TITLE: Effect of extruded flaxseed in broiler diets on blood oxidative stability and meat fatty acid composition

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Einfluss von extrudierter Leinsaat im Futter auf die Oxidationskennwerte im Blut und auf das Fettsäuremuster im Fleisch von Broilern

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Introduction

Flaxseed (*Linum usitatissimum*), also known as linseed, is a rich source of oil in quantities from 35 to 45%, of which more than 70% are unsaturated fatty acids (FAs). Contents of crude protein range in average from 20 to 30%, including all essential amino acids. Furthermore, flaxseed contains fibre, lecithin, vitamins, minerals, etc. (ANJUM et al., 2013; IVANOV et al., 2012). Flaxseed is traditionally incorporated in animal nutrition as seed and as oil, to improve the productive performance and health parameters of animals (CAROPRESE et al., 2010; RAGNI et al., 2014). ANJUM et al. (2013) reported that the digestive tract of the chicken is able to digest flaxseed and absorb nutrients from it in the small intestine. Precisely, the health benefits of flaxseed are related to the ingestion of polyunsaturated fatty acids (PUFA), especially essential alpha-linolenic acid (ALA; C18:3n–3) and linoleic acid (LA; 18:2n-6), which cannot be synthesised in the body of animals, and therefore should be ingested through the diet. Furthermore, broilers are able to metabolise ALA to eicosapentaenoic acid (EPA; C20:5n–3) and docosahexaenoic acid (DHA; C22:6n–3), which are essential FAs for mammals and therefore for humans (BETTI et al., 2009).

In spite of its favourable FA composition and high nutritive value, usage of flaxseed in animal diets is limited, due to the fact that it contains antinutritive components. Whole flaxseed grain used as a feed have to be thermally treated in order to degrade cyanogenic glycosides – antinutritive components which release hydrogen cyanide (HCN) in the body of animals by the

action of an endogenous β-glucosidase enzyme (oxynitrilase). Extrusion shows several advantages in comparison to other technological processes in animal feed production and have thereby become very popular during the last two decades. It is defined as shaping of treated material by force through a specially designed opening, often after previous heating of the material. Generally, extrusion is a high temperature and short time thermal processing technology. Moreover, the fatty acid contents such as ALA and LA are prone to degradation under high temperature thermal processing which is not desirable (IMRAN et al., 2013).

Modern way of life followed by speed and deadlines, tension and stress, as well as health problems and health disorders, is setting new requirements in all fields, especially in that of dietetics. Therefore, in recent years, food science has intensively engaged in research of specific ingredients that positively affect human health or have a preventive effect on decreasing diseases of modern age (SIROA et al., 2008; FONTI-i-FURNOLS and GUERRERO, 2014). A very important group among these ingredients are PUFA, especially n-3 PUFA and the long chain fatty acids EPA and DHA, which are well recognised for their protective effects on hypercholesterolemia, cardiovascular disease, inflammatory diseases, their role in brain health and the nervous system, cancer and various other biological functions (SIMOPOULOS, 1999; CONNOR, 2000; SIMOPOULOS, 2002; HARRIS, 2007; SIMOPOULOS, 2008; CALDER, 2009; ANJUM et al., 2013). The ratio between polyunsaturated (PUFA) and saturated fatty acids (SFA) and the ratio between n-6 and n-3 fatty acids are considered as two important parameters for nutritional evaluation of fat due to their protective role in human health. A way to change FA composition of meat, which implies increasing n-3 PUFA and improvement of mentioned ratios, is modification of the meat FA composition through diet. As the feed has a direct impact on meat quality, it is possible to change the FA composition of meat by using feed enriched with flaxseed (JIMÉNEZ-COLMENERO et al., 2001; JIMÉNEZ-COLMENERO et al., 2006; IVANOV et al., 2010; OKANOVIĆ et al., 2010; PUVAČA et al., 2014).

Poultry meat is widely present in the human diet due to its nutritional value. Its world production increased much more than any other type of meat during the past decade and accounts for 30% of global meat consumption (DAGHIR, 2009; FAO, 2010). Regarding the high presence of poultry meat in human diet, it is very important to improve its nutritional value. Therefore, the objective of this study was to determine the influence of diet enriched with extruded flaxseed on oxidative status of broiler blood and fatty acid composition of chicken meat.

Materials and methods

Experimental design and sample collection

The principles of animal protection were strictly followed and the experimental protocol was approved by the local Ethics Committee. Experiments under *in vivo* conditions were performed on a total of 400 Ross 308 hybrid broiler chickens of both sexes. One–day–old broilers, randomly selected, were divided in 20 experimental units of 20 broilers each. These units were further allotted to 4 treatments in 5 repetitions. All broilers were fed on diets without addition of antibiotics, coccidiostats or growth promoters. The structural and chemical composition of the used mixtures is shown in Table 1. The control treatment broilers were fed on the basal diet, while in the experimental treatments the basal diet were supplemented with extruded flaxseed at 25 g/kg (EFS25), 50 g/kg (EFS50) and 100 g/kg (EFS100) during whole experimental period of 42 days. To minimise lipid peroxidation, the experimental diets were prepared every week and kept in a cold room (4 °C) in airtight containers.

Broilers had free access to feed and water. The lighting program consisted of a period of 23 h light and 1 h of darkness throughout the whole experimental period. The ambient temperature was initially set at 33 °C and gradually decreased by 3 °C per week to 24 °C on the third week and then was kept constant. The broilers were not vaccinated.

