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Comparison of effects of *Echinacea purpurea* juices and *Nigella sativa* seeds on performance, some blood parameters, carcass and meat quality of broilers

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@	at the rate of
%	percentage
°C	degree Celsius
B	Boron (metal)
$BW^{0.75}$	Metabolic body weight
Ca	Calcium (metal)
Cu	Copper (metal)
DM	Dry matter
e.g.	for example
Fe	Iron (metal)
g	gram
g/dl	gram per decilitre
G/l	Giga per litre (10^9)
I.U.	International Unit
K	Potassium (metal)
kg	kilogram
µ	micro
m	meter
mg	milligram
ml	milliliter
MJ	Mega joul
Mn	Manganese (metal)
NaCl	Sodium chloride
$NaHCO_3$	Sodium Bicarbonate
NK	Natural killer (cells)
p	probability
P	Phosphorus (metal)
ppm	parts per million
T/l	Tera per litter (10^{12})
U/l	Units per litre
Vit	Vitamin
Zn	Zink (metal)

Dedicated to my parents and my family

1 INTRODUCTION

1.1 Background and research objectives

Poultry is one of the most successful and fast growing industry that provides high quality protein at economical price. Since ancient times, different strategies have been applied to improve animal productivity and profitability. Most important of them were always directed towards maintaining health, reducing disease outbreak and improving general immunity. Antibiotics have been successfully used in poultry production for more than fifty years for treatment of diseases, prevention from diseases by group medication in case of existing or potential disease. Antibiotics have also been continuously applied at subtherapeutic levels (as antibiotic growth promoters – AGP) in feeds of livestock species for maintenance of health and improving performance. AGP were supposed to increase growth rate as a result of improved gut health, resulting in better nutrient utilization and improved feed conversion efficiency (VISEK, 1978). The use of AGP in animal feeds is prohibited in European Union (EU) since 2006, due to the potential risks associated with their use and development of resistant strains of bacteria, mainly in humans. Now, as a result of ban on use of antibiotics in EU, increasing demand of antibiotic free products in other parts of the world and demand for organic animal products, alternative substances are required to create a safety margin in animal production against unexpected hazards and stressful conditions. The alternatives to antibiotics should 1) be safe and economical, 2) have significant, sustainable and beneficial impact on animal health and production, 3) be easily applied and stored, 4) not contribute to environmental pollution, and 5) be acceptable by consumers. A number of experiments conducted to find alternative substances revealed that no single alternative exists with the effects comparable to antibiotics (NASIR and GRASHORN, 2006).

Phytogenic feed additives (PFA) (often also called as phytobiotics or botanicals) are plant–derived products, used in animal feeding to improve performance of agricultural livestock through amelioration of feed properties, promotion of production performance, and improving the quality of animal origin food (WINDISCH et al., 2008). PFA are available in different forms as herbs (flowering, non-woody, and non persistent plants), spices (herbs with an intensive smell or taste commonly added to human food), essential oils (volatile lipophilic compounds derived by cold expression or by steam or alcohol distillation), or oleoresins (extracts derived by non-aqueous solvents). These pure forms and their different combinations have been used in a number of *in vitro*

and *in vivo* experiments and have shown antibacterial, anti-inflammatory, anti-oxidant and immunopotentiating properties. However, the desired activity of phytogenic products is not always constant, as affected by a number of factors, like level and type of active ingredients (which depends on plant part used), agronomical practices, geographical origin and processing techniques.

Phytogenic feed additives are often claimed to improve the flavour and palatability of feed (by stimulating activity and secretion of digestive enzymes), to show inhibitory action on pathogens, to affect physiopathologies (e.g. anti-inflammatory, anti-diarrhoea properties) and to be active in different body systems (e.g. endocrine and immune system). Beneficial effects of PFA in poultry may arise from activation of feed intake and digestive secretions, immune stimulation, anti-bacterial, coccidiostatic, anthelmantic, antiviral, anti-inflammatory activities and antioxidant properties. Most experimental studies investigated blends of various active compounds and reported their effects only on production performance rather than on physiological impacts. The complex nature and variability of active ingredients in phytogenic preparations need to be investigated in more details focussing especially on their effects on different physiological parameters related to animal health as well as on immune status along with their effects on animal performance and meat quality.

Immunostimulatory effects of various phytogenic compounds have been extensively investigated in human and laboratory trials, which indicate a potential of their beneficial effects in poultry and other livestock species. *Echinacea purpurea* (EP) (family Asteraceae) and *Nigella sativa* (NS) (family Ranunculacea) belong to the group of phytogenic immunostimulants that help in establishment and strengthening of para-immunity and are reported to posses a number of pharmacologically active substances (AKHTAR et al., 2003; NASIR and GRASHORN, 2006; 2009a). Echinacea is popular herbal immune-stimulator in North America and Europe, while NS seeds have been used traditionally in Mediterranean and South Asian countries in humans for their beneficial effects on immune system and as natural remedy in many diseases (NASIR and GRASHORN, 2006; 2009a).

Echinacea and its different preparations contain a variety of active substances like alkamides, glycoproteins, polysaccharides, phenolic compounds, cinnamic acids, essential oils and flavonoids (BARRETT, 2003; SASAGAWA et al., 2006; ZHAI et al., 2007; LIU et al., 2007; NASIR and GRASHORN, 2009a). These substances are effective in treatment of various ailments and proved to be beneficial in improving immunity (WOELKART and BAUER, 2007; BAUER, 1999). Solutions made from herb and root EP powders produced defined reproducible macrophage

activity and proved anti-inflammatory and antioxidant properties (RININGER et al., 2000; ZHAI et al., 2007).

Seeds of NS contain alkaloids, fixed and volatile oils and a variety of pharmacologically active substances like thymoquinone, dithymoquinone, thymol, carvacrol, nigellicine-N-oxide, nigellidine and alpha-hedrin (AL-HOMIDAN et al., 2002; NASIR et al., 2005; ATTIA et al., 2008). NS and its various extracts were found to possess antibacterial activity against gram positive and gram negative bacteria, and they caused inhibition of aflatoxin production (NASIR and GRASHORN, 2006; SINGH et al., 2005). Application of NS seeds has shown some effects on broilers performance (GULER et al., 2006; AL-HOMIDAN et al., 2002; DURANI et al., 2007; ZIAD et al., 2008), layer performance and egg quality (AKHTAR et al., 2003, NASIR et al., 2005; AYDIN et al., 2008). But, the findings of these studies are in-consistent. In contrast to NS, not enough data is available on application of EP in livestock species, especially in poultry. According to BGA commission E (1989) Echinacea should be used prophylactically for several weeks daily. Continuous in-feed application of EP in poultry diets have not shown beneficial effects on performance and meat quality either in cobs form or in dried herbal preparations (ROTH-MAIER et al., 2005; GARDZIELEWSKA et al., 2003; KORELESKI and SWIATKIEWICZ, 2007). On the other side, dietary supplements of EP during starter phase seem to be useful adjuvants for live anticoccidial vaccines and provided weight gain advantage compared to live vaccination alone (ALLEN, 2003). JURCIC et al. (1989) reported that continuous application of Echinacea may lead to over-stimulation and they recommended that Echinacea preparations should be applied intermittently. Some effects of intermittent in-feed EP application on immunity were observed in sows (BÖHMER et al., 2008; MAASS et al., 2005; KUHN et al., 2005), layers (BÖHMER et al., 2008) and broilers (ALLEN, 2003).

In literature no data is available on a comparative testing of effects of NS and EP, administered singly or combined, on broiler health, performance and meat quality. The present experiments were conducted in view of a more practical application of EP and NS in the field. Therefore, the objectives of present study were set to investigate the effects of supplementation of EP juices and grounded NS seeds on performance, metabolism (serum protein, blood picture and some liver and heart associated enzymes in blood), carcass and meat quality in broilers.

1.2 Design of the study

The project was composed of two consecutive parts. The first part was designed to basically analyze the effects of EP juices (applied by drinking water) and grounded NS seeds (applied by feed) on metabolism and health of broilers, whereas, the intention of the second part was to test the practical application of both substances by elucidating their effects on performance of broilers.

Due to limited experimental space the basic trials had to be repeated several times. In total, 5 smaller experiments were conducted. The experiments were carried out in a windowless room with climatic control at the small research unit 'Karlshof', Hohenheim University, Stuttgart, Germany. For the experiments boxes of 1 sqm with solid walls have been available. For 35 days fattening period boxes could be filled by up to 12 birds. Boxes were littered with wood shavings and equipped with nipple drinkers and feed troughs. Water and feed were offered *ad libitum*. Continuous lighting was provided by incandescent bulbs. Environmental temperature was adjusted by heating bulbs starting with 34°C on arrival of day-old chicks and declined thereafter to room temperature (about 24°C). For all experiments day-old chicks of breed Ross 308 were purchased from Brueterei Weser-Ems GmbH Sued, Regenstauf, Germany and were sexed on arrival by the speed of feathering and placed to boxes with equal sex ratio.

The fattening trial was run at the research farm 'Tierhaltung, Tierzuechtung, Kleintierzucht und Bioenergie' of Hohenheim University, Unterer Lindenhof, Eningen, Germany. Day-old broiler chicks of the same breed purchased from the same hatchery as for basic trials were used and were sexed on arrival by the speed of feathering. Chicks were allotted to small pens of 2.2 sqm, with walls of wire net and littered with wood shavings in groups of 20 birds (10 male, 10 female). All boxes have been equipped with automatic bell drinkers and feed troughs. Environmental temperature was adjusted by heating of the whole house, starting with 34°C on bird level at day 1 and declining to about 24°C in third week.

For all experiments antibiotic-free feed, in mash form, was offered *ad libitum* in two phases; starter (0 – 14 days) and grower diets (15 – 35 days). Daily fresh tap water at room temperature was offered *ad libitum* for drinking. The water of treatment groups was supplemented with EP juices according to treatment schedule.

The study includes three preliminary experiments on application of different preparations and levels of EP juices and two preliminary experiments on application of grounded NS seeds in

broilers. Based on the results of these preliminary experiments the major experiment was carried out. The objectives of this experiment was to study the comparative and synergistic effects of fermented EP juice and grounded NS seeds on broiler health, performance, carcass, and meat quality in broilers.

1.2.1 Preliminary experiments using *Echinacea purpurea*

Main aim of preliminary experiments was to compare the effects of intermittent application of two different EP juices through drinking water and to find a suitable dose level for further investigations. Drinking water of treatment groups was supplemented with two different preparations of EP juices: EP-F (EP fermented juice) and EP-A (EP juice on alcohol basis). EP juices were obtained from Berghof-Kräuter GmbH, Heilsbronn, Germany. These juices were extracted from above ground parts of EP plant and preserved either through fermentation or by addition of ethanol.

