



Effect of housing systems on fatty acid structures of liver, abdominal fat and breast muscle in broiler production

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ABSTRACT

This study was conducted to determine fatty acid structures of liver, abdominal fat and breast muscle (*Musculus pectoralis profundus*) in broiler reared in cage (CH) and floor (FH) housing systems. For this purpose, 15 broilers (Ross-308) per replicate having stable live weight were selected in each system and fatty acids analyses were simultaneously taken in summer, autumn and winter seasons by a gas chromatography (GC) system. It was found that the poultry meat had higher ratio of polyunsaturated fatty acids (PUFA), especially omega-6 fatty acids (n-6) related with the diet consumed by chicken. Cage housing system caused accumulation of myristic (C_{14:0}), palmitic (C_{16:0}), stearic (C_{18:0}), arachidic (C_{20:0}) and heneicosanoic (C_{21:0}) fatty acids in the chicken that significantly raised total saturated fatty acid (SFA) ratios of abdominal fat and pectoral muscle tissues. Proportions of total monounsaturated fatty acid (MUFA), total PUFA and PUFA/SFA of abdominal fat tissue were found higher in FH system. Proportions of omega-3 fatty acids (n-3), n-6, n-6/n-3 and PUFA/SFA were similar between groups in liver and muscle tissues. Consequently, cage housing system used by broiler production was inclined to synthesis of saturated fatty acids, especially in fat and breast muscle tissues, and changed fatty acid structure of chicken meat.

Key words: Broiler, Cage housing, Fatty acids, Floor housing

Cage housing systems have been used for years for broilers chicken production because of more dense production, increasing productivity per coop and decreasing cost by making better use of constant outgoings. On the other hand, it causes some welfare problems in the birds, especially inadequate physical activity and behavioral limitation (Shields and Greger 2013, Simsek *et al.* 2014, Özhan *et al.* 2016). Although increasing consumer desire to healthy production, researches mention the effect of these systems on meat quality are insufficient. Housing systems using broiler production alter meat composition, physical properties of meat and sensory attributes (Molee *et al.* 2012, Lin *et al.* 2014). Some studies reported that the cage housing production type increased crude fat content and calorie content of meat in poultry (Lin *et al.* 2014), and also different housing system altered fatty acid structure of meat thanks to changing animal welfare level (Funaro *et al.* 2014) and nutrition regime; the level of omega-3 fatty acids (n-3) was greater in meat of broiler in free-range pastured system

(Molee *et al.* 2012). In addition, functional fatty acids such as long-chain n-3 fatty acids and conjugated linoleic acids have significant biological roles in body. The high level of saturated fatty acids (SFA) and ratios of omega-6 fatty acids (n-6)/n-3 promote the pathogenesis of many chronic diseases (Das 2006). Poultry meat has been noticed one of the main resources of polyunsaturated fatty acids (PUFA), however, fatty acids composition of the poultry meat is affected by lots of factors; especially diet and management (Tougan *et al.* 2013). The aim of the present study was therefore to determine the distribution of fatty acids of liver, abdominal fat and breast muscle tissues, and to compare the accumulation rate of fatty acids in the tissues in broiler reared cage and floor housing systems.

MATERIALS AND METHODS

Experimental design: The study was conducted at cage and floor farms of an integrated commercial company with the approval of Firat University Animal Research Ethic Committee (FUHADEK, Verdict no: 2012/07). Chicks of Ross-308 breed were obtained from hatchery of the facility and randomly placed in poultry houses. Birds were allocated to 2 groups viz. floor housing group (FH), placed in wood shavings deep litter pens (17 birds/m²) and cage housing group (CH), placed in cage storeys with plastic mesh floor material (17.5 chickens/m² each cage unit) (<http://www.kutlusan.com.tr>). Three replicate flocks of each cage

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Table 1. Fatty acid structures of finisher diets in three seasons