At the end of the experimental period, 5 broilers from each treatment (which was 20 broilers in total) were randomly selected and blood samples were collected. After blood sampling, broilers were slaughtered according to the ethical guidelines and standard technological procedure. Carcasses were cut in the basic anatomical parts. White (breast) and red meat (leg) were washed with saline, packaged in polythene zip lock bags and kept frozen at −18°C until analysis.

Extrusion of flaxseed

Before processing, flaxseed was milled on a hammer mill, followed by extrusion using a single screw annular gap expander (OEE 8, AMANDUS KAHL GmbH & Co. KG, Germany) with a length-to-diameter ratio of 8.5:1.0 and capacity of 100 kg/h thereby obtaining an expanded product at 130 ± 1°C. Optimum pre-conditioning was carried out in a double-shaft steam conditioner (Muyang SLHSJ0.2A, China), until the material reached temperature of 80°C, with direct water addition into the feed mash during conditioning (LEVIĆ and SREDANOVIĆ, 2010). Material moisture content after the conditioning process was 25.5%. The conditions of extrusion of flaxseed were as follows: extruder final head temperature (80-120°C), extruder barrel speed

(80-120 rpm) and feeder speed (12-16 rpm) as described by IMRAN et al. (2013). The extruded flaxseed was subsequently added to the broiler diet.

Enzyme assay

Blood samples were collected before slaughtering from the wing vein into heparinised test tubes. The plasma was separated by centrifugation (2000 *g*, 10 min) and stored at –70°C for further analysis. Remaining red blood cells (RBCs) were washed three times with isotonic NaCl (0.15 M) solution prior to use in the biochemical assay. Haemolysates of RBC were prepared by diluting RBCs with ice–cold double distilled H_2O in ratio 1:10, shaken vigorously to force haemolysis and stored at –70°C (FEBEL et al., 2008). Levels of haemoglobin, necessary for the expression of the enzymatic activities in haemolysed blood, were determined using a commercial test ('Dialab', Vienna, Austria) on a spectrophotometer (Multiscan MCC 340, Finland). Protein content was determined by the method of PRAKASH et al. (2010). The SOD (EC 1.15.1.1) activity was determined by the spectrophotometric method based on the inhibition of adrenaline reduction to adrenochrome at pH = 10.2 (KOSTADINOVIĆ et al., 2001). The GSHPx (EC 1.11.1.9) activity was determined by spectrophotometric measurement of absorbance at 412 nm with cumenhydroperoxide as the substrate (CHIU et al., 1976). Activity of the GSHR (EC 1.6.4.2) was determined from the rate of NADPH oxidation, monitored by the absorbance at 340 nm (LUKASZEWICZ-HUSSAIN and MONIUSZKO-JAKONIUK, 2004). The CAT (EC 1.11.1.6) activity was measured by observing decomposition of hydrogen peroxide and one international unit (IU) was equivalent to one mmol H_2O_2 consumed/min/mg protein (AHN et al., 1998).

Fatty acid analysis

The crude fat extracts were used for preparation of fatty acid methyl esters by the transmetylation method, which a recommended method for this type of substrates. This method requires the use of 14% wt. boron trifluoride/methanol solution (Sigma Aldrich, MO, USA) (HAYAT et al., 2009). Fatty acid composition analyses were done on a gas chromatographer (Agilent 7890A system, Agilent Technologies, Santa Clara, CA, USA) with flame ionisation detector (GC-FID), auto-injection module for liquid, equipped with fused silica capillary column (DB-WAX 30 m, 0.25 mm, 0.50 μm). Carrier gas was helium (purity > 99.9997%). Prepared samples (2 μl each) were injected with helium (flow of 3.5 ml/min), which was programmed for operating conditions such as column oven temperature 220 °C for 7.5 minutes, split ratio (50%) with injector and detector temperatures (260°C). The standards of fatty acid methyl esters

purchased from Sigma-Aldrich were also run under the same conditions. Peak areas and total fatty acid profile percentage were calculated for each sample by retention time using Agilent Chem. Station software compared to retention times of authentic standards and these were expressed as percentages of total fatty acid methyl esters.

Statistical analysis

Analyses of variance ANOVA was performed to assess data differences between various groups using Statistics software version 12 (STATISTICA, 2013). Significant differences among treatment means were analysed by Duncan's multiple range tests. The data means were considered significantly different at $P<0.05$.

Results

As it can be seen from the Table 2, where FA composition of control and experimental treatments is presented, the applied extrusion process was successfully used for commercial flaxseed production with significant retention of n-3 FA.

Growth performance of broilers fed on dietary extruded flaxseed

From the results presented in the Table 3 a significantly higher body weight ($P<0.05$) can be seen in the broilers treated with different concentrations of flaxseed compared to the control treatment.

At the end of the second week, chickens in all flaxseed treatments had achieved significantly higher body weights compared to treatment C. At the end of the 5th week the highest body mass was recorded in the treatment with addition of 100 g/kg (EFS100) of flaxseed, followed by treatments EFS50 and EFS25, while in the control treatment the lowest body weight was recorded.

At the end of the third fattening period, addition of flaxseed in treatments EFS25 and EFS50 led to significant differences ($P<0.05$) in body weight in relation to the control and EFS100 treatments.