There is no data available on dose level of EP preparation for poultry and other livestock species. Therefore, basic dosage of EP juices for broilers was adjusted on the basis of human medical recommendations at the rate of 0.25 ml/kg $BW^{0.75}$. Based on the informations in literature that beneficial effects of EP on health and immunity can be obtained by intermittent application, EP juices were supplemented intermittently for a limited time period (3 days), followed by three times (9 days) EP free application. The application of EP juice was repeated three times (on 1-3, 13-15 and 25-27 days) during 35 days of rearing period.

Experiment 1: EP-F and EP-A juices were compared against a negative control (without any supplementation). In total, 108 one-day old broilers were randomly divided into 9 experimental boxes (12 chicks/box with equal sex ratio). Each of two preparations, EP-F and EP-A, were applied (at the rate of 0.25 ml/kg $BW^{0.75}$) to 3 boxes each, randomly, while 3 boxes served as negative control.

Experiment 2: This experiment was performed to compare two different dose levels (0.25 ml/kg $BW^{0.75}$ and 0.50 ml/kg $BW^{0.75}$) of EP-F against a negative control. For this experiment, 72 one-day old broilers (Ross 308, mixed sexes) were randomly divided into 6 boxes (12 chicks/box). Two experimental boxes were supplemented with EP-F juice at the rate of 0.25 ml/kg $BW^{0.75}$ (EP-F25), two experimental boxes with EP-F juice at the rate of 0.50 ml/kg $BW^{0.75}$ (EP-F50), while two remaining boxes served as negative control (without supplementation).

Experiment 3: The third experiment was performed to compare the effects of EP-F25 juice (0.25 ml/kg $BW^{0.75}$) against a negative control (without any supplementation). For this experiment 48 one-day old broiler chicks (Ross 308, mixed sexes) were randomly divided into four groups. Two groups (having 12 birds each) received drinking water supplemented with EP-F juice at the rate of 0.25 ml/kg $BW^{0.75}$ (EP-F25), while other two groups received drinking water without supplementation and served as negative control.

1.2.2 Preliminary experiment using *Nigella sativa*

Two experiments were carried out to study the effects of supplementation of different levels of grounded NS seeds in diets on broiler performance, protein and liver metabolism, and immunity. Egyptian NS seeds were obtained from Alfred Galke GmbH, Gittelde, Germany. Before inclusion in feed, NS seeds were grounded (1mm mesh size) to powdered form and analysed to know proximate composition.

Experiment 1: In first experiment, six experimental rations were prepared with supplementation of 0, 0.5, 1.0, 1.5, 2.0 or 2.5 % grounded NS seeds in standard broiler rations (starter and grower). Effects of treatments on broiler performance, health status and levels of heart and liver associated enzymes in blood were studied to know probable effects on some metabolic pathways.

Experiment 2: This experiment was performed to compare the effects of 1 % grounded NS seeds against a negative control (without any supplementation). For this experiment, 72 one-day old broiler chicks (Ross 308, mixed sexes) were randomly divided into six groups. Three groups (having 12 birds each) received feed supplemented with 1% grounded NS seeds, while other three groups received basal diets without supplementation and served as negative control.

1.2.3 Experiment to study the comparative and synergistic effects of *Echinacea purpurea* and *Nigella sativ*a

This experiment was carried out to study the comparative and synergistic effects of fermented EP juice and powdered NS seeds on broiler health, performance and meat quality. Four treatment combinations were prepared and each treatment was offered to 10 experimental units (200 broilers/treatment). The treatment combinations were as follows: C (control group without any feed or water additive), E (intermittently supplemented with fermented juice of EP through

drinking water), N (continuously supplemented with grounded seeds of NS through feed), and EN (supplemented with EP-F through drinking water intermittently and NS through feed continuously).

For birds in treatment groups E, drinking water was supplemented with fermented juice of EP at the rate of 0.25 ml/kg $BW^{0.75}$, intermittently for 3 days (on 1-3, 13-15, 25-27) followed by 9 treatment free days. Birds in treatment groups N were fed on feeds supplemented with 1 % grounded NS seeds. Birds in treatment groups EN received drinking water supplemented with EP (at the rate of 0.25 ml/kg $BW^{0.75}$, intermittently for 3 days (on 1-3, 13-15, 25-27) followed by 9 treatment free days) and feed continuously supplemented with 1 % NS grounded seeds for 35 days.

1.2.4 Statistical analysis

All data were tested for normal distribution before analysis. Data were subjected to one way/two way analysis of variance using JMP® 5.0.1 program (SALL et al., 2005). Significance of the differences between group means was tested by Student's t-test. In the tables, data are given as means ± SEM (standard error of mean). Significant differences between means are indicated in tables by different small Latin letters ($P<0.05$).

1.3 Structure of thesis

Thesis includes six chapters.

Chapter 1 includes introduction and objectives of study.

Chapter 2 (general discussion) generally explains the results and discussion of different experiments and phytogenic treatments in our study.

Chapter 3 (corresponding to paper 1) describes the potential role of *Echinacea purpurea* in poultry and swine production. This chapter summarises the results of contemporary research done on application of EP in livestock species, especially poultry and swine.

Chapter 4 (corresponding to paper 2) describes the effects of *Echinacea purpurea* fermented juice and *Echinacea purpurea* juice on alcohol basis on broiler health, performance and levels of different blood enzymes related to heart and liver functions.

Chapter 5 (corresponding to paper 3) presents the effects of supplementation of grounded *Nigella sativa* seeds in broiler diets on broiler performance, health and levels of different blood enzymes related to heart and liver functions.

Chapter 6 (corresponding to paper 4) presents the results of comparative and synergistic effects of application of *Echinacea purpurea* fermented juice and grounded *Nigella sativa* seeds on broiler performance, health and meat quality.

Chapter 7 Summary

2 GENERAL DISCUSSION

Many substances have been tested for their potential to substitute in-feed antibiotic growth promoters in poultry diets. Phytogenic substances are considered as one viable alternative due to their activity in different physiological systems. Usually, in livestock production effects of phytogenic substances are only studied in terms of performance parameters and economical importance. But a variety of active ingredients present in different phytogenic substances may have more diverse effects on animal physiology. These active ingredients may possess potential to show effects on different metabolic pathways, activity of different enzyme systems, and immunity along with performance parameters. Especially, improvement of immunity may help birds to cope in a better way with stress situations during fattening and by this may enhance well-being. This aspect gains importance under view of animal welfare issues. *Echinacea purpurea* (EP) and *Nigella sativa* (NS) belong to the group of phytogenic compounds that have been reported to stimulate immune system in laboratory trials as well as in human studies.

Current knowledge on the active substances in EP and NS preparations shows that these phytogenic compounds may also be helpful if applied in poultry as they have shown positive effects during *in vitro* and *in vivo* experiments. However, no study was conducted to evaluate the comparative as well as synergistic effects of EP juices and NS seeds on broiler health, performance and activities of different enzymes related to heart and liver function.

Due to lack of information available on application of EP in livestock species, especially in poultry, initially different dose levels and preparations of EP juices were tested. EP juices were applied through drinking water as there are less chances of binding of active sites of pharmacologically active substances and as they are easily absorbed after intake. Drinking water of treatment groups was supplemented with one of two different preparations of EP juice; EP fermented (EP-F) juice and EP juice on alcohol basis (EP-A). The basic dosage of EP juices for broilers was adjusted on the basis of human medical recommendations at the rate of 0.25 ml/kg $BW^{0.75}$. From use of EP in humans it is well documented that intermittent application has a significant effect on immunity. Therefore, in humans it is recommended that the interval between applications should be two to three times the duration of application (JURCIC et al., 1989). Based on this general knowledge; in the present study EP juices were supplemented intermittently for a limited time period (3 days), followed by three times (9 days) EP free application. The application

of EP juice was repeated three times (on 1-3, 13-15 and 25-27 days) during 35 days of rearing period.

During preliminary experiments better results were obtained by application of EP-F as compared to EP-A. Similarly, BÖHMER et al. (2008) observed better health and immunity in layers by application of EP-F as compared to EP-A. Presence of ethanol in EP-A may interact with active ingredients of EP and affect their activity. Ethanol is also reported to have stimulatory effects on immune system (HAN and PRUETT, 1995). A general disadvantage of feed additives containing alcohol is that they need to be registered as drugs rather than additives for their future use in poultry and other livestock species. Therefore, EP-F was selected for further application in present study. Higher dose levels of EP-F up to 1.5 ml/kg $BW^{0.75}$ were also tested and application of EP-F at the rate of 0.25 ml/kg $BW^{0.75}$ through drinking water was found to be most suitable for present study.

Seeds of NS were grounded to powdered form (1mm mesh size) and supplemented in basal broiler diets. Due to inconsistent reports in literature on application level of NS seeds in broilers, a dosage trial was performed to find a suitable dose level. Different inclusion levels ranging from 0.5 to 2.5 % were tested. Based on the results of dosage trial 1% inclusion level was selected for further study.

The main innovative point in the present study was, to investigate in detail whether EP and NS may have positive and/or negative effects on general metabolism, stress compensation and immunity in broilers. Therefore, activities of serum enzymes related to heart and liver functions, levels of serum proteins, serum cholesterol and serum glucose as well as blood picture were determined as indicators. Results from preliminary experiments on effective supplementation levels of EP and NS were then used in a fattening trial to analyze the effects of these additives on broiler performance and meat quality.

2.1 Effects of *Echinacea purpurea* juices and *Nigella sativa* seeds on serum proteins

Serum proteins (albumin, globulins and total proteins), being synthesised in liver, are used as indicators of metabolic and synthetic activity of liver. Change in immunoglobulin level in blood leads to changes in serum globulin contents, which represent status of immune health. *Echinacea* application has been reported to enhance immunoglobulin production in mouse model, but the increase in antibody titre appeared to be restricted to specific time during the application (REHMAN et al., 1999). Echinacea application might trigger immune system by stimulating

immunoglobulin production, and repeated application can act as booster dose which triggers antibody response.

In the present study, intermittent supplementation of EP juices (through drinking water) showed non significant treatment effects on levels of serum albumin and total proteins in broilers. However, EP-F treated birds showed a trend of higher serum proteins level and especially significantly ($P<0.05$) higher serum globulin level as compared to control (Table 4.4). These results showed that intermittent application of EP-F juice obviously has the potential to increase level of serum globulins. Serum globulins, being used as an indicator of immune response, are source of antibodies (ABDEL-FATTAH et al., 2008) and immunoglobulin production. The present findings are in accordance with previous observations that *Echinacea* application improved serum immunoglobulin concentration, especially IgG concentration (SCHRANNER et al., 1989; REHMAN et al., 1999). In rats *Echinacea* applied intermittently by drinking water rose serum immunoglobulin concentration, increased antibodies (SCHRANNER et al., 1989) and augmented primary and secondary IgG (REHMAN et al. 1999). Positive effects of intermittent EP application on immunoglobulins were also observed in sows and piglets (KUHN et al., 2005). These findings suggest that in poultry immunostimulatory and immunopotentiating properties of *Echinacea* can be obtained by intermittent oral application.

