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Effect of dietary mixture of *Aspergillus* probiotic and selenium nano-particles on growth, nutrient digestibilities, selected blood parameters and muscle fatty acid profile in broiler chickens

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Forty five chickens (15 days old) were assigned to three groups: (1) control group fed a basal diet; group (2) fed the basal diet supplemented with 0.05% *Aspergillus awamori* and group (3) fed mixture of *A. awamori* 0.05% + selenium nano-particles 0.3%. Chickens were allowed free access to feed and raised for 12 days. Body weight gain and breast muscle weight increased in group 2 and 3 when compared to the control. Plasma 3-methylhistidine concentration occurred lower in group 2 and 3 Plasma triglyceride and total cholesterol were decreased by feeding *A. awamori* and the combination of *A. awamori* with selenium nano-particles. Fat content of the breast muscle increased accompanied by decrease of saturated fatty acids (SFA_s) and increase of unsaturated fatty acids (UFA_s). Moreover, decreased muscle TBARS and increased α-tocopherol contents in the breast muscle was observed in both experimental groups. The mRNAs of FAS, GPX and delta-6 desaturase were increased by feeding *A. awamori* and the combination of *A. awamori* with selenium nano-particles. In conclusion, it is suggested that feeding *A. awamori* alone or its combination with selenium nano-particles may improve growth performance, modify the skeletal muscle fatty acid profile and α-tocopherol content, suggesting that they may improve meat quality.

KEY WORDS: *Aspergillus* / broilers / chicks / fatty acids / probiotics

Currently, an important research area is the use of probiotics as feed additives. There are many reports concerning the effect of the use of probiotics on feeding performance [Midilli and Tuncer 2001, Galik *et al.*2011, Saleh *et al.* 2011, 2012a]. *Aspergillus*

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probiotics have been commercially produced for the animal industry and used as feed additives to improve growth performance and animal health [Hideya and Taku 2004]. Selenium (Se) is an essential micronutrient for higher animals and humans [Eisler 2000, Klasing 1998]. The best-known biological roles of selenium are linked to its presence as the functional component in selenoenzymes such as glutathione peroxidase (GPx) and thioredoxin reductase (TrxR). It was demonstrated that Se nano-particles are more efficient than selenite, selenomethionine, and methylselenocysteine in upregulating selenoenzymes in mice and rats, while exhibiting significantly lower acute toxicity [Wang *et al.* 2007, Xu *et al.* 2003, Zhang *et al.* 2008]. Nano-Se can be used as an antioxidant with reduced risk of Se toxicity and kind of chemopreventive agent because the induction of GST by Se is a crucial mechanism for this chemopreventive effect [Wang *et al.* 2007]. Nano-Se supplements administered to turkey and broiler chickens' feed determine an improved production and health and can be a natural way to obtain functional foods, meaning turkey selenium – enriched meat [Yaroshenko *et al.* 2004]. The improvement of growth performance due to the combination of *A. awamori* with nano-Se may be partially due to the increase in metabolisable energy of the feed [Saleh *et al.,* 2012a], or this improvement may be related to the balanced microbial population in the gastrointestinal tract which plays an important role in the health and performance of the broilers [Thongsong *et al.,* 2008, Saleh *et al.* 2013a]. Also, the improvement may be due to cellulase and xylanase produced by *Aspergillus* which is required for the digestion of soluble non-starch polysaccharides and regulation of several enzymatic systems interferring in energetic metabolism by selenium feeding. Several reports have shown that *Aspergillus awamori* and selenium affects lipid metabolism, by reducing serum cholesterol and triglyceride in broilers [Mohan *et al.* 1996, Jin *et al.* 1998, Saleh *et al.* 2012b], layers [Abdulrahim *et al.* 1996] and rats [Fukushima *et al.* 1995, 1996]. Also, the combination of *A. awamori* and nano-Se modified muscle fatty acid content due to the effects of Δ6-, Δ5- and Δ4- desaturase [Kralik *et al.* 2012, Saleh *et al.* 2013b]. Therefore, in order to assess the beneficial effects of *Aspergillus* probiotic with selenium, we investigated the effects of *Aspergillus awamori* fed with nano-selenium upon the growth performance, blood constituents and muscle fatty acids profile in broilers.