Fatty acids, %	Summer	Autumn	Winter
C 16:0	9.62	9.00	7.56
C 16:1n7	0.57	0.87	1.05
C 18:0	0.33	1.09	0.22
C 18:1n9	16.88	13.25	18.51
C 18:2n6	56.90	65.34	62.72
C 18:3n3	5.79	6.45	4.98
C 20:1n9	0.55	1.27	0.47
C 20:2n6	0.60	0.95	1.07
C 20:4n6	7.65	8.23	7.19
C 20:5n3	0.50	1.20	0.63
C 24:1	0.58	-	1.21
SFA	9.95	10.09	7.78
MUFA	18.58	15.39	21.24
PUFA	71.44	74.52	70.98
n-3	6.29	7.65	5.61
n-6	65.15	66.84	65.37
PUFA/SFA	6.54	6.62	9.12
n-6/n-3	10.35	8.73	11.65

SFA, Saturated fatty acid; MUFA, monounsaturated fatty acid, PUFA, Polyunsaturated fatty acid; n-3, Omega-3 fatty acid; n-6, Omega-6 fatty acid. Fatty acids: Butyric acid (C4:0), myristic acid (C14:0), pentadecenoic acid (C15:1), palmitic acid (C16:0), palmitoleic acid (C16:1n-7), heptadecanoic acid (C17:0), heptadecaenoic acid (C17:1), stearic acid (C18:0), oleic acid (C18:1n-9), linoleic acid (C18:2n-6), α -linolenic acid (C18: 3n-3), α -linolenic acid (C18:3n-6), arachidic acid (C20:0), eicosenoic acid (C20:1n-9), eicosadienoic acid (C20:2n-6), dihomo-g-linolenic (C20:3n-6), arachidonic acid (C20:4n-6), eicosapentaenoic acid (C20:5n-3), heneicosanoic acid (C21:0), behenic acid (C22:0), docosapentaenoic acid (C22:5n-3), docosahexaenoic acid (C22:6n-3), lignoceric acid (C24:0), nervonic acid (C24:1).

and floor housing groups were simultaneously examined during summer, autumn and winter seasons in the capacities of 25,000 broilers. Environmental conditions in two systems were organized according to the needs of broiler. Throughout the study, fresh water and feed produced at feed unit of facility in accordance with NRC (1994) standards were automatically distributed as an *ad lib*. Fatty acid profiles of feeds are presented in Table 1. To determine the fatty acids structures of tissues, enough chickens were individually weighed on slaughter day (d 33). A total of 15 broilers per replicate composed of 7 females and 8 males having a live weight of approximately 2.0 and 2.5 kg respectively were picked out and transferred for slaughter from each system. Feathers were plucked from selected chickens at slaughter house with wet plucking method and soon liver, abdominal fat and *Musculus pectoralis profundus* were extracted from carcass and stored -20°C until analyses.

Lipid extraction and preparation of fatty acid methyl esters: Extraction of lipids from tissue specimens and feed were carried out with Hara and Radin (1978) method in which 3:2 (v/v) hexane isopropanol mixture was used. Preparation of fatty acid methyl esters was made according to Christie (1994).

Gas chromatographic analysis of fatty acid methyl esters: Fatty acid methyl esters were analyzed with SHIMADZU GC 17 Ver. Three gas chromatography 25 m long Machery-Nagel (Germany) capillary column with an internal diameter of 0.25 mm and a thickness of 25 micron PERMABOND film was used. During the analysis, column heat was kept at $120-220^{\circ}\text{C}$, injection heat was kept at 240°C and detector heat was kept at 280°C . Column heat program was regulated to 220°C from 120°C , heat increase was set to $5^{\circ}\text{C}/\text{min}$ until 200°C and to $4^{\circ}\text{C}/\text{min}$ from 200 to 220°C and kept at 220°C for 8 min. Nitrogen was used as a carrier gas, while Flame Ionization detector was used as detector. During the analysis, before the analysis of fatty acid methyl esters of the samples, the mixtures were injected to standard fatty acid methyl esters and residence times of each fatty acid were determined. After this treatment, necessary program analysis was made and fatty acid methyl esters mixtures were analyzed.

