EFFECT OF TRITICALE AND NON-STARCH POLYSACCHARIDES (NSP) DEGRADING ENZYMES ON COLOUR AND SENSORY CHARACTERISTICS OF BROILER MEAT

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Abstract. The trial was conducted to investigate the effect of triticale and non-starch polysaccharides (NSP) on the degrading enzymes supplementation to broiler chickens' performance, meat colour and sensory parameters. First of all, in the study, it was determined the amount of phenolic acids in the winter wheat variety *Zentos* and winter triticale variety *SU Agendus*, which were added to the broiler chickens' control and experimental diets. During a 5-week feeding experiment, 600 one-day-old Ross 308 broiler chickens were fed *ad libitum* with a crumbled wheat-soybean meal based diet (C group) supplemented with 15% triticale (T group) and 15% triticale with NSP degrading enzymes (E group; NSP degrading enzymes activities - endo-1.4-β-xylanase 11000 VU/ml and endo-1.4-β-glucanase (cellulase) 3200 DNS units/ml of feed). The sensory evaluation was performed according to a standardized sensory descriptive method. The analysis of phenolic acids in wheat and triticale, indicated that, out of the 5 phenolic acids the major part was made up of ferulic acid, which amounted to 535.71 µg/g DM in the wheat and with triticale – 601.04 µg/g DM. The other major phenolic acid was sinapic. Phenolic acids such as *p*-hydroxybenzoic and vanillic were found in minor quantities. The results of the feeding experiment with broiler chickens indicated that the addition of 15% triticale in combination with NSP degrading enzymes, have no effect on the broiler chickens' performance parameters. The usage of triticale with or without enzymes changed colour profiles of raw and thermally treated breast meat, but didn't have a significant effect on the thigh meat colour characteristics. E group raw and thermally treated breast meat had a higher value of lightness (L*) than the control group (53.89 vs. 48.10 and 83.15 vs. 81.78) ($P<0.05$). Raw and boiled breast meat from T and E groups showed higher values of redness (a*) and yellowness (b*) ($P<0.05$) in comparison to samples from the control group. Sensory evaluation of breast meat samples revealed that the tenderness in T group and colour intensity in E group had a tendency to decrease by 1.87 and increase – 1.25 respectively ($P<0.05$) compared to the control group. No other differences were observed between the control and analysed diets. Therefore, more additional study is required for better investigation of the effect of triticale and non-starch polysaccharides (NSP) degrading enzymes supplementation on broiler chickens' meat colour and sensory parameters.

Keywords: broiler chicken, triticale, enzyme, meat, colour, sensory analysis

Introduction. In recent years triticale (*genus X Triticosecale*) has been used as an interesting energy ingredient for poultry diet due to its similar nutritional composition to corn or wheat (Leeson and Summers, 2005; Barneveld and Cooper, 2002). However, the use of new varieties of triticale in the broiler diet has been associated with controversial results. Several studies suggested that triticale may be incorporated in broiler diets with no major effect to the nutritive value of the diet or bird performance (Çiftci et al., 2003; Pourreza et al., 2007; Zarghi and Golian, 2009), while other findings (Korver et al., 2004; Santos et al., 2008) have shown no effect on the quality of poultry meat. The inclusion of NSP degrading enzymes can increase the digestibility of nutrients and reducing the amount of excreta in broilers (Başer and Ramazan, 2014). Although numerous research articles and reviews have been published on various aspects of enzyme use in the poultry industry (Bedford, 1996; Choct, 2006; Kamyab and Houshmand, 2004; Aksu 2007; Brzoska and Stecka, 2007; Mendes et al., 2013), it is still unclear how these enzymes could remain effective in improving feed utilisation or how they could affect the quality of poultry meat.

In recent years, poultry meat consumption has increased significantly. Chicken meat availability and consumption has increased mainly as a result of its low price, easy processing, diversity, and high nutritive value – it has a high-value protein content and relatively low fat, saturated fatty acids and cholesterol content (Haščík et al., 2012; Starčević et al., 2015). It is also tender and fine-fibred. The nutritional properties of poultry meat are highly valued (Starčević et al., 2015).

