

## Effect of feeding of 10 % prefermented feed on fatty acid profile and oxidation changes in chicken breast meat

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### Abstract

The aim of this work was to evaluate the effect of addition of 10 % fermented feed (FF) into commercial broiler feed on fatty acid profile, chemical composition and oxidative stability of chicken breast meat fat. FF was prepared by the process of fungal solid-state fermentation. Application of FF into the commercial feed mixtures resulted in increased content of gamma-linolenic and oleic acid in broiler breast meat ( $P < 0.05$ ). Oxidative stability of meat fat was significantly higher in experimental broilers group fed diet with the addition of 10% FF ( $P < 0.05$ ) after storage period of samples for seven days at temperature of 4 °C.

Key words: *broiler, meat, fatty acid, fat, fermented feed*

### Introduction

In recent years, there has been an increased interest in methods of manipulating the fatty acid (FA) composition in the meat fat produced by animals. Application of feeds with a high proportion of PUFAs (vegetable oils, fish oil and fish flour) in production animals diet could have impact on the final profile of FA in produced meat, because some FA are being incorporated in the fat tissue through the nutrition. Thus indirectly it is possible to modify the composition of food fatty acids (meat, eggs) and produce functional foods (Amano et al., 1992; Brandelli et al., 2015). Microbial oils produced by different species of biologically active microorganisms are an easily available alternative to fish oils. However, production of microorganisms in the process of classical submerged fermentations requires some conditions which are particularly disadvantageous from an economic point of view. From the feed-processing point of view, therefore, the production of PUFAs are based on the process of semi-dry cultures of lower fibrous fungi. The attractiveness of such cultivations lies in the use of readily available substrates on the basis of by-products from agricultural and food production. In addition to PUFAs production, gamma-linolenic acid (GLA), dihomagamma-linolenic acid (DGLA), arachidonic acid (ARA), lower fimbriae (such as *Cunninghamella*, *Mortierella*, *Thamnidium*) present by their fermentation activity which eliminates the activity of antinutrients (Čertík, 2013). The final product of solid-state fermentation with lower filamentous fungi could be a feed with increased content of PUFAs, which could be beneficial in animal production. These fatty acids can be more easily degradable in the oxidation process due to the increased number of multiple bonds, it is necessary to adapt this material to the requirement of higher stability. In our previous published studies, we confirmed that some types of lower fibrous fungi are able to produce both PUFAs and carotenoid pigments at the same time (Klempová et al., 2013). In the present study, the lower filamentous fungi strain *Umbelopsis isabellina* CCF2412 was chosen as a producer of PUFAs and pigments for preparation of fermented feed (FF). This strain showed high levels of desired products (GLA and beta-carotene) even in solid-state

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fermentation (SSF) conditions. The aim of this work was to investigate the effect of feeding of broiler chickens with the addition of 10% fermented feed to commercial feed mixtures on chemical composition and fatty acid profile of breast meat samples and lipid oxidation stability of meat fat during storage period.

### Material and methods

The experiment included 80 pcs of sexed ROSS 308 one day-old broiler chickens divided into two groups of 40 pcs. The control group (C) was fed with commercial feed mixtures Br1, Br2 and Br3 (DeHeus, Czech Republic). The fattening period of broilers lasted 38 days. In the experimental group, FF was administered to commercial feed mixtures from 10th day of fattening. The amount of 10% commercial feed mixture was replaced by 10 % of FF in experimental broilers diet. The added amount of FF was a reduced dose of a commercial feed mixture. During the fattening (38 days) the chickens had access to feed and water *ad libitum*. On the 39th day, the broilers were stunned, bled and slaughtered. Subsequently, samples of breast meat were collected. Samples were packaged in polyethylene containers and stored in a refrigerator at temperature of 4°C for seven days. Basic chemical analysis of meat samples was performed according to Popelka et al. (2008). Determination of fatty acids was carried out according to Čertík et al. (2008), where the following parameter were set in the analyses: DB-23 column (50% cyanopropyl methylpolysiloxane, length 60 m, diameter 0.25 mm, film thickness 0.25 µm, was automatically injected with 1 µl sample of fatty acid methyl esters, which were analyzed under the following conditions: carrier gas - hydrogen (44 cm / s at 130 ° C), injection temperature - 220 ° C, split - 1:50, FID detector (250 ° C, hydrogen flow: 30 ml / min, oxygen flow: 500 ml / min), temperature regime : 130 ° C - 1 min, 130-170 ° C - 6.5 ° C / min, 170-215 ° C - 2.7 ° C / min, 215 ° C - 7 min, 220-240 ° C - 20 ° C / min, 240 ° C - 2 min). Records were evaluated using ChemStation B0103 (Agilent Technologies, USA) and quantified based on retention times of known C4-C24 fatty acid standards (Sigma, USA). The oxidative stability of fat in broilers breast meat was determined by thiobarbituric (TBA) number method according to Marcinčák et al. (2004). Statistical processing of the results was performed using GraphPad Prism software version 8.3 (GraphPad Software, San Diego, California, USA). The results were expressed as arithmetic mean (x) and standard deviation (sd). The individual results between the groups were compared with each other and statistically evaluated by Student's T-test. P <0.05 was considered a statistically significant difference.

