Research Article

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The effect of silica-calcite sedimentary rock contained in the chicken broiler diet on the overall quality of chicken muscles

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Abstract: Opoka is a silica-calcite sedimentary rock chemically and structurally similar to diatomaceous earth (DE), composed mainly of silicon dioxide (SiO₂), calcium carbonate (CaCO₃), amorphous SiO. Opoka occurs predominantly in South Eastern Europe and Russia. Due to these specific properties investigation on the effect of opoka-enriched diet on chemical composition and overall quality of breast and leg muscles of broilers was initiated. Working samples showed a statistically significant increase in ash content or water content and a decrease in lipid content in the leg muscles of both male and female broilers ($P < 0.01$). Furthermore, the addition of opoka to the diet increased WHC of leg muscles in females and hardness or chewiness of these muscles in both genders ($P < 0.05$). The supplementation of broiler diet with opoka can be effectively applied to modify texture features of leg and breast muscle tissue which might, in turn, serve to regulate the nutritional and technological value of chicken meat.

Keywords: diatomaceous earth; opoka; feed additive; meat quality; broiler.

1 Introduction

The commercial quality of poultry meat is of major importance. It is usually consumed as cuts or as

processed products rather than as whole carcasses. Due to genetic improvement and progress in nutrition and poultry management, meat-type chickens may exhibit very high growth rates and feed efficiency required by the industry. The lines characterized by a quick increase in mass are however sensitive to environmental factors while inappropriate feeding results in a significant decrease in meat quality. A proper diet can serve to regulate the quality and usefulness of meat of monogastric animals. Investigations proved that in the case of poultry, the decrease in calorific value of diet mixture decreases the content of inter-muscular fat hence juiciness of broiler meat [1]. According to the results presented by Mieczkowska et al. [2], Bahman [3] and Saleh et al. [4] amount of fat introduced to the diet regulates the amount of fat in carcass. Generally, an increase in oils in the diet improves the chemical and sensory characteristics of broiler meat [5-8]. Addition of linseed oil in the range of 2-3% of diet mixture causes increase in PUFA n-3 content in lipid fraction of broiler meat which in turn causes desired increase in dietary value of the meat while maintaining good sensory attributes [9-10]. The content of glycogen in muscles of monogastric animals can be diet-regulated as well. According to Lauridsen et al. [11] the amount of glucose in pig muscles was reduced by the use of a high-fat diet applied from the moment animals reached 25 kg of live weight until slaughter. Similar results were obtained in a study with rats [12] and rabbits [13]. Vitamin E supplementation of farm animals' diet improved meat quality, e.g., color and oxidative stability [14] while the adverse effect on WHC value was observed by Rosenfold et al. [15]. Creatine monohydrate diet supplementation was shown to increase weight gain, probably by increased water retention in muscle tissues. Furthermore, myosin synthesis increases with creatine supplementation in chicken muscle cells [16]. Moreover, creatine monohydrate supplementation minimizes potential muscle protein denaturation and sustains water-holding properties

