigs. Studies with the pig whipworm, *Trichuris suis* showed that a dietary supplementation of 6 % inulin did not influence the establishment rate but retarded worm growth (Thomsen et al., 2006), whereas 16 % inulin supplementation decreased establishment, egg excretion and female worm fecundity (Petkevičius et al., 2007) when compared with oat hull meal as source of insoluble NSP. Similar results have also been reported for the pig nodule worm *Oesophagostomum dentatum*. It has repeatedly been shown that lignin rich diets or supplementations of insoluble NSP provided favorable conditions for the establishment of the nodule worm, whereas inulin supplemented diets had a profound devorming effect (Petkevičius et al., 1997; 2001; 2003). In another study with intestinally cannulated pigs, the same authors showed that intracaecal infusion of SCFA and lactic acid reduced worm counts by 92%, suggesting that the anti-parasitic effect of inulin is mediated through its metabolic products such as increased SCFA and lactic acid concentration resulting from intestinal microbial activity (Petkevičius et al., 2004).

As shown by our own as well as by the studies in pigs, the effects of NSP on nematode infections can mainly be attributed to alterations of digesta characteristics and

intestinal microbial fermentation. The favorable effects of I-NSP for *H. gallinarum*- and *A. galli*- infections in birds are in agreement with the reported results of the pig studies. However, in contrast to the effects of inulin in association with *T. suis*- and *O. dentatum*-infections, the inulin rich S-NSP diet in the present study also provided most favorable conditions for *H. gallinarum*- and *A. galli-infections*. The responses of different worm species residing in their specific hosts to the same substance (e.g., inulin) remain further to be investigated.

5.3. Technical issues in determination of nematode egg excretion

Estimations of gastrointestinal nematode infection intensity in the living animals widely rely on the quantification of nematode egg concentration in host animal faeces, and mostly expressed as number of eggs per gram of faeces (EPG). Although *H. gallinarum* has been subject to many studies, egg excretion of this nematode has scarcely been performed (Fine, 1975). This is high probably because of technical difficulties in sampling of the right faecal material. Collection of daily faeces from infected birds did not only provide a valid approach for quantification of the egg excretion of this nematode, but also proved a weakness of EPG. In the mono *H. gallinarum*-experiment (Chapter 3) we demonstrated that EPG is not a valid indicator for estimating infection intensity in case of the total amount of faeces are expected to differ among groups to be compared. EPG rendered unsatisfactory information about the actual infection intensity and the worm fecundity in the birds on the NSP diets. The inclusion of the total daily amount of faeces for the calculation of daily total number of excreted eggs (EPD) eliminates dilution effect of faeces and provides more accurate information about the actual infection status of the host animal as well as for the actual worm fecundity estimates than EPG alone.

5.4. General conclusions

Histomonas meleagridis does not only harm the definitive host, but also its vector, *Heterakis gallinarum*. Insoluble and soluble NSP supplemented diets favor *H. gallinarum* infection while S-NSP additionally intensifies histomonas infection, which then impairs the establishment and development of *H. gallinarum*. The pea bran and chicory root meal used as sources of insoluble and soluble dietary NSP, respectively, favored the establishment of histomonas-free *H. gallinarum* in grower layers. Chicory root meal

additionally enhanced the fecundity of this nematode. Insoluble and soluble dietary NSP retard the growth, alter the gastrointestinal environment, lead to higher weights of splanchnic tissues and elevate the establishment of *A. galli* in grower layers. The enhancing effects of the dietary NSP on the nematode infections are presumably caused by an altered gastrointestinal environment, with S-NSP being more affective than I-NSP. It is concluded that the two natural sources of insoluble and soluble NSP offer no potential as protecting agents against parasitic infections in grower layers. Therefore, suitable measures of precaution should be applied to production systems particularly prone to gastrointestinal parasitic infections and where diets with relatively high NSP-contents are fed.

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ZUSAMMENFASSUNG

Ziel der vorliegenden Arbeit war es, die Hypothese zu prüfen, ob leicht lösliche oder unlösliche Nicht-Stärke-Polysaccharide (NSP) einen Einfluss auf eine *Heterakis gallinarum* beziehungsweise *Ascaridia galli* Infektion bei Aufzuchthennen haben. Da *Heterakis gallinarum* als Vektor für *Histomonas meleagridis*, dem Erreger der Schwarzkopfkrankheit, fungiert, wurde weiterhin untersucht, ob ein NSP-angereichertes Futter Einfluss auf Wechselwirkungen dieser beiden Parasiten hat.

