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Helminth infections in laying hens kept in alternative production systems in Germany – Prevalence, worm burden and genetic resistance

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for you

„So sehr wir dem Licht entgegenstreben,
so sehr wollen wir auch von den Schatten umschlossen werden.“

Zoran Drvenkar

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SUMMARY

The aim of this study was to investigate the spectrum and intensity of helminth infections, as well as to estimate seasonal effects on the prevalence and burden of helminths in organic free range layers in Germany. Furthermore, resistance of six common commercial laying hen strains to an experimental *Ascaridia galli* infection was compared. In a next step genetic parameters of resistance to a natural mixed infection under field condition were estimated for two commercial breeds.

The experiments were conducted between 2007 and 2010 at the Department of Animal Sciences and on a commercial laying hen farm.

For the first trail (**chapter II**), laying hens from organic free range farms were collected between 2007 and 2010. The hens were sacrified and the gastrointestinal tracts were examined for the presence and intensity of helminth infections with standard methods. Hens slaughtered from October to March were included in winter data, whereas hens slaughtered from April to September were included in summer data sets. Almost all hens harboured at least one worm of one helminth species. Average worm burden per hen was 218.4 worms. The most prevalent species were the nematodes *Heterakis gallinarum* (98 %) followed by *Ascaridia gali* (88 %) and *Capillaria* spp. (75.3 %), whereas the overall prevalence of the cestodes was 24.9 %. Total worm burden was significantly higher during the summer season when compared with animals slaughtered during winter season. Risk of being infected with any of the nematodes was higher in summer than in winter. Probability of infection with any of the tapeworm species was higher in the summer than in winter.

For the second experiment (**chapter III**) six genotypes of commonly used commercial laying hens, namely Lohmann Brown (LB), Lohman Silver (LSi), Lohmann LSL classic (LSL), Lohmann Tradition (LT), Tetra SL (TETRA) and ISA Brown (ISA), were compared for their ability to resist an experimental *Ascaridia galli* infection. Laying performance, feed intake, change in the integument and faecal egg counts were determined during the experiment. The hens were infected at the beginning of laying period and slaughtered 105 d after infection i.e., at an age of 35 weeks, to determine their worm counts. Significant differences in average worm counts of the genotypes were quantified. LSL hens had the highest (25.8) and LT hens had the lowest (12.9) worms per hen. Although worm burden of LSL hens did not differ than those of TETRA and ISA, they had higher worm burdens than LSi, LT and LB hens. ISA hens

also had higher worm burdens when compared with LT and LB hens. LSL and ISA hens had higher number of larva than LSi, TETRA, LT and LB hens. No large differences were observed among the genotypes for the performance parameters.

For the third trail (**chapter IV**), groups of Lohmann Brown (LB) and Lohmann Selected Leghorn (LSL) hens were reared under helminth-free conditions and kept afterwards together in a free range system. Mortality rate, body weight development, laying performance and faecal egg counts (FEC) were recorded during a 12 month laying period. At the end of the laying period, 246 LSL and 197 LB hens were necropsied and worms counted following standard methods. LB hens showed a significantly higher average number of adult *H. gallinarum*, *Capillaria spp.* and tapeworms when compared with LSL animals. In total, LB had a significantly higher worm burden than LSL. The estimated heritabilities for total worm burden were on moderate in LSL and high in LB.

It can be concluded that the vast majority of hens in organic production systems is infected with a broad spectrum of helminths. However, within- and between-breed variation and heritability estimates reported in this study suggest, that it is possible to select for helminth resistance in both breeds based on worm counts. Such an approach should be considered sustainable as an explicit genetic progress for resistance against each single nematode species can be achieved from short to long terms. This may be of importance for chickens kept in alternative and organic farming systems.

ZUSAMMENFASSUNG

Das Ziel dieser Arbeit bestand darin, das Spektrum, sowie Befallsextensitäten und -intensitäten von Helmintheninfektionen in deutschen ökologischen Legehennenhaltungen darzustellen und einen etwaigen jahreszeitlichen Einfluss zu evaluieren. Des Weiteren wurde die Resistenz von 6 herkömmlichen Legehennenlinien gegenüber einer künstlich induzierten *Ascaridia galli* Monoinfektion verglichen. In einem weiteren Schritt wurden dann genetische Parameter der Resistenz gegenüber einer natürlichen Mischinfektion für 2 Legehennenherkünfte geschätzt.

Die Versuche wurden im Zeitraum von 2007 bis 2010 am Department für Nutztierwissenschaften der Universität Göttingen, sowie auf beteiligten Legehennenbetrieben durchgeführt.

Für die erste Studie (**Kapitel II**) wurden im gesamten Zeitraum der Arbeit sukzessive Legehennen von ökologisch wirtschaftenden Betrieben geschlachtet und auf das Vorhandensein von Helmintheninfektionen untersucht. Nach Schlachtung der Tiere, wurden dafür der Magen-Darm-Trakt und die Luftröhre entfernt und mit etablierten Standardmethoden auf Präsenz und Anzahl von Helminthen untersucht. In Abhängigkeit vom Zeitpunkt der Schlachtung, erfolgte eine jahreszeitliche Einteilung der gewonnen Daten in Winter (Oktober – März) und Sommer (April – September). 737 der 740 Hennen (99,6 %) beherbergten mindestens einen Wurm einer Helminthenart. Die mittlere Wurmzahl pro Tier betrug 218,4. Die befallshäufigsten Arten waren die Nematoden *Heterakis gallinarum* (98 %), *Ascaridia galli* (88 %) und *Capillaria* spp. (75,3 %). Die Prävalenz von Bandwürmern betrug 24,9 %. Die mittlere Wurmzahl pro Henne war im Sommer signifikant höher als im Winter. Ebenso war die Wahrscheinlichkeit, dass Wirtstiere sich mit Rundwürmern oder Bandwürmern infizieren im Sommer höher als im Winter.

In einem zweiten Versuch (**Kapitel III**) wurden 6 praxisrelevante Legehennenlinien (Lohmann Brown, LB; Lohman Silver, LSi; Lohmann LSL classic, LSL; Lohmann Tradition, LT; Tetra SL, TETRA; ISA Brown, ISA) hinsichtlich ihrer Empfänglichkeit gegenüber einer künstlich herbeigeführten *Ascaridia galli* Infektion untersucht. Während des Versuchs wurden die Legeleistung, die Futteraufnahme, Veränderungen am Integument und die Parasiteneiausscheidung pro Gramm Kot ermittelt. Für den Versuch wurden die Tiere mit Beginn der Eiablage künstlich infiziert und nach einer 15-wöchigen Versuchsphase im Alter von 35 Wochen geschlachtet und die Wurmzahlen ermittelt. Die mittleren Wurmzahlen der verschiedenen Herkünfte

unterschieden sich signifikant. Die Herkunft LSL wies mit 25,8 die höchste und die Herkunft LT mit 12,9 die geringste Wurmzahl pro Henne auf. Der mittlere Wurmbefall der LSL Hennen unterschied sich nicht von dem der Herkunft TETRA und ISA, war jedoch höher als der mittlere Wurmbefall der LSi, LT und LB Gruppen. Die Herkunft ISA beherbergte im Mittel ebenfalls eine größere Anzahl Würmer als LT und LB. Die Anzahl nachgewiesener Larvenstadien bei den LSL und ISA waren im Vergleich zu den LSi, TETRA, LT, und LB Hennen signifikant erhöht. Die Leistungsparameter der jeweiligen Herkünfte bewegten sich im Rahmen des vom Züchter angegeben Leistungsprofils und unterschieden sich folglich nur rassenspezifisch.

Für den dritten Versuch (**Kapitel IV**) wurden Lohmann Brown (LB) und Lohmann Selected Leghorn (LSL) Eintagsküken unter wurmfreien Bedingungen gemeinsam aufgezogen und anschliessend als Mischherde auf einen ökologisch wirtschaftenden Betrieb aufgestallt. Während der 12 monatigen Legeperiode wurden Mortalitäten, Körpergewichtsentwicklung, Legeleistung und Parasiteneiausscheidung (EpG) für jede Herkunft erfasst. Nach Abschluss der Legeperiode wurden insgesamt 246 LSL und 197 LB geschlachtet und die der Magen-Darm-Trakt sowie die Luftröhre auf das Vorhandensein von Parasitenstadien untersucht. Die Hennen der Herkunft LB beherbergten im Vergleich zu den LSL Hennen signifikant mehr adulte Stadien von *Heterakis gallinarum*, *Capillaria* spp. und Bandwürmer. Der mittlere Gesamtwurmbefall war signifikant höher bei den LB Hennen. Die Heritabilitäten für den Parameter ‚Gesamtwurmzahl' waren moderat (LSL) bis hoch (LB).

Basierend auf den Ergebnissen der Arbeit kann gefolgert werden, dass die große Mehrheit der Hennen in ökologisch wirtschaftenden Betrieben mit einer Vielzahl verschiedener Helminthenspezies befallen sind. Die in der Arbeit aufgezeigten Variationen innerhalb und zwischen Rassen, sowie die Werte der geschätzen Heritabilitäten weisen darauf hin, dass eine Selektion auf Resistenz gegenüber einer Helmintheninfektion auf Grundlage des Parameters ‚Gesamtwurmzahl' möglich ist. Bei entsprechender Berücksichtigung in Zuchtprogrammen könnten mittel- bis langfristig nachhaltige Erfolge bezüglich der Resistenz von Legehennenlinien gegenüber Helmintheninfektionen erzielt werden. Dies wäre von übergeordneter Bedeutung für alternative und ökologische Legehennenhaltungsysteme.

CHAPTER I

General Introduction

Foreword

Chicken egg is a precious food for many people all around the world, and consumers are aware of its nutritional properties. A fairly stable demand has been evident, unless there are major incidents that make them reluctant to buy and eat eggs. Egg production systems, like the vast majority of other modern animal husbandry systems, are highly industrialized to increase the quantity and efficiency of production. This implies confined housing, power ventilation, mechanical feeding and automatic egg collection aiming at the reduction of production costs to increase revenues at the market. Therefore the vast majority of hens in important egg producing countries were kept in laying cages which fitted the mentioned requirements best and were (and still are) the most economical way to produce eggs (van Horne, 2006). Furthermore, these cage systems had a positive side effect in providing best conditions for infectious disease prevention (Hulzebosch, 2006).

In the last two decades, consumer demands in several countries worldwide, particularly in European countries, changed towards a less-intensive animal production intending egg productions systems should focus more on animal welfare. Thus, animal welfare received more legislative attention in EU than in many other countries of the world (van Horne and Achterbosch, 2008). Therefore, in 2012 the EU directive 1999/74/EC (Anonymous, 1999) will enter into force, banning conventional cage systems for laying hens EU-wide. As a consequence, the cage systems (excluding enriched cages) are being gradually replaced by alternative egg production systems. In Germany, the percentage of farms with an alternative production system increased from 15% in 2001 to 63% in 2009 (ZMP, 2008; MEG, 2010). Since January 2011, all laying hens have to be kept in alternative husbandry systems as the German government and House of Representatives decided to overrule the directive with a relevant national directive (TierSchNutzV, 2009). On the one hand, alternative production systems are supposed to offer highest animal welfare standards (Tuyttens et al., 2008), on the other hand, the freedom of movement, as an important factor of alternative animal husbandry (Berg, 2001), increases the risk of infection with several parasites, as hens are in contact with faeces allowing helminths to complete their lifecycle. High prevalences and worm counts have been described in several studies (Zeller, 1990; Permin et al., 1999; Kaufmann and Gauly, 2009). Consequently biosecurity in these production systems seems to be fairly poor. However, as such husbandry systems will be state of the art in the foreseeable future, researchers and experts are requested to study impacts, effects

and constrains of such production systems that these alternative production systems are able to meet their high expectations. The current work tries to do so and provides useful information regarding the epidemiology of helminths in German organic free range farms (**19**) and shows possible approaches to improve the current situation in free range husbandry systems (**40** and 57).

1.1 Important helminth parasites in the domestic chicken

Per definition, parasitism is defined as '*an intimate and obligatory relationship between two heterospecific organisms during which the parasite, usually the smaller of the two partners, is metabolically dependent on the host*' (Cheng, 1973). According to this definition, for now and without further restrictions, all parasites are important as these so called 'metabolic dependencies' lower the performance of the host in different ways and thus, economic losses occur. To define which parasites are of major importance in poultry production, their prevalence should be the first criteria. According to several studies the most prevalent infections are with the nematodes *Ascaridia galli, Heterakis gallinarum* and *Capillaria obsignata* (Permin and Hansen, 1998; Permin et al., 1999; Irungu et al., 2004; Kaufmann and Gauly, 2009).

All three nematodes have a direct life cycle (Figure), i.e. no intermediate host is needed to complete their lifecycle (Herd and McNaught, 1975; Norton and Ruff, 2003; McDougald, 2005) which, to some extent, explains the high prevalence rates.