In the present study, supplementation of 1 % NS seeds showed no significant effect on serum albumins, globulins and total proteins. Similarly, AL-HOMIDAN et al. (2002) observed no significant change in contents of total proteins, albumins and globulins by supplementation of 1 and 10 % NS seeds in broiler diets, respectively. However, OSMAN and BARODY (2000) reported significantly ($P<0.05$) increased serum albumin, globulin and total protein concentrations by supplementation of 1 % grounded NS seeds in feed along with SRBC (sheep red blood cell) immunization on 7^{th}, 21^{st}, 28^{th} and 35^{th} day. The missing effect of NS on immunity in own experiments was not surprising as NS was administered by feed continuously. Per definition a permanent stimulation of the immune system will not result in an enhanced immunity due to down regulation of receptors. Therefore, NS supplementation may increase levels of serum proteins only if it is applied along with other immunostimulating treatments. In present experiment not any other immunostimulator or antibiotics was used in feed which might support NS activity in increasing protein synthetic activity of liver as observed by increased contents of serum albumin, globulins and total proteins.

2.2 Effects of *Echinacea purpurea* juices and *Nigella sativa* seeds on serum enzymes (CK, LDH, ALT, γ-GT and ALP)

In serum enzymology, the concentration of the different enzymes related to metabolism and function of heart, liver and kidney are used as indication of their effects on these vital organs. Serum levels of creatine kinase (CK) and lactate dehydrogenase (LDH) are used for clinical diagnosis of circulatory and cardiac disturbances in humans (IMAEDA, 1999). Changes in activities of serum CK and LDH may indicate damage to cardiac muscles (ZHANG et al., 2008). In some diseases their levels are also used as indication of damage caused and positive effects, if any, of applied treatments.

Supplementation of EP juices have shown a trend towards decreasing CK and LDH activities in general and significantly ($P<0.05$) less CK activities in EP-F25 supplemented groups as compared to control (Table 4.5 and 4.6). Non significant decrease in activities of CK and LDH enzymes were observed by application of NS seeds. CK is an ATP dependent enzyme. Its elevated levels in blood of heart patients are considered as first indication of heart attack (ADAMS and APPLE, 2004; RAMSBOTTOM et al., 2008). Increased CK level was also observed in stressed birds and may indicate pathological changes of cardiac muscle and increased risk of sudden death syndrome (SDS) in broilers (ITOH et al., 1997). Certain medications used to reduce the risk of heart attack and to alleviate its pathological effects are directed towards maintaining serum CK level to normal (RAMSBOTTOM et al., 2008). Intermittent application of EP-F has shown a trend towards reduced serum CK level. Obviously, EP-F juice has beneficial effects on cardiac health and well being of the birds by reducing the risks of SDS.

Activities of alanine amino transferase (ALT), gamma-glutamyl transferase (γ-GT) and alkaline phosphatase (ALP) are indicators of liver functions and its metabolic activity. In the present experiments, levels of ALT and γ-GT did not differ significantly ($P>0.05$) between treatments by application of either EP-F or NS. However NS seeds numerically (non significant) decreased ALT contents up to 30 %. Thymoquinone present in NS seeds has been reported to protect hepatocytes and decrease leakage of ALT and AST (DABA and ABDEL-RAHMAN, 1998). But, no significant change in ALT contents was observed by application of 2 and 10 % NS seeds to broilers for 4 and 7 weeks (AL-HOMIDAN et al., 2002). Application of NS seeds up to 1.5 % had shown no negative effect on liver enzymes and showed a trend of reduced activities of ALT, ALP, CK and LDH (NASIR and GRASHORN, 2008). Similarly, no significant treatment effect on liver enzymes was observed by MAASS et al. (2005) by in-feed application of EP

preparations to pigs. Application of EP-F juices up to 1.5 ml/kg $BW^{0.75}$ (6 times of human standard dose) has shown no detrimental effect on different blood parameters (NASIR and GRASHORN, 2007a, b). These results indicate that application of grounded seeds of NS and EP juices do not have any negative effect on liver functions visible by the activity of different enzymes.

2.3 Effects of *Echinacea purpurea* juices and *Nigella sativa* seeds on blood picture (RBC, WBC, Hb, Hematocrit)

Erythrocytes (RBC) are synthesised in bone marrow and they contain haemoglobin (Hb) and their main function is oxygen transport. The proportion of the blood marked by RBC is called as hematocrit. Leucocytes (WBC) are part of immune system; they destroy and remove old or aberrant cells and cellular debris, as well as attack infectious agents and foreign substances.

No significant treatment effect was observed by application of either EP-F or NS on numbers of erythrocytes, leucocytes, Hb and hematocrit. However, both EP-F and NS showed a trend of decreased number of leucocytes. Present results support the previous observations by BÖHMER et al. (2008) and MAASS et al. (2005) in pigs and poultry. Intermittent in-feed supplementation of EP-F juices to layers and pigs have not shown any significant effect on number of lymphocytes and total leucocytes (BÖHMER et al., 2008). Similarly, no significant treatment effect was observed on numbers of RBC and WBC in pigs by continuous in-feed application of *Echinacea* cobs in pigs (MAASS et al., 2005). In γ-irradiated rats EP application have been reported to reverse the detrimental effects of irradiation on Hb, RBC and differential WBC count, but no significant effect of EP application was observed in non-radiated normal rats (ABOUELELLA et al., 2007). Echinacea has been reported to modulate immune system by stimulating various immune cells such as macrophages, monocytes, NK cells and effects on cytokines/chemokines in various *in vitro* and *in vivo* trials (BROUSSEAU and MILLER, 2005; WOELKART and BAUER, 2007).

No significant effect of 1 % NS seeds, meal and oil was observed on Hb and RBC, and WBC in Japanese quails (EL-SAUD, 2000). AL-HOMIDAN et al., (2002) also observed no significant effect of 2 and 10 % NS seeds supplementation on RBC, Hb and PCV. Significant decrease in leukocyte number was observed by application of NS fixed oil to rats for 12 weeks, but these changes were not significant until 8 weeks of NS application (ZAOUI et al., 2002). These results show that there is no negative effect on supplementation of EP-F juice and grounded NS seeds on numbers of RBC, WBC and contents of Hb and hematocrit.

2.4 Effects of *Echinacea purpurea* juices and *Nigella sativa* seeds on blood glucose and cholesterol

Many metabolic changes are reflected in the contents of cholesterol and glucose in blood. Levels of serum glucose and cholesterol rise due to stress and may affect health and performance of birds and meat quality. Application of grounded NS seeds has been reported to reduce elevated levels of serum cholesterol and serum glucose in layer and rats (AKHTAR et al., 2003; MERAL et al., 2004). Similarly, juices of EP have been reported to reduce cholesterol levels in pig meat (HANCZAKOWSKA and SWIATKIEWICZ, 2007). In the present study, no significant treatment effect was observed by application of EP-F or NS on serum glucose and cholesterol levels in broilers. AL-HOMIDAN et al. (2002) also observed no significant change in serum cholesterol contents by application of 2 and 10 % NS seeds for 4 and 7 weeks. Similarly, non significant changes of serum total cholesterol, HDL-cholesterol, but significant decrease in serum LDL-cholesterol and egg yolk cholesterol contents was observed by continuous (12 weeks) in-feed application of 1 % grounded NS seeds in layer diets (AKHTAR et al., 2003). NS and its extracts have been reported to decrease the elevated glucose concentration and protect from diabetic induced disturbances of heart rate and some haematological parameters in alloxan-induced diabetic rabbits (MERAL et al., 2004). In contrast to our findings, ZAOUI et al. (2002) reported that oral treatment of rats with fixed oils of NS (1 ml/kg/12 weeks) decreased serum cholesterol and glucose levels. In present study, the duration of application was 5 weeks, during which no significant effect of EP-F or NS application was observed on contents of serum cholesterol and blood glucose. These effects might be observed if the birds were reared for longer period (12 weeks) with applied treatments. This might be relevant in extensive production and in organic poultry farming.

2.5 Effects of *Echinacea purpurea* juices and *Nigella sativa* seeds on broiler performance

Usually, phytogenic substances are supposed to improve performance of birds by stimulating secretion of digestive enzymes leading to enhanced nutrient digestion and absorption (GEIER and OSTER, 2001; RECOQUILLAY, 2006). Furthermore, the presence of active ingredients and phenolic compounds can reduce numbers of intestinal pathogens, thus minimizing nutrient loss and improving performance. Both effects may result in better intestinal health and may lead to more protein deposition in body tissues. But, the effects of active ingredients on performance and health of animals are in-consistent, as affected by the number of environmental and agronomical factors leading to differences in amount and activities of active ingredients. In literature, no clear-cut and reproducible effect of phytogenic compounds on performance parameters in different classes of livestock has been reported.

In the present study, numerical (non significant) improvement of broiler performance was observed by application of EP juices, while significantly ($P<0.05$) better ADWG was observed by application of EP-F juices (Table 4.4). In preliminary experiments no prophylaxis against coccidiosis was done, which created a challenge environment for birds. In this challenge condition birds supplemented with EP juices showed better growth performance as compared to non supplemented birds. The observed tendencies might indicate that beneficial effects of EP can be obtained by intermittent application of EP-F juices through drinking water. Intermittent application of *Echinacea* preparations has also been reported to be beneficial in improving health by modulation of immune system (JURCIC et al., 1989). BÖHMER et al. (2008) reported some positive effects of intermittent application (2-days application, 12-application free days) of EP-F on layer health and immunity, while KUHN et al. (2005) observed immune stimulating effects in piglets by a repeated 5 days of application of EP juices. During the present experiment, 3-days application of EP-F followed by 9 application free days showed positive effects in terms of better broiler performance and serum globulin contents. In contrast to our observations, ROTH-MAIER et al. (2005) observed no significant effect on performance parameters by continuous application of EP cobs through feed to broilers and layers. Intermittent application of EP-F seems to be more beneficial as compared to continuous application as *Echinacea* has been reported to trigger the weak immune system, which can be better achieved by intermittent application. Present study showed that some positive effects can be achieved by application of EP-F for 3 days followed by 9 treatment free days; however there might be some other treatment regimen that can show more positive effects. There is need for some further research.