Material and methods

Animals, feeding and experimental design

Experiment was conducted in accordance with the guidelines of Kagoshima University.

A hundred of one day old male broiler chickens (Chunky strain) were supplied by a commercial hatchery (KUMIAI HINA CENTER, Kagoshima, Japan). The chicks were housed in an electrically heated battery brooder and provided with water and a commercial starter diet (23% crude protein and 12·8 MJ ME/kg; Nichiwa Sangyou Company) until day 12 of age. On day 12, forty five birds with similar body weight were selected from a group of 100 and housed individually in wire-bottomed

aluminium cages (49 x 39 x 59 cm). The birds were preconditioned for 3 days before the treatment and fed a basal diet. The experimental diets were formulated using mainly ground yellow maize and a soyabean meal, as shown in Table 1. The chicks were divided into three groups (15 birds in each group). Control group fed the basal diet; *A. awamori* group fed the basal diet supplemented with 0.05% *A. awamori* and mix feed group fed the basal diet supplemented with 0.05% *A. awamori* and selenium nano-particles 0.3%. Fatty acids profile in the diets is given in Table 2. *Aspergillus awamori* and selenium nano-particles were mixed in the basal diet. The birds were given the experimental diets from day 15 to 27 days of age. The experiment was conducted in a temperature-controlled room with 14 h light: 10 h dark cycle. Room temperature was kept at 25°C with relative humidity from 50 to 70% throughout the experiment. Body weight was recorded every 3 days and feed intake daily during the experimental period. At the end of the experiment, the birds were slaughtered and dissected to measure the weights of the breast muscle (pectoral superficial muscle), abdominal fat, liver and heart.

Table 1. Composition of basal (control) diet

Ingredient	g/kg
Corn seed	501.9
Lucerne meal	26.4
Soyabean meal	390.1
Corn oil	44.0
L-Lysine HCl	0.10
DL-Methionine	1.80
Mineral mixture[1]	33.1
Vitamin mixture[2]	2.6
Calculated values	
CP (%)	226.0
ME (M.J/kg)	12.89
Ca (%)	11.0
P (%)	4.6
Na (%)	2.6
Cl (%)	2.5

CP– crude protein; ME – metabolisable energy.
[1]The mixture supplied (g/kg feed): calcium hydrogen phosphate 20; calcium carbonate 8.15; sodium chloride 5.1 (mg/kg feed): iron sulfate 400; copper sulfate 31.5; zinc sulfate 176; manganese sulfate 152; sodium iodate 0.55; sodium selenite 0.27.
[2]The mixture supplied (mg/kg feed): retinol 1.4; DL-α-tocopherol acetate 6.5; thiamine hydrochloride 2.6; riboflavin 6.5; pyridoxine hydrochloride 1.30; calcium D-pantothenate 10.4; nicotinic acid 26; (mg/kg feed): menadione sodium bisulfite 650; D-biotin 70; choline chloride 780; pteroylglutamic acid 520; cyanocobalamin 26; cholecalciferol 13.

Table 2. Chemical composition of the diets as related to the saturated, monounsaturated and polyunsaturated fatty acids (g.100 g-1)*

Fatty acid	Control	Diet *Aspergillus awamori* 0.05%	*Aspergillus awamori* 0.05%+ selenium nano-particles 0.3%
Saturated			
C14:0 Myristic acid	0.18	0.03	0.06
Arachidic	na	0.41	0.44
C16:0 Palmitic acid	11.23	10.11	10.27
C18:0 Stearic acid	3.49	3.46	3.34
total	14.90	14.01	14.05
Monounsaturated			
C18:1, ω-9 cis oleic acid	23.01	25.07	26.85
C20:1, ω-9 cis 11 - eicosenoic acid	0.17	0.41	0.82
MUFAs total	23.18	25.48	26.85
Polyunsaturated			
C18:2, ω-6 linoleic acid	49.17	53.32	54.40
C18:3, ω-3 α-Linolenic acid	3.67	4.67	5.28
PUFAs total	52.84	57.99	59.68
ω6/ω3 ratio	13.39	11.41	10.3
Poly/saturated ratio	2.95	4.13	3.24

*The values expressed on a dry matter basis; na – not available.

