EFFECT OF OMEGA-3 FATTY ACIDS AND PHYSICAL EXERCISE ON EGG QUALITY, BONE CHARACTERISTICS AND PHYSIOLOGICAL PARAMETERS IN LAYING HENS

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Ardita JAHJA
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1 GENERAL INTRODUCTION

1.1 BACKGROUND AND MAIN RESEARCH OBJECTIVE

Foods of animal origin such as eggs take an important place in human diets because of their nutritional qualities. Polyunsaturated fatty acids (PUFA) such as 18:3 n-3 and 18:2n-6 are essential and cannot be synthesized in the metabolism of animals and humans; however they can be incorporated into egg or tissue lipids through dietary fat. Incorporation of 18:3n-3 in the laying hens’ diet has been reported to modify the omega-3 (n-3) and omega-6 (n-6) fatty acid composition of egg yolk. The biochemical pathway of fatty acid biosynthesis or tissue deposition may be influenced by the nature of dietary fatty acids. In addition, factors other than diet, such as locomotor activity, may affect lipid metabolism in the laying hens.

Increasing concern on the relationship between animal fats high in saturated fatty acids and coronary heart disease has lead health experts to recommend a reduction in the consumption of animal products in general. However, enrichment of animal product with omega-3-fatty acids may present an opportunity to reduce health risks in humans. The human organism is not able to synthesize the required amounts of polyunsaturated fatty acids (PUFA) by itself (NEWTON, 1996) and, therefore, depends on a sufficient dietary supply for proper functioning of the body (VAN ELSWYK, 1997). Due to the cholesterol lowering (CASTON and LEESON, 1990; JIANG et al., 1991; WATKINS, 1992; FERRIER et al., 1995; SCHEIDELER and FRONING, 1996; SARI et al., 2002) and protective effects of n-3 PUFA against coronary disease (ZEGHICHI-HAMRIS et al., 2010; ADKINS and DARSHAN., 2010), incorporating PUFA and increasing the polyunsaturated to saturated fatty acids ratio of egg yolk or tissue lipids would make poultry products more acceptable to health-conscious consumers. PUFA are mainly needed to synthesize eicosanoids which have an important role to play in controlling cardiovascular, inflammation and immunological processes. Two different types (series) of eicosanoids are synthesized starting either from linoleic (n-6) or linolenic (n-3) fatty acids by using the same enzymes. The optimum ratio between n-6 and n-3 fatty acids in the food is between 5:1 and 10:1 for human nutrition. In Western countries, due to the consumption of food rich in linoleic acid, the dietary content of n-6 fatty acids in relation to n-3 fatty
acids is higher than >25:1 (SINGER, 2000). Therefore, any strategies to increase the content of n-3 fatty acids in human food are appreciated. Traditionally, see fish is a suitable source of n-3 fatty acids, but the consumer acceptance of fish varies distinctly between countries (KROMHOUT et al., 1985). An alternative strategy may be to develop food with higher contents of n-3 PUFA, e.g. ‘functional eggs’ which are enriched with n-3 fatty acids (e.g. FARRELL, 1995; ALBRECHT and KLEIN, 1995; NEWTON, 1996).

Poultry meat and eggs are gaining importance in human nutrition, especially in low income countries of Europe (e.g. Balkan countries), in Africa, Asia and Latin America (FAO, 2010). It has been shown in numerous studies that the level of Omega-3-fatty acids and the ratio of n-6/ n-3 fatty acids poultry products can be improved considerably through special diets (such as poultry meat and eggs enriched with n-3 PUFA). Enriching eggs with ingredients like omega-3 fatty acids has become a broad field of research. Besides sea fish products, several n-3 containing fats, algae products, linseed, rapeseed and herbs have been used as feeding stuffs for enriching eggs with n-3 PUFA (NARAHARI, 2003). Besides nutrition further factors may enhance deposition of n-3 fatty acids in egg yolks. STEINHILBER (2003) found differences in deposition of n-3 fatty acids in egg yolk among different layer breeds and husbandry systems. Eggs from organic and free-range farms contained higher n-3 fatty acids levels than eggs from indoor systems. Yet, it is not clear which factors in free range systems are the main contributors to this enrichment. Since hens in free range show a high level of locomotion, it has been assumed that physical exercise may play a role in this context.

Physical exercise not only influences the physiological mechanisms of bone formation (modelling and remodelling) (WHITHEAD, 2004; RUTTEN et al., 2002) but also the metabolism of fatty acids. This effect has been demonstrated in different animal species: dogs and goats (MCCLELLAND et al., 1995), horses (GOTTLIEB et al., 1989), rabbits (MENG and PEIRCE, 1990) as well as in humans (BROUNS and van der VUSSE, 1998; HELGE et al., 2001).

Bone breakage is a serious welfare problem of laying hens in both intensive and extensive husbandry systems. There are numerous reports on changes in bone criteria and other physiological characteristics of laying hens kept under management systems with varying
levels of locomotor activity. Higher bone strength of hens kept in cages with perches vs. cages without perches has been reported by HUGHES and APPLEBY (1989), DUNCAN et al. (1992) and LEYENDECKER et al. (2005). Similar results have been observed when bone strength of layers in non-cage systems and conventional cage systems were compared (ROWLAND and HARMS, 1970; ROWLAND et al., 1968; NORGARD-NIELSEN, 1990; FLEMING et al., 1994; ABRAHAMSSON and TAUSON, 1995; NEWMAN and LEESON, 1998; LEYENDECKER et al., 2005).