Moreover the development of the food industry leads to the search for new raw materials with improved nutritional properties. According to Yang et al., (2010), the large content of phenolic compounds in wheat grain contains from simple molecules such as phenolic acids to highly polymerised compounds such as tannins and

proanthocyanidins. Positive effects of phenolic compounds used in small doses in animal diet were observed as health promoters rather than inhibitors (Starčević et al., 2015). According Piironen et al., 2009 investigations, the levels of phenolic compounds in rye grain were higher than in many other common grains. The total level of phenolic acids in oats, corn and rice is less than half of the amount in rye or wheat. Phenolic acids have been associated with colour and other sensory characteristics (Robbins 2003). Heinio et al., 2008, believed that the high level of phenolic compounds is one of the reasons for the bitter taste of rye.

However, available literature data on the use of triticale with the appropriate composition of phenolic acids and its combination with NSP degrading enzymes in broiler chicken diets and their impact on meat sensory parameters is still rare. So, this study was carried out with the aim: 1) to determine phenolic acids composition of wheat and triticale varieties used in the experiment with broiler chickens and 2) to evaluate the effect of triticale and non-starch polysaccharides (NSP) degrading enzymes supplementation on broiler chickens' performance, meat colour and sensory parameters.

Materials and Methods

Determination of phenolic acids in wheat and triticale. One gram of grain samples (winter wheat variety *Zentos* and winter triticale variety *SU Agendus*) was weighed into a 100-mL volumetric flask and 25 mL of 0.1 M NaOH added. The slurry was shaken at 40 °C for one hour, cooled to 20 °C, acidified with 2 M HCl to pH 5–6 (indicator paper) and 20 mL of methanol was added. The flask was placed in an ultrasonic bath for 30 minutes, cooled to 20 °C and made up to volume with methanol. The filtrate (0.2 mm membrane filter) was analysed by HPLC (Varian Corporation, USA). Phenolic acid derivatives were separated on Phenomenex Gemini C18, 5 µm, 250 × 4.6 mm (Phenomenex, USA) chromatography column at temperature 30 °C. The mobile phase consisted of H_2O; ACN; acetic acid (88:10:2) 10 µL of derivatives were injected to separation. Separated derivatives were detected at Ex 260 nm – Em 320 nm (Kvasnička et al., 2008).

The design of experiment with broiler chickens. An experiment was carried out with 600 *Ross 308* broiler chickens allotted to 3 groups with 4 replications over a 35-day period. It was a continuation of the previous study with broiler chickens (Alijošius et al., 2016), fed *ad libitum* with a crumbled wheat (variety *Zentos*) – soybean meal compound diet (C group), in which 15% of wheat was replaced by triticale variety *SU Agendus* (T group) and in the diet of E group wheat variety *Zentos* was replaced by 15% of the same triticale variety as used in the previous group and added NSP degrading enzymes (dosage endo-1.4-β-xylanase 11000 VU/ml and endo-1.4 -β-glucanase (cellulase) 3200 DNS units/ml of feed). NSP degrading enzymes mixture (containing mainly endo-1.4-β-xylanase and endo-1.3/1.4-β-glucanase) used in this study was provided by Adisseo (France) and produced from *Penicillium funiculosum*. The dietary treatments consisted of basal diet (C group);

In our previous study (Alijošius et al., 2016) the composition and calculated values of the basal diet were presented. Also the chemical and amino acids composition of triticale and wheat grain, used in the diets were given. The diets were formulated to suit the nutrient and energy requirement for broiler chickens (NRC, 1994).

During the experiment, the body weight (BW), and feed conversion ratio (FCR) were measured. At the end of the trial (35 days) 5 broiler chickens from each group (5 birds × 3 groups of birds = total of 15 birds) were selected and slaughtered according to the recommendation for euthanasia of experimental animals (Close et al., 1997).