Selenium nano-particles preparation

Selenium nano-particles (sample ready for use) was obtained from Professor Mohsen Zommara, of the Department of Dairy Science, Faculty of Agriculture, Kafrelsheikh University, Egypt, and the selenium nano-particles were prepared according to Zommara *et al*. [2007] and Prokisch *et al.* [2008]. Selenium nano-particles were prepared from pure yoghurt cultures of *Streptococcus thermophilus (S. thermophilus) (CNCM I-1670), Lactobacillus delbrueckii subsp. bulgaricus (L. bulgaricus) (NCAIM B 02206)* obtained from the National Collection of Agricultural and Industrial Microorganisms, Budapest, Hungary**.** MRS media were amended with 20 mg. ppm of filter-sterilized (SARTORIUS AG, Germany) selenium Se (IV) (sodium selenite, Na_2SeO_3. $5H_2O$, SIGMA-ALDRICH, Switzerland) and incubated at 40°C up to 24 h. The media were centrifuged at 4500 g for 20 min at room temperature to spin down the bacteria cells. The cell pellets were washed 2 times by Tris-HCl buffer (50 mM, pH 7.5) and finally with ultra pure water to obtain the Se nano-particles fortified cell fraction. The Se content was analysed by inductively coupled plasma mass spectrometer (ICP-MS) (X series, THERMO FISHER SCIENTIFIC, Germany). The obtained selenium nanospheres were within range 100-500 nm range as mentioned by Prokisch *et al.* [2008, 2011] and Eszenyi *et al.* [2011].

Biochemical analyses

Plasma chemistry tests and 3-methylhistidine concentration. Blood was withdrawn into heparinised test tubes, quickly centrifuged at 5,900 g for 10 min at 4°C

to separate plasma, and stored at -30°C until analysed. The plasma 3- methylhistidine concentration was measured by HPLC method according to Hayashi *et al.* [1987]. Protein was determined by macro-corder machine (J-SCIENCE LAB. Co., Ltd, Kyoto, Japan) and energy by bomb calorie meter (YOSHIDA, Tokyo, Japan). Total cholesterol level, triglycerides, HDL and GOT of plasma were determined by automated Fuji DRY-CHEM 3500 (FUJI MEDICAL SYSTEMS, Tokyo, Japan) according to the manufacturer's instructions. Concentration of muscle thiobarbituric acid reactive substance (TBARS) was measured according to Ohkawa *et al.* [1979]. The α-tocopherol concentration of the muscle was determined by SHIMADZU HPLC model LC6A (Tokyo, Japan) with a Shim-Pack CLC-ODS column (6.0 150 mm) according to Faustman *et al.* [1989].

RNA Isolation and Real Time PCR. Total RNA was extracted from a fragment of pectoralis superficial muscle (about 100 mg) using an RNeasy® Fibrous Tissue Mini Kit (QIAGEN, Tokyo, Japan) according to the manufacturer's protocol. RNA concentration and purity were assessed spectrophotometrically using A260 and A280 values of a photometer (BIOPHOTOMETER, EPPENDORF, Hamburg, Germany). The ratio A260/A280 for all samples varied between 1.8 and 2.0. cDNA was synthesized at 800 ng RNA per 20 ml of reaction solution with PRIMESCRIPTRT® reagent Kit (Perfect Real Time, Takara, Shiga, Japan) using the Program Temp Control System PC320 (ASTEC, Fukuoka, Japan), which was set at reverse transcription 37°C for 15 min, inactivation of reverse transcriptase at 85°C for 5 s, and refrigeration at 4°C for 5 min. Real-time PCR primers were prepared as previously described. Gene expression was measured by real-time PCR using the 7300 Real Time PCR system (APPLIED BIOSYSTEMS, Foster, USA) with SYBR® Premix Ex Taq™ (Perfect Real Time, Takara). The thermal cycle was as follows: 1 cycle at 95 °C for 10 s, and 60 cycles at 95°C for 5 s and at 60°C for 31 s. Expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was used as an internal standard and was not found to differ significantly among the two experimental groups. Gene expression results are shown as the per cent of the control value.