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of the meat during the conversion of muscles into the meat [17]. Mineral components play important role in the metabolism of skeletal muscles, and some elements e.g. Na, Ca, K, P and Mg play vital roles in enzymatic processes responsible for normal muscle function and the course and extent of postmortem changes in muscles [18]. According to Schaefer et al. [19] and D'Souza et al. [20] magnesium supplementation may reduce stress responses in animals and have favorable effects on meat quality. Na, K, Ca and Mg contribute to maintaining osmotic pressure and electrolyte balance in cells and tissues, thus playing a major role in regulating the level of meat hydration. P, present in meat in the form of phosphates, also contributes significantly to water holding capacity of meat [21]. Opoka is a silica-calcite sedimentary rock and occurs in South Eastern Europe and Russia. Sedimentary rocks are formed from the erosion of other rocks (igneous, metamorphic, or sedimentary rocks) and cementation, compaction, and re-crystallization into a new rock. Organic sedimentary rocks are formed from material originating from dead plants and animals. This usually occurs in swamp regions with an abundant supply of growing vegetation and low amounts of oxygen. Microscopic investigation showed that opoka is rich in silica of organic origin. Remains of Radiolaria, diatoms, and sponges are frequent. Structure and composition of opoka are quite similar to diatomaceous earth (diatomite) i.e. a siliceous sedimentary rock that consists of fossilized remains of diatoms, a type of hard-shelled algae (Figure 1). In contrast to crystalline limestone, opoka is of amorphous structure making it highly porous [22]. Opoka consists mainly of silicon dioxide (SiO_2) and calcium carbonate (CaCO_3). Other components are calcite, quartz, clay minerals and amorphous SiO . Aluminum oxide (Al_2O_3), iron(III) oxide (Fe_2O_3) as well as other oxides (K, Mg, Mn, Na, Ti, and P) occur at trace level. The main trace element in opoka is strontium (Sr), present in its non-radioactive form. Traces of barium (Ba), tungsten (W), vanadium (V), chromium (Cr), zinc (Zn), and zirconium (Zr) are also present [23]. Diatomite has been used in poultry feeding for a long time. Adeyemo [24] and Adebisi et al. [25] demonstrated the effect of fossil shells on the mineral content in tibia bone of cockerel and broiler, respectively. The addition of diatomaceous earth to broiler diet increases feed efficiency and egg production [26]. Mathis and McDougald [27] found that feeding with DE significantly improved feed conversion in broilers. It has been proved that opoka is completely safe as a feed additive. Unpublished data show that the addition of opoka does not change blood morphology or biochemistry, overall redox state as well as bones' quality of broilers.

Figure 1: Scanning electron microscopic images of remains of minute marine organisms in opoka.

The aim of the present study was to investigate the effect of feeding fast-growing broiler chickens with opoka-enriched feed on meat quality features of breast and leg muscles. To our knowledge, this issue has not been thoroughly investigated so far.

2 Materials and Methods

2.1 Opoka morphology

Diatomaceous earth - opoka used in current investigation originates from deposits of this raw material located near Kraśnik city in Lubelskie District, Poland. Rock material was clean from clay remaining and milled to micrometric particles in the ball mill (Retsch PM 100 CM).

Scanning electron microscopy (SEM) observations of the opoka were done using a Quanta 200 microscope (FEI, Hillsboro, OR, USA).

2.2 Animals and feed

42 one-d-old ROSS 308 broiler chickens (40.9 ± 1.5 g) were divided into 2 groups (control and experimental) each containing 12 randomly selected males and 12 females. The control and experimental groups consumed feed without and with opoka addition, respectively. Each of the two groups was further divided by gender into 2

Table 1: Components and chemical composition of the experimental feeds, %.

Commercial diet ¹	Control groups (CM and CF)		Experimental groups (EM and EF)	
	Starter	Grower	Starter	Grower
	%			
Wheat	10	10	10	10
Maize	48.7	49.7	47.9	48.7
Soy bean meal 46%	32.6	31	32.7	31.4
Soy bean oil	3.5	3.82	3.5	3.85
DL-methionine 98%	0.2	0.1	0.2	0.05
L- Lysine 99%	0.2	0.34	0.2	0.35
Threonine 98,5%	0.1	0.1	0.1	0.1
Chalk	1	1	0.7	0.7
Monocalcium phosphate	2.4	2,6	2.4	2.6
Sodium chloride	0.34	0.34	0.34	0.34
Premix ²	1	1	1	1
Opoka	0	0	1	1
Metabolisable energy MJ/kg ³	12.2	12.35	12.1	12.26
Crude protein g/kg ³	210.0	210.0	200.0	200.0
Calcium*	10.5	10.4	10.6	10.9
Phosphorus*	4.7	5.01	4.7	5.02