At the end of the experimental period, the highest achieved body weight of broilers was in treatment EFS100 (2939 g), followed by treatment EFS50 (2829 g) and EFS25 (2802 g), with statistically significant differences ($P<0.05$) compared to the control treatment (2702 g).

In starter phase, the highest feed intake (881 g) was noticed in treatment EFS100, while the lowest intake was noticed in the control treatment (873 g). In experimental treatments EFS25 and EFS50 feed intake was 877 g and 875 g, respectively, and without significant difference (P>0.05) between them.

Treatments EFS25 and EFS50 achieved a final feed conversion ratio of 1.63 and 1.58 which was significantly (P<0.05) higher than feed conversion ratio of chickens in treatment EFS100 (1.41). However, feed conversion ratio in the control treatment was significantly higher (P<0.05) compared to all flaxseed treatments. Deviation from this trend was not noticed during the experimental period.

The highest mortality rates were recorded in treatment C throughout all fattening periods. Significantly lower (P<0.05) mortality rates were recorded in the flaxseed treatments with addition of 25 g/kg and 50 g/kg of flaxseed, while difference between them were absent during the entire trial. In treatment EFS100 the lowest mortality rate (0.69%) during all fattening periods was noticed with significant difference (P<0.05) compared to all other experimental treatments.

Enzyme assay of broiler blood

Results of antioxidative activity in blood serum from chickens fed with different levels of flaxseed are presented in Table 4. Different treatments of extruded flaxseed had significant (P<0.05) effects on the enzymatic and non-enzymatic antioxidative systems. Treatment EFS10, with maximum level of extruded flaxseed, showed the highest SOD (31.2 μmol/g Hb min) and CAT (44.4 μmol/g Hb min) activity, while EFS5 SOD (29.8 μmol/g Hb min) and CAT (40.5 μmol/g Hb min) were also significantly (P<0.05) affected compared to the control treatment.

The CAT activity is related to the SOD activity since SOD converts superoxide radicals to hydrogen peroxide. More production of superoxide radicals will increase the activity of SOD and hence increase activity of CAT. The highest GSHPx (9.2 μmol/g Hb min) activity was observed in EFS10 and the minimum (8.3 μmol/g Hb min) in the control treatment. The activity of the GSHR increased in treatments containing higher amounts of extruded linseed, from 14.1 to 16.5 μmol/g Hb min in the control and EFS100 treatments, respectively.

Fatty acid composition of broiler meat

The FA compositions of breast and leg meat from control and experimental treatments are shown in Table 5 and Table 6, respectively.

The major n-3 fatty acid, alpha-linolenic acid (ALA; C18:3n–3) had its lowest value 0.015 g/100 g in white meat of control treatment and its highest value 0.037 g/100 g of white meat in EFS50 and EFS100 treatments, while in leg meat the lowest value was also observed in the control treatment 0.079 g/100 g of leg meat and the highest value in EFS100 treatment 0.115 g/100 g of leg meat. The enrichment of ALA in breast meat and leg meat from EFS100 treatment was 2.47 times and 1.46 times higher than in the control treatment, respectively.

Long chain n-3 PUFA, such as EPA, DPA, and DHA, were present in both, white and red (leg) meat. Total long-chain n-3 PUFA (EPA+DPA+DHA) in white meat ranged from 0.024 g /100 g of white meat (control treatment) to 0.051 g/100 g of white meat in treatments EFS50 and EFS100, while in leg meat this content ranged from 0.092 g/100 g of leg meat (control) to 0.150 g/100 g of leg meat (EFS100). The concentration of EPA+DPA+DHA in breast meat and leg meat from EFS100 treatment was 2.13 times and 1.63 times higher than in the control treatment, respectively. Leg meat obtained from chickens fed with extruded flaxseed contained significantly (P<0.05) higher contents of long chain n-3 fatty acids. The predominant long-chain n-6 FA was arachidonic acid, constituting 0.052 (control), 0.077 (EFS25), 0.069 (EFS50), and 0.070 g/100 g of leg meat (EFS100). In white meat n-6/n-3 ratio ranged from 3.10 in EFS100 to 7.29 in the control treatment, while in red meat this ratio ranged from 3.48 in EFS100 to 5.50 in the control treatment. In both, breast and leg meat, the most favourable n-6/n-3 ratio was obtained in the EFS100 treatment. However, the n-6/n-3 ratio was significantly (P<0.05) reduced in meat from the EFS25, EFS50 and EFS100 treatments fed with flaxseed, mainly because of the increase in n-3 FAs (ALA, EPA, DPA, and DHA).

Total MUFA content was highest in breast meat from the EFS50 and EFS100 treatments. The same tendency was observed in leg meat. This was mainly because content of oleic and gondoic acids, as two predominant MUFAs in flaxseed, increased.

Discussion

The results regarding the production performance showed that the broilers were able to utilise the flaxseed-supplemented diets more efficiently than the conventional feed, as the diet preparations containing flaxseed improved broiler performance in terms of higher body weights. It can be inferred from this that the inclusion of flaxseed meal in broiler diets, as represented by feeds EFS25, EFS50 and EFS100 in the current study, had no adverse effects. Moreover, based on the obtained results it could be concluded that significant differences between the various

treatments in terms of enhanced feed intake, feed conversion ratio and live weights showed that flaxseed can be used in compound feed rations for broiler chickens.