Determination of colour and sensory characteristics of broiler meat. Instrumental colour measurements were performed using a spectrophotometer, Konica Minolta, calibrated throughout the study using the reference illuminate C that is close to average daylight. The measurements were averaged and colour parameters for each sample was expressed in terms of CIE L*a*b* values for lightness (L*), redness (a*) and yellowness (b*). $h^*=\arctan(b^*/a^*)$; $C^*=((a^*)^2+(b^*)^2)^{1/2}$ (ISO 11037:2011).

Sensory analysis was done by the Food Institute of Kaunas University of Technology, Sensory analysis laboratory. A sensory panel for the descriptive analysis consisted of 9 assessors experienced in sensory evaluation of different food products. The assessors were selected and trained according to the ISO 8586:2012. The sensory attributes of the boiled chicken meat (breast and thigh) were analysed. A structured numerical scale was used for evaluation of the intensity of each attribute. The left side of scale corresponding to the lowest intensity of attribute was given value of 1, and the right side corresponding to the highest intensity was given value of 15. All sessions were conducted in a climate-controlled sensory analysis laboratory equipped with individual booths according to ISO 8589:2007. A data collection system for automatic data acquisition of the assessors scores and data analysis was used (FIZZ, Biosystems, France). The samples were placed into the special bags for cooking and then added to boiled water bath and stayed boiling for 30 min (breast) or 25 min (thigh) after the water had started boiling, what correspond to enough time to reach internal temperature of 85 °C. The samples were quartered lengthwise and served immediately to panellists along with room temperature water, tea and white bread for neutralisation of receptors. The assessors were instructed to clean the palate with water or tea between the evaluations of each sample. The following characteristics were assessed: intensity of overall odour, boiled chicken odour, non-typical odour, intensity of colour, hardness, chewiness, juiciness, fibrousness, mouthfeel, boiled chicken taste, non-typical taste and aftertaste.

A preliminary acceptability (n=9, consumers aged 22–65) was evaluated by asking which sensory properties could negatively affect the acceptability. The samples for consumers' panel were prepared in similar way as to the sensory analysis. A structured numerical scale was used for the evaluation of the acceptability of odour, taste, texture and overall impression. The left side of scale

corresponding to the "dislike extremely", and the right side corresponding to the "like extremely" ISO 4121:2003. All the sessions were conducted in a climate-controlled sensory analysis laboratory equipped with individual booths.

Statistical analysis. Statistical significance was established using one-way analysis of variance ANOVA (statistical package SPSS 22), and data were reported as a mean of standard deviation. Mean comparison and separation were done using Duncan's *t*-test (P<0.05).

Results and Discussion

The present study showed the differences in a composition of phenolic acids of analysed wheat variety *Zentos* and triticale variety *SU Agendus* (Table 1).

Table 1. **Phenolic acids content in wheat and triticale varieties (µg/g, dry matter (DM))**

	p-hydroxybenzoic	vanillic	*p*-coumaric	ferulic	sinapic
Wheat variety *Zentos*	3.27 [a]	5.74[a]	9.42[a]	535.71[a]	55.03[a]
Triticale variety *SU Agendus*	1.74[b]	3.91[b]	11.52[b]	601.04[b]	87.59[b]
[a, b] - Means in the same column with different letters are significantly different, P<0.05					

The mean amount of *p*-hydroxybenzoic and vanilic acids in the analysed triticale variety was lower, but *p*-coumaric, ferulic and sinapic acids was higher than in the wheat variety (P<0.05). This resulted in a higher total amount of phenolic acids in triticale variety *versus* wheat variety (705.80 *vs.* 609.17 µg/g DM). The results of conducted analysis were in agreement with the results of other researches (Jonnala et al., 2010; Kandil et al., 2012), who declared that more than 90% of the phenolic acids were in the form of ferulic acid but in their study the amount of total phenolic acids in all the triticale lines is higher (845-1501 µg/g bran) than we have determined. The other major phenolic found was p-coumaric acid. Phenolics such as p-hydroxybenzoic and vanillic acids were found in minor quantities. Similar individual phenolic acid compositions were reported for analysed triticale cultivar (Ultima) by Hosseinian and Mazza (2009).

Table 2 shows the data of body weight and feed conversion ratio (FCR) during the overall (1–35 days) experimental period. No significant differences (P>0.05) were observed in body weight and FCR among the three groups.