Most bone fractures are commonly seen in hens selected for egg production and the fractures are attributed to osteoporosis (THORP and MAXWELL, 1993). The metabolic competition for calcium and phosphorus in eggshell formation and bone formation has been considered as important factor of osteoporosis and weak bones in layers. With sexual maturation, the medullary bone develops on the endosteal surface of long bones. The medullary bone has a capacity of remodeling and provides Ca for eggshell formation (DACKE et al., 1993). This usually leads to bone weakness. In addition, limited opportunity for exercise in conventionally caged laying hens reduces osteoplastic processes and thus, increases the susceptibility to bone fractures. This compromises hen’s welfare and has negative consequences for production (JENDRAL et al., 2008).

The extent of physical exercise in different husbandry systems affects structural bone loss and bone strength in laying hens (FLEMING et al., 1994). KNOWLES and BROOM (1990) found that in birds kept in housing system with varying degrees of spatial freedom bone strength was related to the amount of movement. MEYER and SUNDE (1974) studied bone breakage and bone strength in hens kept on floor and in cages. Bone breakage was higher and bone breaking strength lower in caged reared hens. Physical exercise on a treadmill of caged hens reduced the number of broken bones and increased the breaking strength of humerus while breaking strength of the tibia remained unchanged.

Broken bones were found in about 30% of hens before slaughter and the proportion reached 90% in carcasses at the end of the processing line (GREGORY and WILKINS, 1989). Layers kept in aviaries usually show higher bone strength, however, the incidence of bone fractures of hens of these systems was on similar level. Bone breakage in hens from conventional cages occurred during catching and transport, whereas in contrast, the
bone breakages in hens from aviaries mainly were the result of crash landing during switching between levels of the system (BOSCH and VAN NIEKERK, 1994). So, the higher bone strength of hens in aviaries was accompanied by higher risk in this system. This led to the assumption that low bone breaking strength in caged hens is caused by low level of exercise. Bone development also depends on a wide range of other factors. Prostaglandins, for example, derived from polyunsaturated fatty acids (PUFA) of the n-6 and n-3 series play a role in bone development. Prostaglandins of n-6 fatty acids origin inhibit to some extend bone development, while prostaglandins of the n-3 series stimulate osteoblast function and bone development (CHANG et al., 1998).

Calcium, phosphorus, trace elements and vitamins are important nutrients for bone strength (RÄTH et al., 2008). However, we consider that these nutrients are usually adequate in commercial layer diets.

Recent studies also highlighted the beneficial effects of n-3 fatty acids. LIU et al. (2003a, b) found an improved strength of tibia in Japanese quail in response to supplementation of PUFA from fish oil. MCCORMACK et al. (2006) found better breaking strength in bones of broilers when the n-3/n-6 ratio was increased by replacing maize oil by fish oil. Similar results have been reported by MANSOUB et al. (2011). The breaking strength of broiler bones was significantly improved when the omega-3 fatty acid level was increased through fish oil. JOHNSTON et al. (2006) could not find significant effects of n-3 rich diet on bone strength in turkey breeders. BAIRD et al. (2008) modified the n-6/n-3 ratio in the diet of layers kept in individual cages from 47.8: 1 to 4.7: 1. Only the cortical thickness responded significantly to the decrease in n-6/n-3 ratio. There was a curvilinear response with the lowest values at the highest and lowest ratio. The contrasting results of n-3 fatty acids on the chicken bones in the above cited experiments may be due to interactions with other factors.

The relationship between dietary fatty acids and the deposition of fatty acids in eggs and poultry meat is well established. It is also acknowledged that physical exercise improves bone strength in chickens. There is, however little and inconsistent information on the effect of n-3 fatty acids on bone strength and on the effect of physical activity on the modification of the fatty acids in egg yolks. Furthermore, information on the combined
effects of dietary fat source and exercise on metabolic measures in the blood are rare. Therefore, in the present study laying hens were subjected to a controlled physical exercise on a treadmill and fed diets which differed largely in their n-6/ n-3 ratio. The effects of the treatments on egg quality, bone characteristics and physiological criteria have been studied. The results are presented in three papers (Chapter 2)
Summary

The experiment was performed to study the combination of effects of diets and physical body activity (training) on laying hens’ performance, egg weight, yolk proportion and fatty acids profiles. Results of present experiment show that dietary fat source affects egg production rate, egg weight and yolk proportion. In diets palm oil, soybean oil and linseed oil were used as fatty acids sources. Physical activity was provoked on a treadmill.

Exercise on the treadmill reduced feed intake and egg production. No significant effect of dietary fat source and running treatment was observed on laying hens performance. Diets significantly influenced egg weight, yolk proportion and fatty acids profiles either egg weight, yolk proportion or fatty acids profiles indicating a dominating effect of dietary fat source.

Keywords
Laying hens, palm oil, soybean oil, linseed oil, fatty acids, yolk
2.2 JAHJA ET AL.: PHYSICAL ACTIVITY AND BONE CONDITION IN LAYING HENS

EFFECT OF PHYSICAL ACTIVITY OF LAYING HENS ON BONE CONDITION

Einfluss von Bewegungsaktivität auf den Knochenzustand von Legehennen

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Summary

The experiment was performed to study the combination of effects of fatty acids profiles of diets and physical activity (training) on bone several characteristics. Three experimental diets with different fat sources were fed to 12 laying hens each: Palm oil (PO), Soybean oil (SO), and Linseed oil (LO). The fat sources corresponded to a low content of poly unsaturated fatty acids (PUFA) – PO, a high content of omega-6 (n-6) fatty acids – SO, and a high content of n-3 fatty acids (LO). Half of the hens of each dietary treatment (6 x 3 = 18 birds) were exposed to exercise by walking on a running treadmill (EG) through the four wks lasting experiment, whereas, the remaining 18 hens served as a control group (CG) and were permanently kept in the individual cages with very limited walking space. At the end of the experiment different bone characteristics were determined at the tibia by computer tomography, shear force tool and chemical analyses.