Die Untersuchungen wurden zwischen 2007 und 2010 am Department für Nutztierwissenschaften der Universität Göttingen durchgeführt. Es kamen drei verschiedene Fütterungsvarianten zum Einsatz. Der Nährstoffgehalt und die umsetzbare Energie der Grundmischung (CON) entsprachen den Vorgaben und Empfehlungen für Aufzuchtlegehennen. Erbsenschalen und Zichorienwurzelmehl dienten als Komponenten für unlösliche bzw. lösliche NSP. In der Fütterungsvariante I-NSP wurden pro kg CON 100 g Erbsenschalen und in der Fütterungsvariante S-NSP pro kg CON 100 g Zichorienwurzelmehl zugesetzt. Die drei Versuchsmischungen CON, I-NSP und S-NSP wurden pelletiert.

Im ersten Experiment sollte der Einfluss eines mit NSP angereicherten Futtermittels auf die Wechselwirkung zwischen *H. gallinarum* und *H. meleagridis* untersucht werden. Dieses Experiment beinhaltete eine prophylaktische, über das Tränkesystem applizierte Behandlung der Hälfte der Tiere mit Dimetridazol (0,05%) gegen H. *meleagridis*. Die durch diesen Versuch gewonnenen histomonadenfreien weiblichen *H. gallinarum* Würmer dienten als Infektionsmaterial für die anderen Versuchsdurchgänge mit *H. gallinarum*.

Der Einfluss von NSP des Futters auf *H. gallinarum* beziehungsweise *A. galli* wurde in zwei aufeinanderfolgenden Versuchen mit jeweils 3 Wiederholungen pro Nematodenspecies, untersucht. In jedem Durchgang wurden 3 Fütterungsgruppen gebildet, die mit je einer der Versuchsmischungen bis zu einem Alter von 3 Wochen gefüttert wurden. Nach 3 Wochen wurden die Tiere individuell mit Flügelmarken gekennzeichnet und gewogen. Jede Fütterungsgruppe wurde nochmals in eine infizierte und eine nicht-infizierte Gruppe unterteilt. Die infizierten Versuchsgruppen wurden jeweils mit je 200 embryonierten *H. gallinarum*- beziehungsweise 250 embryonierten *A. galli*- Eiern künstlich infiziert. Während der folgenden 8-wöchigen Versuchsphase wurde der Futterverbrauch pro Gruppe täglich erfasst. In den letzten beiden Durchgängen mit *H. gallinarum* wurden zur Bestimmung der täglichen Kotmenge, der Parasiteneiausscheidung pro Gramm Kot (EPG) und der Anzahl

ausgeschiedener Parasiteneier innerhalb eines Zeitraums von 24h (Eier pro Tag, EPD) Tiere in Einzelkäfigen gehalten.

Im *A. galli* Experiment wurden die Tiere zur Bestimmung der Wurmzahl pro Tier 8 Wochen nach der Infektion geschlachtet. Zum Zeitpunkt der Schlachtung erfolgte darüber hinaus eine individuelle Kotprobenentnahme zur EPG-Bestimmung. Weiterhin wurden der pH-Wert sowie der Gehalt flüchtiger Fettsäuren in der Blinddarm-Digesta untersucht.

Im ersten Versuch (*Histomonas* infizierte *H. gallinarum*, **Kapitel 2**) wurde festgestellt, dass – unabhängig von der Fütterung - die Behandlung gegen *Histomonas meleagridis* sowohl das Auftreten von *H. gallinarum* als auch die durchschnittliche Länge der Würmer steigert. Es ergab sich eine Interaktion zwischen der Fütterung und der Dimetridazol-Behandlung. S-NSP führte bei unbehandelten Tieren zu den niedrigsten Wurmzahlen, während die behandelten Tiere die höchste Befallsintensität aufwiesen. Innerhalb aller Fütterungsvarianten hatten behandelte Tiere jeweils höhere Wurmzahlen als unbehandelte. Die Infektion mit *H. gallinarum* beeinträchtigte das Körpergewicht (BW) der Tiere, und die gleichzeitige Infektion mit *Histomonas meleagridis* verstärkte diese Wirkung. Die Behandlung mit Dimetridazol wirkte sich nicht auf die Körpergewichtsentwicklung aus. Beide NSP-Futtermischungen, insbesondere S-NSP, führten zu geringerem Körpergewicht der Tiere.