¹ NRC (1994)² In 1 kg of premix diets: vitamin A 15 000 IU; vitamin D 3000 IU; vitamin E 20 mg; vitamin K₃ 2.7 mg; vitamin B₁ 2.5 mg; vitamin B₆ 0.4 mg; vitamin B₁₂ 0.015 mg; nicotinic acid 25 mg; pantothenic acid 8 mg; folic acid 1.2 mg; Choline chloride 450 mg; DL-methionine 1.0 mg; Mn 74 mg; Fe 30 mg; Zn 45 mg; Cu 4 mg; Co 0.4 mg; I 0.3 mg³ Calculated* Experimental feed were corrected for Ca content from CaCO₃ contained in the rock (169.8 g Ca per 1 kg of rock) or Ca value corrected for opoka CaCO₃ content (169.8 g Ca per 1 kg of rock); Brogowski and Renman, (2004)

subgroups (Table 1) - each subgroup consisted of 12 birds. The chickens were reared in individual pens for 42 days under controlled conditions i.e. temperature range from 32°C to 21°C during the first 12 days (decreasing by 1°C per day) and then constant at 21°C; humidity range 64-70%, light/dark cycle 23/1h during the first 7 days, and then 18/6h for the rest of the experiment [28]. The animals had free access to water and feed. Diets, starter (from 1st to 14th day) and grower (from 15th to 42nd day) were formulated in compliance with [29]. The balance of calcium was done base on literature data that characterizes opoka [23]. The chickens in the control groups (male - CM and female - CF) were fed ad libitum with the standard diet. Animals in the experimental group, both EM and FM, were fed with the standard diet supplemented with 1% of silica-calcite sedimentary rock (opoka), delivered by Feeds and

Concentrates Production Plant in Kcynia (Poland). The feed components are presented in detail in Table 1.

2.3 Analyses

At the end of the experiment, birds were fasted for 24 h with free access to water and then slaughtered. Carcasses were weighed and then chilled at 4°C for 12 h. 24 h post-slaughter, samples of breast and leg muscles were analyzed for pH₂₄ with an Elmetron CP-411 pH-meter using the combined electrode [30]. Water holding capacity was determined according to the Grau and Hamm [31] filter paper method with modification by Pohja and Niinivaara [32]. A sample of meat placed on a filter paper (Whatman 1), was put between two glass plates and pressed with a

2 kg press. After 5 min, the surface occupied by meat and muscle juice was determined. Results were presented as the area size of the pressed muscle juice deduced by the surface occupied by meat and expressed as area size per 1 g of raw material (cm²/g). Chemical composition (water, protein, fat, and ash) using standard methods [33]; color of breast skin and both breast (*M. pectoralis superficialis*) and thigh (*M. biceps femoris*) meat of chilled carcasses were measured using a reflectance colorimeter (Chroma Meter CR, **b, Minolta, Japan). Values were recorded according to the CIE [34] L* a* b* system, where L*, a*, and b* represent lightness (or brightness), redness-greenness and yellowness-blueness, respectively; texture parameters - using a Zwick testing machine type 1120; collagen content - according to PN ISO 3496 [35].

2.4 Statistical analyses

The results obtained were statistically processed by two-way ANOVA and Duncan's multiple range tests using the Statgraphic 4.1 Plus software package (StatPoint, Inc., USA). The differences with $P < 0.05$ were considered significant.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and Discussion

The current study was aimed at evaluating the effect of 1% silica-calcite sedimentary rock addition to feed on chemical composition and texture parameters of breast and legs meat of chicken broilers.

3.1 Opoka characterization

Scanning Electron Microscope was used to observe opoka. Figure 1. shows the image of amorphous silica had round biofossils and the diameter of the exoskeletons ranged from 10 μm to 50 μm .