The application of training for the locomotion activity in laying hens has affected some bone characteristics. The same was true for the dietary fat source. But, the overall effect of
diets on bone characteristics was not significant. Nevertheless, there is a trend of higher level of total area and corticalis area in LO group as compared with PO and SO groups.

Physical exercise did not significantly affect bone characteristics. But there was a trend of higher level of total area and corticalis area in the LO group as compared with PO and SO group. Total density and cortical density showed the opposite tendency. Significant diet x exercise interactions were observed for total area (TOT_A), corticalis area (CORT_A) and corticalis density (CORT_DEN). In tendency, diet PO resulted in lower TOT_A, CRT_A and SSI, but in higher TOT_DEN and SSI. Running on the treadmill resulted in lower TOT_A and CORT_A for diets LO and PO, whereas, higher values were observed for birds with exercise fed on diet SO. The relation was opposite for corticalis density. Here, lower values were observed for birds without exercise fed on diets LO and PO. CORT_DEN was lower for birds with exercise fed on diet SO. It is concluded that in hens fed on diets low in n-3 fatty acids physical exercise can improve bones condition.

**Keywords**

Laying hens, physical activity, bone development, feeding mixture, fat sources
2.3 EFFECT OF DIETARY FATTY ACID PROFILES AND PHYSICAL ACTIVITY ON PHYSIOLOGICAL PARAMETERS IN LAYING HENS

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Introduction

The effects of dietary fat sources on performance and on fatty acid profiles of egg yolks are well investigated in laying hens (e.g. NEWTON, 1996). STEINHILBER (2003) concluded that physical activity of laying hens in a free range husbandry system resulted in the observed increased incorporation of long-chain poly-unsaturated fatty acids (PUFA), mainly of class omega-3 (n-3), into egg yolks. In an experiment where laying hens were fed with diets differing distinctly in their fatty acids profiles and were subjected to physical exercise on a treadmill no clear effects on performance of hens was observed but fatty acids profiles of egg yolks reflected the used dietary fat sources (JAHJA et al., 2011). In other studies it was reported that dietary fatty acids profiles also affect the metabolism in the hens. High dietary contents of n-3 PUFA reduce the blood cholesterol level of laying hens (GRASHORN, 1994; BASMACIOGLU et al., 2003; SVEDOVA et al., 2008), but do not affect the plasma cholesterol level in humans consuming daily several n-3 PUFA enriched eggs (FERRIER et al., 1995). Changing the n-6/n-3 ratio in the diet directly influences the eicosanoid (e.g. thromboxans, prostaglandins, leucotriens) metabolism shifting it either to the production of series-2 eicosanoids (n-6 pathway) or series 3 eicosanoids (n-3 pathway)(VAN ELSWYK, 1994; HERMIER, 1993; BLANCH and GRASHORN, 1996). F2 prostaglandin e.g. increases the aggregation of blood platelets whereas F3 prostaglandin counteracts. This effect was be proven by YEH et al. (2009) and
PITA et al. (2011) when feeding laying hens with diets containing high contents of n-3 PUFA. Furthermore, JAHJA et al. (2013) observed higher bone total area, corticalis area, and bone strength for higher n-6/n-3 ratios (0.8 vs 8.5 and 10.3) which was in agreement with observations of WHITEHEAD (2007).

In contrast to the lipid metabolism information on the effects of dietary fatty acids profiles on other metabolic processes (e.g. liver functioning, stress compensation) is lacking.

Physical exercise has clear metabolic effects, depending both on intensity and type of exercise. Moderate exercise appears to be of first importance (TURCOTTE, 1999). In contrast, a long term training protocol results in distinct changes in substrate metabolism (GEOR et al., 2002). Several data have been published on training-induced physiological changes in humans (BROUNS and VAN DER VUSSE, 1998; HELGE et al., 2001), horses (GOTTLIEB et al., 1989), dogs, goats (MC CLELLAND et al., 1995) and rabbits (MENG and PIERCE, 1990), but rarely in laying hens. According to VINCENT and BRACKENBURY (1988) and BRACKENBURY et al. (1993) training increases the use of free fatty acids for energy generation and improves the nutrient supply of thigh muscles (BRACKENBURY et al., 1990) as indicated by several blood characteristics.

To better understanding the effects of dietary fat sources and physical exercise on the metabolism in the hen studies on metabolic indicators in the blood seem to be feasible. Especially, metabolic activity of the liver is of interest as this organ is the main site for lipid metabolism. Alanin-Aminotransferase (ALAT) is a measure for hepatocellular injury. Significantly elevated levels of ALAT indicate the existence of heart failure or/and damage or liver functioning problems. Total cholesterol (CHOL) level is measured in order to indicate normal functioning of lipid metabolism and actual stress status. Creatinkinase (CK) is involved in energy metabolism in cells. Increased values are observed under stress and after excessive activity (HALLBACH, 2006).

The objectives of the present study have thus been to investigate the effects of dietary fat sources in combination with physical exercise on heart and liver weight and on some physiological indicators in laying hens.
Materials and methods

Birds and housing
In total, 36 young brown non-beak-trimmed laying hens (Bovans Brown) were used in the experiment. Hens were randomly taken from group pens of 120 birds each. At 22 wks of age the hens were moved to individual cages (area 2,250 cm², 38-48 cm in height). Each cage was equipped with a feeder (18.5 cm wide) and two drinking nipples. Complete layer diet and water were available ad libitum (for details see JAHJA et al., 2011).