Statistical analysis: Effects of floor and cage housing systems on fatty acid structures of liver, abdominal fat and muscle tissues in broiler chicken were evaluated by independent-samples t test after test of normality. P-values were given in the tables showing total effect of the housing systems. All analyses were performed by using SPSS for Windows (2012). The results were considered as significant when P values were lower than 0.05.

RESULTS AND DISCUSSION

This study examined the effects of housing systems on fatty acid profiles of broiler meat (Tables 2–4). When total fatty acids structure of the tissues was examined, PUFA ratios of the chicken tissues were found higher from MUFA and SFA as reported by previous studies (Simsek *et al.* 2009b, Narciso-Gaytán *et al.* 2011). When n-6, n-3 and n-6/n-3 fatty acids were investigated; percentage of n-6 fatty acids and the ratio of n-6/n-3 were very high in the tissues. This elevated value of n-6 fatty acids in the chicken meat can be associated with feed ingredients consumed by the chicken (Table 1), because broiler meat fatty acids are significantly influenced by fatty acid composition of feed (Zdunczyk and Jankowski 2013).

Eicosadienoic acid (C20:2n-6) and eicosapentaenoic acid (C20:5n-3) were higher in CH system (Table 2). Linoleic acid (C18:2n-6), docosapentaenoic acid (C22:5n-3) and lignoceric acid (C24:0) were higher in FH system, while nervonic acid (C24:1) was lower in liver tissue. There was no significant effect of housing system on total SFA, monounsaturated (MUFA), PUFA, n-6, n-3 fatty acids and ratios of PUFA/SFA and n-6/n-3 in this tissue. Housing systems significantly affected fatty acid structure of abdominal fat (Table 3). Myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), linoleic acid (C18:2n-6), arachidic acid (C20:0), eicosadienoic acid (C20: 2 n-6) and heneicosanoic acid (C21:0) were found higher in CH system. However, palmitoleic acid (C16:1n-7) and eicosenoic acid (C20:1n-9) were increased in FH system. Total SFA ratio was found to be higher in CH system, while

Table 2. Effect of floor and cage housing systems on fatty acid structures of liver

Fatty acids, %	Floor housing (FH)	Cage housing (CH)	Effect of housing systems (P)
C15:1	0.44±0.01	0.31±0.01	NS
C16:0	16.10±0.27	16.44±0.21	NS
C16:1n7	1.20±0.06	1.11±0.04	NS
C17:0	0.18±0.02	0.18±0.01	NS
C17:1	0.69±0.03	0.57±0.03	NS
C18:0	21.03±0.87	20.93±0.56	NS
C18:1n9	8.31±0.50	8.45±0.74	NS
C18:2n6c	22.04±1.00	21.52±1.48	NS
C18:2n6t	1.06±0.05	0.68±0.02	*
C18:3n6	0.52±0.03	0.46±0.03	NS
C18:3n3	0.71±0.08	0.86±0.06	NS
C20:2n6	0.33±0.02	0.52±0.02	***
C20:3n6	0.69±0.03	0.81±0.05	NS
C20:4n6	16.25±0.44	16.66±0.47	NS
C20:5n3	0.62±0.03	0.73±0.03	*
C22:0	0.80±0.05	0.79±0.05	NS
C22:5n3	1.97±0.50	1.42±0.10	*
C22:6n3	4.71±0.08	4.53±0.07	NS
C24:0	1.45±0.07	1.04±0.06	***
C24:1	0.90±0.10	1.99±0.38	***
SFA	39.56±0.72	39.38±0.67	NS
MUFA	11.54±0.50	12.43±0.80	NS
PUFA	48.90±1.36	48.19±0.54	NS
n-6	40.89±1.25	40.65±0.55	NS
n-3	8.01±0.24	7.54±0.30	NS
PUFA/SFA	1.23±0.03	1.22±0.02	NS
n-6/n-3	5.10±0.25	5.39±0.23	NS