Powdered NS seeds posses a number of pharmacologically active substances (like thymoquinone, dithymoquinone, thymol and carvacrol) that are supposed to enhance feed digestion and absorption by stimulating secretion of digestive enzymes, leading to better feed utilization and assimilation. Supplementation of NS seeds up to 2 % have shown no negative effect on performance, health and blood parameters of broilers, while better FCR was obtained during starter phase (NASIR and GRASHORN, 2008). DURRANI et al. (2007) observed better broiler performance by supplementation of 4 % NS seeds, while Guler et al. (2006) and ZIAD et al. (2008) observed improved FCR by supplementation of 1 and 1.5 % NS seeds, respectively.

In fattening trial, no significant effect of EP-F or NS application on BWG, ADWG, and FCR was observed. Comparing the results of present experiment with performance data given by breeder (AVIAGEN, 2007) higher LBW on 35^{th} day, higher cut-up weights and nearly same FCR was observed. This shows that in present experiment, performance of the birds was at their

maximum, leaving no or very little room for further improvement. Similarly no improvement was observed by BOTSOGLOU et al. (2002), by supplementation of phytogenic products to broilers. These findings are also supported by studies of LEE et al. (2003) who demonstrated that well nourished healthy chicks respond less to antibiotics when they are housed in a carefully cleaned and disinfected place.

2.6 Effects of *Echinacea purpurea* juices and *Nigella sativa* seeds on broiler carcass and meat quality

In fattening trial, results of slaughter characteristics show that broilers supplemented with EP-F juice have significantly ($P<0.05$) lower carcass percentage as compared to control and other treatment groups. No significant treatment effect was observed on percentage of abdominal fat, but liver percentage was significantly lower in EP treated birds as compared to control. NS treated birds had highest breast percentage, which might be due to their probable effect on protein metabolism and improving growth rate.

Feed additives have been reported to influence physical and chemical characteristics as well as sensory and microbial quality of meat. In present experiment (Table 6.5), meat dry matter and crude protein of EP-F and NS treated birds was significantly ($P<0.05$) less as compared to control and EN group. GARDZIELEWSKA et al. (2003) observed no significant effect on broiler meat quality by continuous supplementation of 1 % EP herb through feed. No effects on pig meat and carcass quality were observed by intermittent application of EP (KUHN et al., 2005). Similarly, KORELESKI and SWIATKIEWCZ (2007) observed no effect of EP juice (supplemented continuously at the rate of 560mg/kg feed from 22 – 42 days of age) on changes in fatty acid composition during frozen storage of broiler meat. NS supplemented groups showed significantly ($P<0.05$) higher crude ash contents indicating better availability of minerals by supplementation of NS seeds. Physical parameters of meat quality (grill and cooking losses, colour (L*, a* and b* values), shear force value and electrical conductivity) were not significantly different. However, meat samples of birds supplemented with NS showed numerically less (7.62 %) shear force value and higher cooking losses (8.2 %) as compared to control and other treatment groups. Reduction of shear force value due to inclusion of NS seeds in diets could be due to tenderizing effects of active ingredients of NS. Fat percentage in meat samples of NS treated birds was also numerically higher as compared to control, which might made tissues soft and reduced shear force value.

2.7 Synergistic effects of *Echinacea purpurea* juices and *Nigella sativa* seeds

There is lack of studies using more than one phytogenic immunostimulants in animals, especially in poultry. Combined application of two immunostimulants can have synergistic as well as antagonistic effects on health and performance of treated animals. NS seeds or their extracts have been reported to have anti-microbial, antioxidant, anti-inflammatory, anti-carcinogenic, anti-hepatoxic, and immunostimulatory effects due to presence of pharmacologically active substances like thymoquinone, dithymoquinone, thymohydroquinone (EL-BEITAWI et al., 2008; NASIR and GRASHORN, 2006). Similarly, various extracts and preparation made from *Echinacea* have shown anti-inflammatory, antioxidant and antibacterial activities along with their effects on macrophage activation, PMN (polymarphonuclear) granulocyte modulation and stimulation of NK cells due to presence of various active substances such as alkamides, polysaccharides, glycoproteins and derivatives of caffeic acid (HINZ et al., 2007; WOELKART and BAUER, 2007; THYGESEN et al., 2007; SHARMA et al., 2008).

In fattening trial synergistic or antagonistic effects of NS and EP-F were studied by their combined application. NS was continuously supplemented through feed and EP-F was supplemented intermittently (3 application days followed by 9 application free days). No negative effects on broiler health and performance were observed by combined application of EP-F and NS seeds. Reduced mortality and numerical (nearly significant) improvement was observed in LBW, on 35^{th} day, total WG and ADWG. Moreover, combined application resulted in significantly ($P<0.05$) higher meat crude protein contents as compared to both EP-F and NS. This might be due to synergistic effects of active ingredients of both compounds leading to better protein metabolism These results indicate that continuous application of NS along with intermittent application of EP-F has no negative effects on broiler performance. Combined application of different phytogenic preparations can be used for further research.

CONCLUSIONS AND RECOMMENDATIONS

The effects of phytogenic compounds are more diverse than the effects of antibiotics on feed digestion, gut physiology and microbial population. The results of present study show that immunostimulating phytogenic compounds like EP and NS do posses potential to improve broiler health, performance and immunity distinctly. Some positive effects of *Echinacea* on broiler health and performance can be obtained by intermittent application (3 days treatment – 9 days interval) through drinking water as compared to continuous application through feed. Intermittent application of EP juices through drinking water is practicable, cost and labour efficient as compared to in-feed application. Comparing the effects of EP-F and EP-A, better performance and improved health was observed by application of EP-F juices. This might be due to appropriate amount of alkamides and absence of alcohol in EP-F juice. Presence of ethanol in EP-A can also affect the activity of active ingredients of EP, and ethanol is also reported to have stimulatory effect on immune system. Application of 0.25 ml/kg $BW^{0.75}$ EP-F juice has shown more positive effects as compared to 0.50 ml/kg $BW^{0.75}$. EP-F has shown positive effects on broiler performance in terms of improved ADWG and on health in terms of reducing levels of CK and a trend of reducing LDH level. This shows that EP-F supplementation has potential to reduce the risk of Sudden death syndrome which is the most common cause of loss in fast growing broilers. Maintaining the activity of other heart and liver related enzymes by application of EP-F shows that it can be applied for reducing mortality and improving immunity and health of broilers. No toxic effect of EP-F application was observed as indicated by activities of serum enzymes and blood parameters.

Application of grounded NS seeds (up to 2 %) has shown no negative effect on broiler health and performance. Numerically better FCR during starter phase was obtained by application of 1 % NS seeds in broiler diets, but these effects were decreased during grower phase. Less number of mortalities was observed in NS treated birds. This showed that NS seeds possess potential to improve performance as well as health and immunity of the birds, leading to more profitability. NS supplementation didn't show any negative effect on activities of heart and liver related enzymes and resulted in numerically better FCR.

Significant beneficial effects of EP and NS application on performance and meat quality were not obtained because birds performed at their maximum and there were fewer chances for improvement. However, the combined application of EP and NS has more beneficial effects on

health and performance of animals. No negative or antagonistic effects of combined application of phytogenic compounds have been observed.

In future, it is recommended that effects of phytogenic substances are not only studied on performance parameters, but physiological impacts of feed additives on different body systems also have to be investigated. Synergistic and antagonistic effect of combined treatments should be studied in more details for future combined application. Intermittent application of EP-F through drinking water in broilers can be used for obtaining better health and performance. However, research is required to find mechanism of action of active ingredients of EP and NS on different physiological systems and performance parameters in more details under practical conditions.

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3 ECHINACEA: A POTENTIAL FEED AND WATER ADDITIVE IN POULTRY AND SWINE PRODUCTION

Echinacea: Ein potentieller Futter- und Trinkwasserzusatz in der Geflügel- und Schweinerzeugung

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Abstract

Feed additives with performance and health stimulating effects are widely used in animal production. For a long time antibiotics have been the dominating substances in animal nutrition with distinct growth promoting effects. After the ban imposed on the subtherapeutic use of antibiotics in animal feeds in European Union (EU) and the increasing demand of organic animal products, alternative substances are required, which can maintain health and improve performance of livestock without any residual effects. *E. purpurea* is one of the most promising phytogenic additives due to its immuno-stimulatory and potentiatory properties. Echinacea has been widely investigated in laboratory animals, as well as it is under continuous study in human trials for its potential clinical use. It contains a variety of active substances like alkamides, glycoproteins, polysaccharides, phenolic compounds, cinnamic acids, essential oils and flavonoids, which are effective in treatment of various ailments and improving immunity and health. The toxicity of Echinacea is reported to be very low. Present research on the use of Echinacea in livestock is limited, but it can be used as a guiding line and rationale for its use in livestock. In this review, the extent of present knowledge and need for future studies are discussed in the light of effectiveness of active substances of *E. purpurea* in improving health, performance and immunity of various livestock species.

Key words

Echinacea purpurea, alternative to antibiotics, phytogenic compounds, immunostimulator, swine, poultry.

4 EFFECTS OF INTERMITTENT APPLICATION OF DIFFERENT *ECHINACEA PURPUREA* JUICES ON BROILERS PERFORMANCE AND SOME BLOOD PARAMETERS

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Abstract

Three experiments were performed to study the effects of intermittent oral application of two different preparations of *Echinacea purpurea* (EP) juices on broiler performance, blood picture, liver and heart associated enzymes. Results of present experiments show that EP juices do not have any harmful effect on broiler health and performance. Numerically better broiler performance was observed in EP treated groups as compared to control, except for ADWG, which was significantly ($P<0.05$) higher for EP-F treated group in experiment 2. Significant ($P<0.05$) improvement of serum globulin contents by application of EP-F through drinking water for 3 days followed by 9 treatment free days, showed that EP-F juice has a potential to improve immunity of birds. Reduced creatine kinase levels show that application of EP juices can also have beneficial effect on cardiac muscles and can reduce the risks of sudden death syndrome in fast growing broilers. No negative effects of EP supplementation on liver function could be observed.