### Results and discussion

In the FF as well as in the mixture of 10% FF and commercial feed mixtures, all the required bioactive substances were detected (GLA). GLA was also present in other commercial mixtures (BR2, BR3) with the addition of a FF to ensure their intake throughout the duration of the experiment. Table 1 shows the chemical composition of the breast meat. The statistical significant differences were observed in increased dry matter, fat and total protein content of meat samples of experimental group, when compared to control (P < 0.05).

Table 1 The chemical composition of breast muscle samples

	Dry matter (%)	Fat (%)	Total proteins (%)
Control group	24.78 ± 0.15 <sup>a</sup>	3.40 ± 0.20 <sup>a</sup>	22.02 ± 0.40 <sup>a</sup>
Experimental group	26.05 ± 0.51 <sup>b</sup>	4.04 ± 0.76 <sup>b</sup>	23.00 ± 0.42 <sup>b</sup>

<sup>a, b</sup> – statistically significant difference between means (P < 0.05)

Within the experiment, meat analyzes focused on fatty acid content were performed. The results of the breast muscle analyzes are shown in Table 2. A significant increase in gamma-linolenic (GLA) and oleic acid (OA) (P < 0.05) acid was recorded in the fat of the breast tissue. On the other hand, the ARA and EPA decreased significantly (P < 0.05) in the experimental group. The n-6 / n-3 ratio was not affected by the fermented feed. The present study firstly demonstrated the use of *Umbelopsis Isabellina* strain, which was used in the process of solid state fermentation to prepare fermented feed, which was applied in broilers nutrition. Because of that, the comparison with previous published studies was complicated. It is therefore difficult to compare our results with other studies. In similar study, Bača

Table 2 Fatty acid profile of breast meat

Fatty acids (%)	Breast	
	C	E
C16:0, palmitic acid	22.32 ± 0.19	22.58 ± 0.34
C18:0, stearic acid	10.16 ± 0.12	9.77 ± 0.15
C18:1-9c, oleic acid	33.55 ± 0.32 <sup>b</sup>	35.76 ± 0.42 <sup>a</sup>
C18:1-11c, vaccenic acid	3.89 ± 0.58	3.11 ± 0.04
C18:2, linolenic acid	17.03 ± 0.19 <sup>b</sup>	18.32 ± 0.15 <sup>a</sup>
C18:3, gamma-linolenic acid	0.13 ± 0.01 <sup>b</sup>	0.26 ± 0.01 <sup>a</sup>
C18:3, alfa-linolenic acid	0.97 ± 0.19	1.12 ± 0.03
C20:3, dihomogamma-linolenic acid	0.89 ± 0.29	0.58 ± 0.13
C20:4, arachidonic acid	2.59 ± 0.05 <sup>b</sup>	1.87 ± 0.59 <sup>a</sup>
C20:5, eicosapentaenoic acid	0.35 ± 0.14 <sup>b</sup>	0.20 ± 0.01 <sup>a</sup>
C22:5, docosapentaenoic acid	0.02 ± 0.01	0.02 ± 0.01
C22:6, docosahexaenoic acid	0.37 ± 0.02	0.34 ± 0.01
Σ SFA	33.50 ± 0.15	33.02 ± 0.51
Σ PUFA	66.50 ± 1.08	66.98 ± 0.22
Σ PUFA n-3	1.81 ± 0.08	1.76 ± 0.18
Σ PUFA n-6	20.63 ± 2.32	20.94 ± 0.65
n-6/n-3 ratio	11.40 ± 0.55	11.87 ± 0.07