The host infection starts with the ingestion of an embryonated egg, containing an infective L 3 –Larvae. This is similar for all of the three mentioned nematode species. In the case of *A. galli* the larvae hatch around 24 hours either in the proventriculus or the duodenum of the host, where it lives freely in the lumen for around 9 nine days, and then penetrates the mucosa for the tissue phase (histotropic phase). This tissue phase lasts for 7 to 50 days depending on the infection dose (Herd and McNaught, 1975) and is causing inflammatory reactions and injures hosts intestinal cells (Ramadan and Abou Znada, 1991). After several moltings, *A. galli* reaches maturity and female worms start producing eggs (prepatent period) at an age of 5 to 8 weeks depending on hosts immune status, age and length of the histotropic phase (Anderson, 1992; Idi, 2004). Favourable predilection site is the upper part of the intestine around the Lieberkuhn´s glands were this nematode feeds on digesta. Average lengths of the adults vary between 5 to 8 cm in

male and between 6 to 12 cm in female worms (Idi, 2004; Ramadan and Abou Znada, 1992), making *A. galli* the largest nematode parasite described in poultry.

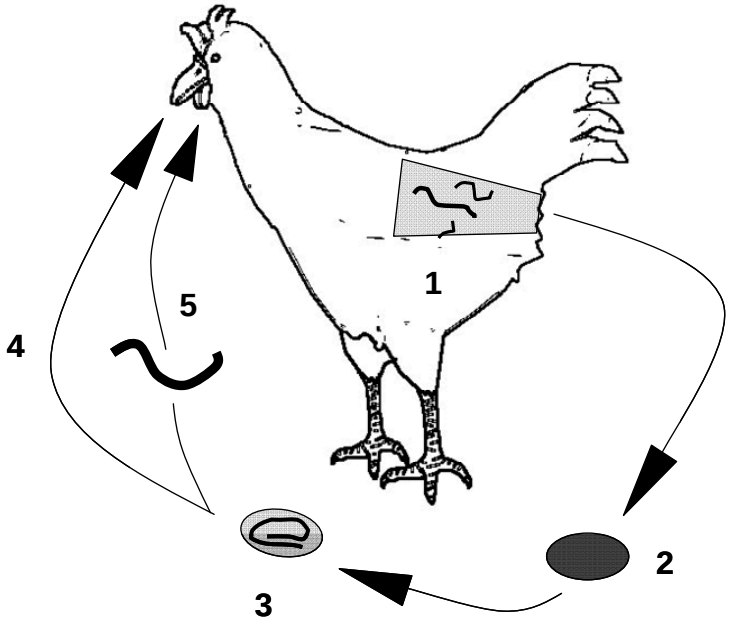

Figure. Life cycle of *H. gallinarum* as a representative of the genera nematoda. Mature female worms produce eggs (1) which are excreted with the hen's droppings (2). Eggs embryonate in the soil or litter (3) and the embryonated eggs, containing an infective L 3 –larva, are ingested by hens, either directly (4) or indirectly with the intake of an earthworm as a potential transport host (5).

Heterakis gallinarum larvae hatches in the upper intestine and within the following 24 hours they reach the caeca representing the final predilection site (Norton and Ruff, 2003). It is not fully known, if the life cycle of *H. gallinarum* includes a tissue phase. Some authors described a histropic phase (Hsü, 1940; Van Grembergen, 1954), whereas others state just a rare occurrence of a tissue phase (Norton and Ruff, 2003) if at all (Bauer, 2006). However, the fact that larval stages are closely associated and occasionally embedded in cecal tissue (Norton and Ruff, 2003), it may lead to misinterpretation and confusion surrounding this phenomenon. Prepatent period of *H. gallinarum* varies between 21 to 34 days (Fine, 1975; Bauer, 2006). Average lengths of the adults vary between 7 to 13 mm in male and between 10 to 15 mm in female worms (Norton and Ruff, 2003). Similar lengths and time frame for prepatent period are described for the 'hairlike' *Capillaria obsignata* (Wakelin, 1965; Norton and Ruff,

2003). As one may assume by the nickname, a specific characteristic of the *Capillaria* - species is their width ranging between 33 to 53 µm.

Going back to the initial definition, all described nematodes have a negative impact on the performance, expressed in weight depression or retarded weight gain, as they feed on host digesta and / or damage intestinal and cecal mucosa (Levine, 1938; Reid and Carmon, 1958; Norten and Ruff 2003; Kilpinen et al., 2005) and therefore have adverse effects on the absorption of nutrients (Hurwitz et al., 1972; Walker and Farrell, 1976). *A. galli* seems to have a higher pathogenicity compared to the other two described nematodes due to their size, their impact on the host during tissue phase (Ramadan and Abou Znada, 1991) and their immunosuppressive effect (Sharma, 1997; Malviya et al., 1998; Roepstorff et al., 1999). When hens suffer from heavy infections and thus, space becomes a limiting factor in the small intestine, migration of worms into the oviduct and in hen´s eggs has been observed (Reid et al., 1973). Next to the mentioned direct impacts on host animal, indirect impacts and losses may occur due to the fact that *H. gallinarum* as well as *A. galli* can act as vector or carrier for other pathogens. *H. gallinarum* is regarded as relatively less pathogenic parasite (Taylor et al., 2007) but its ability to transmit *Histomonas meleagridis*, the causative agent of 'Blackhead diseases', increases the importance of this nematode (McDougald, 2005). *Ascaridia galli* is reported to act as a vector for *Salmonella enterica* (Chadfield et al., 2001) and alter the effects of a concurrent infection with *Pasteurella multocida,* the causative agent of fowl cholera (Dahl et al., 2002).

When speaking about important endoparasites in poultry, cestodes have to be mentioned, as they are highly prevalent, especially in production systems with outdoor access (Permin et al., 1999; Kaufmann and Gauly, 2009). Compared to the nematodes, pathogenicity of tapeworms is low and the major concern for the egg producers is a potential blockage of the small intestine lumen when birds are heavily infected with large tapeworm species (McDougald, 2003). All tapeworm species have an indirect life cycle, i. e. they require intermediate host(s), (e.g. beetles, snails, flies, ants) to complete their life cycles. Thus, prevalence of tapeworms depends on the abundance of intermediate host and therefore underlies seasonal changes (Riddle, 1983; Black and Krasfur, 1986a,b; Pfinner and Luka, 2000; Yamazaki et al., 2002; Salam et al., 2010).

The use of anthelmintics, especially with broad-spectrum benzimidazole, has been proved to be effective against poultry helminthiasis (Ssenyonga, 1982; Kirsch, 1983). But as the use of anthelmintics is limited in the alternative and organic production systems, alternative control strategies are needed to be adopted. Precondition for a development of alternatives is the knowledge of the current spectrum, prevalence and intensity of helminth infection in free range production systems for laying hens (**chapter II**).

1.2 Resistance to parasitic infections

Parasitic infections in livestock are costly, due to medication, vaccination, secondary infections and other direct or indirect losses caused by death of animals and/or a depressed performance (Chubb and Wakelin, 1963; Okulewicz and Zlotorzycka, 1985; Ramadan and Adou Znada, 1991; Chadfield et al., 2001; Dahl et al., 2002; Kilpinen et al., 2005; Permin et al., 2006 Gauly et al., 2007; Daş et al., 2010).

Regardless of the substantial losses, infections are also an animal welfare and hygienic issue affecting both, the producers (including the animals) as well as the consumer (Craig, 1993; Waller, 1994; Sangster, 1999; Jackson and Miller, 2006).

The change in consumers demand also led to changes in the legislative and therefore, drug-use regulations changed gradually over the last years. However, this approach makes it necessary to search for alternative control tools for infection and disease control. As livestock are reported to differ in their ability to resist parasitic infection (Owen and Axford, 1991) the scene is set and interest in breeding hosts to resist parasitic infections increases, considerably.

Diseases resistance is a result of the interaction between a genotype or individual and the environment (Warner et al., 1987). If adverse environmental factors including pathogenic agents can not be isolated from the animal, or vice versa, the individual or genotype will adopt their natural resistance to the challenging agent. Resistance is based on variation observed within and between genotypes (Gray, 1991; Eady et al., 1996) allowing animal producers to apply different strategies to take advantage of the diversity (Woolaston and Baker, 1996). Three main strategies are described in small ruminats:

- choice and/or substitution of breeds (Baker et al., 1993)
- within breed selection (Albers and Gray, 1986)

- crossbreeding (Baker et al., 1993)

These mentioned strategies could be adapted to laying hen husbandry, as between and within-breed differences were described in earlier studies and estimated heritabilities of indicator traits were on a useful level (Ackert et al., 1935; Permin et al., 1997; Permin and Ranvig 2001; Gauly et al., 2002; Gauly et al., 2008). The current manuscript ties up on the older studies aiming to compare a larger variety of common, commercial laying hen breeds regarding their susceptibility to experimental *Ascaridia galli* infection in order to be able to recommend certain breeds for free range systems (**chapter III**). This may enables producers to take advantage of one the above mentioned strategies by choosing a relevant breed. To take full advantage of genetic variation, improvements in a certain trait, i.e. worm burden, should be done by selecting animals. Therefore, determination of the trait's heritability is essential (**chapter IV**).

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CHAPTER II

Helminth infections in laying hens kept in organic free range systems in Germany

Abstract

This study describes the spectrum and intensity of helminth infections in laying hens kept in organic production systems in Germany. A total of 740 laying hens from 18 organic free range farms were collected between 2007 and 2010. The hens were sacrified and the gastrointestinal tracts were examined for the presence and intensity of helminth infections with standard methods. Three nematode (*Ascaridia galli, Heterakis gallinarum, Capillaria spp.*) and four cestode (*Raillietina cesticillus, Hymenolepis cantaniana, Hymenolepis carioca, Choanotaenia infundibulum*) species were found. Almost all hens (99.6 %, N = 737) harboured at least one helminth species. Average worm burden per hen was 218.4 worms. The most prevalent species were the nematodes *Heterakis gallinarum* (98 %) followed by *Ascaridia galli* (88 %) and *Capillaria* spp. (75.3 %), whereas the overall prevalence of the cestodes was 24.9 %. Total worm burden was significantly higher during the summer season when compared with animals slaughtered during winter season (254 vs. 191, $P < 0.0001$). The most dominat helminth species was *Heterakis gallinarum* averaging 190 worms per hen in the summer and 129 in winter season, respectively ($P < 0.0001$). Average *Ascaridia galli* burden was 25 in summer and 26 in winter seasons, respectively ($P = 0.1160$). Risk of being infected with any of the nematodes was 1.5 times higher in summer than in winter ($\Psi = 1.49$, $P < 0.0319$). Probability of infection with any of the tapeworm species was 4.5 times higher in the summer than in winter ($P < 0.0001$).

It is concluded that the vast majority of the hens are subclinically infected with the helminth species. The prevalence as well as intensity of the helminth infections, particularly with tapeworms, considerably increases in summer. The present results indicate that it is essential to adopt alternative control strategies in order to lower infection risk and to limit the potential consequences to perform an appropriate animal husbandry.

2.1 Introduction

Recent changes in consumer prospects regarding a sustainable animal production and welfare has led to the ban of the conventional cages for laying hens in the European Union after 2012 (Anonymous, 1999). Thus, alternative production systems have gained popularity and percentages of hens kept in such systems increased over the last couple of years (ZMP, 2008; MEG, 2010). There is strong evidence that different production systems harbour different risk of parasite infections for animals. Parasitic infections, particularly in floor husbandry systems with or without outdoor access, are re-emerging. A study from Demark showed that the prevalence of the nematode *Ascaridia galli* was 64 % in free range / organic systems, 42 % in deep-litter systems and 5 % in conventional cages (Permin et al., 1999). The birds get infected by ingestion of infective parasite stages present in soil and litter and / or by eating intermediate or transport hosts. Infections with endoparasites have severe consequences on the host as well as the production systems as reported by several studies. Parasites may obstruct the small intestine and cause death (Ramadan and Znada, 1991). They can act as vectors and lead to secondary infections e.g. *E. coli*, (Okulewicz and Zlotorzycka, 1985; Chadfield et al., 2001; Dahl et al., 2002; McDougald, 2005; Permin et al., 2006). Furthermore they have adverse effects on behavior patterns, growth and nutrient utilization of chickens (Chubb and Wakelin, 1963; Gauly et al., 2007; Daş et al., 2010a). The control of endoparasites in various species is heavily dependent on the use of anthelmintics. In general, the use of anthelmintics in layers is very limited due to economic concerns as well as environmental and hygiene regarding development of drug resistance (Jackson and Miller, 2006) and chemical residues in animal products (Craig, 1993; Waller, 1994; Sangster, 1999). Particularly in organic production systems use of anthelmintics is strictly restricted as prophylactic treatments are prohibited. The changes in the production systems and in the climate may alter population dynamics of endoparasites which accumulates the importance of helminth infections in the future. To improve and maintain the performance and productivity of the hens, and to adopt alternative control strategies against endoparasites (Heckendorn et al., 2009) it is essential to determine the spectrum as well as intensity of the parasitic agents, which has not so far been performed for organic layers in Germany.

Therefore, the aim of the present study was to investigate the spectrum and intensity of helminth infections, as well as to estimate seasonal effects on the prevalence and burden of helminths in organic free range layers in Germany.

2.2 Materials and methods

2.2.1 Farm and animal sampling

A total of 740 laying hens of 5 genotypes (Lohmann Brown, Lohmann Selected Leghorn, Isa Brown, Tetra Brown, Lohmann Tradition) were collected from 18 commercial free range farms in Germany. All farms were certified as organic farms according to the guidelines of the European Union and national guidelines (2092/91/EEC; 834/2007/EC; Bioland, 2010). Farms were located throughout Germany with a focus on the central region.