Im Versuch mit *H. gallinarum* ohne *Histomonas meleagridis* Infektion (**Kapitel 3**) steigerte die NSP-Fütterung im Vergleich zur Kontrolle sowohl die Befallhäufigkeit als auch die Befallsstärke. Ein Einfluss des Futters auf die durchschnittliche Wurmlänge konnte hingegen nicht beobachtet werden. Die NSP-Fütterung führte zu gesteigerter täglicher Kotmenge. EPG, EPD und die Fruchtbarkeit der weiblichen Würmer (EPD: Anzahl weiblicher Würmer) stiegen unter dem Einfluß von S-NSP, während I-NSP das Verhältnis von EPG : weiblichen Würmern verringerte. Die Anzahl ausgeschiedener Parasiteneier pro Tag stieg in der Reihenfolge CON < I-NSP < S-NSP. Sowohl die NSP-Fütterung als auch die Infektion führten zu geringerer Körpergewichtsentwicklung. Die Infektion beeinträchtigte zusätzlich die Futterverwertung. Die Blinddärme der mit NSP, insbesondere der mit S-NSP gefütterten Tiere waren durchschnittlich länger als die der Kontrolltiere. Die Fütterung und die Infektion beeinflussten das Gewicht der Blinddärme. S-NSP und die Infektion führten zu schwereren Blinddärmen. Die Infektion steigerte sowohl das Gesamt- (inklusive Darminhalt) als auch das Nettogewicht (ausgewaschen/gespült) der Blinddärme. Im Vergleich zu CON und I-NSP führte S-NSP zu niedrigeren pH-Werten, zu niedrigerer Acetat- und höherer Butyratkonzentration sowie zu größerem Pool an flüchtigen Fettsäuren (VFA) im Blinddarm.

Die Infektion erhöhte die pH Werte und verminderte die Butyratkonzentration sowie den VFA Pool im Blinddarm.

Im Versuch mit *Ascaridia galli* (**Kapitel 4**) erhöhte die NSP-Fütterung und insbesondere die mit S-NSP die Befallshäufigkeit. Im Gegensatz zum Versuch mit *H. gallinarum* wurde kein Einfluss auf die Wurmlänge und die Wurmfruchtbarkeit festgestellt. Die mit *A. galli* infizierten Tiere wiesen unabhängig von der Fütterung, eine verminderte Körpergewichtsentwicklung und eine ungünstigere Futterverwertung auf. Auch die NSP-Fütterung, und wiederum vor allem S-NSP, beeinträchtigte die Körpergewichtsentwicklung der Tiere. S-NSP bewirkte außerdem niedrigere pH Werte im Blinddarm, während die Infektion mit *Ascaridia galli* diesbezüglich keinen Einfluss hatte. Die Infektion und die Fütterung erhöhten die Mengen an VFA im Blinddarm. S-NSP steigerte die Mengen an VFA am stärksten. Im Vergleich zu CON führte die NSP-Fütterung, insbesondere S-NSP, zur Verdickung zur Gewichtserhöhung des Dünndarmgewebes.

Die Ergebnisse zeigen, dass *H. meleagridis* nicht nur den Endwirt schädigt, sondern auch den Vektor *H. gallinarum*. Sowohl unlösliche als auch lösliche NSP fördern die Etablierung einer *H. gallinarum* Infektion. Lösliche NSP fördern und intensivieren zusätzlich das Auftreten von *Histomonas meleagridis*. Dies hat wiederum nachteilige Auswirkungen auf die Etablierung und Entwicklung von *Heterakis gallinarum*. Sowohl Erbsenschalen als auch Zichorienwurzelmehl als Quellen unlöslicher bzw. löslicher NSP fördern die Infektion mit Histomonas–freien *H. gallinarum* und mit *A.* galli. Zichorienwurzelmehl steigert zusätzlich die Fruchtbarkeit von *H. gallinarum*. Die verwendeten unlöslichen und löslichen NSP beeinträchtigen das Wachstum der Tiere, reichern sich im Gastrointestinaltrakt an und fördern so möglicherweise die Empfänglichkeit gegenüber Infektionen mit Nematoden.

Die Ergebnisse lassen schließen, dass Erbsenschalen und Zichorienwurzelmehl als Quellen unlöslicher bzw. löslicher NSP ungeeignet sind, der Etablierung und Entwicklung von Nematodeninfektionen entgegenzuwirken. Insbesondere in alternativen Haltungssystemen, in denen Tiere einen erhöhten Infektionsdruck ausgesetzt sind und vermehrt NSP aufnehmen (können/), muss nach anderen Bekämpfungsmöglichkeiten gesucht werden.

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