Inclusion of 15% triticale and its combination with (NSP) degrading enzymes into broiler diets, inclusion has no effect on body weight compared to the control group. Chickens fed with compound feed with inclusion of triticale had no difference to body weight from the values obtained in feeding broilers with wheat (Korver et al., 2004).

Table 2. **The effect of triticale and non-starch polysaccharides (NSP) degrading enzymes supplementation on broiler chickens' performance parameters**

Broiler chickens age in days	***Groups***		
	C	**T**	**E**
	Body weight (g)		
35	2658.79±286.34	2647.63±234.53	2641.02±231.86
	Feed conversion ratio (kg/kg)		
1-35	1.51±0.03	1.55±0.03	1.51±0.06

However, the data obtained by Moharrery et al. (2015) indicates that triticale has great potential as feed for chickens: the group which fed the diet with triticale had the higher body weight. Józefiak et al. (2007) and Santos et al. (2008) also reported higher body weights of chickens which were fed with triticale than diets with only triticale, rye or wheat. Also Narasimha et al., (2015) and Pinheiro et al. (2004) didn't find any difference between the performance parameters of chickens fed diets with NSP enzymes.

Instrumental colour analysis of raw breast meat samples (Tables 3) has shown that the lightness (L*), redness (a*) and yellowness (b*) values were higher in T and E groups (*P*<0.05) compared to the control group. The same differences in colour characteristics L*, a*, b* were determined after the thermal treatment. However, boiled meat samples of control group had higher C value. Meat colour is affected by the level of pigments in bird diet and organism properties. It has a direct effect on consumers' perception as quality and safety indicator. Our study revealed that usage of triticale with or without enzymes changed the colour profiles of both raw and thermally treated meat. It could be related to the higher amount of phenolic acids in the triticale variety.

After evaluating the samples of raw and boiled meat of the thigh (Table 4), established that the composition of feed didn't have a significant effect on this type of meat colour characteristics. The cooked samples did not differ from each other by the instrumentally determined colour characteristics, but it should be noted that the distribution of the values is quite broad, which may most likely be explained by the fact that the samples were small and rather heterogeneous (small thighs).

Table 3. **Mean CIEL*a*b* values of raw meat of the chicken breast**

Parameters	*Groups*		
	C	**T**	**E**
	Raw meat		
L*	48.10±1.44[a]	51.33±1.67[b]	53.89±1.89[b]
a*	1.19±0.07[a]	2.78±1.20[b]	1.95±0.18[b]
b*	1.10±0.48[a]	2.50±0.08[b]	2.86±0.44[b]
h	0.72±0.18	0.76±0.20	0.97±0.09
C	1.64±0.38[a]	3.79±0.87[b]	3.47±0.34[b]
	Boiled meat		
L*	81.78±0.23[a]	84.76±0.69[b]	83.15±0.55[b]
a*	1.74±0.04[b]	3.94±0.50[a]	3.61±0.44[a]
b*	12.10±0.06	11.22±0.19	11.44±0.54
h	1.27±0.00	1.32±0.04	1.35±0.03
C	12.66±0.07[b]	11.60±0.26[a]	11.73±0.62[a]
[a, b] Mean values for each meat type in each row with different subscripts differ significantly, P<0.05			

Table 4. Mean CIEL*a*b* values of raw meat of the chicken thigh

Parameters	*Groups*		
	C	**T**	**E**
	Raw meat		
L*	52.74±3.41	50.21±5.57	53.76±1.93
a*	11.35±2.81	12.48±2.75	13.81±1.29
b*	9.93±2.87	7.89±4.07	11.76±1.37
h	0.72±0.14	0.54±0.14	0.73±0.07
C	15.17±3.46	14.85±4.40	17.70±0.63
	Boiled meat		
L*	71.19±3.84	71.28±6.37	75.54±1.77
a*	6.87±0.99	6.17±0.87	5.38±0.45
b*	12.94±0.35	12.08±0.22	12.56±0.23
h	1.08±0.06	1.10±0.07	1.17±0.03
C	14.68±0.46	13.57±0.16	13.67±0.35