On average 41 hens per farm were randomly selected to perform necropsies. The animals were sampled either in the last third or at the end of the laying period. Therefore, age of hens varied between 54 and 72 weeks.

Hens slaughtered from October to March were included in winter data (N = 417), whereas hens slaughtered from April to September were included in summer data sets (N = 323).

2.2.2 Necropsy, parasite processing and species identification

After slaughtering, the gastrointestinal tracts and tracheas were removed, opened longitudinally, and washed in tap water following the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines for evaluating the effectiveness of anthelmintics in chickens and turkey (Yazwinski et al., 2003). The separated contents were poured into a sieve with a mesh aperture of 100 µm, washed and examined for the presence of adult helminths.

All visible parasites were collected first and then the content of the gastrointestinal tract and the scraped mucosa were examined under 20x dissecting microscope. All species were counted and stored in tap water until differentiation on the same day. Identification of nematodes started with cleaning the worms in physiological saline solution. Afterwards they were examined under a light microscope at 40x magnification and differentiated based on the morphological characteristics as described by Soulsby (1982) and Norton and Ruff (2003).

Cestode harvest was done by submerging the intestine in water to float the worm and increase its visibility. In some cases, the scolices were strongly attached to the mucosa. To liberate the scolice, the attachment points were located; the intestine was

cut around the attachment point and refrigerated in water for 2 h. After thawing, scolices were released easily out of the mucosa using fine needles. Cestodes were identified using the helminthological keys according to Soulsby (1982), Schmidt (1986), Jones et al. (1994) and McDougald (2003). Staining-destaining techniques with Carmine dye were performed for identification of testes and cirrus.

All adult *A. galli* and *H. gallinarum* worms found were sexed as determined by Hartwich (1975). Furthermore, for each hen, a maximum of randomly selected ten worms per worm species and sex were measured for length using a ruler.

2.2.3 Statistical analyses

Prevalences of mono species-specific and mixed helminths infections was calculated with the Freq procedure of SAS (2010). Effect of season on the incidence of each helminth species was analyzed using the GENMOD procedure of SAS with a logit link function as shown in the following model.

$$\eta_i = \log [p_i / (1 - p_i)] = m + \tau_i$$

i= seasons; winter, summer

where;

p_{ij}= the proportion of infected birds on season i

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

τ_i = the effect of season i

The GENMOD procedure fits to the generalized linear models and is suited for variables with binary (0,1) outcomes (Kaps and Lamberson, 2004). Based on the output of the GENMOD procedure, the odds ratios (Ψ) as the probability of being infected with a given species at one of the seasons were estimated.

Because the species specific and the total worm burden data were not normally distributed (Kolmogorow-Smirnow, $P < 0.05$) and positively skewed (Skewness > 0), worm burden data (y) were log-transformed using the following function:
[log(y)=Log10(y+10)].

Effects of season on species specific worm burden as well as on total worm burdens were estimated with one-way ANOVA using proc GLM of SAS. Sex ratio (female/male) and average male and female worm length for *Heterakis gallinarum* and *Ascaridia galli* were analyzed using the same one-way ANOVA model as mentioned

above. Sex ratio was calculated in cases where both genders of the same worm species were present in the same animal.

2.3 Results

2.3.1 Prevalence of helminths infections

Out of 740 hens, 737 (99.6 %) harboured at least one worm of one helminth species. In total, three nematode and four cestode species were. The most prevalent species were the nematodes, *Heterakis gallinarum* (98 %), *Ascaridia galli* (88 %) and *Capillaria spp*. (75.3 %). Prevalence of the cestodes was 17.8 %, 8.2 %, 3.8 %, and 0,5 % for *Raillietina cesticillus*, *Hymenolepis cantaniana, Hymenolepis carioca* and *Choanotaenia infundibulum,* respectively (Table 1). A small proportion of the hens (4.9 %) were infected with only one helminth species, while 22.4 % harboured two and 54.6 % harboured three species. Almost 20 % of the hens had helminth infections with four or more species.

2.3.2 Average worm burdens

The intensity of infection was highest for *Heterakis gallinarum* with an average of 155.6 worms per hen. Average worm counts for *Capillaria spp*, *Ascaridia galli* and cestodes were 29.8, 25.7 and 8.2, respectively (Table 2). The hens harboured an average of 218.4 worms of which 210.3 were nematodes.

The sex ratio (female : male worms) for *A. galli* was 1.74 : 1, this of *H.. gallinarum* 1.35 : 1, respectively. The average worm length for female and male *A. galli* worms were 7.4 and 5.5 cm and for *H. gallinarum* 9.5 and *7*.9 mm, respectively (Table 2).

2.3.3 Seasonal effects on prevalence and worm burdens

The intensity and prevalence of infections with different helminths species varied in the two seasons, whereas the total prevalence did not significantly differ between summer and winter season ($P = 0.0632$, Table 1) but total worm burden was significantly higher in summer season when compared with winter (254 vs. 190.9, $P < 0.0001$, Figure 1). The prevalence as well as average worm burden per hen of different

tapeworm species was higher in summer season. Prevalences of nematode species were all significantly higher in summer but, with exception for *H. gallinarum,* their worm burden did not differ between the seasons.

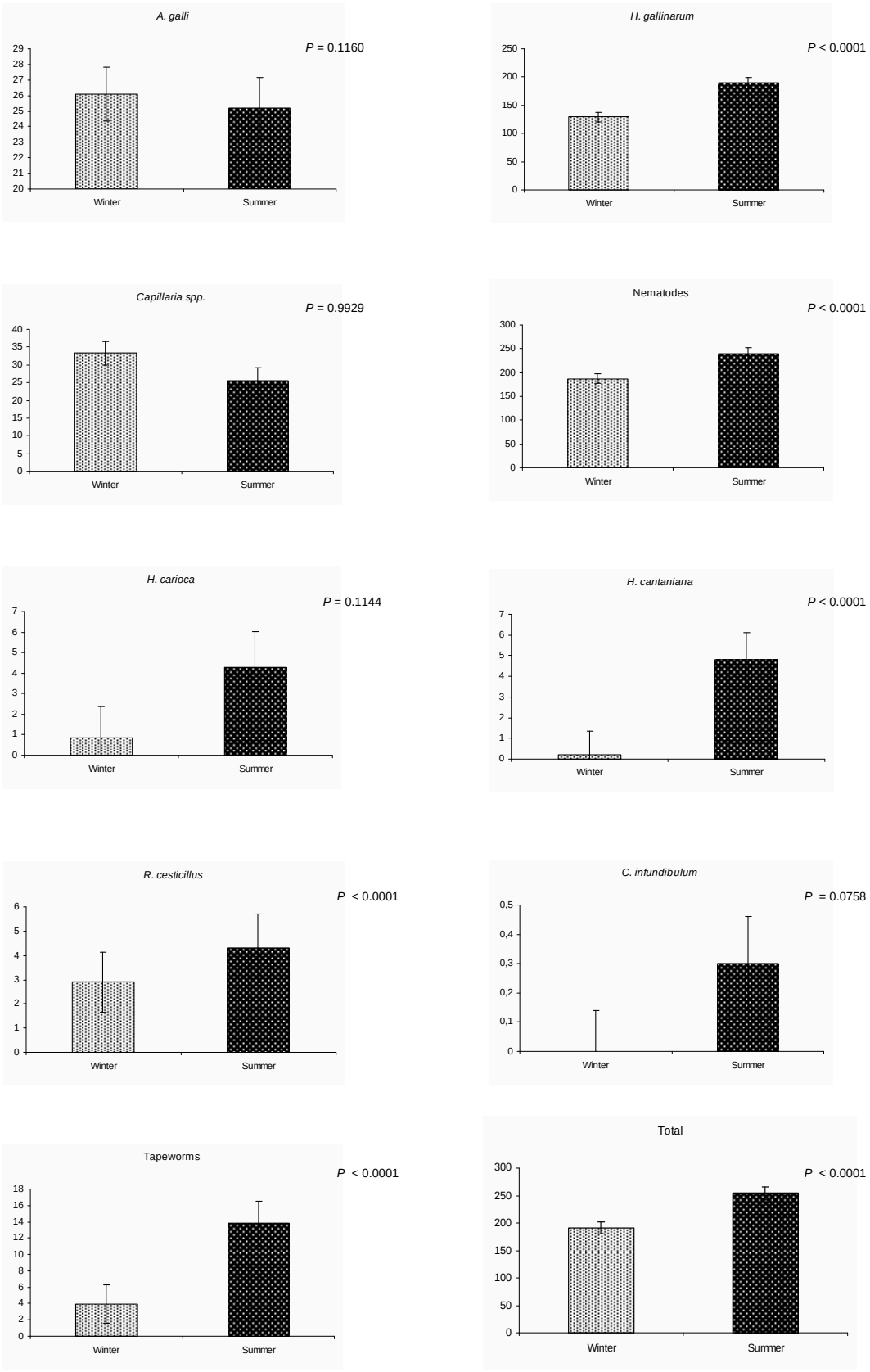

Figure 1. Seasonal effects on species specific worm burden* of the hens presented as LSMeans and standard errors (SE) on the error bars*.

*: LSMeans and SE represent untransformed data, *P*- values are based on the transformed data.

Figure 2 shows the occurrence of multiple infections during the different seasons. Animals sampled in winter were infected with less helminth species than those sampled in summer season. The number of harboured helminth species per hen differed between the two seasons. In summer 33.8 % (N = 109) of the animals had a mixed infection with more than 3 species, whereas it were 5.3 % (N = 22) in winter. However, in both seasons, the majority of the hens harboured 3 helminth species. Percentages of animals harbouring 3 species were 59.2 in winter and 48.6 in summer, respectively.

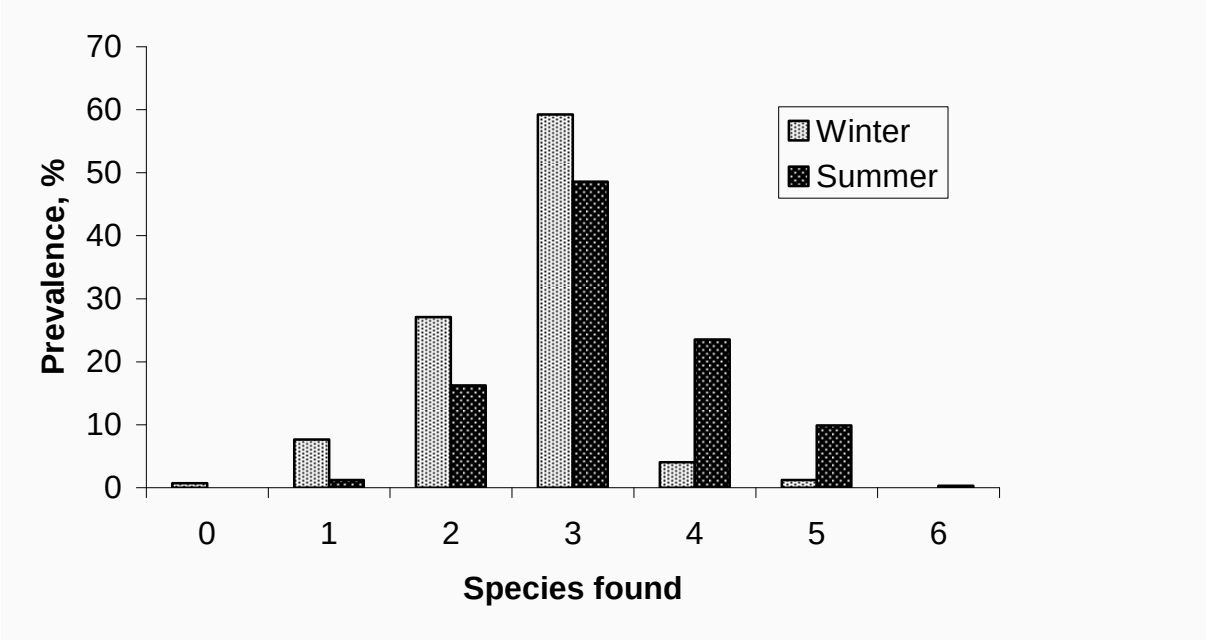

Figure 2. Season dependent, cumulative prevalences of the species found.

The risk of getting an infection with nematodes as well as cestodes was significantly higher in the summer season. The probability for an animal to be infected in summer was 1.5 times ($P = 0.0319$) and 4.5 times ($P < 0.0001$) higher for nematode and cestode infections, respectively (Table 1).

Table 1. Overall and season dependent prevalence of helminth species (N = 740), and the odds ratios (Ψ) as the probability of being infected in summer in comparison to winter season.

Species	Overall, % (N = 740)	Winter, % (N=417)	Summer, % (N = 323)	Season effect (Pr > ChiSq)	Ψ
A. galli	87.97	83.21	94.12	<.0001	3.23
H. gallinarum	97.97	96.88	99.38	0.0104	5.16
Capillaria spp	75.28	70.78	80.80	0.0018	1.74
H. carioca	3.78	2.88	4.95	0.1442	1.80
H. cantaniana	8.24	4.32	13.31	<.0001	3.40
R. cesticillus	17.84	7.19	31.58	<.0001	5.95
C. infundibulum	0.54	0.00	1.24	0.0099	1.93
Nematodes	99.46	99.04	100.00	0.0319	1.50
Tapeworms	24.86	12.95	40.25	<.0001	4.53
Total	99.59	99.28	100.00	0.0632	1.12

Table 2. Descriptive statistics for the worm burden data (N=740).