In pathobiochemistry, free radicals that appear during oxidative stress may cause various cellular damages. Free radicals are defined as atoms or molecules which have one or more unpaired electrons that make them highly reactive (PANDEY and RIZVI, 2010). Since the existence of free radicals is an indispensable constituent of all aerobic cells, during the evolution many different mechanisms have emerged that eliminates these molecular species or reduce their harmful effects. These protective mechanisms include enzymatic, non–enzymatic and secondary antioxidative protection. The most important enzymes are superoxide dismutase (SOD) (EC 1.15.1.1), catalase (CAT) (EC 1.11.1.6.), glutathione peroxidase (GSHPx) and glutathione reductase (GSHR) (EC 1.6.4.2.). Higher GSHPx values indicate that higher oxidation, as lipid oxidation is positively correlated with the GSHPx activity. GSHPx activity can be used as an indicator of meat oxidative stability. The higher GSHPx activities observed for the different treatments of extruded flaxseed may be due to the higher content of PUFA in the diet. The results obtained in this study are in agreement with MARACHIELLO et al. (1999). GSHR activity is induced by the unsaturated and polyunsaturated FAs compared to the saturated FAs. The increase of GSHR activity in red blood cells is a positive feedback mechanism in response to rising lipid peroxidation (RODRIGUEZ-MARTINEZ and RUIZ-TORRES, 2001).

The n-6/n-3 ratio is one of the very important indicators in fatty acid composition analysis. It has been estimated that a Western diet is deficient in n-3 fatty acids, with a ratio of n-6 to n-3 of 15-20/1, instead of 1/1 (SIMOPOULOS, 2008). Nutritional advice is that this ratio should be less than 4 (SCOLLAN et al., 2006). According to published investigations, a low n-6/n-3 ratio has suppressive effects on many diseases, such as cardiovascular disease, cancer, inflammatory and autoimmune diseases (SCOLLAN et al., 2006).

In this study, it was found that the fatty acid composition of the feed is reflected in the fatty acid composition of the meat and this is confirmed by others (IVANOV et al., 2010; TAULESCU et al., 2010; JIMÉNEZ-COLMENERO et al., 2006). In chickens fed with flaxseed, the deposition of total n-3 fatty acids in all portions increased, which was connected to the increase of α-linolenic acid (ALA), but also eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in both types of meat samples. The EPA and DHA deposition is a consequence of *in vivo* metabolism with ALA serving as a precursor for their synthesis through the desaturation-chain elongation pathway within the liver. Due to the complexity of this biosynthesis (SPRECHER, 2000), the increase of DHA in meat products would be expected to be lower than that of ALA.

Considering the positive health effects of ALA consumption, as reported in the Lyon Heart Study and the Mediterranean Alpha-Linolenic Enriched Groningen Dietary Intervention Trial (BEMELMANS et al., 2002), the ALA content supplied through various meat products could have positive impact on human health. The increase in n-3 fatty acid deposition in the meat from chickens fed with flaxseed was at the expense of decreased concentrations of n-6 fatty acids in all meat portions, and SFA and MUFA in the investigated tissues. The pectoral muscle (breast meat) presents lower values of n-6 fatty acids compared to thigh muscle (leg meat), probably due to competition for the Δ-5 and Δ-6 desaturation of n-3 and n-6 acids in tissues.

An important parameter in meat fatty acid composition analysis is the ratio between PUFA and SFA. The average ratio of PUFA/SFA recommended by the British Department of Health is more than 0.45, and WHO/FAO experts have reported guidelines for a 'balanced diet' in which suggested ratio of PUFA/SFA should be above 0.4 (WOOD et al., 2008; WOOD et al., 2003; HMSO, 1994). The PUFA/SFA ratio obtained in this study ranged from 0.97 to 1.33, which is above the WHO/FAO recommended value for all samples of breast and leg meat.

Conclusions

In conclusion, since flaxseed is rich in essential n-3 and n-6 fatty acids, it is documented that it can be incorporated into poultry diets as a source of essential fatty acids. Different treatments of extruded flaxseed in this experiment had significant effects on enzymatic and non-enzymatic antioxidative systems in the blood. Also, treatment with flaxseed at 100 g/kg of diet showed the highest SOD, CAT, GSHPx and GSHR activity. Moreover, the addition of 100 g/kg of flaxseed in the diet increased the n-3 fatty acids (ALA, DHA and EPA) in breast and leg meat, making these products favourable in terms of human health. Finally, most favourable n-6/n-3 ratio for both breast and leg meat was obtained in the treatment supplemented with extruded flaxseed at 100 g/kg.

Summary

The aim of the present study was to investigate the influence of flaxseed enriched diets on oxidative status of blood and meat fatty acid composition in broilers. A total of 400 day–old Ross 308 hybrid broilers were randomly allotted to 4 experimental treatments and fed with diets containing extruded flaxseed grain at 0 g/kg (control), 25 g/kg (EFS25), 50 g/kg (EFS50) and 100 g/kg (EFS100), respectively for 42 days. Extruded flaxseed had significant effects on the

enzymatic and non-enzymatic antioxidative systems in broiler blood. Treatment EFS100, showed the highest (31.2 µmol/gHb min) SOD activity, CAT activity (44.4 µmol/gHb min), GSHPx (9.2 µmol/gHb min) and GSHR activity (16.5 µmol/gHb min). Diets enriched with extruded flaxseed led to a significant increase in α-linolenic acid (ALA; C18:3n-3) in breast and leg meat, which is a major n-3 fatty acid. Long chain n-3 fatty acids, such as eicosapentaenoic acid (C20:5n-3), docosapentaenoic acid (C22:5n-3) and docosahexaenoic acid (C22:6n-3), were also present in both, breast and leg meat. In both breast and leg meat the most favourable lowest n-6/n-3 ratio was obtained in the EFS100 treatment, but in EFS25 and EFS50 this ratio also was significantly reduced.