Fatty acids analysis. Lipids were extracted from the breast muscle with a mixture of chloroform and methanol (2:1) in a separatory funnel. The funnel was shaken carefully for 15 min and left to stand for 4 h to separate the organic layer. The organic layer was collected, passed through a glass funnel containing anhydrous sodium sulfate and evaporated to near dryness using a vacuum evaporator [Radwan 1979]. Muscle total fat content was measured using the method described by Folch *et al.* [1957]. Fatty acid profiles were analysed by *gas chromatography-mass spectrometry* (*GC-MS)* [Saleh *et al.* 2012b].

Statistical. The data were analysed by one-way analysis of variance (ANOVA) with the General Linear Model using SPSS Statistics 17.0 (Statistical Packages for the Social Sciences, released 23 August 2008). The significant differences among means of treatments were compared by Tukey's multiple test. $P<0.05$ was set as limit of significance.

Results and discussion

The effect of feeding *A. awamori* with or without selenium nano-particles on body weight gain, feed intake, feed conversion ratio, breast muscle weight, abdominal fat weight and digestibilities of protein and energy are summarized in Table 3. Feeding compound of *A. awamori* and selenium increased the body weight gain significantly ($P<0.05$) while feed intake decreased. Thus, feed conversion ratio was significantly improved in both experimental groups. Protein and energy digestibilities increased significantly ($P<0.05$) in both experimental groups compared to control group.

Table 3. Effect of feeding *Aspergillus awamori* with or without selenium anano-particles on growth parameters in broilers (means with standard errors)

Trait	Treatment		
	control	*A. awamori* 0.05%	*A. awamori* 0.05% + selenium 0.3%
Initial BW (g)	377±3.2	377±6.4	377±5.5
Live weight at slaughter (g)	1072±6^{b}	1133±8^{a}	1156±7^{a}
BWG (g/15 day)	695±8^{c}	756±11^{b}	779±12^{a}
FI (g/15 day)	1163±13^{a}	989±11^{b}	995±15^{b}
Feed conversion	1.67±0.8^{a}	1.30±1.1^{b}	1.27±0.9^{b}
BMW (g/100 g BW)	24.0±1^{b}	26.7±3^{ab}	27.9±5^{a}
Abdominal fat (g/100 g BW)	1.49±0.53^{a}	0.89±0.64^{ab}	0.78±0.44^{b}
Protein digestibility (%)	59±3^{b}	67±3^{a}	69±5^{a}
Energy utilization (%)	66±3^{b}	73±4^{a}	77±4^{a}

^{a,b,c}Within rows means bearing different superscripts differ significantly at $P<0.05$.
BW – body weight; BWG – body weight gain; FI – feed intake; BMW – breast muscle weight.

Table 4. Effect of feeding *Aspergillus awamori* with or without selenium nano-particles on selected blood ingredients in broilers (means with standard errors)

Blood ingredient	Treatment		
	control	*A. awamori* 0.05%	*A. awamori* 0.05% + selenium 0.3%
Total cholesterol (mg/dL)	146±10^{a}	119±8^{b}	110±8^{b}
Plasma TG (mg/dL)	31±2^{a}	21±3^{b}	19±2^{b}
Plasma HDL (mg/dL)	67±8	76±5^{ab}	81±6^{a}
Plasma GOT (U/l)	189±11^{a}	167±11^{b}	152±9^{c}
Plasma glucose (mg/dL)	236±12^{a}	198±9^{b}	189±7^{b}
Plasma TP(mg/dL)	2.9±1.3^{b}	3.3±1.3^{ab}	3.3±1.4^{a}
Plasma 3-MH (μmol/mL)	30.1±2^{a}	21.3±1^{b}	19.8±3^{b}

^{a,b,c}Within rows means bearing different superscripts differ significantly at $P<0.05$.
TG – trigliceride; HDL – high density lipoprotein; GOT – glutamic oxalacetic transaminase; TP – total protein; 3-MH – 3-methylhistidine.