	Mean	SD	Min	Max
A. galli	25.7	35.2	0	310
Sex ratio, ♀:♂	1.74	1.31	0.17	12
Length (cm), ♂	5.5	1	1	8.5
Lenght (cm), ♀	7.4	1.5	0.9	13
H. gallinarum	155.6	179.3	0	1420
Sex ratio, ♀:♂	1.35	0.79	0.11	9
Length (mm), ♂	7.9	1.1	3	11.7
Lenght (mm), ♀	9.5	1.2	2.5	12.1
*Capillaria spp.**	29.8	64.6	0	1237
H. carioca	2.3	31.3	0	798
H. cantaniana	2.2	23.5	0	525
R. cesticillus	3.5	25.3	0	589
C. infundibulum	0.1	2.9	0	76
Nematodes	210.3	211.3	0	1455
Cestodes	8.2	48.1	0	798
Total worm burden	218.4	218.3	0	1470

*N=730

2.4 Discussion

The spectrum, prevalences and worm counts of helminth species in the present study refer for the first time for layers in organic productions systems in Germany. The spectrum of the encountred helminths is mainly in accordance with other studies from Europe (Permin et al., 1999; Zeller, 1990; Morgenstern and Lobsiger, 1993), USA (Wilson et al., 1994; Robel et al., 2003), Africa (Hassouni and Belghyti, 2006; Permin et al., 2002; Magwisha et al, 2002; Matur et al., 2010) and Asia (Rabbi et al, 2006; Puttalakshamma et al., 2008; Köse et al., 2009). However, prevalence and worm counts in the current study are on a higher level when compared to the above-mentioned

previous studies that examined chickens from semi-intensive, extensive or even backyard systems. Due to certain management factors, parasitic infection rates differ among different production systems (Zeller, 1990; Morgenstern and Lobsiger, 1993; Permin et al., 1999). Incidence of infection and worm counts increases from cage systems over deep litter systems to free range systems. As shown by our survey, chickens in the organic farms do not only harbour a large spectrum of helminths, but also the intensity of infections is high.

The large spectrum and intense helminth infections can not only be attributed to the fact that biosecurity in free range systems is poor, but also draw attention to the distinctive properties of organic farming that appear to provide favourable conditions for helminth infections. Organic egg production systems imply different housing and feeding conditions for the animals. The obligate outdoor access increases the risk of infection with several parasites, as hens are exposed to a natural environment that allows helminths to complete their life cyles (Norten and Ruff, 2003).

Most of the farms surveyed in the present study intend to reach the maximum flock size and stocking rates which are allowed by the law. Therefore, the degree of intensification for these farms can be considered as high. It is reasonable to expect higher risk of helminth infections as the flock size increases. Higher stocking rates in outdoor areas seems to have no effect on helminth infections in laying hens (Permin et al., 1998; Heckendorn et al., 2009), whereas significantly higher faecal egg counts, and worm burdens were recorded in pigs (Mejer et al., 1998). Even no direct relationship between stocking rate and helminth infections has been reported for laying hens, it was shown that a higher stocking rate in outdoor runs result in deterioration of the run vegetation (Heckendorn et al., 2009). Limited availability of vegetation may lead to an intensified foraging behaviour, which increases the risk of helminth transmission as infective stages are present in soil and litter (Maurer et al., 2009). Furthermore, most farms sampled in this study are using the same pasture without rotation. This intensive use of a single pasture may accumulate infective parasite stages over years leading to the high prevalences and worm counts found in the present study (Thomson et al., 2001).

Sustainable and economic organic egg production heavily relies on the best possible nutrient supply, particularly with essential amino acids (Sundrum et al., 2005). Organic laying hens must be fed primarily on diets based on the organically produced feedstuffs. Chemically extracted soybean meal and synthetic amino acids are banned by

the council regulation (804/99/EC). Therefore, the hens are often fed fiber rich energy diluted diets in order to benefit from compensatorily increased feed intake, that guarantee adequate amount of essential amino acids (Deerberg, 2004; Sundrum et al., 2005; van de Weerd et al., 2009). However, it has repeatedly been shown that energy diluted diets favour establishment of both *H. gallinarum* and *A. galli* in chickens (Daş et al., 2010b;c;d) as well as fecundity of *H. gallinarum* (Daş et al., 2010d) when compared with a standard diet. On the other hand, inadequate intake of single amino acids, i.e. lysine, may also increase incidence of infections with A. galli (Daş et al., 2010d) probably due to an impaired immune response (Konashi et al., 2000; Li et al., 2007).

The present results revealed a seasonal effect on the risk of occurrence and intensity of helminth infections. The summer season in Germany provides warm and relatively humid conditions which is beneficial for development of parasite eggs and transmission (Roepstorff and Murell, 1997; Larsen and Roepstorff, 1999). This finding is of major interest as hens have nearly unrestricted outdoor access and are therefore exposed to infective stages, vectors as well as potential transport, paratenic and intermediate hosts. Transport or paratenic hosts, such as earthworms, may play a major role in transmission of eggs and infective stages of *Heterakis gallinarum* (Ackert, 1917; Madsen, 1962; Lund et al., 1963, 1966). The majority of hens in winter season harboured up to 3 species. Just 5.3 % of hens had a mixed infection with more then 3 species, whereas in summer season the spectrum was higher with 33.8 % of hens harbouring more then 3 species. The cut off after 3 species (Figure 2) can be explained with occurrence of cestodes. In order to complete their lifecycle cestode species are depended on intermediate hosts such as various species of beetles, slugs, snails and flies (Norten and Ruff, 2003). As intermediate hosts are not active in natural environment due to overwintering (Riddle, 1983; Black and Krasfur, 1986a;b; Pfinner and Luka, 2000; Yamazaki et al., 2002) cestode occurrence depends on presence of intermediate hosts in the stable and is therefore reduced. Spectrum of infections with helminths in winter season is therefore dominated by nematodes with their relatively short and direct lifecycle.

Organic production systems are supposed to offer the very highest animal welfare standards. As shown by the present study, the hens are intensively infected with a large spectrum of helminths. Effects of parasitic infections on animal welfare, performance as well as on the farm economy remain to be further investigated. Losses

due to a high morbidity might be considered of greater economic impact than high worm counts that cause mortality in a few birds.

It is concluded that the vast majority of the hens are subclinically infected with the helminth species. The prevalence as well as intensity of the helminth infections, particularly with tapeworms, considerably increases in summer.

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CHAPTER III

Resistance of six commercial laying hen strains to an experimental Ascaridia galli infection

Abstract

Six genotypes of commonly used commercial laying hens, namely Lohmann Brown (LB), Lohman Silver (LSi), Lohmann LSL classic (LSL), Lohmann Tradition (LT), Tetra SL (TETRA) and ISA Brown (ISA), were compared for their ability to resist an experimental *Ascaridia galli* infection. Laying performance, feed intake, change in the integument and faecal egg counts were determined during the experiment. The hens were infected at the beginning of the laying period and slaughtered 105 d after infection i.e., at an age of 35 weeks, to determine their worm counts.

No large differences were observed among the genotypes for the performance parameters. However, significant differences in average worm counts of the genotypes were quantified ($P = 0.008$). LSL hens revealed the highest (25.8) and LT hens the lowest worms per hen (12.9). Although worm burden of LSL hens did not differ from those of TETRA and ISA ($P > 0.05$), they had higher worm burdens than LSi, LT and LB hens ($P < 0.05$). ISA hens also had higher worm burdens when compared with LT and LB hens ($P < 0.05$). LSL and ISA hens had higher number of larva than LSi, TETRA, LT and LB hens ($P < 0.05$). Number of female worms, length of the worm and worm sex ratio did not differ significantly between the genotypes ($P > 0.05$).

The results suggest that there is a considerable variation in the responses of most commonly used chicken genotypes to the nematode infection. Although no large differences were observed in the performance of the genotypes, LT and LB hens were more resistant than LSL and ISA hens when exposed to an experimental *A. galli* infection. It is concluded that genetic differences in the responses of the breeds to the nematode infection may contribute to the efforts for selecting more suitable breeds for alternative floor production systems, where hens face poor biosecurity.

3.1 Introduction

Recent changes in consumer demands and animal welfare have led to the ban of the conventional cages for laying hens. Therefore, alternative production systems have gained popularity and percentages of hens kept in such systems increased over the last couple of years. Alternative production systems are supposed to offer higher animal welfare standards (Tuyttens et al., 2008). However, studies have shown that alternative production systems, especially floor husbandry systems with or without outdoor access, are highly connected with poor biosecurity and therefore appear to provide favourable conditions for helminth infections (Zeller, 1990; Permin et al., 1999; Kaufmann and Gauly, 2009). The obligate outdoor access increases the risk of infection with several parasites, as hens are exposed to a natural environment that allows helminths to complete their life cycles. Consequently, direct and indirect losses have been described (Chubb and Wakelin, 1963; Ramadan and Znada, 1991; Kilpinen et al., 2005; Daş et al., 2010).

Ascaridia galli can be classified as one of the most important and pathogenic nematode in chickens due to their relatively large size (7-12 cm), histotropic phase, the role as a vector for *Salmonella spp*. and at long last, their high prevalence, observed in floor and free-range husbandry systems (Permin et al., 1999; Kaufmann and Gauly, 2009). As the application of medication against helminths entails several problems (Craig, 1993; Waller, 1994; Sangster, 1999; Jackson and Miller, 2006), alternative control strategies are needed to be adopted. One promising approach may be the diversity of animal genetics as studies showed that different chicken genotypes profoundly differ in their susceptibility to helminth infections (Permin and Ranvig, 2001; Gauly et al., 2002; Kaufmann et al., 2010). It is clear, that there is a considerable variation within and between individuals of different breeds. However, as most of the studies focused on comparisons of two genotypes (Permin and Ranvig, 2001; Gauly et al., 2002), availability of information about resistance of a larger variety of different laying hen strains is limited. Therefore, the aim of the present study was, to compare resistance of six common commercial laying hen strains to an experimental *Ascaridia galli* infection.

3.2 Materials and methods

3.2.1 Animals and management

The commercial lines Lohmann Brown (LB), Lohmann Silver (LSi), Isa Brown (ISA), Tetra SL (TETRA), Lohmann Tradition (LT) and Lohmann Selected Leghorn classic (LSL) were used. One day old chicks were marked with wing tags and raised together as one flock for 19 weeks under helminth free conditions in a floor husbandry system at a commercial raising farm. The farm was certified as an organic farm according to the guidelines of the European Union and national guidelines (Bioland, 2010).

Maximum stocking density during the raising period was 18 kg of body weight per m^2. At an age of seven days all animals were vaccinated with an anticoccidial vaccine (Paracox®-8, Essex, Germany). No anthelmintic treatment was given during the trial.

At 19 weeks of age, the hens were brought to the experimental stable at the Department of Animal Sciences, University of Goettingen. The hens were then allocated to six groups according to the genotypes. Hens of each genotype were kept in separated pens within the same experimental stable. Number of animals per genotype ranged between 50 and 57. The hens were allowed to adopt to the new husbandry conditions for 7 days until start of the experimental trail with artificial infection at an age of 20 weeks. On this day all hens were weighed and their integument was evaluated. All management and husbandry conditions but outdoor access fitted the mandatory guidelines for organic egg production. In order to avoid natural helminth infections, outdoor access was not provided. During the experimental period, an automatic 15 h / day light program was installed. Wood shavings were used as litter and were replaced twice a week. A commercial, organic diet (17 % crude protein, 10.9 MJ ME / kg DM) and drinking water were offered *ad libitum*. Group based laying performance and feed consumption was determined daily and weekly, respectively.

3.2.2 Experimental infection

The hens were infected at an age of 20 weeks after one week of adaptation period to new husbandry conditions. Infection material was produced at the Department of Animal Sciences, University of Goettingen. Female *Ascaridia galli* worms harvested

from the intestines of naturally infected chickens were used as source of the eggs. Therefore, the uteri of mature female worms were cut and eggs squeezed out using a pastel on tea sieve. Residual material and worm tissue on the screen were flushed and removed, and eggs collected in a petri dish. Harvested eggs were incubated in a media containing 0.1 % potassium dichromate for 2 weeks at room temperature. After incubation, egg culture was stored in a fridge at 4°C until day of infection. For the infection, the culture media was diluted with tap water and egg concentration (egg / ml suspension) within this suspension was determined, after several repetitions, using a McMaster egg counting slide. Eggs only with clear morphological formations were classified as embryonated. The infection dose was adjusted to 500 eggs / 0.25 ml suspension. Each hen was then infected orally by administering 0.25 ml suspension using a 5 cm cannula with olive tip. Hens were slaughtered 105 d *post-infectionem* (*p.i.*).