3.2 Chemical composition of meat due to gender

Many factors can influence poultry meat quality, including sex and strain [36-39]. Literature data show that female meat is of greater fragility as it contains less collagen. Breast muscles contain 0.68-0.80% and 0.6-0.65% of

collagen for males and females, respectively. Leg muscles contain 0.92-1.15% and 0.7-0.85% of collagen for males and females, respectively [40]. On the other hand, Northcutt et al. [39] reported no difference in breast fillet shear values between sexes. Mobini et al. [41] showed that both males and females have a similar number of collagen-rich fiber tendons in breast muscles (*Pectoralis superficialis*), while in thigh muscles (*Quadriceps femoris*) the amount of fiber tendon is greater in females. The current study confirms the differences between genders. Leg and breast muscles of males were richer in collagen in comparison with females, although differences were quite small (Table 2). Current studies indicated a greater percentage abundance of crude lipid in leg muscles of females compared with leg muscles of male birds in the control group. The same was reported by Sanz et al. [42], Haro [43] and Bogosavljevic-Boskovic et al. [44]. In terms of protein content, the results of the study did not show differences between genders, contrary to those reported by Bogosavljevic-Boskovic et al. [44] who reported higher protein content in leg muscles of male compared to female broilers. In the current study, the percentage abundance of water in the control group was smaller in female leg muscles compared to male leg muscles (Table 2). Bogosavljevic-Boskovic et al. [44] reported the same: higher values of the dry matter content of leg muscle in females compared to males. According to Tumova and Teimouri [45], the difference observed between genders was associated with metabolic differences, higher competitiveness among males, different fat deposition capacity, different nutritional requirements and a higher hormonal effect in female broilers.

3.3 Chemical composition of meat due to opoka addition to feed

The changes in the chemical composition of both male and female muscles due to opoka addition were similar in both genders. Small differences between genders were statistically insignificant. The current study shows that a 1% addition of opoka to feed can significantly change the chemical composition of muscles and, in turn, change the technological properties of meat. Our data show an increased abundance of collagen in breast muscles of animals fed with opoka addition, which is probably due to the presence of silicon in this material. It has been suggested that silicon, together with vitamin C and AKG, participates in hydroxyproline synthesis of the hydroxylation process of procollagen. However, a quantitative optimal dose of silicon has never been established or even suggested, either for animals or humans [46]. It is conceivable that

Table 2: Effect of gender and supplement of opoka on chemical composition of breast muscles of broilers (Two-way analysis of variance followed by Duncan's test).

Components (%)	Group				Anova				
	male		female		gender		opoka		g x o
	opoka	control	opoka	control	SEM	p-value	SEM	p-value	p-value
Water	76.24	75.82	74.73	75.51	0.284	0.055	0.284	0.748	0.173
Crude protein	20.73	20.93	21.53	20.73	0.357	0.568	0.357	0.568	0.351
Crude lipid	1.86	2.13	2.50	2.63	0.178	0.054	0.178	0.449	0.798
Crude ash	1.00	1.06	1.10	1.00	0.012	0.347	0.012	0.347	0.001
Collagen	0.63	0.53	0.53	0.43	0.023	0.017	0.23	0.017	1.000
Collagen w/s protein	2.93	2.56	2.46	2.1	0.096	0.009	0.096	0.027	1.000

g x o – interaction between gender and opoka

SEM – standard error of mean

the amount of silicon in a standard diet of intensively mass-increasing animals is too small. Content of collagen in breast muscles of broilers plays a significant role in terms of meat quality, both technological and nutritional. Collagen gives toughness to meat which is caused by the formation of a complete network by hydroxylic groups of lysine and proline [47]. On the other hand, the presence of an incomplete network of collagen, characteristic for young animals, increases the fragility of meat which is a feature strongly desired by consumers. Petracci et al. [48] showed that white-striped fillets contained low-quality protein due to the high content of collagen in muscles of non-standard chickens. In their studies, white-striped breast muscles contained 1.37% of collagen while the maximum level of this component ensuring proper quality is 1.30%. In the current study the level of collagen in breast muscles was around half of the Petracci et al. [48] results and was within the proper range (0.6-0.8%) given by other authors [40]. It suggests that the addition of opoka upregulates the level of collagen in muscles. The ratio of collagen to protein is a very important parameter in terms of meat quality. The higher the ratio the worse is the quality of meat Petracci et al. [48]. The ratio of collagen to total protein for both genders was significantly higher in breast meat (Table 2) obtained from the opoka-fed chickens in comparison with breast meat from the control group. In the case of leg muscles, females also showed an increase in this ratio while the beneficial effect of a decrease was observed in males (Table 3). Those observations point at a reduced nutritional and consumer value of proteins in breast muscles and female leg muscles. The explanation of this phenomenon may be lower digestibility of amino acids contained in proteins caused by the presence of opoka, which in turn may cause