The effect of feeding *A. awamori* with or without selenium nano-particles on plasma traits (total cholesterol, triglyceride, HDL, GOT, glucose, total protein and 3-methylhistidine are summarized in Table 4. Plasma total cholesterol, triglyceride, glucose and 3-MH levels occurred lower (P<0.05) by feeding the compound of *A. awamori* and selenium nano-particles compared to control group. Also, plasma GOT, as index of liver functions, decreased by feeding mixture of *Aspergillus* with selenium, while plasma HDL-cholesterol and total protein increased.

Effects of feeding *A. awamori* with or without selenium nano-particles on muscle total fat, α-tocopherol, TBARS and drip loss are shown in Table 5. Muscle total fat and α-tocopherol increased significantly when the birds were fed the mixture of *A. awamori* and selenium on the other hand, muscle TBARS and drip loss decreased (P < 0.05) compared to control group.

Table 5. Effect of feeding *Aspergillus awamori* with or without selenium nano-particles on selected meat quality traits in breast muscle of broilers (means with standard errors)

Item	Treatment		
	control	*A. awamori* 0.05%	*A. awamori* 0.05% + selenium 0.3%
Muscle fat (g/100 g muscle)	1.3±0.7[b]	2.3±0.8[a]	2.9±0.8[a]
Muscle α-tocopherol (mg/100 g muscle)	0.3±0.02[c]	0.4±0.03[b]	0.4±0.07[a]
Muscle TBARS (nmolMDA/g muscle)	29±2[a]	19±3[ab]	14±1[b]
Muscle drip loss (%)	8.6±1.2[a]	8±0.9[a]	5.7±0.7[b]

[a,b,c]Within rows means bearing different superscripts differ significantly at P<0.05.

Effects of feeding *A. awamori* with or without selenium nano-particles on breast muscle fatty acids profile are presented in Table 6. The level of myristic, palmitic and stearic acids as saturated fatty acids (SFA) decreased (P<0.05) in both experimental groups compared to control group, but an arachidic acid per cent was not affected So, total saturated fatty acids were decreased by feeding *A. awamori* and significantly by the mixture of *A. awamori* and selenium. Oleic and eicosenoic acids as monounsaturated fatty acids (MUFA) increased (P<0.05) and total MUFA were significantly higher (P<0.05) in both groups compared to control group. Linoleic, linolenic fatty acids and total polyunsaturated fatty acids (PUFA) increased significantly (P<0.05) in both experimental groups. The ω6/ω3 ratio was found lower. On the other hand, polyunsaturated / saturated fatty acids relation was increased by feeding *Aspergillus awamori* with selenium nano-particles.

The effect of feeding *A. awamori* with or without selenium nano-particles on mRNAs of FAS, delta-6 desaturase and GPX contents of muscle are shown in Figure 1. The mRNAs of FAS (1A), delta-6 desaturase (1B) and GPX (1C) were increased (P<0.05) by feeding *A. awamori* with selenium compared to control group.

Table 6. Effect of feeding *Aspergillus awamori* with or without selenium nano-particles on fatty acids profile in breast muscle (g.100 g-1)* (means with standard errors)

Fatty acid	Treatment		
	control	*Aspergillus awamori* 0.05%	*Aspergillus awamori* 0.05%+ selenium nano-particles 0.3%
Saturated			
C14:0 Myristic acid	0.23±0.008[a]	0.14±0.007[b]	0.15±0.007[b]
Arachidic	0.42±0.006	0.41±0.009	0.44±0.009
C16:0 Palmitic acid	17.23±1.2[a]	8.11±0.98[b]	6.27±0.99[b]
C18:0 Stearic acid	2.89±0.19[a]	1.46±0.89[b]	1.34±0.79[b]
total	20.77±2.7[a]	10.12±0.98[b]	8.2±1.01[c]
Monounsaturated			
C18:1, ω-9 cis oleic acid	17.02±1.9[b]	19.17±1.02[a]	23.88±1.09[a]
C20:1, ω-9 cis 11 - eicosenoic acid	0.18±0.003[c]	0.38±0.004[b]	0.76±0.005[a]
MUFAs total	17.2±1.7[b]	19.55±1.03[a]	24.64±1.1[a]
Polyunsaturated			
C18:2, ω-6 linoleic acid	48.11±3.8[b]	58.36±4.5[a]	58.40±4.9[a]
C18:3, ω-3 α-Linolenic acid	4.57±0.60[b]	5.97±0.98[a]	6.98±0.97[a]
PUFAs total	52.68±3.9[b]	64.33±4.9[a]	65.38±5.1[a]
ω6/ω3 ratio	10.52±0.98[a]	9.77±0.8[b]	8.36±0.9[b]
Poly/saturated ratio	2.53±0.26[c]	5.76±0.8[b]	7.97±0.6[a]