Keywords

Broilers, flaxseed, nutrition, extrusion, blood, lipid oxidation, meat, fatty acid profile

Zusammenfassung

Einfluss von extrudierter Leinsaat im Futter auf die Oxidationskennwerte im Blut und auf das Fettsäuremuster im Fleisch von Broilern

Das Ziel der Studie war die Untersuchung des Einflusses des Einsatzes von extrudierter Leinsaat im Futter auf den oxidativen Status im Blut und das Fettsäuremuster im Fleisch von Broilern. Hierzu wurden 400 Ross 308 Eintagsküken zufällig auf vier Behandlungsgruppen verteilt. Diese wurden über 42 Tage mit Futterrationen gefüttert, die 0 (Kontrolle), 25 (EFS25), 50 (EFS50) oder 100 (EFS100) g extrudierte Leinsaat/kg Futter enthielten. Die unterschiedlichen Einsatzmengen an extrudierter Leinsaat wirkten sich signifikant auf die enzymatischen und die nicht-enzymatischen anti-oxidativen Regelsysteme im Blut aus. Die Behandlung EFS100 wies die höchsten Aktivitäten von SOD (31,2 µmol/gHb min), CAT (44,4 µmol/gHb min), GSHPx (9,2 µmol/gHb min) und GSHR (16,5 µmol/gHb min) auf. Der Einsatz der extrudierten Leinsaat führte zu einer signifikanten Zunahme an α-Linolensäure (ALA; C18:3n-3) im Brust- und Schenkelfleisch. Ferner wurden deutliche Gehalte an den langkettigen Omega-3-Fettsäuren Eicosapentaen- (C20:5n-3), Docosapentaen- (C22:5n-3) und Docoshexaensäure (C22:6n-3) im Brust- und Schenkelfleisch gefunden. Sowohl beim Brust- als auch beim Schenkelfleisch wurde das günstigste n-6/n-3-Verhältnis für die Behandlung EFS100 ermittelt. Das Verhältnis war aber auch bei den Behandlungen EFS25 und EFS50 signifikant günstiger als in der Kontrollgruppe.

Stichworte

Broiler, Fütterung, Leinsaat, Extrudieren, Blut, Fettoxidation, Fleisch, Fettsäurenmuster

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Table 1. Composition and nutritive content of isoenergetic and isonitrogenic broiler diets supplemented with different concentrations of flaxseed (%)

Zusammensetzung und Nährstoffgehalte der isoenergetischen und isonitrogenen Broilerfutterrationen mit unterschiedlichen Anteilen an Leinsaat (%)

Ingredients	Experimental diets											
	Control			EFS25			EFS50			EFS100		
	S[1]	G[2]	F[3]	S[1]	G[2]	F[3]	S[1]	G[2]	F[3]	S[1]	G[2]	F[3]
Maize	45.6	44.8	60.4	44.3	43.5	63.9	42.9	42.1	62.9	40.3	39.4	60.9
Wheat	10	15	5	10	15	-	10	15	-	10	15	-
Soy cake	25.2	26.8	-	24.1	25.7	-	22.9	24.6	-	20.6	22.4	-
Fullfat soya	15	10	28.3	15	10	27	15	10	25.5	15	10	22.3
Flaxseed	-	-	-	2.5	2.5	2.5	5	5	5	10	10	10
Sunflower meal	-	-	3	-	-	3	-	-	3	-	-	3
MCP	1.3	1.1	0.3	1.3	1.1	0.4	1.3	1.1	0.3	1.2	1.0	0.3
NaCl	0.2	-	0.1	0.2	-	0.1	0.2	-	0.1	0.2	-	0.1
Chalk	0.5	0.7	1.2	0.4	0.7	1.2	0.4	0.7	1.2	0.4	0.7	1.2
Lysine	0.2	-	0.1	0.2	-	0.3	0.2	-	0.4	0.2	-	0.6
Methionine	0.3	-	-	0.3	-	-	0.3	-	-	0.3	-	-
Minazel Plus[4]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Premix[5]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Nutrients (% of as-fed basis)												
Dry matter*	86.2	86.5	85.9	86.8	86.1	86.4	86.3	86.9	85.1	86.4	86.6	86.8
Moisture*	13.8	13.5	14.1	13.2	13.9	13.6	13.7	13.1	14.9	13.6	13.4	13.2
Crude fat*	3.8	3.9	4.7	3.9	3.7	4.3	3.9	4.0	4.4	3.7	3.8	4.5
Crude fibre*	3.5	3.5	3.8	3.4	3.6	3.7	3.5	3.1	3.2	3.5	3.9	3.4
Ash*	5.3	4.8	5.6	5.1	5.0	5.6	4.9	4.2	5.1	4.8	4.9	5.6
Crude protein*	21.0	20.7	17.3	21.1	20.4	17.0	21.2	20.0	17.8	21.0	20.6	17.1
Methionine + Cysteine	0.64	0.71	0.72	0.67	0.72	0.80	0.68	0.77	0.77	0.67	0.78	0.72
Calcium*	0.81	0.90	1.10	0.81	0.92	1.17	0.80	0.96	1.10	0.82	0.91	1.10
Phosphorus*	0.38	0.38	0.33	0.36	0.39	0.39	0.36	0.34	0.33	0.34	0.35	0.36
Lysine	1.15	1.16	1.16	1.16	1.12	1.11	1.15	1.11	1.19	1.13	1.19	1.12
Threonine	0.81	0.85	0.90	0.81	0.81	0.95	0.79	0.87	0.90	0.78	0.81	0.90
ME (MJ/kg)	12.5	12.8	13.3	12.5	12.9	13.4	12.5	12.8	13.4	12.5	12.9	13.3