Keywords

Broiler, nutrition, *Echinacea purpurea,* performance, serum proteins, creatine kinase

5 EFFECTS OF SUPPLEMENTATION OF GROUNDED *NIGELLA SATIVA* SEEDS ON BROILER PERFORMANCE AND SOME BLOOD PARAMETERS

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5.1 Abstract

Two experiments were performed to study the effects of grounded *Nigella sativa* (NS) supplementation on broiler performance, activities of liver and heart related serum enzymes and blood picture. In first experiment, basal broiler diets were supplemented with grounded NS seeds at the rate of 0, 0.5, 1.0, 1.5, 2.0 and 2.5 %. In second experiment, effects of supplementation of 1 % NS seeds were compared against a negative control. Three randomly selected groups received feeds supplemented with 1 % NS seeds, while three groups served as negative control. Data were collected for weekly feed consumption, body weight gain, and daily mortality. Blood samples were collected during slaughtering (35^{th} day). Results of first experiment showed non significant improvement of dressing percentage in groups receiving 0.5, 1.0 and 1.5 % NS seeds. Birds supplemented with 1 and 1.5% NS seeds showed better FCR (feed conversion ratio) during starter phase, while birds supplemented with 2 % NS seeds showed better FCR during grower phase. No significant treatment effect was observed for levels of serum total protein, albumin, globulin, gamma-glutamyl transferase (γ-GT), leucocytes and erythrocytes. Results of second experiment showed numerically (non significant) better FCR during starter and grower phase of NS (1%) treated birds as compared to control. No significant effect of NS supplementation on serum proteins, levels of alanine amino transferase (ALT), γ-GT, alkaline phosphatase (ALP), creatine kinase (CK) and lactate dehydrogenase (LDH) was found. Number of erythrocytes and leucocytes and contents of glucose and cholesterol were also not significantly different between the treatment groups. It was concluded that supplementation of grounded NS seeds in broiler diets at the doses studied does not lead to a negative effect on the growth performance and different blood parameters. Best results on performance and blood parameters were observed by supplementation of NS seeds at 1% in feeds.

(**Key words:** Broilers, Nutrition, *Nigella sativa*, Performance, Serum proteins, Liver enzymes)

5.2 Introduction

Phytogenic compounds posses a variety of active ingredients which have been reported beneficial for maintaining health and treatment of different diseases in human as well as in veterinary practice. Therapeutic and prophylactic use of phytogenic substances is increasing globally in livestock farming. After the ban on use of antibiotic growth promoters in EU in 2006, there is growing research for exploring hidden benefits of phytogenic compounds for their future role as alternative to antibiotics in livestock production. Phytogenic substances and extracts have

wide range of activities in animals, as e.g. on digestive, immune and endocrine systems. Furthermore, they show physio-pathological (anti-inflammatory, anti-oxidative) and anti-microbial activities (static and cidal) (Nasir and Grashorn, 2009).

Nigella sativa Linn. (NS) (family Ranunculacea), commonly known as black seed, is an annual herb of Mediterranean region, South and Central Asia and is now also cultivated in Europe. NS plant and its seeds contain alkaloids, fixed and volatile oils, pharmacologically active substances (thymoquinone, dithymoquinone, thymol, carvacrol, nigellicine-N-oxide, nigellidine and alpha-hedrin), and antioxidants (selenium, DL- α-tocopherol, DL-γ- tocopherol, and trans-retinol) (Al-Saleh et al., 2006; Nasir et al., 2005). NS seeds and their various extracts were found to possess antibacterial activity against gram positive and gram negative bacteria, and caused inhibition of aflatoxin production (Nasir and Grashorn, 2006; Singh et al., 2005; Ferdous et al., 1992). Significantly ($p<0.05$) better dressing percentage along with improved feed intake and weight gain was observed by supplementation of 4 % NS seeds to broilers diets (Durrani et al., 2007). Application of NS seeds and its preparations have shown some effects on broilers performance (Siddig and Abdelati, 2001; Al-Homidan et al., 2002; Ziad et al., 2008), comparable results to antibiotics (Guler et al., 2006) and antiviral activity against Newcastle disease virus (Yaseen, 2003), reduction in egg cholesterol levels and better egg quality in layers (Akhtar et al., 2003; Nasir et al., 2005; El-Bagir et al., 2006; Aydin et al., 2008).

Extracts and preparations made from NS seeds might have more diverse effects in metabolism due to presence of a variety of active substances (especially thymoquinone) which can be observed in activities of different heart and liver related serum enzymes and in blood biochemistry. Therefore, the study of the activities of different serum enzymes related to the functioning and health of heart and liver may give more detailed information for future role of phytogenic compounds in poultry nutrition. Effects of different feed additives on synthetic activity of liver are indicated by serum proteins and hepatotoxicity can be observed by the activities of alanine amino transferase (ALT), gamma-glutamyl transferase (γ-GT) and alkaline phosphatase (ALP). Increased activities of serum creatine kinase (CK) and lactate dehydrogenase (LDH) are results of damage caused to cardiac and other smooth muscles (ZHANG et al., 2008); changes in levels of glucose and cholesterol may indicate stress. Therefore, the objectives of the present study were set to investigate the effects of grounded NS seeds on broiler performance and metabolism (serum protein, blood picture and some liver and heart associated enzymes in blood).

5.3 Material and methods

5.3.1 Animals, housing and diets

For both experiments, one-day old broilers (Ross 308) were purchased from Brueterei Weser-Ems GmbH Sued, Regenstauf, Germany. After arrival at the experimental station of Hohenheim University broilers were feather-sexed, weighed individually, wing-tagged and distributed to experimental boxes having equal sex ratio. Twelve broilers were kept in one experimental box with about $1m^2$ of area. Experimental boxes were littered with wood shavings and were provided with nipple drinkers and trough feeders. Temperature and relative humidity of the room was according to the recommendations of broiler breeder company (Aviagen, 2002). A continuous 24-h lighting program was applied. Birds were vaccinated routinely against Newcastle Disease (ND) and Infectious Bronchitis (IB), but no type of medication was administered during the entire experimental period and no prophylaxis against coccidiosis was done.

Antibiotic-free feed, in mash form, was offered *ad libitum* in two phases; starter (0 – 14 days) and grower diets (15 – 35 days) (Table 1). Feed was offered daily and residual feed was measured at weekly intervals. Daily fresh tap water at room temperature was offered *ad libitum* for drinking.

5.3.2 Experimental treatment

Egyptian NS seeds were obtained from Alfred Galke GmbH, Gittelde, Germany. Before inclusion in the feed, NS seeds were grounded to powdered form (1mm mesh size) and analysed in the laboratory of research station 'Tierhaltung, Tierzuechtung, Kleintierzucht und Bioenergie' of Hohenheim University, Unterer Lindenhof, Eningen, Germany according to Naumann and Bassler (1976). NS seeds contained dry matter 5.6 %, crude protein 21.5 %, crude fat 43.2 %, crude ash 4.0 %, crude fibre 11.3 %, Ca 0.54 %, P 0.51 % and metabolisable energy 18.75 MJ/kg (WPSA, 1984).

5.3.3 Experimental design

Two experiments were performed to study the effects of supplementation of grounded NS seeds in broiler diets.

Experiment 1. This experiment was performed to compare the effects of different dose levels of NS seeds supplementation on broiler performance and different blood parameters. In total 72, one-day old broiler chicks were divided into six groups and fed on diets supplemented with 0, 0.5, 1.0, 1.5, 2.0 or 2.5 % grounded NS seeds for 35 days.

Experiment 2. This experiment was performed to study the effects of supplementation of 1 % NS seeds in broiler diets against a negative control (without supplementation). In total 72, one-day old broiler chicks (Ross 308) were divided into six groups. Three randomly selected groups received feeds supplemented with 1 % NS seeds, while three remaining groups served as negative control.

5.3.4 Data collection, analysis and statistics

Birds were weighed individually at the start of experiment and at weekly intervals. During the experiments daily feed intake and weekly weight gains were recorded. Feed consumption was recorded on sub-group basis during a 35-day experimental period and feed conversion ratio (FCR) was calculated (kg feed consumed/kg of live weight gain). Mortalities were recorded when occurred. At the end of the experimental period all birds were slaughtered to determine the carcass weight.

Blood samples of six birds per replicate (selected randomly) were collected during slaughtering. Two blood samples per bird were collected from jugular veins into two different tubes. Blood in EDTA tubes (having ethylenediaminetetraacetic acid (EDTA) as anti-coagulant) was collected for determination of blood picture and contents of cholesterol and glucose, while whole blood samples for determination of serum proteins and liver enzymes were collected without anticoagulant. Serum was separated after centrifugation of clotted whole blood at 3,500 rpm for 20 minutes. Serum and EDTA blood were kept at 4°C till further analysis. Blood samples were analysed by Vet Med Labor GmbH (Ludwigsburg, Germany) to determine the contents of total protein, albumin, globulin, glucose and cholesterol; levels of ALT, γ- GT, ALP, LDH, CK and numbers of leucocytes and erythrocytes. Blood was analysed photometrically for contents of

proteins/enzymes by using Moduler (Roche). Quantitative determination of blood cells from un-coagulated blood (in EDTA tubes) was carried out by using the Coulter counter.

Statistical analysis

Data were subjected to one-way analysis of variance using JMP® 5.0.1 program (Sall et al., 2005). All data were tested for normal distribution before analysis. One-way ANOVA model was used for all experiments. Significance of differences between group means was tested by Student's t-test. In the tables data are given as mean±SEM (standard error of the mean). Values with different superscripts differ significantly ($p<0.05$) between treatments.

5.4 Results

5.4.1 Experiment 1

The results of experiment 1 (Table 2) showed that mean final body weight, total weight gain, ADWG and carcass weight were not significantly ($P<0.05$) different between treatments except birds supplemented with 0.5% NS seeds which showed least values. Dressing percentage of birds supplemented with 0.5% NS seeds was highest, which decreased by increase in inclusion levels. FCR in all NS treated groups was better than in control group during starter phase, with birds fed on diets supplemented with 1.0 and 1.5% NS seeds being best (Figure 1). This influence diminished during the grower phase. Abdominal fat percentage was less in birds receiving feeds supplemented with 2 % NS seeds, while maximum for birds fed on diets supplemented with 1 % NS seeds. One bird died in control group, group supplemented with 1.5 and 2.0% NS seeds during first week of experiment. No further mortality was observed during remaining experimental period.

The results of blood analysis (Table 3) showed no significant treatment effect on contents of serum total protein, albumin, globulin and levels of γ-GT, leucocytes and erythrocytes. Higher (non significant) level of leucocytes were observed at 2.0 % and 2.5 % NS seed supplementation. The level of ALT in serum of birds receiving 0.5 % NS seeds was significantly ($p<0.05$) higher than in control group, but further higher level of NS seeds supplementation showed non significant effect on ALT level with birds supplemented with 1 % NS seeds showing minimum values. Birds

fed on diets supplemented with 0.5 % NS seeds showed higher (non significant) levels of CK and LDH than control, which were also not followed by other groups supplemented with higher levels of NS seeds. No significant treatment effect was observed on contents of glucose and cholesterol and numbers of erythrocytes and leucocytes.

5.4.2 Experiment 2

Results of performance parameters during experiment 2 are shown in table 4, which indicate that there was no significant ($p>0.05$) effect of 1% NS supplementation on final body weight, total weight gain, ADWG, feed consumption and FCR. However, FCR of NS treated groups was (numerically) 3.8 % better than the control groups during starter phase and 3.3 % during grower phase. No significant treatment effect was observed on carcass and abdominal fat percentage. One bird died from control group during the experiment, while no mortality was observed in treatment groups.