*The values expressed on a dry matter basis.
[a,b,c]Within rows means bearing different superscripts differ significantly at P<0.05.

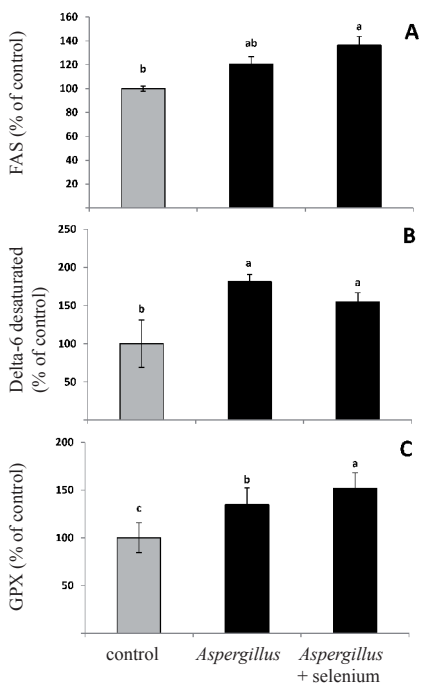

Fig.1. Effect of feeding *Aspergillus awamori* with or without selenium nano-particles on mRNAs of FAS (A), delta-6 desaturase (B) and GPX (C) contents in breast muscle. Values are expressed as % of the control values (means±S.D). [abc] Means with different superscripts differ significantly at P<0.05.

The major aim of this study was to show how the growth performance and the fatty acid muscle profile can be modified by feeding the mixture of *A. awamori* as an effective probiotic and selenium nano-particles as antioxidative agent. The nano-sized red elemental Se (nanoSe) was shown to lower acute toxicity as compared to selenite in mice; however bioavailability of selenite was similar in terms of inducing seleno-enzymes in cultured cells and in Se-deficient rats [Zhang *et al.* 2001]. The improvement in weight gain and feed efficiency resulting from *Aspergillus* and selenium feeding may be partially due to the increase of metabolisable energy of the feed [Saleh *et al.* 2012a]. Organic selenium is also involved in regulation of several enzymatic systems interferring in energetic metabolism, spermatozoa functions, and synthesis of prostaglandins and metabolism of the essential fatty acids, purinic and pyrimidinic bases and organism's general immunity as well [Sara and Odagiu 2008, Ebeid 2012]. Breast muscle weight was increased, while abdominal fat weight was decreased ($P<0.05$) by the fungus and selenium. Also Yamamoto *et al*. [2007] reported that when broilers were fed the diets containing 0.05 and 1% of Koji-feed, carcass weight was significantly increased and the breast muscle weight tended to rise. That effect seems to be due to a growth promoter reported by Kamizono *et al.* [2010]. The growth promoting effect of the fungus can be explained by its effect on plasma 3-methylhistidine concentration. The plasma 3-methylhisitidine concentration was decreased by the fungus, indicating a decreased rate of skeletal muscle protein degradation. In the present experiment both protein and energy digestibilities were significantly increased by the fungus and selenium. The results are in accordance with earlier findings indicating that *Aspergillus awamori* increases digestibility in broilers [Saleh *et al.* 2011, 2012a]. Saleh *et al.* [2005] reported that exogenous enzymes contained in Koji-feed might stimulate digestion and improve growth. Furthermore, Aryanti *et al.* [1999] found that *A. awamori* possesses the ability to digest raw starch. These may be the reason for the efficient feed utilization due to *Aspergillus* and selenium feeding. It is also possible that *Aspergillus* could improve nutritive quality of soybean meal since trypsin inhibitor contained in soybean is degraded by *Aspergillus* [Hong *et al.* 2004]. On the other hand, selenium nano-particles have important biological functions and are essential element for animal growth. Moreover, selenium is an important auxiliary factor for the key enzyme of 5′-deiodinase, which synthesizes triiodothyronine (T3) in animals. Triiodothyronine is a main hormone that regulates animal growth by controlling the body's energy and protein anabolism. Se deficiency can cause the reduction of T3 synthesis and growth inhibition [Preter 2000]. In addition, through the interaction between Se and glutathione peroxidase, it can prevent the lipid structure of animal cell membranes from being destroyed by oxidative damage, and then affect growth. Previous studies have shown that livestock diets supplemented with different doses of Se have different impact on the body weight gain and feed utilization [Wolter 1999, Lawler *et al.* 2004, Vignola *et al.* 2009]. Also, Choct *et al.* [2004] reported that increased dietary selenium content markedly reduced feed conversion ratio (FCR) as a result of significantly lower feed intakes of birds while maintaining the same weight gain.