Treatments
Hens were fed on diets low in PUFA – palm oil (PO), rich in n-6 fatty acids – soybean oil (SO) or rich in n-3 fatty acids – linseed oil (LO). Half of the hens (6 birds) of each dietary treatment were exposed to exercise by walking on a running treadmill (EG) through the four wks lasting experiment. The remaining hens were kept in individual cages with limited walking space (CG). Details of treatments are given by JAHJA et al. (2011).

Data collection
At the end of the experiment laying hens were slaughtered for collecting blood serum, tibia bones (JAHJA et al., 2013) and for determining liver and heart weight. After 12 h starvation hens were stunned by hitting the head and killed by cutting the blood vessels at the neck. Blood was immediately collected in tubes without coagulant for retrieval of blood serum. After clotting of the blood tubes were centrifuged at 2,500 rpm for 15 minutes. The serum was then transferred to serum tubes for further analyses.

After debleeding, carcasses were scalded and mechanically defeathered. During evisceration hearts and livers were withdrawn and weights of carcass, heart and liver were determined with an accuracy of 0.1 g. Relative weights of hearts and livers were calculated by dividing weights through carcass weight.

Laboratory analysis
Contents of main nutrients and fatty acids in diets have been analyzed as described previously (JAHJA et al., 2011).
Blood serum was sent refrigerated to Vet Med Laboratory GmbH, Ludwigsburg, Germany, for analysis of contents of Alanin-Aminotransferase (ALAT), cholesterol (CHOL) and creatinkinase (CK).

**Statistical analysis**

Prior to statistical analysis data were tested for deviation from normal distribution. As no significant deviation was observed data were subjected to a two-way analysis of variance using the software package SAS (JMP, 2007).

**Results and discussion**

Diet SO contained the highest level of n-6 fatty acids, while LO contained the highest level of n-3 fatty acids (Table 1). The n6/n3 ratios decreased from PO to SO and LO (10.3, 8.5 and 0.8 %, respectively). Effects on performance, fatty acids profiles of egg yolks and bone condition have been published previously (JAHJA et al., 2011; JAHJA et al., 2013).

Diets significantly reduced life body weight (Table 2) and in tendency egg production rate (JAHJA et al., 2011) in laying hens fed on PO diet. This may be caused by the low content of linoleic acid (0.7 %; Table 1) in diet PO. HAERTEL (1972) has shown that linoleic acid has some additional effects beside the fat effect in the intermediary metabolism resulting in improved performance. For an optimal performance level a content of 1.0 % linoleic acid is recommended for laying hens (GFE, 1999). In contrast to the dietary fat source exercise did not affect body weight. As interactions between diet and exercise were not significant for body weight the dietary content of linoleic acid seems to be the reason for observed differences in body weights.

Carcass weights mainly reflect treatment effects on body weight (Table 2). In contrast to body weight effects of diets on carcass weight were not significant. But, relative organ weights have been partly influenced by diet and/or exercise. In tendency, relative heart weights have been higher for diets PO and SO and for exercised hens. The interaction between diets and exercise is significant and indicates that training is improving heart functioning. The biggest difference is visible between treatments LO-CG and SO-EG. The
high content of linoleic acid in diet SO results mainly in the formation of series-2 eicosanoids which e.g. increase heart rate and blood pressure (FARRELL, 1995). In combination with the expected increased metabolic activity due to improved performance (HAERTEL, 1972) and with the training effect the high relative heart weight in treatment SO-EG may be explained. Both, metabolic and physical activity require oxygen and energy which has to be transported to the target tissues by blood.

Relative liver weights have mainly been affected by diet (Table 2). Feeding PO diet resulted in a significantly higher relative liver weight. According to HAERTEL (1972) a low content of linoleic acid results in an increased fat catabolism and in an enlargement of the liver due to an accelerated de novo fat synthesis from sugar. Unfortunately, the chemical composition of the liver was not determined in the present experiment. Exercise in general enlarges energy demand resulting in increased metabolic activity and efficacy as already indicated. Therefore, training on the treadmill reduced relative liver weights in tendency. These effects are also reflected by diet x training interactions. Especially, training of hens fed on LO diets resulted in a significantly lower relative liver weight. Probably, the high content of n-3 fatty acids in diet LO improved the metabolic efficacy due to the favorable n-6/n-3 ratio.

No standard serum ALAT levels are known for chicken. Therefore, an assessment whether the observed values indicate pathological changes is not possible. In general, treatments did not significantly affect ALAT levels in blood (Table 2) but distinct differences were observed between dietary treatments. The highest ALAT level has been determined for diet SO which may reflect the high metabolic activity of this group. In general, exercise on the treadmill resulted in lower blood ALAT levels. Probably, high metabolic activity of hens fed with diet SO resulted in a higher stress to the liver, but exercise may relieve the liver. This assumption seems to be confirmed by the diet x exercise interaction. Exercise did not result in distinct differences in ALAT levels for diets LO and PO, but clearly reduced ALAT levels for diet SO. This seems to be in contrast to the assumption of BRACKENBURY et al. (1993) that exercise increases liver activity. This should also be visible by increased ALAT values. In the present experiment feeding diet SO resulted in
distinctly higher ALAT levels for hens without exercise. Obviously, exercise improves the metabolic efficiency in the liver and reduces liver load.