Results of blood analysis on 35th day are presented in table 5. These results show that no significant effect was observed on contents of serum proteins (total proteins, albumin and globulins). Activity of ALT was 33% lower (non significant) in NS treated birds as compared to control. No significant difference in levels of γ-GT, ALP, CK, LDH, glucose, cholesterol; and numbers of erythrocytes and leucocytes was observed between control and NS treated birds.

5.5 Discussion

In literature, results of various experiments conducted to study the effects of application of phytogenic feed additives on poultry performance are controversial. This variation might be due to variation in composition and concentration of active ingredients and parts of plants used. There are also differences in results based on the phytogenic compounds obtained from different sources. Results of present experiment showed that NS supplementation has some positive effects on performance of the birds in terms of numerically better (non significant) FCR, especially during starter phase. Supplementation of 1 and 1.5 % NS (experiment 1) and 1 % NS seeds (experiment 2) showed better FCR during starter phase than other treatment levels and control groups. Positive effects of NS supplementation on FCR were reduced during grower phase. Our findings support the results obtained by other researchers. Osman and El-Barody (1999) also observed that positive effects of NS supplementation (0.8 % and 1.0 %) at 4th and 6th were less as compared to at 2nd

weeks of age. Similarly, Guler et al., (2006) observed 5 % improvement in FCR (d 1–42) by supplementation of 1 % NS seeds to broiler diets; however positive effects on FCR were more visible during starter phase (d 1–21). Durrani et al., (2007) observed improved ($p<0.05$) dressing percentage by supplementation of 4 % NS seeds to broiler diets. Osman and Barody (1999) and Ziad et al., (2008) also observed improved FCR by supplementation of broiler finisher diets with 1 and 1.5% grounded NS seeds, respectively.

Improvement of FCR (especially during starter phase) might be due to stimulation of digestive enzymes followed by better digestion and utilization of feed. In the life of broilers initial 2-3 weeks (starter phase) is a critical period. At this time immune system is not fully functional and there are more chances of adverse effects of pathogenic bacteria on health and subsequent performance during grower phase. NS seeds might have helped establishing better gut microflora and reducing the colonization of gut by pathogenic bacteria, as NS seeds have been reported to show antibacterial and antifungal properties (Singh et al., 2005; Ferdous et al., 1992; El-Sayed et al., 2000). Beneficial effects of NS supplementation during grower phase are less but they do exist in terms of better nutrient utilization by stimulating the activity of digestive enzymes. Essential oils of NS seeds have been reported to stimulate secretion and activities of digestive enzymes in the intestinal mucosa and pancreas that improve the digestion of dietary nutrients and feed efficiency. Al-Homidan et al., (2002) and Durrani et al., (2007) observed improved broiler performance by supplementation of 2 and 4 % NS seeds. Such variation may be related to chemical composition and availability of active ingredients in NS seeds obtained from different sources.

No significant effect of NS supplementation on mortality was observed in present experiments, but a trend of reduced mortality was observed in NS treated birds as compared to control. Guler et al., (2006) also observed no significant effect on mortality by supplementation of NS seeds upto 3 %. However, improved immunity in broilers was observed by NS supplementation (Durrani et al., 2007; Yaseen, 2003; Osman and Barody, 1999). Application of grounded NS seeds in broilers have been reported to prevent from lipid peroxidation, increasing the activity of immune potential and antioxidant defence system and protect from liver damage (Sogut et al., 2008).

Active ingredients of NS seeds (especially thymoquinone) have also been reported to show hepatoprotective effect by regulating the activity of different enzymes and protein synthesis. El-Soud (2000) observed significant ($p<0.05$) improvement in serum albumin, α_2-globulin, total proteins contents in quails on 35^{th} day by supplementation of 1 % NS. But in present experiments no significant effect on serum proteins (total serum protein, albumin and globulin) was observed.

Serum enzymes ALT, γ-GT, ALP, CK and LDH are commonly elevated following cellular damage of smooth muscles (heart, liver and kidneys) as a result of enzyme leakage from cells to blood. In present study, no negative effects of 1 and 1.5 % (experiment 1) and 1 % (experiment 2) NS supplementation on serum enzymes related to heart and liver functions were observed. Similarly, no significant change was observed by application of ethanol-methanol extract of NS in rats, but in cisplatin (anti-cancer drug used in chemotherapy and reported to cause damage to liver and kidney) treated rats NS supplementation reduced the toxic effects of drug on liver and kidney metabolism by maintaining levels of serum proteins and enzymes to normal (El-Daly, 1996).

In experiment 2, activity of ALT was 33 % lower (non significant) in NS treated birds than birds in control groups, which indicates that NS supplementation tends to reduce damage to vital organs reducing the release of ALT in circulation. However, in contrast to our findings significantly ($p<0.05$) higher level of ALT and γ-GT and lower levels of ALP were observed by Zaoui and co-workers (2002) and Al-Jishi and Hozaifa (2002) in rats after oral administration of aqueous extract of NS, but these changes were non toxic. Serum γ-GT and ALT concentration were increased following administration of NS aqueous extracts, without degenerative changes in hepatocytes (Tenekoon et al., 1991).

In both experiments, no significant treatment effects were observed on blood pictures, blood glucose and cholesterol levels. Increased number of leucocytes was observed by Osman and Barody (1999) in broilers, El-Soud et al., (2000) in quails, but no change was observed by El-Daly (1996) in rats.

The results of present experiments showed that supplementation of NS seeds in broiler diets does not have any negative effect on activities of serum enzymes related to heart and liver functions. No significant effects on blood picture, contents of serum cholesterol and glucose were observed. NS seeds posses potential to improve performance of birds in terms of better FCR, but further research is required to identify suitable dose levels based on concentrations of active ingredients.

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Table 5.1. Composition of the experimental diets

Ingredient (%)	Starter diet	Grower diet
Wheat	56.5	60.0
Soybean meal	33.5	29.3
Soybean oil	5.00	3.46
Palm fat	-	2.66
Limestone	1.12	1.06
Mono Ca Phosphate	0.08	1.46
Mineral premix [1]	0.08	0.08
Cholincholride	0.20	0.20
Methionine	0.20	0.17
L-Lysin-HCl	0.35	0.30
$NaHCO_3$	0.28	0.27
NaCl	0.10	0.35
Luprosil	0.40	0.53
Loxidan TD 100	0.015	0.015
Vitamin premix [1]	0.20	0.20

Table 5.1 (continued) Analysed values of nutrients (%) in experimental diets

Nutrient (%)	Starter diet		Grower diet	
	Without NS	With NS	Without NS	With NS
Dry matter	88.8	88.9	89.2	89.1
Crude protein	22.1	22.2	21.3	19.9
Crude fat	6.89	7.57	7.71	7.98
Crude ash	6.07	6.09	5.73	5.61
Crude fibre	2.80	2.62	2.62	2.78
NFE	51.0	50.1	51.8	52.8
Methionine [2]	0.50	0.50	1.24	1.24
Lysine [2]	1.39	1.39	0.45	0.45
Ca	0.98	0.97	0.92	0.91
P	0.84	0.82	0.68	0.71
ME (MJ/kg DM) [3]	12.3	12.3	12.6	12.4

[1]Supplements per kg of feed: Vit A 12600 I.U., Vit D_3 3150 I.U., Vit E 41 mg, Vit B_1 3 mg, Vit B_2 6mg, Vit B_{12} 32 µg, Niacin 53 mg, Pantothenic acid 13 mg, Folic acid 1050 µg, Biotin 105 µg, Fe 81 mg, Mn 108 mg , Zn 72 mg, Cu 14 mg, Iodine 1.44 mg, Selenium 0.45mg.

[2] Calculated values

[3] Calculation of ME (Metabolizable Energy) based on WPSA (1984)

Table 5.2. Expt. 1- Effects of supplementation of different levels of NS on broiler performance (mean ± SEM)

NS %	Final wt (g)	Total WG (g)	ADWG (g)	Carcass wt (g)	Dressing %	Abdominal fat %
0.0%	2228^{a} ±89	2179^{a}±89	62^{a}±2.5	1560^{a}±62	70.0^{ab}±0.4	1.66^{abc}±0.16
0.5%	1936^{b}±73	1889^{b}±73	54^{b}±2.1	1378^{b}±60	71.1^{a}±0.6	1.91^{ab}±0.15
1.0%	2073^{ab}±69	2027^{ab}±69	58^{ab}±2.0	1465^{ab}±50	70.7^{a}±0.3	2.02^{a}±0.19
1.5%	2064^{ab}±87	2019^{ab}±87	58^{ab}±2.5	1460^{ab}±59	70.8^{a}±0.6	1.46^{bc}±0.12
2.0%	2109^{ab}±103	2063^{ab}±104	59^{ab}±3.0	1475^{ab}±79	69.8^{ab}±0.5	1.30^{c} ±0.18
2.5%	2003^{ab}±63	1955^{ab}±63	56^{ab}±1.8	1384^{ab}±59	68.9^{b}±1.0	1.69^{abc}±0.14
p value	0.24	0.24	0.24	0.37	0.14	0.03

^{a,b,c} Means in a column without a common superscript are significantly different (P< 0.05).