Plasma cholesterol, triglycerides, GOT and glucose, all were decreased, while plasma HDL-cholesterol and total protein were increased by the *Aspergillus* with selenium nano-particles in the present experiment. This was due to lipolysis which increased by selenium intake [Oppenheimer 1991]. Kim *et al.* [2003] also found that *Aspergillus oryzae* at 0.1% of diet significantly lowered serum cholesterol and triglyceride in broiler chickens. The mechanism underlying the cholesterol lowering effect of *Aspergillus* could be related to an inhibitor of 3-hydroxyl-3- methylglutaryl-coenzyme (HMG-CoA) reductase [Hajjaj *et al.* 2005]. HMG-CoA reductase inhibitor (statin) was extracted from a probiotic [Endo 1985]. Statin is recognized as safe and is widely used to treat patients suffering from hypercholesterolemia [Serruys *et al.* 2004]. HMG-CoA reductase inhibitor may be responsible for decrease in carcass fat deposition. *Aspergillus* plus selenium may affect fat deposition by influencing the activities of hormone sensitive lipase and malate dehydrogenase enzyme in adipose tissues [Mersmann 1998]. Hormone-sensitive lipase (HSL) is the enzyme that initiates the catabolism of triacylglycerol in adipocytes. Because HSL is present in an inactive form, it has to be activated in the terminal event of the cascade mechanism, beginning with the formation of a hormone-receptor complex (e.g., epinephrine and the β-adrenergic receptor) at the cell surface and ending with the phosphorylation of HSL [Mersmann 1998, Saleh *et al.* 2013b]. Otherwise, selenium nano-particles supplementation increases the production of 15-deoxy-Δ-12, 14-prostaglandin J2 [Ricote *et al.* 1998, Touyz and Schiffrin 2006, Vunta *et al.* 2007], a known peroxisome proliferator-activated receptor-γ ligand. Activation of peroxisome proliferator-activated receptor-γ can reduce the concentration of sterol regulatory element-binding protein-2, resulting in a reduction of cholesterol synthesis [Klopotek *et al.* 2006].

In this study breast muscle TBARS decreased, while muscle α-tocopherol and total fat content increased by fungus with selenium supplementation and drip loss increased. There was a negative correlation found between muscle TBARS and α-tocopherol content [Maraschiello *et al.* 1999]. These results indicate that *A. awamori* produces antioxidative substances. In fact, Kaminishi *et al.* [1999] reported that several strains of *Aspergillus* produce antioxdative substances. Also, there is correlation between GSH-Px activity and selenium content of tissues of poultry [Daun and Akesson 2004]. Mckenzie et al. [1987] reported that GPX activities in children were lower than activities observed in New Zealand adults, reflecting their lower blood selenium concentrations. One of the most important functions of selenium in animals consists, as already outlined, of its antioxidant role. This antioxidant activity is enhanced by the interrelations between selenium and vitamin E, liposoluble vitamin from cell and cellular organnelles membranes with role in cellular mechanisms of defence against peroxidation of the membrane phospholipids [Ebeid 2012]. Selenium, component of glutation-peroxidase acts through secondary mechanism of defence, as consequence of incapacity of vitamin E to destroy all metabolic peroxides. As cellular component of glutation-peroxidase, selenium acts together with vitamin E for reducing the cellular stress [Hess *et al.* 2003, Ebeid 2012]. The existence of a tight relation between the