Blood cholesterol level was significantly higher for diet PO (Table 2). This is in agreement with the observations of BASMACIOGLU et al. (2003), GRASHORN (1994), SARI et al. (2002) and SVEDOVA et al. (2008) that high contents of n-3 fatty acids and more favorable n-6/n-3 ratios in diets reduce blood cholesterol levels. Exercise resulted in a slight increase in serum cholesterol level. This may be a side-effect of training what is also visible by the interaction. Activity enhances the energy metabolism (BRACKENBURY et al., 1990; BRACKENBURY et al., 1993; VINCENT and BRACKENBURY, 1988) what is paralleled by higher blood cholesterol levels for all dietary fat sources due to the increased insulin secretion (DOENECKE et al., 2005). The distinctly higher cholesterol level of PO-CG and PO-EG is caused by the low content of PUFA, mainly n-3 fatty acids, in the this diet. For diet PO, exercise may partly compensate the unfavorable lipid metabolism. This is in agreement with the observed higher relative liver weight and a confirmation of the proposed higher lipid content due to de novo fatty acids formation.

No significant treatment effects could be observed on blood creatinkinase (CK) levels (Table 2). CK is involved in lipid metabolism and is an indicator of stress (IMAEDA, 1999). Higher CK levels for treatments PO and SO may indicate enhanced lipid metabolism and/or increased formation of series-2 eicosanoids, respectively. Comparable CK levels for exercised and non-exercised hens do not point at a general stress situation due to training. But, based on diet x training interactions exercise seems to have a positive effect for treatments PO and SO. The negative effect for treatment LO is difficult to explain. Maybe, this is a specific effect of the series-3 eicosanoids derived from α-linolenic acid (n-3) which enhance metabolic efficacy and stabilize the cardio-vascular system. In general, interactions for serum CK levels do not follow interactions for serum cholesterol levels. Especially, low serum CK levels for treatment SO-EG indicate that CK is not a primary source for energy supply.

Conclusions

Diet and exercise mainly affected performance characteristics of hens and fatty acids profiles of egg yolks, whereas, effects on blood characteristics have been less clear. In
general, blood characteristics are more affected by dietary fat sources than by physical exercise. But, exercise can improve metabolic efficacy if dietary PUFA content is low. Nevertheless, diets with a higher content of poly-unsaturated fatty acids seem to have a more beneficial effect on the metabolism. LO results in the highest metabolic efficacy, whereas, SO results in the highest metabolic turnover. In general, PO has a negative impact on the metabolism of the hen and this may not be fully compensated by exercise.

Summary

Thirty six young Bovans Brown laying hens were used to determine the effect of treadmill exercise and dietary fat source (palm oil - PO, soybean oil - SO, linseed oil - LO) on performance, some relative organ weights and some blood indicators of metabolism. Results show that mainly dietary fat source affects weight of laying hens, relative liver weight and serum cholesterol level. Die PO resulted in lower body weight, increased relative liver weight and increased serum cholesterol level. Hens fed with diet SO (rich in n-6 fatty acids) showed the highest serum ALAT level indicating an accelerated metabolic activity. There were no significant effects of exercise in a treadmill on determined characteristics. But, in tendency exercise increased relative heart weight and decreased relative liver weight. Observed interactions between dietary fat and exercise reveal that exercise can compensate negative side-effects of an increased metabolic activity for diets SO and LO, whereas, the unfavorable effects of a diet with a low content of linoleic acid (PO) cannot be equalized.

Keywords
Laying hens, palm oil, soybean oil, linseed oil, fatty acids, and heart

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Table 1. Fatty acids profiles of experimental diets

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>PO</th>
<th>SO</th>
<th>LO</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 16:0, mg/g</td>
<td>19.7</td>
<td>4.7</td>
<td>3.0</td>
</tr>
<tr>
<td>C 18:0, mg/g</td>
<td>3.6</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>C 18:1n9c, mg/g</td>
<td>5.6</td>
<td>9.8</td>
<td>8.4</td>
</tr>
<tr>
<td>C 18:2n6c, mg/g</td>
<td>7.3</td>
<td>22.4</td>
<td>11.1</td>
</tr>
<tr>
<td>C18:3n3, mg/g</td>
<td>0.58</td>
<td>2.5</td>
<td>14.2</td>
</tr>
<tr>
<td>SAT, %</td>
<td>64.9</td>
<td>14.1</td>
<td>12.0</td>
</tr>
<tr>
<td>MUFA, %</td>
<td>13.8</td>
<td>20.3</td>
<td>20.8</td>
</tr>
<tr>
<td>PUFA, %</td>
<td>21.1</td>
<td>55.6</td>
<td>67.1</td>
</tr>
<tr>
<td>n6, %</td>
<td>19.3</td>
<td>49.7</td>
<td>29.2</td>
</tr>
<tr>
<td>n3, %</td>
<td>1.9</td>
<td>5.8</td>
<td>37.8</td>
</tr>
<tr>
<td>n6/n3</td>
<td>10.3</td>
<td>8.5</td>
<td>0.8</td>
</tr>
</tbody>
</table>
Table 2. Effect of diet and exercise on body weight, carcass weight, relative organ weight and blood serum characteristics (ALAT, Cholesterol, CK)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (kg)</th>
<th>Carcass weight (g)</th>
<th>Heart (%)</th>
<th>Liver (%)</th>
<th>ALAT (U/I)</th>
<th>Chol (mg/dcl)</th>
<th>CK (U/I)</th>
</tr>
</thead>
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<td>Diet</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LO</td>
<td>1.74 a</td>
<td>1093</td>
<td>0.42</td>
<td>1.37b</td>
<td>3.09</td>
<td>67.4ab</td>
<td>3746</td>
</tr>
<tr>
<td>PO</td>
<td>1.63 b</td>
<td>1022</td>
<td>0.46</td>
<td>1.60a</td>
<td>3.66</td>
<td>83.8a</td>
<td>4104</td>
</tr>
<tr>
<td>SO</td>
<td>1.76 a</td>
<td>1065</td>
<td>0.47</td>
<td>1.43ab</td>
<td>4.70</td>
<td>63.8b</td>
<td>4283</td>
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<tr>
<td>CG</td>
<td>1.71</td>
<td>1067</td>
<td>0.43</td>
<td>1.50</td>
<td>4.12</td>
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<tr>
<td>EG</td>
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<td>1053</td>
<td>0.47</td>
<td>1.44</td>
<td>3.51</td>
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<td>LO – CG</td>
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<td>1100</td>
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3 GENERAL DISCUSSION AND CONCLUSIONS