deficiency in some essential amino acids (e.g. tryptophan, sulfur amino acids, and lysine) in connective tissues as compared to myofibrillar and sarcoplasmic proteins [49]. Suggested lower digestibility should be followed by the excretion of amino acids present in feed. However, the biochemical results of blood, especially the level of total proteins in plasma, carcasses, and muscles of animals fed with opoka did not differ from controls (unpublished data). If the hypothesized lowered digestion and excretion had occurred it would confirm the observations reported by Shurson et al. [50] who studied zeolites which, like opoka, represent a large group of minerals containing hydrated aluminosilicates characterized by ion-exchange and adsorption properties and large surface area facilitating adsorption. The authors showed that daily nitrogen levels in pig feces increased due to a reduced digestibility of nitrogen caused by the increased level of zeolite A or clinoptilolite in the feed. The next effect of opoka in feed demonstrated itself in the chemical composition of breast and leg muscles, e.i. the content of crude lipid water and ash for both genders (Table 2 and 3, respectively). A decrease in fat content resulting from the addition of opoka to feed is an unexpected and exciting phenomenon. Two different explanations are suggested here. First is the described below change in fat metabolism caused by the presence of microelements. The second explanation for this phenomenon is that opoka lowers fat available from the feed. This is supported by the potential ability of undissociated opoka molecules to bind non-polar molecules e.g. fat, followed by their excretion. Preliminary studies of lipid adsorption showed that natural minerals e.g. clinoptilolite (similar to opoka) are strong lipid sorbents, which would support the suggested above explanation [51]. It is, however, unclear why this

Table 3: Effect of gender and supplement of opoka on chemical composition of leg muscles of broilers (Two-way analysis of variance followed by Duncan's test).

Components (%)	Group				Anova				
	male		female		gender		opoka		g x o
	opoka	control	opoka	control	SEM	p-value	SEM	p-value	
Water	74.06	72.83	73.56	71.70	0.235	0.039	0.235	0.016	0.369
Crude protein	18.40	18.06	18.33	18.00	0.132	0.729	0.132	0.111	1.000
Crude lipid	6.36	8.03	6.96	9.16	0.228	0.027	0.228	0.000	0.433
Crude ash	1.00	0.90	1.00	0.93	0.012	0.346	0.012	0.001	0.347
collagen	1.37	1.35	1.17	1.08	0.049	0.009	0.049	0.473	0.621
Collagen w/s protein	7.47	7.56	6.36	5.98	0.282	0.009	0.282	0.731	0.572

g x o – interaction between gender and opoka

SEM – standard error of mean

effect of mineral feed additive shows better in leg muscles compared to breast muscles where the decrease in crude lipids is smaller (see Table 2 and 3). One valid suggestion could be that the factor determining alteration of the chemical composition of muscles after opoka addition is the different rate of metabolism occurring in different muscles.