antioxidants intake (e.g. of selenium and vitamin E) and superior quality of poultry meat was found. A supplementary contribution of selenium in humans and animals may determine the decrease of the cancer incidence by 50%, heal the male infertility and lead to the decrease of abortion per cent, double the immune capacity of the cells involved in immune response and lead to decrease of mortality produced by cardiac diseases [Leng *et al.* 2003]. Important are the metabolic mechanisms of which selenium is involved in the process of improvement of the broiler chickens' meat quality [Sara and Odagiu 2008]. The antioxidant system includes natural, synthetic and various *in vivo* antioxidant enzymes that are present in the body [Sies 1991]. A normal metabolism produces free radicals, but excessive free radicals damage the cells. The SOD, GSH-Px and CAT are three major free radical enzyme scavengers [McCord 1979]. The T-AOC reflects the total antioxidant capacity of the body. Although Se is not a component of SOD or CAT, the ability to synthesize various antioxidant enzymes declines under Se-deficient conditions in animals, while the increase of lipid peroxidation affects vitality. Therefore increase in GSH-Px and CAT activities enhance the ability to eliminate free radicals. A decrease of the MDA content in the tissues is related to the enhancement of antioxidant enzymatic activity resulting from the provision of supplemental dietary Se. The results of this study showed that diets supplemented with selenium nano-particles improve antioxidant activities.

PUFA concentration in the chicken muscle were increased by feeding *Aspergillus awamori* and selenium nano-particles. This is important as they play significant role in reducing the incidence of life-style diseases such as coronary artery diseases, hypertension and diabetes, as well as certain inflammatory diseases such as arthritis and dermatitis in humans [Simopoulos 2000]. Several studies have shown that oleic and linoleic acids are the most common unsaturated fatty acids produced by *Aspergillus*, whhile linoleic acid is a major constituent of fungal lipids [Mazur *et al.* 1991, Calvo *et al.* 2001, Richard *et al.* 2004, Tsitsigiannis *et al.* 2004]. Richard *et al.* [2004] reported the *Aspergillus* to produce desaturase which converts SFA_s to UFA_s. Ignatius *et al.* [2010] found that *Aspergillus terreus* produces linolenic acid, while production of other PUFAs has not been studied. It is probable that the increases in oleic, linoleic and linolenic acids in the muscle are the result of the intestinal activities of *A. awamori*. On the other hand Haug *et al.* [2007] determined a significant effect of selenium contained in broiler feed on the content of eicosapentaenoic, docosapentaenoic and docosahexaenoic acids of thigh muscles, *i.e.* increased content of selenium in feed affected the increase of fatty acid content in thigh muscles ($P<0.05$). Kralik *et al.* [2012] showed a trend of increase in the content of eicosapentaenoic and docosapentaenoic fatty acids in breast muscle tissue, while Haug *et al.* [2007] reported the similar increase in thigh muscle tissue. These authors explained that higher content of selenium in feed affected the activity of Δ6-, Δ5- and Δ4-desaturase, which catalyze elongation and desaturation of short-chain fatty acids into long-chain fatty acids, or that intake led to reduced speed of long-chain fatty acids degradation in peroxidation processes Kralik *et al.* [2012].

Delta 6-fatty acid desaturase (D6DES) effected on the synthesis of PUFAs [Garcia *et al.* 2002]. In the present study mRNAs of fatty acid synthesis, delta 6-fatty acid desaturase and GPX were all increased by *A. awamori* and selenium. These results are in accordance with author's earlier study which indicated that mRNAs of ACC, FAS and delta-6 desaturase were increased by feeding koji [Saleh *et al.* 2012b].

In conclusion, this study clearly shows that growth performance is improved by increased body weight gain and improved feed conversion ratio as well. The muscle lipid profile can be modified by increased unsaturated fatty acid and decreased saturated fatty acid by the combined addition of *A. awamori* and selenium nano-particles to broiler diets.

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