[1]Starter diet- 1-14 days; [2]Grower diet-15-35 days; [3]Finisher diet-36-42 days; [4]Mycotoxins adsorbent – Minazel Plus® Patent co. Serbia; [5]In 1 kg of Premix: Vitamin A (E672) 12000 IU, Vitamin D3 (E671) 3000 IU, D3 (25-OH)(E670a) 2000 IU, Vitamin E (DL-α tocopherol) 75 mg, Vitamin K3 3 mg, Vitamin B1 3 mg, Vitamin B2 8 mg, Vitamin B6 5 mg, Vitamin B12 0,016 mg, Niacine 60 mg, Pantothenic acid 15 mg, Folic acid 2 mg, Biotine 0,2 mg, Choline chloride 800 mg, Beaten 570 mg, Mn 100 mg, Fe 40 mg, Zn 100 mg, Cu 16 mg, J 1,25 mg, Se 0,3 mg, Glucanase 10 FBG, Phytase 10000 FYT, Xylanase 150 BXU, Antioxidants 125 mg. *Analysed content.

Table 2. Fatty acid composition of control and experimental diets supplemented with different concentrations of extruded flaxseed

Fettsäuremuster der Kontroll- und der Versuchsrationen mit unterschiedlichen Anteilen an extrudierter Leinsaat

Fatty acid (%)	Experimental diets												Flaxseed
	Control			EFS25			EFS50			EFS100			
	S [1]	G[2]	F[3]	S [1]	G[2]	F[3]	S [1]	G[2]	F[3]	S [1]	G[2]	F[3]	
16:0	9.05	9.10	9.00	9.15	9.12	9.12	8.74	8.73	8.69	8.09	8.08	8.07	5.30
18:0	5.10	5.05	5.30	5.17	5.21	5.21	5.28	5.25	5.25	5.29	5.35	5.32	4.31
18:1	33.36	33.35	33.31	32.27	32.29	32.30	34.03	33.97	34.06	34.71	34.73	34.76	23.1
18:2n-6	50.3	50.2	50.2	49.1	49.1	49.1	46.2	46.2	46.2	45.6	45.7	45.7	17.0
18:3n-3	2.24	2.30	2.24	4.30	4.25	4.26	5.74	5.82	5.84	6.28	6.14	6.18	50.3
Σ SFA	14.2	14.2	14.3	14.3	14.3	14.3	14.0	14.0	13.9	13.4	13.4	13.4	9.61
Σ MUFA	33.4	33.4	33.3	32.3	32.3	32.3	34.0	34.0	34.1	34.7	34.7	34.8	23.1
Σ PUFA	52.5	52.5	52.4	53.4	53.4	53.4	52.0	52.1	52.0	51.9	51.8	51.9	67.3
Σ n-3	2.24	2.30	2.24	4.30	4.25	4.26	5.74	5.82	5.84	6.28	6.14	6.18	50.31
Σ n-6	50.3	50.2	50.2	49.1	49.1	49.1	46.2	46.2	46.2	45.6	45.7	45.7	17.0
n-6/n-3	22.4	21.8	22.4	11.4	11.6	11.5	8.05	7.94	7.90	7.27	7.44	7.39	0.34
Total fat	4.61	4.65	4.60	4.69	4.62	4.70	4.71	4.72	7.76	4.79	4.80	4.84	38.8

[1]Starter diet: 1-14 days; [2]Grower diet:15-35 days; [3]Finisher diet:36-42 days;

Control, EFS25, EFS50, EFS100 represent the maize-soybean meal basal diet (control), or basal diet containing flaxseed at 25 g/kg (EFS25), 50 g/kg (EFS50) or 100 g/kg (EFS100);

Results are given as mean values (n = 5);

Fatty acid content was expressed as percentages of total fatty acid methyl esters.

Table 3. Effect of supplemented flaxseed on growth performances of broilers (accumulated values over the relevant periods)

Einfluss der Anteile an Leinsaat im Futter auf das Wachstum der Broiler (akkumulierte Werte über die jeweiligen Versuchsperioden)