Leg muscles bear more burdens compared to breast muscles, especially in caged animals. Chemical composition in different muscles depends on their affinity for chemical elements, for example, a higher amount of ash in leg muscles. The higher ash content in the leg muscles than the pectoral one is due to the higher content of iron, and therefore myoglobin and hemoglobin in these muscles. Leg muscles also have a higher level of zinc because dark muscles have more metabolic activity than light muscles. As a material rich in various elements, opoka can be a source of scarce elements and thus facilitate an increase in the rate of metabolism and therefore decrease in intermuscular fat. One such scarce element is chromium. According to Brogowski and Renman [23], there is about 30.1 mg of Cr per kg of opoka. Studies on production animals revealed a beneficial effect of supplementation of feed with chromium (3.85-7.70 mmol/day as chromium picolinate) on the quality and quantity of muscles, their accretion as well as a decrease in intramuscular fat [52]. These positive effects have generally been observed when animals were previously or concurrently exposed to stress, e.g. poor-quality dietary protein or transport. Chromium has biological functions, e.g. it potentiates the action of insulin and increases lipid and protein metabolism, however the exact mechanism of potentiating remains unknown. It is hypothesized that chromium is a potentially anabolic element that promotes

accretion of muscle mass and enhances strength gain during resistance training. The use of supplemental chromium in the form of chromium picolinate has also been reported to improve glucose utilization in cattle [53] and carcass protein deposition in pigs [54]. The dry matter of opoka contains 0.63% of MgO [23]. Literature data show a clear relation between magnesium deficiency in diet and plasma lipid disorders [55, 56]. It has also been shown that a fat-rich diet decreases magnesium absorption. Rayssiguier et al. [57] reported that in the case of magnesium-deficient rats, hypertriglyceridemia occurs, most probably as a consequence of increased synthesis of triglycerides in the liver, decreased removal of lipids from blood or a combination of both. Apple et al. [58] reported that intramuscular lipid content in the Longissimus muscle was reduced in lambs fed magnesium mica for an extended period of time. Magnesium present in opoka might also influence the chemical composition of muscles. As mentioned above, the reason for a decreased level of fat in the legs muscles of opoka feed animals may be related to lipid metabolism in the liver, the main organ in the distribution pathway of lipids and magnesium homeostasis. It may be suggested that opoka-fed broilers studied in this work metabolize lipids more effectively because of its trace elements e.g. chromium and magnesium, which in turn results in a significant decrease in fat in leg muscles (Table 3) and smaller but statistically significant decrease in fat in breast muscles (Table 2) of both genders.

Table 4: Effect of gender and supplement of opoka on texture parameter of breast muscles of broilers. (Two-way analysis of variance followed by Duncan's test).

Parametry	Group				Anova				
	male		female		gender		opoka		g x o
	opoka	control	opoka	control	SEM	p-value	SEM	p-value	p-value
pH	5.88	5.94	5.87	5.91	0.027	0.633	0.027	0.211	0.922
Cooking losses (%)	9.03	8.95	9.31	8.20	0.743	0.826	0.743	0.574	0.628
WHC (cm ² /g)	13.15	10.98	12.85	11.33	0.831	0.983	0.831	0.133	0.785
Adhesiveness	0.49	0.50	0.51	0.48	0.007	0.623	0.007	0.330	0.150
Springiness	0.64	0.63	0.61	0.64	0.009	0.375	0.009	0.316	0.264
Hardness (N)	113.40	102.14	93.47	105.59	2.854	0.916	2.854	0.054	0.009
Chewiness (N)	35.58	31.85	29.33	32.90	1.278	0.967	1.278	0.166	0.057
L*	56.87	55.46	57.21	53.25	0.613	0.292	0.613	0.006	0.158
a*	1.45	0.94	1.66	1.43	0.211	0.258	0.211	0.232	0.638
b*	13.65	13.42	13.19	13.19	0.431	0.573	0.431	0.855	0.860

g x o – interaction between gender and opoka

SEM – standard error of mean

L* - lightness; a* - redness-greenness and b* - yellowness-blueness

3.4 Technological quality of meat due to opoka addition to feed

Technological quality refers to several meat attributes, mainly the sensory i.e. color, tenderness and the physicochemical properties i.e. pH₂₄, cooking loss, water-holding capacity, adhesiveness, springiness, hardness, chewiness. Listed here parameters/markers are related to the chemical composition of meat discussed above. Increased level of water and ash and decreased level of fat in leg muscles of both genders of animals fed with opoka generally improved the technological quality of meat.