NS: *Nigella sativa*, WG: weight gain, ADWG: average daily weight gain

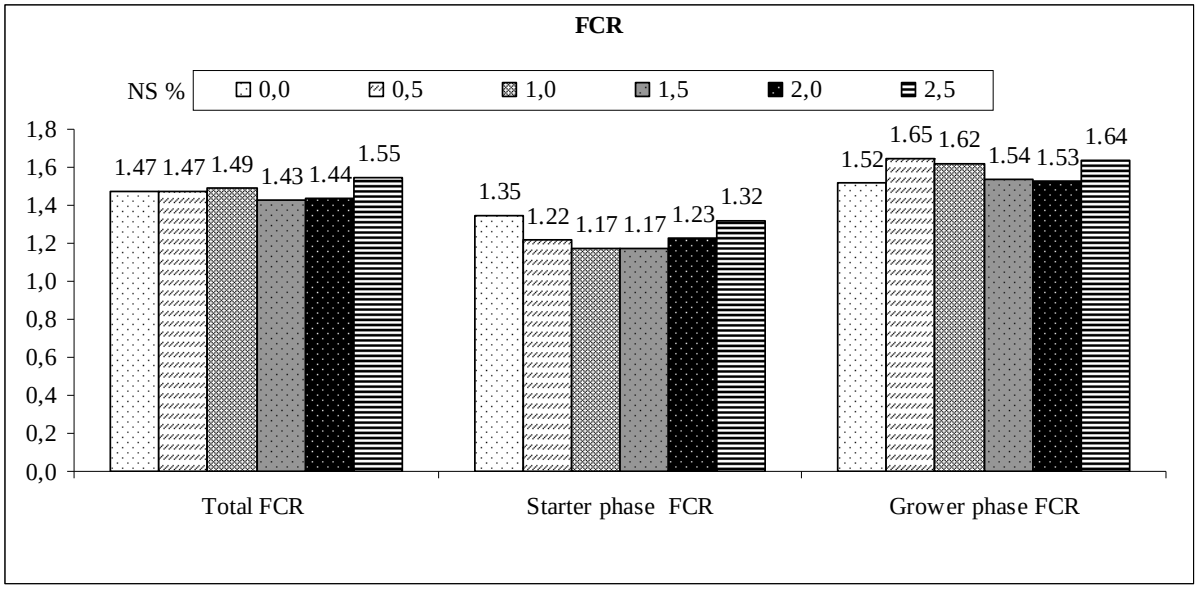

Figure 5.1. Expt. 1-Effects of supplementation of different levels of *Nigella sativa* seeds on feed conversion ratio (FCR) during different phases

Table 5.3. Expt. 1- Effects of different levels of grounded *Nigella sativa* seeds on different blood parameters on 35th day (mean ± SEM)

Parameters	***Nigella sativa* %**						p value
	0	0.5	1.0	1.5	2.0	2.5	
T. Protein (mg/dl)	3.03±0.03	3.23±0.20	2.98±0.15	3.10±0.12	3.07±0.03	3.17±0.09	0.73
Albumin (mg/dl)	1.50±0.00	1.50±0.06	1.48±0.05	1.47±0.03	1.43±0.07	1.53±0.03	0.75
Globulin (mg/dl)	1.53±0.03	1.73±0.14	1.50±0.10	1.63±0.09	1.63±0.03	1.63±0.07	0.54
ALT (U/l)	2.27^{b} ±0.98	4.43^{a}±0.62	1.48^{b}±0.28	2.77^{b} ±0.12	2.70^{b}±0.21	2.50^{b}±0.06	0.02
γ-GT (U/l)	26.0 ±2.1	25.7±3.2	31.7±1.3	27.7±2.7	31.0±7.0	23.7±6.0	0.30
ALP (U/l)	2276±365	2895±344	2353±561	3249±1138	4416±998	1878±999	0.31
CK (10^3 U/l)	19.3±2.7	36.4±12.0	16.5±5.7	13.3±0.66	19.7±1.1	15.9±2.2	0.14
LDH (10^3 U/l)	1.34±0.2	2.0±0.65	1.16±0.19	1.11±0.01	1.42±0.08	0.99±0.04	0.22
Glucose (mg/dl)	231±0.8	216±23	218±9.9	207±8.4	236±13.8	235±4.7	0.51
Cholesterol (mg/dl)	120±1.0	135±14	123±5.2	131±8.7	128±2.2	132±3.8	0.68
Erythrocytes (T/l)	2.24±0.61	2.70±0.21	2.07±0.19	2.42±0.43	2.22±0.23	1.70±0.80	0.69
Leucocytes (G/l)	9.67±3.1	8.33±2.7	9.58±5.2	9.90±1.3	19.3±7.8	12.5±7.5	0.66

^{a,b,c} Means within a row without a common superscript are significantly different (P< 0.05).

Table 5.4. Expt. 2- Effects 1% *Nigella sativa* seeds on broiler performance (mean ± SEM)

Parameter	Treatments		p value
	Control	1% NS	
Final wt. (g)	2059 ± 45	2012 ± 44	0.45
Total weight gain (g)	2019 ± 45	1972 ± 44	0.45
ADWG (g)	57.7 ±1.27	56.3 ±1.25	0.45
Feed consumption (g)	3413 ± 148	3234 ± 84	0.35
FCR (0-35)	1.62 ± 0.08	1.59 ± 0.05	0.74
FCR (0 – 14)	1.30 ± 0.04	1.25 ± 0.05	0.53
FCR (15 – 35)	1.79 ± 0.14	1.73 ± 0.06	0.74
Carcass weight (g)	1463 ± 33	1423 ±31	0.38
Carcass %	71.1 ± 0.23	70.7 ± 0.31	0.38
Abdominal fat (g)	21.6 ± 1.3	23.1 ± 1.4	0.43
Abdominal Fat %	1.05 ± 0.06	1.16 ± 0.17	0.40
Mortality (Number)	1	0	-

Table 5.5. Expt. 2- Effects of 1% *Nigella sativa* seeds on different blood parameters on 35th day (mean ± SEM)

Parameter	Treatments		
	Control	1% NS	p value
Total protein (mg/dl)	3.26±0.07	3.14±0.11	0.38
Albumin (mg/dl)	1.76±0.08	1.67±0.08	0.40
Globulin (mg/dl)	1.50±0.04	1.48±0.07	0.77
ALT(U/l)	2.55±0.52	1.70±0.29	0.18
γ-GT (U/l)	26.0±1.95	26.7±1.09	0.76
ALP (U/l)	2053±329	1945±302	0.81
CK (10^3 U/l)	31.4±5.8	30.2±5.9	0.89
LDH (10^3U/l)	1.89±2.8	1.75±2.2	0.91
Glucose (mg/dl)	211±5.2	210±5.7	0.83
Cholesterol (mg/dl)	138±5.0	133±4.1	0.41
Erythrocytes (T/l)	2.21±0.22	2.83±0.44	0.22
Leucocytes (G/l)	10.1±0.57	4.6±1.6	0.12

6 EFFECTS OF *ECHINACEA PURPUREA* AND *NIGELLA SATIVA* SUPPLEMENTATION ON BROILER HEALTH, PERFORMANCE AND MEAT QUALITY

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6.1 Abstract

Present experiment was performed to study the effects of *Echinacea purpurea* (EP) and *Nigella sativa* (NS) on broiler performance, carcass and meat quality. Four treatment combinations were prepared as: C (control group without any feed or water additive), E (drinking water intermittently supplemented with fermented juice of EP), N (feed supplemented with grounded seeds of NS) and EN (drinking water intermittently supplemented with EP and feed supplemented with NS). No significant treatment effect was observed on weight gain, average daily weight gain, feed conversion ratio and fat percentage. Carcass yield in group C was significantly ($P<0.05$) higher than in E group. Significantly ($P<0.05$) higher breast percentage in group N was observed. Crude protein contents were significantly ($P<0.05$) higher in meat samples of C and EN groups. Grill losses were significantly ($P<0.05$) lower in E and cooking losses were significantly ($P<0.05$) higher in N treated birds. No significant treatment effect was observed on meat colour, electrical conductivity and shear force value. Near significant treatment effects of combined application of NS and EP showed a potential of synergistic effects in improving broiler performance.

Keywords

Broilers, phytogenic feed additives, *Echinacea purpurea*, *Nigella sativa*, performance, meat quality

7 SUMMARY

Use of phytogenic substances in animal production is increasing due to their beneficial effects on feed digestion, promotion of production performance and improving product quality. Phytogenic substances contain a number of pharmacologically active substances which have shown their activities in different body systems and, therefore, they are considered as one viable alternative to in-feed antibiotics. The effects of phytogenic substances on feed digestion, gut physiology and microbial population are more diverse than the effects of antibiotics. Usually, in livestock production effects of phytogenic substances are only studied in terms of performance parameters and economical importance. Presence of a variety of active ingredients indicates that phytogenic substances may have effects on different metabolic pathways, activity of different enzyme systems, and immunity along with performance parameters. Especially, improvement of immunity may help birds to cope in a better way with stress situations during fattening and by this may enhance well-being as well as quality of products of animal origin. *Echinacea purpurea* (EP) and *Nigella sativa* (NS) belong to the group of phytogenic compounds that have been reported to stimulate immune system in laboratory trials as well as in human studies. Positive effects of EP and NS have been observed in a number of *in vitro* and *in vivo* experiments, which show that their application in poultry can be beneficial for maintaining health, improving immunity and performance parameters. However, no study was conducted to evaluate the comparative as well as synergistic effects of EP juices and NS seeds on broiler health, performance and activities of different enzymes related to heart and liver functions.

The present study was conducted in view of a more practical application of EP and NS in the field. Therefore, the objectives of this thesis were set to investigate the effects of supplementation of EP juices and grounded NS seeds on performance, metabolism (serum protein, blood picture and some liver and heart associated enzymes in blood), carcass and meat quality in broilers (Ross 308). For this purpose five preliminary trials were performed to investigate the effects on bird's metabolism. Based on the results obtained from preliminary trials, a fattening trial was carried out to study the comparative and synergistic effects of EP juice and NS seeds on performance, carcass and meat quality.

Three preliminary experiments were performed using two different preparations of EP juices; EP-F (EP fermented juice) and EP-A (EP juice on alcohol basis). Initially, effects of EP-F

and EP-A were compared along with testing different dose levels. Based on the information available in literature that only intermittent application of EP can show positive effects on non specific immunity and health, EP juices were orally supplemented intermittently for a limited time period (3 days), followed by three times (9 days) EP free application. The application of EP juice was repeated three times (on 1-3, 13-15 and 25-27 days) during 35 days of rearing period. The results of these preliminary experiments showed that EP juices do not have any negative effect on broiler health and performance. Comparing the effects of EP-F and EP-A, better performance and improved health was observed by application of EP-F juices. Application of 0.25 ml/kg $BW^{0.75}$ EP-F juice has shown more positive effects as compared to other treatment levels. EP-F treated birds showed significantly better average daily weight gain and significant improvement of serum globulins, indicating beneficial treatment effect on broiler performance as well as immunity. Levels of serum CK (creatine kinase) were significantly reduced in EP-F treated birds as compared to control, which shows that EP-F posses the potential to reduce incidence of mortality related to dysfunction of the cardio-vascular system in broilers. No toxic effect of EP-F application was observed on blood picture and activities of other serum enzymes related to heart and liver function.

Two preliminary experiments were performed using grounded NS seeds. In first experiment different levels (0, 0.5, 1.0, 1.5, 2.0, and 2.5 %) of grounded NS seeds were supplemented in basal broiler diets. 1 % Supplementation level of NS was found to be most suitable, which was used in second preliminary experiment against a negative control. Numerically better FCR during starter phase was obtained by application of 1 % NS seeds in broiler diets, but these effects were decreased during grower phase. Less number of mortalities was observed in NS treated birds as compared to control. NS supplementation has not shown any negative or distinct positive effect on activities of heart and liver related enzymes.