Table 5. Mean sensory characteristics scores (n=5×3 replicates) for the chicken meat samples (scale from 1 to 15)

Parameters	**Breast**			**Thigh**		
	Groups					
	C	**T**	**E**	**C**	**T**	**E**
Overall odour intensity	13.63	13.50	13.00	13.13	13.13	12.88
Boiled chicken odour	12.75	12.88	12.38	12.38	12.13	12.13
Non typical odour	1.25	1.25	1.38	1.38	1.63	1.50
Colour intensity	5.25[ab]	3.88[a]	6.5[b]	9.75	8.25	7.63
Tenderness	8.25[b]	6.38[a]	7.25[ab]	8.75	8.50	7.38
Fibrousness	10.13	9.75	10.25	7.25	7.63	7.88
Juiciness	5.63	6.00	5.25	8.13	7.75	7.88
Chewiness	10.25[b]	9.75[ab]	8.88[a]	9.50	9.50	9.13
Crunchiness	8.25	8.75	9.38	7.50	7.75	7.25
Mouthfeel	4.38	4.25	3.75	7.63	7.63	6.88
Overall taste intensity	13.25	13.00	13.00	12.88	12.63	12.75
Boiled chicken taste	12.50	12.50	12.38	11.88	11.75	11.88
Non typical taste	1.13	1.25	1.25	1.75	1.75	1.38
Residual taste	7.50	7.38	7.75	7.38	7.25	7.38
Odour acceptability	13.88	13.88	13.63	13.75	13.50	13.63
Taste acceptability	13.75	13.75	13.50	13.25	13.25	13.50
Texture acceptability	12.38	13.88	12.88	13.50	13.00	13.75
General acceptability	13.38	13.88	13.50	13.75	13.38	13.50
[a, b,] Mean values for each meat type in each row with different subscripts differ significantly, P<0.05						

The mean sensory values for tested properties are presented in the Table 5. Sensory evaluation of breast meat samples revealed that effect of feed composition to odour properties was insignificant (P>0.05). All samples were characterised by intensive odour typical for cooked chicken meat. Usage of triticale and enzymatic combinations, no side odour was detected, however was get the higher colour intensity (P<0.05). Previous studies (Kliseviciute et al., 2014) revealed that supplementation of broiler chickens feed by whole triticale (amount till 25%) had no significant effect to taste and odour properties of meat, however meat tenderness and fibrousness increased with increasing amount of triticale. In our study, sensory data are opposite, meat from control group was tenderer than meat from T group. The addition of enzyme resulted in meat tenderness similar to control sample (Table 5). Withal, modification of birds' diet with triticale has a significant effect to the quantitative composition of fatty acids (Osek et al., 2010) and this can also change textural properties of meat (Al-Hajo et al., 2016), especially breast meat samples. Meat tenderness is mostly affected by muscle fibre size, lipids content and composition, collagen content and solubility (Baeza, 2013). Although texture of samples differed, another explanation of this the variation could be the fact that during evaluation, assessors tested meat of different birds, or meat sample of the same bird, but from various parts of breast or thigh.

Impact of feed composition to taste characteristics of breast meat has not been detected. Preliminary assessment of acceptability revealed no negative changes in meat to appear, which could reduce meat acceptability, considering consumers' perception.

Conclusions

In our study, it was determined that the replacement of 15% wheat (variety *Zentos)* with triticale (variety *SU Agendus*) in the broiler chickens' diets had a significant effect on higher colour characteristics of raw breast meat samples. These findings could be explained with the higher level of total phenolic acids accumulated in triticale than in wheat. It also influenced the breast meat tenderness. No other differences were observed between the control and analysed broiler diets. However, the addition of 15% triticale and its combination with NSP degrading enzymes in broiler chickens' diets have no effect on final body weight and feed utilisation of broiler chickens in comparison to the control group. Therefore, more studies are required for the better investigation of the effect of triticale and non-starch polysaccharides (NSP) degrading enzymes on broiler chickens' meat colour and sensory parameters.

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Received 7 May 2018
Accepted 24 May 2018