3.5 WHC (Water Holding Capacity)

The role of magnesium-rich opoka is also significant because of muscle parameters and resulting in meat features. The presence of magnesium in opoka at a significant level is probably the reason for the increased WHC factor determined in leg muscles of male and female broilers fed with opoka-supplemented diet (Table 4). According to literature data, magnesium-supplemented diet improved water-binding capacity of pig meat (58-60), similarly as in our results. According to Houston and Harper [61], magnesium regulates pH and intracellular levels of Ca, Na, K. Close relationship and mutual interaction between Mg and K ions due to the level of their concentration in

muscles is also well known [62, 63]. Both elements are vital for physiological functions: they activate over 300 enzymes, take part in ATP synthesis, participate in the glycolysis process, fatty acids degradation and citric acid cycle [64]. Magnesium cations in water solution bind to water molecules more strongly than calcium, potassium or sodium ions due to their higher ionic potential [65]. Thus, hydrated magnesium cations are difficult to dehydrate [66]. Increased level of WHC determined in leg muscles of both genders can, therefore, be explained by a high level of magnesium in opoka that travels from feed to the body via the digestive system. An increased amount of ash in leg muscle samples for both genders was also registered in this study. This result, again, is most probably due to the presence of magnesium compounds that hold water in the intracellular liquid. Therefore the total amount of water in leg muscles of the opoka-fed animals was higher than in the control group (Tables 1 and 2, for breast and leg muscles respectively) with exception of breast muscles in females. There were no differences in cooking loss percentage between broiler breast and leg meat for both, the opoka and control dietary treatment.

3.6 Hardness and chewiness

Mobini et al. [41] reports higher tenderness in male broiler chickens than in females. Although more fat and

Table 5: Effect of gender and supplement of opoka on texture parameter of leg muscles of broilers. (Two-way analysis of variance followed by Duncan's test).

Parametry	Group				Anova				
	male		female		gender		opoka		g x o
	opoka	control	opoka	control	SEM	p-value	SEM	p-value	p-value
pH	6.28	6.26	6.26	6.20	0.021	0.192	0.021	0.192	0.465
Cooking losses (%)	12.03	13.9	12.2	13.1	0.582	0.668	0.582	0.099	0.531
WHC (cm ² /g)	9.60	9.98	8.36	10.95	0.473	0.844	0.473	0.038	0.116
Adhesiveness	0.48	0.46	0.51	0.48	0.007	0.046	0.007	0.064	0.4234
Springiness	0.66	0.63	0.65	0.62	0.010	0.733	0.010	0.064	0.820
Hardness (N)	89.77	74.67	85.02	62.9	2.571	0.034	2.571	0.000	0.342
Chewiness (N)	28.54	21.81	28.18	18.88	1.124	0.313	1.124	0.000	0.428
L*	55.66	58.89	55.16	56.91	0.514	0.103	0.514	0.003	0.320
a*	3.78	3.86	3.65	4.38	0.175	0.123	0.175	0.022	0.616
b*	18.55	17.73	18.43	18.48	0.220	0.326	0.220	0.326	0.179