3.2.3 Faecal sampling and scoring of the integument

The condition of the integument was scored using a methodology adopted from Tauson et al. (1984) and Keppler et al. (2001). Therefore, 6 body regions (neck, wings, back, tail, vent and breast) were scored using a scale from 1-4, where 1 represented the best and 4 the worst condition. Furthermore, injuries were evaluated by scoring the same regions plus the comb with two scores (1 = no injuries, 2 = injured). Evaluation of the feather and injuries was performed by one person, and it took around 30 seconds per hen.

Ascaridia galli egg excretion in selected individuals of each group was determined at 7, 10 and 13 wk *p.i.*. One the first sampling date (7 wk *p.i.*), 20 hens per group were selected randomly, later the same hens were repeatedly examined at wk 10 and 13. Each hen was placed in single cage in order to defecate. Single droppings were collected and egg count per gram of faeces (EPG) determined using McMaster methodology with a sensitivity of 50 eggs per gram of faeces (MAFF, 1986). Faeces samples were taken at same daytimes to avoid variation in egg counts due to fluctuation of nematode egg excretion within one day (Oju and Mpoame, 2006).

3.2.4 Slaughtering and parasite processing

All hens were slaughtered group wise after weighing and electrical stunning 105 days *p.i.* at an age of 35 weeks. After slaughter gastrointestinal tracts were removed, opened longitudinally, and washed in tap water following the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines for evaluating the effectiveness of anthelmintics in chickens and turkey (Yazwinski et al., 2003). The contents were poured into a sieve with a mesh aperture of 100 µm, washed and examined for the presence of *A. galli*. Adult and visible parasites were collected first and stored in tap water until further examinations. The residuals on the screen were transferred into one or two Petri dishes, depending on the amount of content, and examined for the presence of immature worms under stereomicroscope.

All visible parasites were collected first, and then the content of the gastrointestinal tract and the scraped mucosa was examined under a 20x dissecting microscope. Parasites were counted and stored until differentiation in tap water. Afterwards they were examined under a light microscope at a 40x magnification and the sex was differentiated based on morphological keys according to Soulsby (1982) and Norton and Ruff (2003). For each hen, a maximum of ten adult, randomly selected *A. galli* per worm sex were measured for length using a ruler.

3.2.5 Statistical analyses

Performance data were collected based on separately housed group of genotypes, therefore no statistical analyses were performed, but the descriptive means were given.

Because of non-normal distributions, worm counts and number of eggs per gram of faeces (EPG) were log-transformed with the following function; Log_Y= log10(Y+10). Effect of breed on the log-transformed worm counts were analyzed with a one way ANOVA using the GLM procedure of SAS (2010). Tukey test, as a post-hoc test after a significant ANOVA p-value, was employed to separate group differences. Because EPG was quantified from faecal samples of identified (known) chickens at three different time points, EPG data were analyzed with the following model and using the Mixed procedure for repeated measurements.

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

where;

Y_{ijkl}= observation.

μ = the overall mean

α_i = the effect of genotype (i = 1- 6)

β_j = the effect of sampling time (j = 1- 3)

$(\alpha\beta)_{ij}$ = the interaction effect between genotype and sampling time (ij = 1 - 18)

a_k = random effect of repeatedly sampled hen

ε_{ijkl} = residual random error.

All hens had the best possible plumage score and no injuries on the infection day. Thus, the change of overall integument from the start to termination of the experiment was statistically evaluated. Sum of both feather and injury scores were combined in order to create an index for evaluating the whole integument condition of a bird. For this, single scores for each body region were summed up and divided by the number of scored regions. For the injury quotient, total injury score was divided by 7. Plumage and injury quotient were summed up and divided by 2 in order to get a quotient which evaluates the integument (IQ). Because the distribution of IQ was not normal, differences (Δ) between the integument quotients of each hen at the first and the last day of the experiment (slaughter) was used to estimate the effect of genotype on the overall feather and injuries differences during the course of infection. Furthermore, correlations between changes in the integument conditions (Δ) and total worm burdens of the hens were calculated for each genotype, separately.

3.3 Results

3.3.1. Performance parameters

Genotype based average performance parameters are summarized in Table 1. There were no large differences among the genotypes between their average laying performance (85 - 89 %), egg weights (55 - 59 g) and feed intake (114.7 - 122.1 g/d). TETRA hens had the most favourable feed conversion rate (feed intake per kg egg mass), whereas LSi hens had the less favourable feed conversion rate.

Table 1. Average performance parameters of the genotypes.

	LSi	TETRA	ISA	LT	LB	LSL
Laying performance (%)	86	87	89	85	88	85
Egg weight (g)	55.6	59	58.7	59.3	58.3	58.1
Feed intake, g / hen day	120.8	114.7	122.1	117	116.4	118
Feed intake g / g egg mass	2.48	2.17	2.32	2.32	2.26	2.39
Slaughter weight, g	2029	1909	2037	2042	1925	1753

3.3.2 A. galli infection parameters

A total of 285 hens (92.2 %) hens were *Ascaridia galli* positive. Prevalence of the infection in LSi, Tetra, LB and LSL group were 84 %, 89.1 %, 84 % and 96.2 %, whereas it was 100 % in both ISA and LT hens (Table 2).

Differences between total worm counts of the genotypes were significant (P = 0.008; Table 2). LSL hens had the highest (25.8) and LT hens had the lowest (12.9) worms per bird. Although worm burden of LSL hens did not differ between those of TETRA and ISA ($P > 0.05$), they had higher worm burdens than LSi, LT and LB hens ($P < 0.05$). ISA hens also had higher worm burdens compared to LT and LB hens ($P < 0.05$). LSL and ISA hens had higher number of larva than LSi, TETRA, LT and LB hens ($P < 0.05$). Number of male worms were lower in LB hens than hens of all the other genotpyes ($P < 0.05$). Number of female worms as well as worm lengths and worm sex ratio did not differ significantly between the groups ($P > 0.05$).

There was a significant interaction between effects of genotype and sampling time on the EPG counts ($P < 0.0001$). LSL hens had higher EPG counts than hens of all the other genotypes 7 weeks after infection ($P < 0.05$; Figure), but no significant differences ($P > 0.05$) between the genotypes at weeks 10 and 13 could be quantified. Fecal egg counts of all groups decreased slightly during the trail.

There was a significant effect of genotype on the overall difference in the feather and injuries (Δ) during the course of infection ($P < 0.001$). LSL hens had higher Δ values than the hens of other genotypes ($P < 0.05$). No significant ($P > 0.05$) correlation between Δ and total worm burden was found within any of the genotypes. Correlation coefficients between Δ and total worm burden ranged from r = -0.25 to r = 0.08 ($P >$ 0.05) for different genotypes.

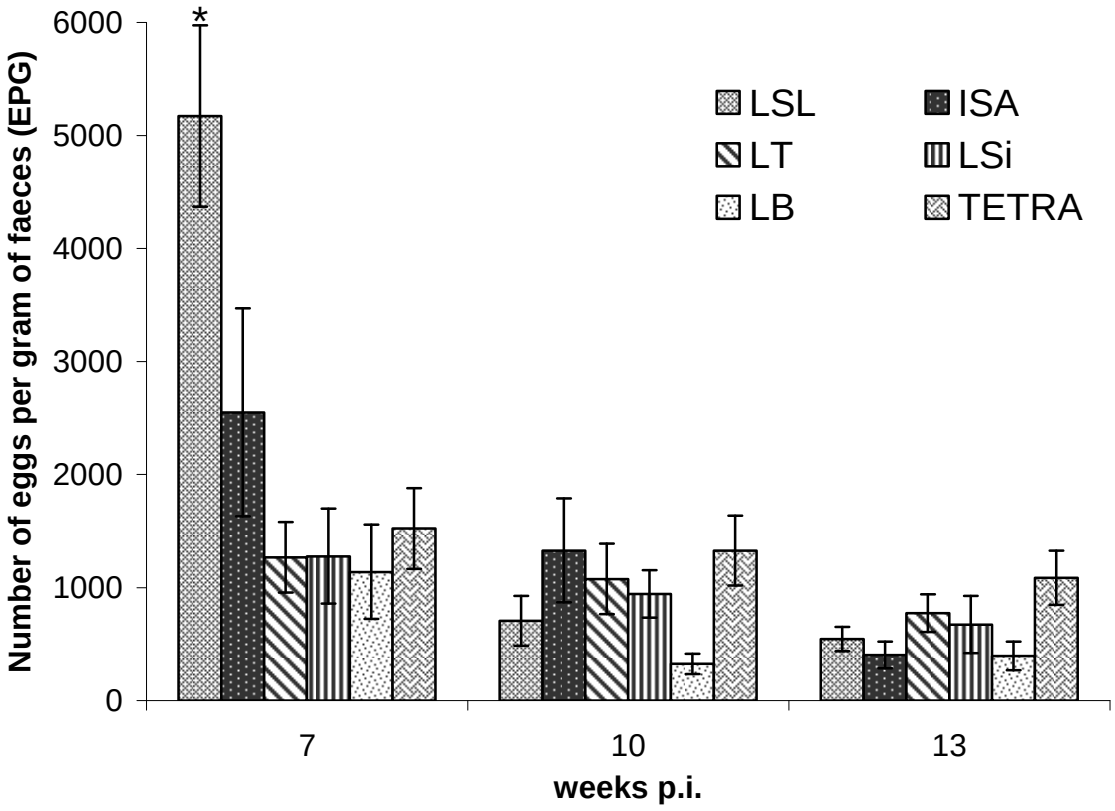

Figure. *Ascaridia galli* egg excretion of 20 hens per genotype over the experimental weeks *p.i*. (Means and SE on the error bars).

(*): EPG was significantly higher in LSL than the other genotypes at 7 wk *p.i*. (Tukey, $P < 0.05$ after a significant interaction between effects of genotype and sampling time, $P < 0.0001$).

Table 2. Prevelance, worm counts, length of the worms and sex ratio in the experimental genotypes (mean ± SE).*

	LSi	TETRA	ISA	LT	LB	LSL	p-value
Prevalence (%)	84	89.1	100	100	84	96.2	-
Total worms	$16.2^{ab} \pm 2.87$	$20.5^{abc} \pm 2.73$	$23.8^{bc} \pm 2.78$	$12.9^{a} \pm 2.89$	$13.8^{a} \pm 2.87$	$25.8^{c} \pm 2.81$	0.0008
- Larva	$5.8^{a} \pm 0.98$	$4.9^{a} \pm 2.27$	$9.6^{b} \pm 1.53$	$4.6^{a} \pm 0.85$	$6.5^{a} \pm 1.16$	$15.6^{b} \pm 2.90$	<0.0001
- ♂-worms	$4.6^{a} \pm 1.18$	$7.1^{a} \pm 1.33$	$7.1^{a} \pm 1.04$	$4.1^{a} \pm 0.66$	$3.5^{b} \pm 0.87$	$4.8^{a} \pm 0.83$	0.0215
- ♀-worms	5.7 ± 1.30	8.5 ± 1.64	7.0 ± 1.17	4.2 ± 0.69	3.8 ± 0.80	5.3 ± 0.91	0.0674
♂-length	7.0 ± 0.16	7.1 ± 0.14	6.8 ± 0.19	6.8 ± 0.18	7.3 ± 0.15	6.7 ± 0.20	0.1908
♀-length	8.9 ± 0.31	8.9 ± 0.28	9.5 ± 0.27	8.6 ± 0.28	9.7 ± 0.39	9.5 ± 0.34	0.1371
Sex ratio (♂: ♀)	1.6 ± 0.29	1.4 ± 0.17	1.2 ± 0.12	1.3 ± 0.14	1.4 ± 0.17	1.3 ± 0.18	0.6529
Δ-IQ	$0.03^{a} \pm 0.01$	$0.03^{a} \pm 0.01$	$0.03^{a} \pm 0.01$	$0.06^{a} \pm 0.01$	$0.06^{a} \pm 0.01$	$0.14^{b} \pm 0.01$	<0.001

(*): Statistical analyses are based on the transformed data, but the presented values are based on raw data.

$^{a;b;c}$: Different superscripts within the same line represents significant differences (Tukey; $P < 0.005$).

3.4 Discussion

Alternative production systems, particularly organic farming, profoundly differ from the conventional production systems in the environmental conditions provided to the animals. The use of large number of available modern breeds, which have been developed with the aid of intense selection programs for conventional production, appears to be inevitable in organic systems (Magnusson, 2001). One of the main constrains in organic farming is that animals are exposed to many pathogenic agents including parasitic infections. Therefore it is crucially important to determine which genotypes can perform best together with exhibiting a natural superiority when exposed to one of the most common nematodes found in alternative systems. We compared six genotypes of commonly used chickens for their ability to resist an experimental *A. galli* infection.

Although average daily feed intake level of the genotypes seems to be higher, their performance did not show any major deviations from the information given by the breeder companies (www.isapoultry.com; www.tetraamericana.com; www.ltz.de). Though *A. galli* infection can induce negative effects on the host animal feed intake and performance (Ramadan and Abou Znada, 1991; Permin and Hansen, 1998; Daenicke et al., 2009; Daş et al., 2010), due to lack of uninfected control groups, it remained unclear whether feed utilization and performance of the genotypes was influenced by the infection in the present study.