Exp. time	Parameter	Experimental treatment				Pooled SEM	Overall *P*
		Control	EFS25	EFS50	EFS100		
Starter 0-14 d	Body weight (g)	532[a]	577[b]	583[b]	595[b]	0.48	<0.001
	Feed intake (g)	872.5[a]	877.0[b]	874.5[b]	880.6[c]	0.73	0.002
	Feed conversion ratio	1.64[b]	1.52[a]	1.50[a]	1.48[a]	0.02	<0.001
	Mortality (%)	0.58[c]	0.31[b]	0.29[b]	0.21[a]	0.01	<0.001
Grower 15-35 d	Body weight (g)	1046[a]	1058[b]	1062[b]	1109[c]	0.34	0.004
	Feed intake (g)	1799[c]	1788[b]	1752[b]	1641[a]	0.22	<0.001
	Feed conversion ratio	1.72[c]	1.69[b]	1.65[b]	1.48[a]	0.15	<0.001
	Mortality (%)	0.47[c]	0.32[a]	0.40[b]	0.33[a]	0.06	<0.001
Finisher 36-42 d	Body weight (g)	1124[a]	1167[b]	1184[b]	1235[c]	0.75	0.012
	Feed intake (g)	2023[c]	1891[b]	1847[b]	1630.2[a]	0.93	<0.001
	Feed conversion ratio	1.80[c]	1.62[b]	1.56[b]	1.32[a]	0.12	<0.001
	Mortality (%)	0.68[c]	0.25[b]	0.22[b]	0.18[a]	0.01	<0.001
Entire period 0-42 d	Body weight (g)	2702[a]	2802[b]	2829[b]	2939[c]	0.98	<0.001
	Feed intake (g)	4695[c]	4556[b]	4474[b]	4152[a]	1.21	0.002
	Feed conversion ratio	1.74[c]	1.63[b]	1.58[b]	1.41[a]	0.41	<0.001
	Mortality (%)	1.78[c]	0.85[b]	0.81[b]	0.69[a]	0.02	<0.001

[a-c] Treatment means with no common superscript letter in the same column differ significantly ($P < 0.05$). Starter diet: 1-14 days; Grower diet:15-35 days; Finisher diet:36-42 days; Feed intake and Feed conversion ratio values are as-fed values.

Table 4. Oxidative status of blood serum in chickens fed on diets supplemented with different concentrations of extruded flaxseed

Oxidativer Status im Blutserum von Masthühnern nach Fütterung der Kontroll- und der Versuchsrationen mit unterschiedlichen Anteilen an extrudierter Leinsaat

Treatments[1]	SOD µmol/g Hb min*	CAT µmol/g Hb min*	GSHPx µmol/g Hb min*	GSHR µmol/g Hb min*
Control	26.2^{c}	35.4^{c}	8.3^{a}	14.1^{c}
EFS25	27.6^{b}	36.9^{c}	8.5^{a}	15.3^{b}
EFS50	29.8^{a}	40.5^{b}	8.9^{a}	15.6^{ab}
EFS100	31.2^{a}	44.4^{a}	9.2^{a}	16.5^{a}
Pooled SEM	1.22	0.79	0.42	1.25
Overall *P*	0.002	0.002	0.076	0.042

Results are given as mean ± standard deviation (n = 5); a-d Means within a column with no common superscript letter differ significantly at $P < 0.05$; *Units for the enzymes are given in terms of micromoles of a certain compound per g of haemoglobin per minute; SOD: superoxid-dismutase; CAT: catalase; GSHPX: glutathione-peroxidase; GSHR: glutathione-reductase; [1]Control, EFS25, EFS50, EFS100 represent the maize-soybean meal basal diet (control), or basal diet containing flaxseed at 25 g/kg (EFS25), 50 g/kg (EFS50) or 100 g/kg (EFS100).

Table 5. Fatty acid composition of breast meat (*Musculus pectoralis*) from chickens fed on diets supplemented with different concentrations of extruded flaxseed (g/100 g of breast meat)
Fettsäuremuster im Brustfleisch (*Musculus pectoralis*) der Masthühner nach Fütterung der Kontroll- und der Versuchsrationen mit unterschiedlichen Anteilen an extrudierter Leinsaat (g/100 g Brustfleisch)

Fatty acid (g/100 g)	Experimental diet[1]				Pooled SEM	Overall *P*
	Control	EFS25	EFS50	EFS100		
16:0	0.145^{ab}	0.157^{b}	0.128^{a}	0.164^{c}	0.04	0.002
16:1	0.019^{a}	0.028^{b}	0.030^{b}	0.031^{b}	0.01	0.041
18:0	0.055^{c}	0.030^{a}	0.042^{b}	0.052^{b}	0.01	<0.001
18:1	0.131^{a}	0.161^{b}	0.179^{c}	0.180^{bc}	0.07	0.004
18:2n-6	0.111^{a}	0.139^{b}	0.144^{b}	0.132^{a}	0.02	0.036
18:3n-3	0.015^{a}	0.030^{b}	0.037^{b}	0.037^{b}	0.00	0.044
20:1n-9	0.002^{a}	0.002^{b}	0.002^{b}	0.002^{b}	0.00	0.039
20:2n-6	0.007^{c}	0.002^{a}	0.004^{b}	0.005^{b}	0.00	0.021
20:3n-6	0.005^{a}	0.005^{a}	0.005^{a}	0.006^{a}	0.00	0.006
20:4n-6	0.044^{b}	0.011^{a}	0.010^{a}	0.010^{a}	0.00	0.033
20:5n-3	0.001^{a}	0.001^{a}	0.001^{a}	0.002^{b}	0.00	0.042
22:4n-6	0.004^{b}	0.003^{a}	0.003^{a}	0.003^{a}	0.00	0.005
22:5n-6	0.003^{b}	0.003^{b}	0.002^{a}	0.002^{a}	0.00	<0.001
22:5n-3	0.004^{a}	0.005^{a}	0.006^{b}	0.006^{b}	0.00	0.009
22:6n-3	0.002^{a}	0.003^{a}	0.006^{b}	0.006^{b}	0.00	0.018
Σ SFA	0.199^{a}	0.187^{b}	0.170^{bc}	0.217^{ab}	0.06	0.028
Σ MUFA	0.133^{a}	0.163^{b}	0.181^{c}	0.182^{bc}	0.03	0.007
Σ PUFA	0.198^{bc}	0.202^{b}	0.219^{c}	0.210^{a}	0.09	0.002
PUFA/SFA	0.99^{b}	1.08^{b}	1.29^{a}	0.97^{b}	0.06	0.059
Σ n-3	0.024^{a}	0.039^{b}	0.051^{c}	0.051^{bc}	0.02	0.004
Σ n-6	0.175^{c}	0.163^{b}	0.169^{b}	0.158^{a}	0.05	0.030
n-6/n-3	7.29^{b}	4.18^{a}	3.31^{a}	3.10^{a}	1.06	0.014
Σ other FAs	0.019^{a}	0.028^{a}	0.030^{a}	0.031^{a}	0.01	0.052
Σ of total FAs	0.55^{a}	0.58^{b}	0.60^{b}	0.64^{c}	0.12	0.039