3.1 GENERAL DISCUSSION

Foods of animal origin and mainly poultry products, especially eggs due to their high nutritive value, take a very important place in human diets. Enrichment of eggs with omega-3 fatty acids is gaining importance due to increasing demand for healthy food. At the same time animal welfare and health condition of poultry is in focus in intensive production systems. One critical point is insufficient available space for locomotion. As indicated by STEINHILBER (2003) enhanced activity of hens may improve the deposition of n-3 fatty acids into egg yolks. Therefore, the question arises whether induced exercise may improve deposition of n-3 fatty acids to egg yolks, performance and bone health in laying hens. It may be expected, that both the nutritive value of the egg may be enhanced for human consumption and at the same time welfare and health of the laying hen may be improved. On the basis of this hypothesis the present project was conducted.

Diets significantly influenced egg weight, yolk proportion and fatty acids profiles. The highest egg weight was observed for diet SO and the highest yolk proportion for diet LO. The distinct effect of SO on egg production and egg weight is probably caused by the high content of linoleic acid which is assumed to exhibit extra-caloric effects (HAERTEL, 1972). The lowest egg production rate in group PO may be the result of the low content of linoleic and linolenic acid in the diet.

Contents of SAT and MUFA were significantly higher in eggs of group PO, whereas, eggs of treatments LO and SO showed a comparable content of PUFA which was significantly higher than for PO. The changes in fatty acids profile of yolks are in accordance with expectations. Palm fat is rich in saturated fatty acids and thus SAT and MUFA are the dominant fatty acids classes in yolks. The n-6 and n-3 contents were significantly highest in SO eggs and in LO eggs, respectively. SO is rich in linoleic acid and contains as well distinct amounts of linolenic acid thus resulting in higher contents of n-6 and n-3 fatty acids in yolks than for PO. The total PUFA content did not differ between SO and LO. In the same way diet SO resulted in a double as high n-6 content in yolks than for PO and LO, whereas, the content of n-3 was about four times higher in yolks of LO than in SO and 10
times higher than in PO. Despite the clear differences in n-6 and n-3 contents in yolks between groups PO and SO the n-6 to n-3 ratio was. In contrast, the n-6 to n-3 ratio was significantly improved for treatment LO and was eight times lower than for PO and SO. In general, the result was quite interesting that n-6 to n-3 ratios of all egg yolks were within the recommended range for human consumption and were distinctly better than the values given for today’s situation in human nutrition (e.g. SINGER, 2000). Thus, it may be concluded that chicken eggs are a suitable source of fatty acids in human food.

Dietary fat source affected laying hens weight and cholesterol level in serum. It was also shown in previous studies that diets rich in omega 3 PUFA reduced cholesterol levels (CASTON and LESSON, 1990; JIANG et al., 1991; WATKINS, 1992; FERRIER et al., 1995; SCHEIDELER and FRONING, 1996; BASMACIOGLU et al., 2003; SVEDOVA et al., 2008). Dietary fat source with higher content of linoleic acid exhibits extra-caloric effects (HAERTEL, 1972) resulting in higher body weight of laying hens. The enhanced metabolism of hens fed with SO diet is reflected by higher ALAT and CK values in the blood, standing for a higher liver activity. In contrast, fat source with lower content of linoleic and linolenic acid in the diet (PO) causes a decrease of laying hens’ body weight and an increase of cholesterol level in blood. At the same time relative liver weight was also significantly increased for diet PO due to the increased lipid catabolism and do novo fatty acid synthesis from sugar (HAERTEL, 1972). This was paralleled by high serum CK levels and increased ALAT levels. As expected, dietary fat sources did not affect relative heart weight.

GREGORY and WILKINS (1989) stated that the high incidence of bone breakage in laying hens under commercial conditions in cage systems indicates that bone strength is generally not satisfactory. The present study revealed that although the overall effect of the diets on bone characteristics was not significant, there was a consistent trend showing a greater Total Area, Corticalis Area and higher SSI with decreasing dietary ratio of n-6/n-3 fatty acids in the control birds. The negative effect of the high n-6/n-3 fatty acid ratio on the bone formation was paralleled by increased bone breaking strength. The cortical density area, however, showed the opposite tendency. The positive effect of n-3 fatty acids on Corticalis Area, breaking strength, bone ash and bone mass which has been reported in
growing broilers (MCCORMACK et al., 2006; MANZOUB et al., 2011) and by LIU et al. (2004) in Japanese Quail may not be expressed in laying hens birds. BAIRD et al. (2008) did not find significant effects of dietary changes in the n-6/n-3 ratio from 48/1 to 5/1 on the breaking strength in the tibia bones of laying hens kept in individual cages. The cortical thickness responded significantly to the n-6/n-3 ratio with a inverse U-shape pattern. The highest and lowest level of n-6/n-3 ratio showed the lowest value. The moment of inertia, which corresponds with the SSI, showed the same tendency as the cortical thickness. Considering that the management conditions of this experiment (individual cages) were similar to the conditions of the control birds in our experiment there was a similarity of the response with regard to the cortical density in our results and the cortical thickness reported by BAIRD et al. (2008), but the Corticalis Area and total bone area showed an opposite trend. The opposite direction of the response of Corticalis Area and cortical density in our result may be explained by a compensatory effect. The reduced bone mass as expressed in the Corticalis Area and the total bone area which may lead to a reduced bone strength was obviously compensated by a higher density. This effect also explains the missing response of the breaking strength to the dietary treatments in our own results as well as in the results of BAIRD et al. (2008).