g x o – interaction between gender and opoka

SEM – standard error of mean

L* - lightness; a* - redness-greenness and b* - yellowness-blueness

less water have been reported in leg muscles of males than females, the increased hardness of male leg muscles was related to an increased percentage of collagen in animals fed with opoka. Opoka addition did not produce different results due to gender except hardness of breast muscles that was significantly increased in the case of males. The current study shows increased hardness and chewiness of leg meat of both males and females after the addition of opoka to feed. Better water – holding capacity followed by increased hardness and chewiness results in better compactness and firmness of meat - very desired parameters in the industry. The hardness of meat of increased chewiness depends on fat and water content, as well as on the muscle fiber structure [67]. Mallek et al. [68] reported that zeolite (chemically similar to opoka), added at 1% to the diet of chickens caused a significant increase in thigh meat hardness and chewiness. All these textural changes can be explained in terms of the influence of natural zeolite as well as opoka on the gelling process of proteins. Zeolite – muscle protein interaction leads to a change in texture and microstructure of the thigh meat. Apart from zeolite, the effects of other additives on the functional properties of meat products, e.g. carrageenan, have been a subject of numerous studies. The changes in textural parameters can also be explained by the change in water holding capacity. When zeolite is added, a reduction in the compactness of a protein gel network allows better

binding of water and makes the meat more tender [68]. It is suggested that the effect of better water-holding in leg muscles of opoka – fed animals is due to increased affinity of opoka components to proteins of leg muscles than those of breast muscles. In the case of adhesiveness and springiness, there was no difference between broiler breast and leg meat of the opoka and control groups.

3.7 Color

The color of broiler meat depends solely on concentration, form and chemical reactions of myoglobin [69]. Darker color related to the higher content of the oxidized form of myoglobin (higher content of Fe³⁺ cations) is less desired by consumers [70]. On the other hand, juicy, tender but not too pale meat is desirable [71]. A significant difference in a* (redness) between the opoka (3.78; 3.65) and the control groups (3.86; 4.38) was observed. Young et al. [72] showed that increased intensity of red color in leg muscles (4.7) was due to stress before slaughter. Red color intensity was much lower in the case of animals without stress (3.4). The decrease in a* parameter of leg muscles due to opoka ranges within the allowed limits and might indicate an unknown mechanism of decreasing effects caused by before-slaughter stress. The decreased intensity in red color was accompanied by the decreased lightness

of leg muscles. The effect of opoka addition to feeding is a lighter meat of breasts and legs. Several researchers have demonstrated a significant negative correlation between breast meat lightness and its pH_{24} [73] or pH [74, 75]. There was however no such correlation in our study. Van Laack et al. [76] reported that breasts considered to be regular had L^* values of 55 and those considered to be pale had L^* values of 60, and stated that high L^* values and low pH (<5.7) were indicative of broiler breast meat that was pale in color with low water-holding capacity. The L^* and pH_{24} values of breast muscles did not change with the addition of opoka. However, in the case of leg muscle lightness was close to the norm described by van Laack et al. [76]. No influence of opoka on meat pH_{24} was reported in the current study. Conversely, water holding capacity increased. The latter parameter is known to affect the color and tenderness of fillets [77]. No differences between a^* , or b^* (redness, and yellowness) of broiler breast meat from the opoka and control groups were reported.

4 Conclusions

The results of the current study suggest great potential of opoka used as an additive in order to modify the chemical composition of meat with better nutritional properties. Leg muscles of broilers fed with opoka contained less fat and more water and therefore were better for culinary purposes. Increased level of collagen is important as it plays a significant role in developing muscles as support and nutritional tissue. The correct growth of animals, especially in intense production, requires collagen. Increased level of collagen improves the technological quality of meat: better compactness and decreased tendency to break apart. Opoka influenced leg muscles more than it did breast muscles; it increased their WHC and color. This may be related to opoka acting to decrease before-slaughtering stress. Opoka's biological properties remain far from explained. The work started in the current study should be continued in order to work out the optimal percentage of additive which would eliminate or attenuate the effect of a decreased metabolism of feed components crucial for growth rate and effective production.

Author contributions. MM conceived and designed the study, performed the research, worked on the analysis and interpretation of results, and provided the final version of paper; TN interpreted data, discussed results and drafted the paper; AŁ participated in the design of the experiment and helped to draft the paper; DP carried out measurements and performed statistical analysis;

LA carried out measurements and performed statistical analysis; MC performed the research and analyzed the data; TF performed the research; PK analyzed data and critically revised the paper. All authors have read and approved the final version of the manuscript.

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