Results are given as mean values (n = 5); SEM: standard error (n = 5); a-c Means within a row with no common superscript letter differ significantly at $P < 0.05$; [1]Control, EFS25, EFS50, EFS100 represent the maize-soybean meal basal diet (control), or basal diet containing flaxseed at 25 g/kg (EFS25), 50 g/kg (EFS50) or 100 g/kg (EFS100).

Table 6. Fatty acid composition of leg meat (*M. tibialis anterior*) from chickens fed on diets supplemented with different concentrations of extruded flaxseed (g/100 g of leg meat)
Fettsäuremuster im Schenkelfleisch (*Musculus tibialis anterior*) der Masthühner nach Fütterung der Kontroll- und der Versuchsrationen mit unterschiedlichen Anteilen an extrudierter Leinsaat (g/100 g Brustfleisch)

Fatty acid (g/100 g)	Experimental diet[1]				Pooled SEM	Overall *P*
	Control	EFS25	EFS50	EFS100		
16:0	0.364^{c}	0.375^{a}	0.387^{ab}	0.407^{bc}	0.09	0.007
16:1	0.085^{b}	0.084^{a}	0.089^{ab}	0.099^{c}	0.04	0.002
18:0	0.104^{a}	0.138^{b}	0.106^{a}	0.112^{a}	0.06	0.029
18:1	0.475^{a}	0.490^{c}	0.507^{abc}	0.526^{bc}	0.23	0.006
18:2n-6	0.418^{c}	0.391^{b}	0.419^{b}	0.413^{a}	0.24	0.001
18:3n-3	0.079^{a}	0.090^{ab}	0.096^{b}	0.115^{c}	0.02	<0.001
20:1n-9	0.005^{a}	0.007^{ab}	0.010^{b}	0.014^{c}	0.00	0.004
20:2n-6	0.023^{bc}	0.022^{a}	0.026^{c}	0.025^{ab}	0.01	0.012
20:3n-6	0.007^{a}	0.009^{ab}	0.013^{c}	0.012^{abc}	0.00	0.026
20:4n-6	0.052^{a}	0.077^{b}	0.069^{b}	0.070^{b}	0.03	0.037
20:5n-3	0.001^{a}	0.002^{a}	0.002^{a}	0.005^{b}	0.00	0.043
22:4n-6	0.003^{b}	0.004^{b}	0.003^{b}	0.002^{a}	0.00	0.045
22:5n-6	0.002^{b}	0.003^{b}	0.001^{a}	0.002^{a}	0.04	0.033
22:5n-3	0.010^{a}	0.015^{b}	0.016^{b}	0.018^{b}	0.00	0.041
22:6n-3	0.02^{a}	0.004^{a}	0.008^{b}	0.011^{b}	0.00	0.035
Σ SFA	0.468^{a}	0.513^{b}	0.492^{a}	0.518^{a}	0.29	0.014
Σ MUFA	0.479^{b}	0.496^{a}	0.517^{b}	0.541^{b}	0.19	0.029
Σ PUFA	0.598^{ab}	0.617^{a}	0.652^{b}	0.672^{b}	0.14	0.032
PUFA/SFA	1.28^{ab}	1.20^{b}	1.33^{a}	1.30^{a}	0.44	0.044
Σ n-3	0.092^{a}	0.110^{b}	0.122^{bc}	0.150^{c}	0.06	0.002
Σ n-6	0.506^{b}	0.506^{ab}	0.531^{b}	0.522^{a}	0.11	0.010
n-6/n-3	5.50^{c}	4.60^{bc}	4.35^{ab}	3.48^{a}	1.01	0.028
Σ other FAs	0.085^{a}	0.084^{a}	0.089^{a}	0.099^{a}	0.02	0.062
Σ of total FAs	1.63^{a}	1.71^{b}	1.75^{b}	1.83^{c}	0.47	0.003

Results are given as mean values (n = 5); SEM: standard error (n = 5); a-c Means within a row with no common superscript letter differ significantly at $P < 0.05$; [1]Control, EFS25, EFS50, EFS100 represent the maize-soybean meal basal diet (control), or basal diet containing flaxseed at 25 g/kg (EFS25), 50 g/kg (EFS50) or 100 g/kg (EFS100).