Exercise of laying hens does not result in differences for egg weight, yolk proportion or fatty acids profiles indicating a dominating effect of dietary fat source (JAHJA et al., 2011). But, exercise of laying hens in treadmill caused a reduction of feed intake and egg production. Obviously, activity of hens increases production costs without additional benefits for product quality. A small effect of exercise on yolk proportion was visible by the interaction diet x exercise. Exercise significantly improved yolk proportion for diet LO but decreased it for diet SO. This can be explained by the observed differences in egg production, which has been lower for treatment LO-EG and higher for treatment SO-EG. The negative correlation between egg production rate and yolk proportion is well documented (e.g. GRASHORN, 2012).

Exercise contributed in tendency to animal welfare as there was a significant diet x exercise interaction for corticalis area and cortical density. The means between exercised and non-exercised hens were not significantly different in the LO and PO diets. In hens fed
the SO diet, physical exercise significantly increased the Corticalis Aerea and decreased the cortical density. This particular effect of SO cannot be explained by the differences in the n-6/n-3 ratio, since it was similar in the SO and PO diet. The high level of linoleic acid in the SO diet may be the cause for this particular effect.

Surprisingly, exercise did not affect physiological parameters in blood. But, it was observed that exercise may compensate negative side-effects of an accelerated metabolism when feeding diets rich in linoleic acids (SO) and/or of an increased lipid catabolism when feeding diets poor in linoleic acids (PO). In general, high contents of PUFA in diets (SO, LO) have a more beneficial effect on the metabolism of the hen than diets low in PUFA (PO).

3.2 CONCLUSIONS

In the frame of the thesis, the combined effects of diets with different contents of omega-3 fatty acids and of exercise on performance, yolk fatty acids profiles, bone characteristics and indicators of metabolism have been investigated. Based on the obtained results, it can be concluded that

1. The dietary fat source affected clearly egg weight, yolk proportion and yolk fatty acids profile. A minor effect on egg production was observed. In contrast to diets exercise on the treadmill caused a reduced feed intake and egg production, but did not affect either egg weight, yolk or yolk fatty acids profiles. Therefore, dietary fat source has a main effect on performance and product quality, whereas, exercise in tendency increases production costs.

2. There was a consistent trend showing a greater Total Area, Corticalis Area and higher SSI with increasing dietary ratio of n-3: n-6 fatty acids, although the overall effect of the diets on bone characteristics was not significant. The positive effect of the high n-6: n-3 fatty acid ratio on bone formation was paralleled by increased bone breaking strength. The observed negative effect of high n-6/n-3 ratio of diet SO on Total area and Corticalis Area was alleviated by exercise. Hence physical exercise seems to compensate for low levels of n-3 fatty acids. Physical exercise improved cortical density in combination with the diet with a higher n-6/n-3 ratio (PO) and decreased it
in combination with the diet with a low n-6/n-3 ratio (LO). But, there was an increase of bone area for the LO diet (lower n-6/n-3 ratio) and a decrease of bone area for diets SO and PO (higher n-3/n-6 ratio). While the production of bone mass was stimulated by a high n-6/n-3 fatty acid ratio, physical exercise can ensure the bones resistance to injuries under n-3 deficient situations. The generally low response of adult hens to dietary fatty acids and to exercise in the present study may also be caused by the short experimental period and the advancing age of the experimental birds.

3. The dietary fat source affected laying hens’ weight and some blood values. In general, blood characteristics are more affected by dietary fat than by exercise. Exercise may improve metabolic efficiency if the dietary PUFA content is low, but cannot compensate it. In contrast, diets rich in PUFA result in the highest metabolic turnover and efficiency.
4. SUMMARY (ZUSAMMENFASSUNG)

4.1. Summary

Foods of animal origin such as eggs take an important place in human diets. Polyunsaturated fatty acids (PUFA) such as 18:3n-3 and 18:2n-6 cannot be synthesized in the metabolism of animals and humans; however they can be incorporated into eggs or meat through dietary fat. Enriching eggs with ingredients like omega-3 fatty acids has become a broad field of research. Besides sea fish products, several n-3 fats, algae products, linseed, rapeseed and herbs have been used as feeding stuffs for enriching eggs with n-3 PUFA. Besides nutrition other factors may enhance deposition of n-3 fatty acids in egg yolks. Differences in deposition of n-3 fatty acids in egg yolks between eggs from organic/free-range and indoor production have been reported. Egg yolks from hens kept in free range contained higher n-3 fatty acids levels. It has been assumed that physical exercise may play a role in this context.

Physical exercise not only influences the metabolism of fatty acids but also the mechanisms of bone formation. This effect has been demonstrated in different animal species: dogs and goats, horses, rabbits as well as in humans. Bone breakage is a serious welfare problem of laying hens in both intensive and extensive husbandry systems. Most bone fractures commonly occur in hens selected for egg production and the fractures are attributed to osteoporosis. Broken bones were found in about 30% of hens before slaughter and the proportion reached 90% in carcasses at the end of the processing line. Recent studies also highlighted the beneficial effects of n-3 fatty acids on bone strength of Japanese Quail and growing chickens. Therefore, the present study has been undertaken to elucidate the relationships between different sources of dietary fatty acids and physical exercise in laying hens on performance, egg quality, bone characteristics and some other physiological criteria of the fatty acid metabolism.