Compared to similar studies, average worm burdens of the genotypes were relatively high (Permin and Ranvig, 2001; Gauly et al., 2002). This may be explained by the higher infection dose and the longer duration of the present experiment. As the hens were slaughtered 13 wk after infection and *A. galli* requires 5 - 8 wk for the prepatent period (Anderson, 1992; Norten and Ruff, 2003) the nematode must have been able to complete its lifecycle and thus, in agreement with Gauly et al. (2005), imposing risk of re-infections with second generation eggs.

The examined genotypes showed a considerable variation in their responses to the nematode infection, as measured by the worm counts per hen. LSL hens were less resistant than LT, LB and LSi hens to the nematode infection. LT and LB hens were also more resistant than ISA hens. In agreement with others (Permin and Ranvig, 2001; Gauly et al., 2002) LSL hens are the most susceptible genotype whereas LB and LT appear to be most resistant genotypes. Although LSL hens had the total higher worm

counts, their adult worm counts (female and male worms) did not highly differ than those of the other breeds, whereas they harboured higher number of larvae. This observation may not only indicate a less effective immune response of this genotype to the re-infections, but may also indicate that production is prioritized over resistance by the genetic background of this breed. Since LSL hens had the lowest body weights, their egg weights and laying performance were similar to those of the most resistant breeds (LT and LB). Therefore ability of the LSL hens to perform well despite the heavier nematode infection may be considered as higher level of resilience.

A. galli egg excretion 7 wk after infection was on a high level but decreased during the trail. Juvenile hens appear to be more susceptible to endoparasite infections compared to older hens. Studies have shown that young hens up to an age of 3 month are highly most susceptible to *A. galli* infections (Idi et al., 2004; Gauly et al., 2005). In the current study, the experimental infection was performed at an age where the hens are about to start egg production. This is a somewhat critical point as serum levels of estradiol and progesterone increase about seven days before start laying (Proszkowiec and Rzasa, 2001). Both hormones are known to depress the immune system which affects the susceptibility to a coetaneous *A.galli* infection (Gauly et al., 2005). This may explain the high EpG counts at the first sampling date. After this first contact with the pathogen *A. galli*, hens then might have developed a resistance (Ackert et al., 1935; Tongson und McGraw, 1967; Marcos-Atxutegi et al., 2009).

Although re-infection occurred, EpG counts decreased throughout the experiment. This could be explained with the 'self-cure' phenomenon which is considered as an immunological reaction of the host leading to an expel of adult worms (Soulsby and Stewart, 1960; Gray, 1973; Permin and Ranvig, 2001).

The overall difference in the integument condition of the hens during the infected period was worse in LSL hens than in the hens of other genotypes. Because LSL hens had the highest worm counts, change in the integument of LSL hens may be attributed to the infection. Gauly et al. (2007) reported that *A. galli* infection increases the level of serum testosterone which then favours antagonistic behaviours and in turn results in an increased tendency of feather pecking in chickens (Gauly et al., 2007). However, within genotype correlations between the change in integument and the worm burden of the birds indicate that such an effect may not be linear. Non-significant and low correlation coefficients also indicate that there is no practically observable linear relation between appearance and worm burden of the birds.

3.5 Conclusion

Our results suggest that there is a considerable variation in the responses of most commonly used chicken genotypes to the nematode infection. Although no large differences were observed in the performance of the genotypes, LT and LB hens were more resistant than LSL and ISA hens when exposed to an experimental *A. galli* infection. It is concluded that genetic differences in the responses of the genotypes to the nematode infection may contribute to the efforts for selecting more suitable genotypes for alternative floor production systems, where hens face poor biosecurity.

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CHAPTER IV

Genetic resistance to natural helminth infections in two chicken layer lines

Abstract

Groups of Lohmann Brown (LB) and Lohmann Selected Leghorn (LSL) hens were reared under helminth-free conditions and kept afterwards together in a free range system. Mortality rate, body weight development, laying performance and faecal egg counts (FEC) were recorded during a 12 month laying period. At the end of the laying period, 246 LSL and 197 LB hens were necropsied and worms counted following the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines (Yazwinski et al., 2003). In addition adult *Heterakis gallinarum* and *Ascaridia galli* were sexed and measured for length.

Significant ($P < 0.01$) differences were observed in mortality rates between LSL and LB animals (12.9 vs. 5.7 %). LSL hens showed significantly ($P < 0.05$) higher FEC when compared with LB hens at almost all dates of monitoring. Almost all animals became infected with at least one helminth species. The most prevalent species were *H. gallinarum*, *Capillaria spp.* and *A. galli*. LB hens showed a significantly ($P < 0.05$) higher average number of adult *H. gallinarum*, *Capillaria spp.* and tapeworms when compared with LSL animals. However, number of adult *A. galli* was in tendency lower in these animals. In total, LB had a significantly ($P < 0.05$) higher worm burden than LSL (192.3 vs. 94.3). The estimated heritabilities for total worm burden were 0.23 (SE ± 0.12) in LSL and 0.75 (SE ± 0.21) in LB, respectively. The number of all different helminth species was positively correlated.

The sex ratio of *H. gallinarum* and *A. galli* and the average worm lengths were not significantly ($P > 0.05$) different between the genotypes.

There was no significant phenotypic correlation between body weight and worm burden in LSL, whereas it was the case in LB ($r = 0.17$, $P < 0.05$).

Based on the estimated heritabilities it is possible to select for helminth resistance in both genotypes.

4.1 Introduction

The ban on the conventional cages for laying hens has led to reemerging of a range of different parasitic infections in alternative farming systems (Fossum et al. 2009). The economically most important endoparasites of poultry are *Eimeria* species and helminths (Voss, 1999; Norton and Ruff, 2003; McDougald, 2003; Daugschies, 2006; Bauer, 2006). Among these helminths, *Ascaridia galli*, *Capillaria spp*. and *Heterakis gallinarum* are the most prevalent species (Permin and Hansen, 1998; Permin et al., 1999; Irungu et al., 2004; Kaufmann and Gauly, 2009). They can damage the intestinal mucosa, sometimes leading to weight depression (Kilpinen et al., 2005), haemorrhages, anaemia and severe diarrhoea. Heavy *A. galli* infections may obstruct the small intestine and cause death (Ramadan and Znada, 1991). Furthermore, parasites can act as vectors and lead to secondary infections e.g. *E. coli*, (Okulewicz and Zlotorzycka, 1985; Chadfield et al., 2001; Dahl et al., 2002; Permin et al., 2006). Furthermore they have adverse effects on behaviour patterns, growth and nutrient utilization of chickens (Chubb and Wakelin, 1963; Gauly et al., 2007; Daş et al., 2010).

The use of anthelmintics is very limited in layers regarding economic concerns as well as environmental and hygiene regarding development of drug resistance (Jackson and Miller, 2006) and chemical residues (Craig, 1993; Waller, 1994; Sangster, 1999). Therefore alternative control strategies need to be adopted (Heckendorn et al., 2009).

There is a large body of evidence that there exists a genetic basis for resistance to gastrointestinal nematodes in various species (Barger, 1993; Stear and Murray, 1994; Pralomkarn et al., 1997; Berthelot et al., 1998; Gasbarre and Miller, 2000; Gauly et al., 2002c).

Gauly et al. (2002a) estimated heritabilities for mean log FEC in white (Lohmann LSL) and brown (Lohmann Brown) laying hens artificially infected with embryonated *A. galli* eggs at an age of 20 weeks between 0.13 and 0.19 for white and 0.0 and 0.10 for brown layers. LB animals were more resistant when compared with LSL. The same authors recently estimated heritabilities for logarithm (ln) worm burden in two chicken layer lines when artificially infected with 100 embryonated *H. gallinarum* eggs at an age of 8 weeks. Estimates were 0.41 (SE ± 0.09) in White Leghorn (WL) and 0.31 (SE ± 0.13) in New Hampshire (NH), respectively (Gauly et al., 2008). WL showed a significantly (P = 0.011) higher number of worms when compared

with NH animals. As both studies are based on experimental mono-infections under controlled conditions, it is not known whether a natural mixed infection and/or environmental changes may affect the estimates of heritabilities. In contrast to controlled conditions, natural conditions are highly unpredictable. According to Ezenwa (2003) group size and host social behavior influences infection risk and has effects on host parasite load. Once an infection occurred there is evidence for heterologous synergistic interactions between helminths being mediated by immunosupression (Christensen et al., 1987). On the other hand, as the technique of giving all animals certain number of larvae in experimental infections eliminates possible between-hens differences in larval intake and restricts possibility of re-infections, ensured infections under natural conditions may be considered as more appropriate testing conditions. However, to our knowledge no genetic parameters for helminths resistance in chickens under natural conditions have been estimated so far. Therefore, the aim of this study was to estimate the heritability of worm burden in two chicken genotypes infected with various helminths under in a free range system.

4.2 Materials and methods

4.2.1 Animals and management

One day old female chicks with defined origin were used in the study. The chicks originated from two different commercial lines (Lohmann Selected Leghorn (LSL, N = 339); Lohmann Brown (LB, N = 254) maintained from Lohmann Tierzucht GmbH, Cuxhaven, Germany. Within each line, offspring were produced by mating each of 20 sires, representing different sire families, to 10 dams each. From both lines an average of 17 daughters per sire were used in the study. In maximum two animals descended from one hen. At the first day of age all animals were marked with numbered wing tags and raised together in a floor system with other 337 LB chicks descending from the same sires. Maximum stocking density during raising period was 18 kg of body weight per m^2.

At an age of 19 weeks the animals were brought to a commercial layer farm and kept in an organic free range system. The selected farm proved to be naturally contaminated with helminths as layers form this farm were sampled for a prevalence

study half a year before. All hens (N = 930) were kept together as a flock for the whole laying period. Average stocking density was 6 animals per m^2. The animals were helminth-free at that time as confirmed by faecal examinations.

A commercial diet and water were provided *ad libitum*. The energy levels of the diets were between 11.4 and 12.0 MJME (1 - 19 weeks of age) and 11.0 MJME (> 20 weeks of age), respectively. The protein levels ranged from 21.0 to 18.5 % (1 - 19 weeks of age) and 18.0 to 14.5 % until slaughtering.

The light program followed the recommendations for commercial layers.

At an age of seven days all animals were vaccinated with an anticoccidial vaccine for chickens (Paracox®-8, Essex, Germany). The vaccine was administered directly into the water in bell-drinkers. Beside this no vaccinations and anthelmintic treatments were given during the trial.

4.2.2 Mortality rate, clinical examinations and performance

Mortality rate (%) was recorded during the whole laying period. Number of eggs (white and brown) belonging to the different commercial weight categories (S < 53 g, M 63 – 53 g, L 73 – 63 g, XL > 73 g) were recorded on a daily basis. Based on this, the average laying performance for both lines was estimated. Furthermore, beginning with an age of 20 weeks, 20 animals per line were randomly selected every second month and weighed on an electronic scale with a precision of ± 5 g.

4.2.3 Faecal egg counts (FEC)

After the above mentioned weighing procedure individual faecal samples were collected from those animals to quantify FEC. Therefore, each hen was housed separately in a cage for a short time and fresh droppings were taken from the cage bottom. Individual faecal samples were examined by a modified McMaster technique with saturated sodium chloride solution using the MSD counting chamber, adapted to detect minimum egg counts of 50 eggs per gram of faeces. *H. gallinarum* /*A. galli,* and *Capillaria spp*. eggs were counted separately.

4.2.4 Worm burden

246 LSL and 197 LB hens were harvested at the end of the laying period (12 months). The gastrointestinal tracts and tracheas were removed, opened longitudinally, and washed in tap water following the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines for evaluating the effectiveness of anthelmintics in chickens and turkey (Yazwinski et al., 2003). The contents were poured into a sieve with a mesh aperture of 100 µm, washed and examined for the presence of adult helminths. The residuals on the screen were examined for the presence of adult and immature worms under stereomicroscope. All adult worms were counted. However, adult *Acuaria hamulosa*, which were partly encapsulated in the gizzards could not be counted.

4.2.5 Parasite processing and identification

All visible parasites were collected first, and then the content of the gastrointestinal tract and the scraped mucosa was examined under 20x dissecting microscope. Parasites were counted and stored until differentiation in tap water. Identification of nematodes started with cleaning the worms in normal saline solution. Afterwards they were examined under a light microscope at 40x magnification and differentiated based on various morphological parameters according to Soulsby (1982) and Norton and Ruff (2003).

The collection of cestodes was done by submerging the intestine in water to float the worm and increase its visibility. In some cases, the scolices were strongly attached to the mucosa. To liberate the scolice, the attachment points were located; the intestine was cut around the attachment point and refrigerated in water for 2 h. After thawing, scolices were released easily out of the mucosa using fine needles. Cestodes were identified using the helminthological keys according to Soulsby (1982), Schmidt (1986), Jones et al. (1994) and McDougald (2003). Staining-destaining techniques with Carmine dye were done for exact identification of testes and cirrus.

All adult *A. galli* and *H. gallinarum* worms found were sexed as determined by Hartwich (1975). Furthermore, for each hen, a maximum of ten randomly selected worms per worm sex of this species were measured for length using a ruler.