C – control group, E – experimental group; Σ SFA – saturated fatty acids, Σ PUFA – polyunsaturated fatty acid, <sup>a,b</sup> – statistically significant difference between means ( $P < 0.05$ ).

et al. (2014) prepared fermented feed by the use of lower filamentous fungi *Thamnidium elegans* and in 3 % concentration applied in broilers nutrition observed reduction of GLA and other PUFAs (ALA, DPA, DHA).

In recent studies, the most of authors used linseed oil or olive oil to modify the FA profile of fat in produced meat (Lopez-Ferrer, et al., 2001b; Pietras and Orczewska-Dudek, 2013; Akinola et al., 2015). They observed similar ratio between n-3 and n-6 PUFA in samples, which resulted in increased fatty acid content in muscles (except the study where linseed oil was used). ALA, which was the major fatty acid in feed, was the largest. Feeding of 10 % of the organic product in this study showed an increase in fatty acids corresponding to the composition of the feed mixture with the fermented feed. This means that feeding the fermented cereal bioproduct increases gamma-linolenic acid and oleic acid. Although GLA belongs to the n-6 group, they have a significant anti-inflammatory role in the human body. Feeding of diets rich in PUFA in broilers could cause their deposit in the carcass. However, the high content of these fatty acids in the modified meat (mainly stored in the phospholipid cell membrane) affects lipid oxidation, and consequently affects color, taste, texture, nutritional value, and ultimately, deteriorating oxidative stability of the meat during refrigeration storage. These findings of adverse effects on the oxidative stability of meat have been confirmed by several authors, including Cortinas et al., (2005). Kouba and Mourot (2011) reported that the use of vegetable oils as a rich source of

Table 3 Lipid oxidation of the fat after storage (7 days, 4 °C) expressed as the amount of malondialdehyde (MDA) content

MDA (mg.kg-1)	1. day	5. day	7. day
Control group	0.140 ± 0.039	0.166 ± 0.017	0.645 ± 0.207 <sup>a</sup>
Experimental group	0.172 ± 0.019	0.206 ± 0.027	0.399 ± 0.147 <sup>b</sup>

<sup>a,b</sup> – statistically significant difference between means ( $P < 0.05$ )

ALA in feed significantly alters the composition of fatty acids of storage and intermuscular fat, thereby increasing the linoleic acid and  $\alpha$ -linolenic acid content and decreasing the monounsaturated fatty acids content in both types of fat. Ayerza et al. (2002) reported that the application of 10 or 20% Chia seeds as a source of ALA in broiler diets, results in an increase in LA and ALA in the muscle while reducing the monounsaturated fatty acids and non-saturated fatty acids.

It is assumed that the oxidative stability of broiler meat will decrease with the application of fermented feed with higher content of PUFAs in broilers nutrition. Many studies connected oxidative instability in meat and meat products with increasing concentration of PUFAs (Guillevic et al., 2009; Betti et al., 2009). Unsaturated fats are easily subjected to oxidation. Peroxides and aldehydes are product which are responsible for decreasing quality of meat during their storage (Betti et al., 2009). Although the TBA values in the experimental group fed diet with FF were not significantly higher, when compared to the control. On the 7th day of storage, these values were significantly lower in the experimental group ( $P < 0.05$ ). The sensitivity of poultry meat to lipid oxidation may be affected by the presence of antioxidants. Therefore, antioxidants in feed are used to improve the oxidative stability of meat lipids and to extend meat storage (Králík et al., 2013). In our case, beta-carotene produced by *Umbelopsis Isabellina* was a source of antioxidant.

### Conclusion

Fermented feed obtained from milling by-product at a dose of 10%, positively affected the composition of the fatty acid profile in chicken breasts. The proportion of gamma-linolenic acid and oleic acid increased markedly and the n-6 / n-3 PUFAs ratio in the fat of the breast muscle was not affected. FF also positively affected lipid oxidation process in meat samples after storage at temperature of 4 °C for seven days.

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