In total of 36 brown laying hens (Bovans Brown) were used in the experiment. Three experimental diets which differed only in the fat source, namely Palm oil (PO), Soybean oil (SO), and Linseed oil (LO) were used. The fat sources corresponded to a low content of poly unsaturated fatty acids (PUFA) – PO, a high content of omega-6 (n-6) fatty acids –
SO, and a high content of n-3 fatty acids – LO. Experimental diets were fed to 12 hens each. Half of the hens of each dietary group (6 birds) were exposed to exercise by walking on a treadmill (EG), whereas, the remaining 6 hens in the control group (CG) were kept in individual cages. EG birds were exposed to a running treadmill every d for the whole experimental period (4 wk). The speed of the treadmill belt was adjusted from 0.5 to 0.7 miles/h. On the first day of the experiment the birds walked 5 min with the speed of 0.5 miles/h. Then duration of walking was prolonged for 2 min/day until day eleven and the speed was increased progressively. After eleven days until the end of experiment the hens walked 25 min/day. On the first day the distance walked was 67 m and increased up to 469 m/day on eleventh day. So the experimental design was 3 diets x 2 activities x 6 birds = 36 hens. At the end of the experiment eggs were collected to determine yolk fatty acids profiles and hens were slaughtered to collect blood indicators for lipid metabolism, tibia bones were removed to determine bone characteristics and relative weights of hearts and livers were calculated.

Diets significantly influenced egg weight, yolk proportion and fatty acids profiles. The highest egg weight was observed for SO and the highest yolk proportion for LO. Contents of SAT and MUFA were significantly higher in eggs of group PO, whereas, LO and SO showed a higher content of PUFA. Eggs of treatment PO showed the highest proportion of palmitic acid and oleic acid, whereas, eggs of treatments SO and LO showed the highest proportions of linoleic acid and linolenic acid, respectively. The n-6 and n-3 contents were significantly highest in PO eggs and in SO eggs, respectively. The n-6 to n-3 ratio did not differ between PO and SO, but was eight times higher than for LO. Exercise of the birds did not result in differences in egg weight, yolk proportion or fatty acids profiles indicating a dominating effect of dietary fat source. The interaction of exercise x diet was significant for the yolk proportion only.

Neither diet nor physical exercise did significantly affect bone characteristics determined by computer tomography, but, there was a consistent trend of higher level of total area and corticalis area in the LO group as compared with PO and SO group. Total density and cortical density showed the opposite tendency. Significant diet x exercise interactions were observed for total area, corticalis area and corticalis density. Running on the treadmill
resulted in lower total area and corticalis area for diets LO and PO, whereas, higher values were observed for birds with exercise fed on diet SO. The relation was opposite for corticalis density. Here, lower values were observed for birds without exercise fed on diets LO and PO. Bone breaking strength, dimensions and composition of tibia were not significantly influenced by the main factors or their interaction. In general, differences between diets and exercise treatments have been relatively small. But, tibia of birds fed on diet PO showed the highest ash, Ca and P contents on a dry matter basis.

Diet PO resulted in lower body weight, increased relative liver weight and increased serum cholesterol level. Hens fed with diet SO showed the highest serum ALAT level indicating an accelerated metabolic activity. There were no significant effects of exercise on other characteristics. But, in tendency exercise increased relative heart weight and decreased relative liver weight. Interactions between dietary fat and exercise revealed that exercise can compensate negative side-effects of an increased metabolic activity for diets SO and LO, whereas, the unfavorable effects of a diet with a low content of linoleic acid (PO) cannot be removed.

In summary, the pattern of fatty acid in the egg yolk has been modified by the different diets as expected. Physical exercise, in contrast did not show any influence on the fatty acid contents of the egg yolk. The higher levels of omega-3 fatty acids in eggs from free range, which have been found in earlier studies, are obviously not caused by the higher physical exercise under these conditions. The effect of physical exercise and diet on bone stability is not caused by the individual factors but by their interactions. While physical exercise in the LO and PO diet reduced the bone area and increased the bone density, the opposite effect was observed in the SO diet. Since lower bone density was compensated by the larger bone area the treatments did not affect bone breaking strength.
4.2. Zusammenfassung


Das Ziel der vorliegenden Studie war deshalb, die Auswirkungen der Laufaktivität und der Fettsäurequelle im Futter auf die Leistung, die Eiqualität, die Knochenmerkmale sowie eine Reihe Indikatoren für den Leberstoffwechsel bei den Legehennen zu untersuchen.

Rationen auf die Ergebnisse. Die Interaktion von Laufraining und Ration war nur für den Dotteranteil signifikant.


Zusammenfassend kann gesagt werden, dass das Fettsäuremuster im Eidotter wie erwartet durch die Rationen verändert wurde. Die Bewegungsaktivität hatte dagegen keinen Effekt.
auf das Fettsäuremuster im Dotter. Die in früheren Untersuchungen gefundenen erhöhten Anteile an Omega-3 Fettsäuren in Eiern, die von Hennen in Auslaufhaltung produziert wurden, sind somit nicht auf die erhöhte Bewegungsaktivität zurückzuführen. Der Einfluss der Bewegungsaktivität und der Fettsäuremuster im Futter auf die Knochenstabilität ist nicht durch die Wirkung der einzelnen Faktoren, sondern durch deren Wechselwirkung bestimmt. Während in der Lein- und Palmöl-Gruppe die Knochenfläche durch Lauftraining verringert und die Knochendichte vergrößert wurden, war dies bei der Sojaöl-Gruppe umgekehrt. Die Verringerung der Knochenfläche wurde jeweils durch eine erhöhte Knochendichte kompensiert, weswegen die Knochenbruchfestigkeit nicht beeinflusst war.
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Where I would be without you my mother? – THANK YOU

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