4.2.6 Statistical Analysis

Statistical analyses were performed applying mixed model methodology as available in the statistical package SAS Version 9.1 (Little et al., 1999). Worm burden data were log transformed [log(worm burden+10)] to get approximately normally distributed data. Log(worm burden+10) transformation was superior to other transformations as indicated by the values of skewness and kurtosis. All analysis regarding parasitological paramaters were performed using the log transformed data. Data related to worm burden were analyzed with a general linear model including the fixed effect of breed and the random effect of the sire within breed. For worm count analysis the following model was used: $Y_{ijk} = \mu + br_i + sire(br)_{ij} + e_{ijk}$ (Y_{ijk}= observation for the trait, μ = overall mean effect, br_i = effect of breed, $sire(br)_{ij}$ = random sire effect nested within breed, e_{ijk} = random residual effect).

Data related to FEC were log transformed [log(FEC+25)] and analyzed with a general linear model including the effect of genotype. The analyses were done for each sampling time separately. The same model was applied for the body weight data that were taken at different sampling dates. Worm sex ratio and worm length data were also analyzed with the same model that included effect of genotype. Analyses were performed for *A. galli* and *H. gallinarum* separately.

Differences for the prevalence of each worm species between the genotypes were analyzed with the Chi-square test. The same Chi-square test was also used for the mortality data.

Heritabilities stratified by breed were estimated within an animal model using REML-methodology and the program VCE4, version 4.2.5 (Neumaier and Groeneveld, 1998). The following animal model was used: $Y_{ijk} = \mu + a_i + e_{ijk}$ (Y_{ijk}= observation for the trait, μ = overall mean effect, a_i = random additiv genetic animal effect, e_{ijk} = random residual effect).

Heritabilities and genetic correlations for the whole dataset, i.e. including animals of both breeds, were estimated applying the following animal model: $Y_{ijk} = \mu + a_i + br_i + e_{ijk}$ (Y_{ijk}= observation for the traits, μ = overall mean effect, a_i = random additiv genetic animal effect, br_i = fixed effect of breed; 1 = LB, 2 = LSL, e_{ijk} = random residual effect).

4.3 Results

4.3.1 Mortality rates and performance

Significant differences ($P < 0.01$) were observed in the mortality rates between LSL and LB animals (12.9 vs. 5.7 %). Mortalities were almost continuous distributed over all laying months in both lines.

Laying performance was not significantly different between the lines during the entire laying period. The average laying performance was 79.3 % in LB and 79.7 % in LSL hens and the estimated average number of eggs per average hen was 271 for LB and 279 for LSL, respectively.

Percentage of eggs belonging to the different commercial weight categories for LB and LSL were 1.9 and 1.6 for S, 43.9 and 43.8 for M, 47.4 and 48.9 for L and 3.2 and 2 for XL eggs, respectively. Differences between the lines were not significant. Distribution and percentage of break eggs (LB 2.3 %, LSL 1.9 %) were not significantly different between the lines.

Body weights of LB animals were significantly ($P < 0.05$) higher when compared with LSL animals at all ages. The average body weight at the beginning and end of the laying period were 1743 (SE ± 31) and 2068 g (SE ± 59) in LB and 1605 (SE ± 25) and 1792 g (SE ± 49) in LSL, respectively.

4.3.2 Faecal egg counts (FEC)

FEC increased from 0 (sampling at the time of housing) to an average of 402 in LB and 851 in LSL at the time of third sampling (month 5 to 6), respectively (Table 1). Afterwards FEC decreased in both lines. 3rd and 4rd samples were significantly ($P < 0.05$) higher in LSL when compared with LB hens.

Table 1. Means (± SE) of faecal egg counts in LB and LSL hens (N = 20 per breed and sampling date) during the laying period.

Laying month	LB	LSL
1	0	0
3	154 ± 35.3	183 ± 65.7
5	402[a] ± 173.3	851[b] ± 213.2
7	374 [a] ± 137.8	713 [b] ± 162
9	166 ± 53.7	210 ± 48
11	251 ± 130.6	181 ± 50.5

[a, b] Means presented in the same line with different superscripts are significantly different ($P < 0.05$).

4.3.3 Worm burden and species

99.2 % (N = 244) of the LSL and 98.5 % (N = 194) of the examined LB hens were helminth positive. The following species were found: *Ascaridia galli, Heterakis gallinarum, Capillaria spp., Acuaria hamulosa, Raillietina cesticillus, Hymenolepis cantaniana, Hymenolepis carioca* and *Choanotaenia infundibulum*

The prevalence of the different species in both lines is given in table 2. The prevalence of *Capillaria* spp., *Acuaria hamulosa* and Tapeworms was significantly ($P< 0.05$) higher in LB hens, whereas prevalence of *Ascaridia galli* was significantly higher in LSL hens, respectively. Tapeworms were not further differentiated as their number didn't differ significantly between the species.

Number of adult *A. galli* worms tended ($P = 0.08$) to be higher in LSL hens than in LB hens (9.9 vs. 7.3).

However, LB hens harboured significantly ($P < 0.05$) higher numbers of adult *H. gallinarum* (162 vs. 76.5), *Capillaria spp.* (20.7 vs. 7.1) and tapeworms (2.3 vs. 0.8). Therefore the total mean worm burden was significantly ($P < 0.05$) higher in LB than LSL (192.3 vs. 94.3; Table 3).

50.5 % of the LB hens carried less then 100, 27.4 % between 100 and 250 and 21.9 % more then 250 worms. The distribution was 65.9, 27.3 and 6.9 % in LSL, respectively. *Acuaria hamulosa* was not included in the calculations.

The sex ratio (male : female) of *H. gallinarum* was 1.0 : 1.52 in the LB and 1.0 : 1.49 in the LSL, this of *A. galli* was 1.0 : 1.42 LB and 1.0 : 1.41 in LSL, respectively.

The differences in both lines were not significant ($P > 0.05$). The average length of male and female *H. gallinarum* worms were 7.3 to 7.4 (SE ± 0.09) and 8.5 to 8.7 mm (SE ± 0.1). The average length of male and female *A. galli* worms were 4.9 to 5.0 (SE ± 0.16) and 6.9 to 7.0 cm (SE ± 0.23), respectively. Differences between the lines were for both species not significant ($P > 0.05$).

Table 2. Prevalence (%) of different helminth species in LB (N = 197) and LSL (N =246) hens naturally infected.

Species	**LB**	**LSL**
Ascaridia galli	70.1[a]	78.5[b]
Heterakis gallinarum	96.5	98
Capillaria spp.	86.3[a]	58.1[b]
Acuaria hamulosa	41.8[a]	16.9[b]
Tapeworms (all)	37.6[a]	21.1[b]
Total	98.5	99.2

[a, b] Means presented in the same line with different superscripts are significantly different (Chi-square; $P < 0.05$).

Table 3. Mean worm burden (± S.E.), minimum (X), maximum (Y) and number of worms in LB and LSL hens.

	LB		**LSL**	
Species	Mean ± SE	X – Y	Mean ± SE	X – Y
Ascaridia galli	7.3 ± 0.9	0 - 73	9.9 ± 0.8	0 - 81
Heterakis gallinarum	162[a] ± 14.6	0 - 2509	76.5[b] ± 13.1	0 - 728
Capillaria spp.	20.7[a] ± 1.3	0 -168	7.11[b] ± 1.5	0 - 78
Tapeworms (all)	2.3[a] ± 0.3	0 - 48	0.8[b] ± 0.3	0 - 12
Total worm burden	192.3[a] ± 15.3	0 - 2696	94.3[b] ± 13.7	0 - 789

[a, b] Means presented in the same line with different superscripts are significantly different ($P < 0.05$).

4.3.4 Phenotypic Correlations and genetic parameters

The estimated phenotypic correlations between different worm species, total worm burden and body weights are given for LB and LSL in Table 4. Number of *H. gallinarum* was highly correlated (r = 0.94 - 0.96) with total worm burden in both lines. However, the numbers of all different species were positively correlated.

Phenotypic correlations between body weights at time of harvesting and worm burden were for most species not significantly different from zero. However, for *H. gallinarum* and total worm burden significantly positive correlations were estimated for LB animals. Genetic correlation and body weight was also positive (r = 0.18; Table 5).

Table 4. Phenotypic correlations between different worm species, total worm burden and body weights in LB / LSL hens.

	Ascaridia galli	*Heterakis gallinarum*	*Capillaria spp.*	Tape-worms (all)	Body weights
Total worm burden	0.36***/ 0.45***	0.94***/ 0.96***	0.44***/ 0.41***	0.12/ 0.16*	0.17*/ 0.03
Ascaridia galli	-	0.22**/ 0.25***	0.35***/ 0.50***	0.22**/ 0.15*	-0.05/ -0.06
Heterakis gallinarum		-	0.21**/ 0.23**	0.01/ 0.10	0.18*/ 0.04
Capillaria spp.			-	0.11/ 0.18**	-0.05/ -0.14*
Tape-worms (all)				-	0.17*/ 0.03

**** P < 0.001*; *** P < 0.01*; ** P < 0.05*

The estimated heritabilities for total worm burden were 0.23 (SE ± 0.12) in the LSL, 0.75 (SE ± 0.21) in the LB hens and 0.66 (SE ± 0.13) over both genotypes, respectively. Heritability estimated for the worm number of the different species ranged between 0.01 and 0.69 (Table 6). Estimated heritabilties for body weights at slaughtering were 0.65 (± 0.14) for LB and 0.40 (± 0.12) for LSL, respectively.

Table 5. Genetic correlations (SE) estimates for the no. of worms in LB and LSL hens (N=443).

	Ascaridia galli	*Heterakis gallinarum*	*Capillaria spp.*	Tape-worms (all)	Total worm burden
Body weight	- 0.06	0.18	0.01	0.18	0.18
	(0,13)	(0.06)	(0,17)	(0,16)	(0.16)
Ascaridia galli		0.37	-0,15	-0,78	0.35
		(0.06)	(0,27)	(0,04)	(0.27)
Heterakis gallinarum			0.84	- 0.19	0.67
			(0,15)	(0.23)	(0.07)
Capillaria spp.				0.31	0.86*
				(0,35)	
Tape-worms (all)					-0.35
					(0,73)

*Standard error not returned due to nonconvergence of the model

Table 6. Heritabilities (± SE) estimates for the no. of worms in LB and LSL hens (N=443).

Helminth species	LB	LSL	LSL and LB
Ascaridia galli	0.11 (± 0.07)	0.13 (± 0.06)	0.10 (± 0.06)
Heterakis gallinarum	0.69 (± 0.20)	0.30 (± 0.11)	0.68 (± 0.07)
Capillaria spp.	0.18 (± 0.07)	0.01 (± 0.02)	0.08 (± 0.04)
Tapeworms (all)	0.28 (± 0.12)	0.05 (± 0.05)	0.08 (± 0.05)
Total	**0.75** (± 0.21)	**0.23** (± 0.12)	**0.66** (± 0.13)

4.4 Discussion

Laying performance was not significantly different between the lines during the entire laying period. The values are still in accordance with other reports and breeders information. The commercial breeder is expecting in alternative housing systems from LSL hens 302 to 312 eggs per hen housed in 12 month lay and 295 to 305 from LB, respectively (Lohmann Tierzucht GmbH, www.ltz.de). Peak production should reach in both lines 92 to 95 %, which was not the case in the study. Especially LSL hens underachieved. Probably this was caused by the high level of production at the beginning. Normally at an age of 140 to 150 days hens should only reach a production level of 50 % in alternative housing systems. However, total laying performance was still in the normal range of alternative systems for both lines (LB 79.3 %, LSL 79.7 %). After 12 months of lay egg production it still reached almost 70 %, which is in accordance with earlier studies (Whay et al., 2007). Percentage of eggs belonging to the different commercial weight categories and percentage of break eggs was for both lines in a normal range as average egg weights were.

Body weights of LB animals were significantly ($P < 0.05$) higher when compared with LSL at all ages. Body weight for LSL hens raised and kept in alternative housing should be at 18 weeks 1.2 - 1.3 kg and at the end of production 1.7 - 1.9 kg. For

LB the weights should be 1.6 - 1.7 kg and 1.9 - 2.1 kg, respectively (Lohmann Tierzucht GmbH, www.ltz.de). Both lines were in this range.

Lohmann Tierzucht GmbH is given liveability rates for both lines kept in alternative housing during laying period between 94 and 96 %. In the study rates were 87.1 for LSL and 94.3 % for LB, respectively. Losses (deaths and culls) can range in free range systems from 1.8 to 21.4 % (median 6.95 %) (Whay et al., 2007). Some authors suggests that genotype x environment interactions are existing which have to be considered when alternative housing systems are proposed (Singh et al., 2009). Most of the time losses in laying hens are caused by maladaptive behaviour like feather pecking that may result in cannibalism and ultimately death of the victims. Genetic differences were shown (Bolhuis et al., 2009). Beside cannibalism, common causes of mortality in necropsied laying hens include colibacillosis, erysipelas, coccidiosis, red mite infestation and lymphoid leucosis (Fossum et al., 2009). To what extent internal parasites had an impact in the study remains unclear. However, because mortalities were equally distributed over the whole laying period internal parasites as an alone reason can partly be excluded. Cannibalism may have played a more important role as reported by the farmer.