In the fattening trial, four treatment combinations were tested as: control (without any feed or water additive), E (drinking water intermittently supplemented with EP-F), N (feed supplemented with grounded seeds of NS) and EN (drinking water intermittently supplemented with EP-F and feed supplemented with NS). The results showed that application of EP-F juice as well as NS seeds has no negative effect on broiler performance, carcass and meat quality. Birds performed to their maximum potential and supplementation of EP-F and NS helped birds to perform better. Combined application of EP and NS resulted in reduced mortality and numerical (non significant) improvement in weight gain and feed consumption. Carcass percentage was not significantly different from birds supplemented with either EP-F or NS, but significantly less than in control. Combined application resulted in significantly ($P<0.05$) higher meat crude protein as

compared to EP-F and NS treatments. In general, a synergistic positive effect of simultaneous application of EP and NS can be expected on performance, carcass and meat quality in broilers.

Based on the results obtained during present study, it can be concluded that intermittent application of EP-F (through drinking water) and continuous application of NS seeds (through feed) has no negative effect on broiler performance, carcass and meat quality. Beneficial effects of *Echinacea* supplementation on broiler performance, health and immunity can be obtained by intermittent application of EP-F through drinking water. However, there might be some other treatments regimens that can show even more positive results. Combined application of EP-F and NS seeds has shown a trend of improved broiler performance and meat quality, which indicate synergistic effects of active ingredients of both phytogenic compounds. However, research is required to find mechanism of action of active ingredients of EP and NS on different physiological systems and performance parameters in more details under practical conditions.

8 ZUSAMMENFASSUNG

In der Mastgeflügelproduktion nimmt der Einsatz an phytogenen Futterzusatzstoffen auf Grund der beobachteten günstigen Effekte auf die Mastleistung und Produktqualität ständig zu. Phytogene Substanzen enthalten eine Vielzahl an pharmakologisch wirksamen Substanzen, die nachweislich die verschiedenen Körpersysteme beeinflussen können. Sie werden daher als eine mögliche Alternative zu den Leistungsförderern mit Antibiotikacharakter gesehen. Die Auswirkung der phytogenen Substanzen auf den Stoffwechsel, die Verdauungsphysiologie und die Keimflora im Darm sind allerdings komplexer als bei den Antibiotika. In der Tierproduktion wurden bisher in der Regel die Effekte der phytogenen Substanzen nur im Hinblick auf die Mastleistung und somit auf die Wirtschaftlichkeit der Erzeugung untersucht. Das Vorhandensein einer großen Anzahl an aktiven Inhaltsstoffen deutet aber darauf hin, dass phytogene Substanzen erhebliche Auswirkungen auf unterschiedliche Stoffwechselvorgänge haben dürften. So sind vor allem Wirkungen auf die unterschiedlichen Enzymsysteme und das Immunsystem zu erwarten. Eine Verbesserung des Immunstatus kann z.B. dazu beitragen, dass die Tiere besser mit Stresssituationen fertig werden, wodurch sowohl das Wohlbefinden als auch die Qualität der Produkte gesteigert werden. *Echinacea purpurea* (EP) und *Nigella sativa* (NS) gehören zu dieser Gruppe der phytogenen Zusatzstoffe, für die sowohl in Laborstudien als auch in Studien beim Menschen ein immunostimulierender Effekt belegt wurde. Auch beim Geflügel wurde inzwischen die positive Wirkung von EP und NS in einer ganzen Reihe an *in vitro* und *in vivo* Versuchen bestätigt. Das Kernergebnis dieser Versuche war, dass sich diese Substanzen günstig auf den Erhalt der Gesundheit und auf das Immunsystem auswirken, wodurch auch die Leistung günstig beeinflusst wird. Allerdings fehlen beim Geflügel bisher noch Studien, in denen die Auswirkung von EP und NS auf die Funktion verschiedener Organsysteme (Kreislauf, Leber), auf das Immunsystem, auf die Gesundheit und auf die Leistung sowohl einzeln als auch in Kombination untersucht wurde.

Die vorliegende Studie wurde daher im Hinblick auf eine mehr praktische Anwendung von EP und NS in der Praxis konzipiert. Das Ziel war entsprechend, die Effekte

eines Zusatzes von EP und NS auf die Leistung, den Stoffwechsel (Serumproteine, Blutbild, einige Leber- und Herzspezifische Enzyme im Blut), die Schlachtkörper- und die Fleischqualität bei Masthühnern zu untersuchen. Die Auswirkungen von EP und NS auf den Stoffwechsel der Tiere wurde im ersten Teil der Studie in fünf Detailversuchen analysiert. Auf der Basis der Ergebnisse der Detailversuche wurde dann ein Mastversuch durchgeführt, in dem die Effekte von *Echinacea* und der *Nigella* einzelnen und in Kombination auf die Leistung, die Schlachtkörper- und die Fleischqualität betrachtet wurde.

In drei Detailversuchen wurden zwei verschiedene Echinacea-Produkte verwendet: EP-F (EP, fermentierter Extrakt) und EP-A (EP, Extrakt auf Alkoholbasis). Zunächst wurde EP-F mit EP-A verglichen, wobei verschiedene Dosierungen getestet wurden. Im einschlägigen Schrifttum ist belegt, dass nur durch einen intermittierenden Einsatz von *Echinacea* positive Effekte auf die unspezifische Immunität und die Gesundheit erzielt werden können. Daher wurden in den eigenen Untersuchungen die Extrakte nur über eine begrenzte Zeitperiode (3 Tage), gefolgt von einer Applikationsfreien Periode (9 Tage), eingesetzt. Die Anwendung des Extraktes wurde dreimal während der 35 tätigen Aufzuchtperiode wiederholt (1.-3., 13.-15. und 25.-27 LT). Die Ergebnisse der Detailversuche zeigen, dass sich der EP-Extrakt weder auf die Gesundheit noch auf die Leistung der Tiere negativ ausgewirkt hat. Der Vergleich von EP-F mit EP-A ergab, dass mit dem fermentierten Extrakt eine bessere Mastleistung und ein günstigerer Gesundheitsstatus erzielt wurden. Die Dosierung von 0,25 ml EP-F/$kg^{0,75}$ Körpergewicht zeigte im Vergleich zu den anderen Dosierungen mehr positive Effekte. Generell wiesen die Tiere der *Echinacea*-Behandlung bessere durchschnittliche tägliche Zunahmen und einen signifikant höheren Serumprotein-Spiegel auf. Dies deutet auf positive Effekte von *Echinacea* sowohl auf die Leistung als auch auf das Immunsystem der Broiler hin. In ähnlicher Weise wurde bei den EP-F Tieren auch eine signifikante Reduzierung des Serum-CK-Spiegels beobachtet. Unter Umständen kann EP-F bei Masthühnern auf diesem Weg das Risiko von Mortalität auf Grund von Funktionsstörungen des Herz-Kreislaufsystems vermindern. Nachdem weder des Blutbild noch die Aktivität der Herz- und Leberspezifischen Serumproteine verändert waren, kann der Schluss gezogen werden, dass der Einsatz von EP-F zu keiner Beeinträchtigung des Gesamtstoffwechsels führt.

In zwei weiteren Detailversuchen wurde die Wirkung von gemahlenen *Nigella sativa* Samen untersucht. Im ersten Versuch wurde NS den Rationen in verschiedenen Dosierungen (0 – 0,5 – 1,0 – 1,5 – 2,0 – 2,5 %) zugesetzt. Die 1 %ige Dosierung führte zu den günstigsten Resultaten und wurde daher im zweiten Versuch gegen eine Kontrolle ohne Zusätze getestet. Während der Starterphase führte der 1 %igen NS-Zusatz zu einer tendenziell besseren Futterverwertung. Dieser Effekt verschwand allerdings in der Growerphase. Generell war beim Einsatz von NS die Mortalität geringer als in der Kontrollgruppe. NS hatte weder negative noch deutliche positive Effekte auf die Aktivität von Herz- und Leberspezifischen Serumenzymen.

Der Mastversuch umfasste vier Behandlungen: Kontrolle (ohne Wasser- oder Futterzusatz), EP-F (intermittierender Zusatz von *Echinacea* zum Trinkwasser), NS (Zusatz von 1 % *Nigella* zum Futter) und EN (intermittierender Zusatz von *Echinacea* zum Trinkwasser und Zusatz von 1 % *Nigella* zum Futter). Die Leistung, die Schlachtkörper- und die Fleischqualität der Broiler wurde durch die Zusätze von *Echinacea* und *Nigella* nicht nachteilig beeinflusst. Generell wurde im Versuch das vom Züchter angegebene Leistungsniveau erreicht und die Zugabe von EP-F und NS führte nur zu unwesentlichen Verbesserungen. Der kombinierte Einsatz von *Echinacea* und *Nigella* bewirkte einen leichten Rückgang in der Mortalität und eine tendenzielle (nicht signifikant) Verbesserung in den Zunahmen und im Futterverbrauch. Die Schlachtausbeute unterschied sich zwar nicht von den Behandlungen EP-F und NS, war aber signifikant geringer als in der Kontrollgruppe. Dagegen führte der kombinierte Einsatz von EP-F und NS im Vergleich zum getrennten Einsatz zu einem signifikant ($P<0,05$) höheren Proteingehalt im Fleisch. Der gemeinsame Einsatz von EP-F und NS hatte somit insgesamt einen positiven Effekt auf die Leistung und die Fleischqualität der Masthühner.

Auf der Basis der Ergebnisse der vorliegenden Studie kann der Schluss gezogen werden, dass der intermittierende Einsatz des fermentierten *Echinacea*-Extraktes über das Trinkwasser und der kontinuierliche Einsatz von *Nigella* über das Futter zu keinen negativen Effekten auf die Mastleistung, die Schlachtkörper- und die Fleischqualität führt. Der intermittierende, alleinige Einsatz von *Echinacea* über das Trinkwasser scheint sich positiv auf die Leistung, die Gesundheit und das Immunsystem der Tiere auszuwirken.

Allerdings können andere Behandlungsregime, die in der vorliegenden Untersuchung nicht berücksichtigt wurden, eventuell zu noch positiveren Ergebnissen beitragen. Die beobachtete positive Wirkung des kombinierten Einsatzes von EP-F und NS auf die Mast- und die Schlachtleistung deuten auf synergistische Effekte der in den beiden Produkten enthaltenen, pharmakologisch wirksamen Inhaltsstoffe hin. Allerdings muss in Zukunft die Wirkung der aktiven Inhaltsstoffe von EP-F und NS auf die Stoffwechselphysiologie und die Leistungsparameter unter praktischen Bedingungen noch detaillierter untersucht werden.

Declaration

I assure that this doctoral thesis is the result of my personal work and that no other than the indicated aids have been used for its completion. All quotations and statements that have been used are indicated. Furthermore I assure that the work has not been used, neither completely nor in parts, for achieving any other academic degree.

Stuttgart-Hohenheim, April 2009

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