P, Statistical significance; NS, Non-significant; *, P<0.05; ***, P<0.001

total MUFA and PUFA/SFA ratios of abdominal fat were higher in FH system. In addition, pentadecenoic acid (C15:1), palmitic acid (C16:0), heptadecaenoic acid (C17:1) and stearic acid (C18:0) were found higher in pectoral muscle in CH system; while docosapentaenoic acid (C22:5n-3) and docosahexaenoic acid (C22:6n-3) were higher in FH system. Total SFA ratio of breast muscle was significantly increased in CH system. The MUFA, PUFA, n-6, n-3, PUFA/SFA and n-6/n-3 ratios were similar between groups (Table 4). The accumulation of myristic (C_{14:0}), palmitic (C_{16:0}), stearic (C_{18:0}), arachidic (C_{20:0}) and heneicosanoic (C_{21:0}) fatty acids in abdominal fat (Table 3) and the accumulation of palmitic (C_{16:0}) and stearic (C_{18:0}) acids in pectoral muscle (Table 4) significantly increased the total SFA ratios of these tissues. Indeed, decreasing desaturase activity of liver under stress conditions was reported in a previous study (Gao *et al.* 2010). Higher proportion of saturated fatty acids in fat and muscle tissues of the caged broilers may explain insufficient desaturase activity of liver on these tissues, only balanced activity in liver tissue. On the other hand, lipid oxidation was considered to be the major cause of deterioration of fatty acids in the tissues. The oxidation primarily influences

Table 3. Effect of floor and cage housing systems on fatty acid structures of abdominal fat

Fatty acids, %	Floor housing (FH)	Cage housing (CH)	Effect of housing Systems (P)
C14:0	0.37±0.02	0.76±0.01	**
C16:0	16.62±0.18	17.61±0.26	**
C16:1n7	4.04±0.39	2.89±0.12	***
C17:0	2.23±0.07	2.17±0.06	NS
C18:0	4.90±0.15	5.49±0.13	***
C18:1n9	30.84±0.54	29.67±0.37	NS
C18:2n6c	32.90±0.54	32.65±0.57	NS
C18:2n6t	1.93±0.03	2.20±0.06	**
C18:3n6	0.94±0.02	0.86±0.02	NS
C18:3n3	3.58±0.06	3.60±0.10	NS
C20:0	0.31±0.00	1.02±0.02	*
C20:1n9	0.59±0.02	0.20±0.00	*
C20:2n6	0.19±0.01	0.23±0.01	*
C21:0	0.18±0.01	0.23±0.01	*
C20:3n6	0.10±0.00	0.10±0.00	NS
C20:4n6	0.28±0.01	0.32±0.03	NS
SFA	24.61±0.25	27.28±0.31	**
MUFA	35.47±0.52	32.76±0.47	*
PUFA	39.92±0.58	39.96±0.62	NS
n-6	36.34±0.46	36.36±0.55	NS
n-3	3.58±0.08	3.60±0.06	NS
PUFA/SFA	1.62±0.03	1.46±0.04	*
n-6/n-3	10.15±0.58	10.10±0.30	NS

P, Statistical significance; NS, Non-significant; *, P<0.05; **, P<0.01; ***, P<0.001

highly unsaturated fatty acid components ending with the exchange in the proportion of the poultry meat fatty acids. Excessive production of reactive oxygen metabolites by chronic stress could alter the fatty acid profiles by increasing the fatty acid saturation in broilers, leading to higher degree of accumulation of saturated fatty acids (Gao *et al.* 2010). Increasing proportion of saturated fatty acids in the present study may also be related with fatty acid saturation in stressed broiler under limited, less mobile, cage condition. Broilers reared in FH have more chance to practice behaviors such as pecking and scratching (Fouad *et al.* 2008), and this regular physical activity may be another factor responsible for alteration of fatty acid profiles of abdominal fat and muscle tissues by increasing desaturase enzyme activity of liver (Mickleborough 2013). The other approach to this accumulation is progressive energy demand of the body in stress condition. Increasing energy demand for adaptation period may change PUFA ratios of tissues by causing to spend of unsaturated fatty acids, especially long chain PUFA (Mickleborough 2013). Similar to these findings, Shim *et al.* (2006) reported that broiler reared under chronic heat stress had higher proportion of total SFA ratio in liver tissue and taurine supplementation, known to