FEC values in this study were high probably dominated by the eggs of *A. galli*. This worm shows a much higher fecundity when compared with *H. gallinarum* (Fine, 1975; Tompkins and Hudson, 1998) and *Capillaria* spp. (Permin et al., 1997). Permin and Ranvig (2001) showed that chickens are able to expel adult *A. galli* worms with the aid of phenomena known as self-cure. Correspondingly, Marcos-Atxutegi et al. (2009) have shown that *A. galli* stimulates a strong antibody response as well as an intense inflammatory reaction. IgG antibodies against embryonated eggs of *A. galli* and adult worms were detected in both blood and egg from infected hens during a period of 105 days after the infection. This may indicate that decrease of FEC after six months was probably caused by the development of host immunity. The differences observed between FEC of two genotypes overlap this period of time. Differences between development of immunity against different worm species may explain differences observed between FEC of the two genotypes. As shown in the last faecal sampling, there was no significant difference between FEC in both genotypes. This is in agreement with the non significant *A. galli* worm burden of the breeds at slaughter, and indicates that *H. gallinarum* eggs do not account for an important part of FEC derived from the randomly taken faecal samples. This is supported by the fact that eggs of *H.*

gallinarum are passed through periodically dropped caecal feces (Clarke, 1979), and thus are often not counted in non-caecal droppings (Fine, 1975). Such differences in the egg shedding patterns of different worm species indicate the limits of using FEC as indicator for worm burden under conditions of mixed infections.

99.2 % of all LSL and 98.5 % of the examined LB hens were helminth positive at the time of slaughtering. The helminth prevalence (Abdelqader et al., 2008; Maurer et al., 2009) and the range of the species found are in accordance with earlier studies (Poulsen et al., 2000). LSL animals had in tendency more *A. galli* worms when compared with LB hens (7.3 vs. 9.9). Under the conditions of a single artificial infection LB showed higher resistance (Permin und Ranvig, 2001; Gauly et al., 2002a). However, such procedures may under- or over-estimate the true difference between breeds and individuals because factors such as feeding behaviour also influence total worm burden (Gauly et al., 2002a). The different development of protective immunity in the hens (Marcos-Atxutegi et al., 2009) might explain the differences between the hens, lines and sires. However, LB animals showed significantly more *Capillaria spp.* when compared with LSL. Even if most of these species also occurs in the small intestine nothing is known about immune mechanism in birds. Maybe the degree of immunity is differently developed in the different parts of the intestine. This seems also to be the case for *H. gallinarum* and tapeworms and may explain why LB animals showed significantly more adult worms of these species, which is not in agreement with earlier studies where heavy breeds proved to be more resistant when compared with White Leghorns (Ackert et al., 1935). However, correlations of number of worms of different species were significantly positive. This may indicate that somehow resistance is acting the same way within the lines even if helminths are located in very different parts of the intestine or immunity itself is in a better position if a single worm is decreasing in numbers. This will be benefical for selection. Similar results were found in sheep (Kemper et al., 2009).

The significant positive phenotypic and genetic correlation between total worm burden and body weights in LB hens disagrees with favourable or neutral relationships found earlier. However, earlier studies showed that estimates of genetic correlations between parasitological parameters (e.g. worm burden, FEC) and performance traits, such as body weight, can vary in a big range (Bishop et al., 1996; Mandonnet et al., 2001). Bishop et al. (1996) estimated a negative genetic correlation between FEC and body weight in sheep, whereas Mandonnet et al. (2001) estimated moderate positive

genetic correlations, respectively. According to Bishop and Stear (2003) the observed genetic relationships between disease resistance and performance can be seen as a balance of the costs versus the beneficial consequences of being resistant. Increasing costs of resistance will tend to make the relationship more unfavourable.

The reasons for high variations of genetic correlation values in this study remain to be discussed. The relatively high standard errors of the estimated genetic parameters were probably caused by the limited number of animals which were used.

Total worm load seems to be relatively high. Gauly et al. (2005) concluded from their studies that age does not play a major role in resistance to *A. galli* infections in layers, whereas a bird's hormonal and immune status, related to laying activity, seems to have a significant negative impact on resistance. The authors conclude that this may be of importance when hens are brought into an environment with higher risks of re-infection, such as free range, when they are about to start laying. In such a case, the hens would tend to establish a higher worm burden and FEC. This was probably the case in animals used in the study. Furthermore nutrients might preferentially have been allocated to growth or performance traits instead of immunity as it has been proposed by Coop and Kyriazakis (1999) that the function of reproduction (in this case laying) is prioritized over the expression of immunity in adult animals whereas young animals may give priority to the development and expression of immunity. According to the host-parasite model of Doeschl-Wilson et al. (2008), higher mean worm burden corresponds with higher heritabilities when full priority is given for growth over immunity.

The sex ratio of *H. gallinarum* and *A. galli* in both lines was almost equal what is in agreement with earlier findings like lengths and weights of both worm sexes are (Gauly et al., 2001; 2008). However, the proportion of female worms was higher when compared with results from studies dealing with experimental infections (Duß, 2005; Homann, 2007; Gauly et al., 2008). Maybe the success rate of the development of eggs cultivated in media is sex related. On the other hand, possible differences between lifespan of female and male worms may also cause such an imbalanced worm population in natural infections. Higher female: male ratios were reported for dominant nematodes found in the house rat and were thought to be because of different lifespans of the genders (Singhvi and Johnson, 1977).

Estimated heritabilities of mean worm counts were reasonably high. They are mainly in agreement with earlier studies (Gauly et al., 2002a, 2008) beside the relatively

extreme value in LB hens for *H. gallinarum*. However, even if the values are over-estimated in the case of LB or under-estimated for LSL this clearly proves the existence of genetic resistance or variation in chickens. Furthermore, the values estimated over both genotypes agree with heritabilities estimated for nematode resistance in sheep, where breeders have started to integrate this parameter into breeding programs (Gray, 1997; Kominakis and Theodoropoulos, 1999; Vanimisetti et al., 2004).

In conclusion, heritability estimates reported in this study suggest that it is possible to select for helminth resistance in both genotypes based on worm counts. Such an approach should be considered sustainable as an explicit genetic progress for resistance against each single nematode species can be achieved from short to long terms. This may be of importance for chickens kept in alternative and organic farming systems. However, before including resistance parameters into breeding programs for chicken, direct and correlated effects of resistance on traits of economical value have to be calculated under local husbandry conditions.

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CHAPTER V

General discussion

5.1 Spectrum and intensity of helminth infections

The spectrum, prevalences and worm counts of helminth species in the present study were referred for the first time for layers in organic productions systems in Germany. It is mentioned earlier, that parasitic infection rates differ among certain production systems (Zeller, 1990; Morgenstern und Lobsiger, 1993; Permin et al., 1999). Therein, incidence of infection increases from cage systems over deep litter systems to free range farms. Results from the current work (**chapter II**) indicate that prevalence and intensity of helminth infections are even higher in organic free range systems. 99.6 % of the examined hens harboured in average around 218 worms. Those numbers can not only be attributed to the fact that biosecurity in free range systems is poor (Tuyttens et al., 2008), but also draw attention to the distinctive properties of organic farming that appear to provide favourable conditions for helminth infections. The environment is probably more conducive of the free living stages of parasites and host animals are in close and constant contact to faeces. Thus, helminths can easily complete their life cyles no matter whether they are direct or indirect. Due to outdoor access, the parasititic stages as well as the hens are in contact with intermediated host. Parasite eggs are able to remain viable for months under cool and moist conditions (Norton and Ruff, 2003). Therefore, hens face a high infection risk as the prevalent agents are present and the conditions of getting a helminth infection are favourable. Organic production systems may also alter the intensity of helminth infections due certain regulations in law as laying hens must be fed primarily on diets based on the organically produced feedstuffs. Chemically extracted soybean meal and synthetic amino acids are banned by the council regulation (No. 1804/99). Therefore, the hens are often fed fiber rich energy diluted diets in order to benefit from compensatorily increased feed intake, that guarantee adequate amount of essential amino acids (Deerberg et al., 2004; Sundrum et al., 2005; Van de Weerd et al., 2009). However, it has repeatedly been shown that energy diluted diets favour establishment of both *H. gallinarum* and *A. galli* in chickens (Daş et al., 2010b;c;d) as well as fecundity of *H. gallinarum* (Daş et al., 2010d) when compared with a standard diet. On the other hand, inadequate intake of single amino acids, i.e. lysine, may also increase incidence of infections with *A. galli* (Daş et al., 2010d) probably due to an impaired immune response (Konashi et al., 2000; Li et al., 2007). This may explain the broad and highly prevalent helminth spectrum, detected in laying hens obtained from organic farms in Germany (**chapter II**). Beside the high occurrence of heavy helminth infections,

organic egg production systems face other problematic issues which should be taken into account when evaluating such husbandry systems (Berg, 2001; Thamsborg, 2001; Sundrum, 2001; Zeltner and Hirt; 2003, 2008; Fossum et al., 2009; Kaufmann-Bart and Hoop, 2009)

When considering animal health as a major part of animal welfare we may conclude that the situation in laying hen welfare and husbandry is far from being ideal.

5.2 Genetic variation of breeds

As postulated in the general introduction (**chapter I**), the current efforts in animal breeding should offer contributions to improve the situation for free range laying hens in the future by implementation of the following classical three strategies (Baker et al., 1993; Albers and Gray 1986):

- choice and/or substitution of breeds,
- within breed selection and
- crossbreeding.

The results presented in chapter III indicate differences between 6 common, commercial laying hen genotypes in their responses to an experimental *A. galli* infection. Hens of the genotype Lohmann Brown and Lohmann Tradition appear to be most and Lohmann LSL the least resistant. Regarding resistance to *A. galli* infection, farmers may use these results to apply the mentioned strategy one and choose one of the resistant lines for egg production under free range conditions. Some authors suggest, when alternative housing systems are proposed, genotype x environment interactions should be considered (Singh et al., 2009). Such an interaction seems to be the case for the evaluated genotypes in the present study as shown by the response of LB hens to experimental (**chapter III**) and natural infections. LB hens seem to be more resistant to experimental *A. galli* infection compared to LSL hens (**chapter III**; Gauly et al., 2002). However, when exposed to natural mixed infections, LB hens still had lower *A. galli* worm counts but a higher total worm burden when compared with LSL (**chapter IV**). Thus, presence of other infections and environmental effects (Kaufmann, unpublished) has to be taken into account when choosing and/or substituting genotypes for egg productions. But this

finding may also indicate that farmers may have a considerable impact on hen´s health and welfare when running a favourable management.

Another strategy, considered to improve parasite burdens of hens, is selection within breeds. Heritability of total worm burden, as a direct measure of infection intensity, was high for LB and LSL in the study described in **chapter IV**. Even if these values may be considered as overestimated, this clearly proves an existing genetic variation in both lines. When selecting on this single trait (total worm burden) rapid genetic progresses are expected (Falconer, 1989). Compared with other species, generation interval in poultry is short, implying possibly less time and lower costs for the selection efforts. The combined results indicate that the genetic background of LB hens may be favourable regarding their ability to resist helminth infections as they were most resistant to artificial *A. galli* infection and showed promising heritabilities for the trait 'worm counts' in the field.

Because of the lack of uninfected control animals in the present investigations, it remained unclear whether responses of the breeds to the parasitic infections would affect their performance. Similarly, direct effects of the parasitic infections on the most important performance parameters remained unclear.

Improving resistance to certain pathogens via selection programs requires knowledge about the covariation with production traits (Woolaston and Baker, 1996). In the current work, it was possible to estimate genetic correlations between worm burden and body weight of the hens at slaughter day. Estimates for the breed LB and LSL were 0.31 and -0.41, respectively. This indicates that a direct selection for parasite resistance would either result in selecting animals with lower or higher body weight, respectively. The significant positive phenotypic and genetic correlation between total worm burden and body weights in LB hens disagrees with favourable relationships found earlier (Ackert et al., 1935). However, it has also been showed that estimates of genetic correlations between parasitological parameters (e.g. worm burden, FEC) and performance traits, such as body weight, can vary in a big range (Bishop et al., 1996; Mandonnet et al., 2001). Favourable, moderate and unfavourable correlations were also reported for other species (Eady et al., 1994; Bishop et al., 1996; Mandonnet et al., 2001). The observed genetic relationships between disease resistance and performance can be seen as a balance of the costs versus the beneficial consequences of being resistant. Increasing costs of resistance will tend to make the relationship more unfavourable (Bishop and

Stear, 2003). Therefore, it may be possible that the genetic relation between resistance and performance traits is parabola-shaped as it is described for the relationship between bodyweight and laying performance (McDaniel et al., 1981; Siegel und Dunnington, 1985; Pym, 1985). What clearly remain to be discussed are the observed high variations of the genetic parameters. The high standard errors of the estimations are relatively high probably due to the limited number of animals which were used.

However, further investigations on the genetic relationship between parasite resistance and performance traits in different hen strains are essential.

5.3 Conclusion

Laying hens in organic production systems are infected with a broad spectrum of helminths. Prevalence of infections and infection intensity were on a high level, so it is concluded that organic production systems provide favourable condition for helmith infections and hens are exposed to a high infection risk. Responses to helminth infections differed between- and within-breeds. As the heritability of the trait 'worm count' is on a useful level, selection to parasite resistance can be applied in order to gradually improve the conditions in organic egg productions systems.

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