Table 4. Effect of floor and cage housing systems on fatty acid structures of breast muscle

Fatty acids, %	Floor housing (FH)	Cage housing (CH)	Effect of housing systems (P)
C4:0	0.97±0.09	1.45±0.17	NS
C15:1	3.38±0.33	4.21±0.34	*
C16:0	17.80±0.31	18.36±0.23	**
C16:1n7	1.99±0.17	1.92±0.15	NS
C17:1	1.35±0.16	1.76±0.14	*
C18:0	10.70±0.67	11.71±0.58	*
C18:1n9	19.94±0.74	19.10±1.16	NS
C18:2n6c	24.74±1.12	24.45±1.60	NS
C18:2n6t	0.78±0.05	0.87±0.06	NS
C18:3n3	1.73±0.75	1.68±0.13	NS
C20:2n6	1.60±0.07	1.19±0.07	NS
C20:3n6	1.10±0.08	0.94±0.06	NS
C20:4n6	7.32±0.62	7.06±0.75	NS
C20:5n3	0.56±0.14	0.33±0.02	NS
C21:0	1.13±0.31	1.11±0.09	NS
C22:5n3	1.21±0.11	0.46±0.10	*
C22:6n3	2.45±0.51	1.39±0.10	*
C24:0	1.24±0.16	2.00±0.12	NS
SFA	31.84±0.96	34.63±0.82	**
MUFA	26.66±0.97	26.99±1.18	NS
PUFA	41.49±1.06	38.37±0.53	NS
n-6	35.54±0.89	34.51±1.43	NS
n-3	5.95±0.58	3.86±0.13	NS
PUFA/SFA	1.30±0.06	1.10±0.04	NS
n-6/n-3	5.97±0.40	8.94±0.33	NS

P, Statistical significance; NS, Non-significant; *P<0.05; **P<0.01.

play an antioxidant role, resulted in significant decrease in the proportion of saturated fatty acids.

Increasing levels of docosapentaenoic (C22:5n3) acid in liver tissue (Table 2), docosapentaenoic acid (C22:5n3) and docosahexaenoic acid (C22:6n-3) in pectoral muscle tissue (Table 4) and total MUFA, PUFA and PUFA/SFA in abdominal fat tissue (Table 3) were associated with improved welfare conditions and physical activity in FH system than CH. Similarly, it was stressed that animal welfare and nutrition management in free-range and organic production system caused a higher proportion of n-3 fatty acids compared to intensively-reared chicken meat (Funaro *et al.* 2014, Molee *et al.* 2012). Physical activity could also increase polyunsaturated fatty acids by reducing fatness in a bird reared by floor system. It was detected that SFA and MUFA fatty acids increase faster with increasing fatness than those of PUFA, resulting in a decrease in relative ratios of PUFA and PUFA/SFA (Toplu *et al.* 2016). Simsek *et al.* (2009b) reported that improved housing design caused decrease in total SFA and MUFA ratios of total carcass, breast and thigh meat, and increased total PUFA, n-3 and n-6 fatty acids, related with animal welfare and fatness in broiler. Mickleborough (2013) clarified that low dose

regular physical exercise could enhance antioxidant status of tissues by activating antioxidant enzyme activity; conversely, intense/exhaustive exercise could lead to muscle fatigue and significantly affect fatty acid profiles of the tissues. In agreement with this finding, Simsek *et al.* (2009a) indicated that lower stocking density decreased total SFA and MUFA ratios of chicken meat associated with movement space and increased total PUFA, n-3 and n-6 fatty acids in *ad libitum-fed* groups. These fatty acids structures were inversely impressed under lower stocking density with limited *pair-fed* groups associated with over muscle load.

The findings of the present study clearly suggest that although cage housing system has some economic benefits, however, fatty acid structure of tissue was deteriorated in caged chicken as a result of activation of saturated fatty acid synthesis, especially in muscle and fat tissue.

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