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PROTEIN QUALITY CHICKEN MEAT AFTER FEEDING WITH ACTIVE SUBSTANCES OF CITRUS FRUITS AND DICLAZURIL AND SALINOMYCIN SODIUM

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ABSTRACT
The purpose of this study was an experimental investigation of the influence of active substances obtained mainly from citrus fruits in the experimental feed mixtures, and diclazuril and salinomycin sodium in the control feed mixtures of broiler chickens on productive efficiency and protein quality of the breast and thigh muscles. *In vivo* experiment was carried out with hybrid chickens Cobb 500. Basic feed mixtures were equal a soy cereal type for experimental and control group. Indicators of productive efficiency were measured and calculated, and protein, lysine and methionine contents in the breast and thigh muscles were measured by the method of FT IR, Nicolet 6700. Active substances obtained mainly from citrus fruits confirmed a statistically significant (p <0.05) positive effect on the body weight gain; tended to slightly increase feed intake per bird, protein, energy, lysine and methionine intake per bird; slightly decrease feed intake per 1 kg of body weight gain, protein, energy, lysine and methionine intake per 1 kg of body weight gain; slightly increase protein efficiency ratio and energy efficiency ratio. Additive substances used in the feed mixtures did not have a statistically significant effect on protein, lysine and methionine contents in the breast and thigh muscles but displayed a strong positive, statistically significant relation between lysine and methionine in them.

Keywords: chicken breast; chicken thigh; protein; lysine; methionine; additive substance

INTRODUCTION
Citrus (*Citrus* L. from Rutaceae) is one of the most favorite fruit, which contains active phytochemicals that can help to protect health. Besides, it is good sources of vitamin C, folic acid, potassium and pectin **(Anagnostopoulou et al., 2006; Guimarães et al., 2009)** and it was reported that citrus fruits, citrus fruit extracts and citrus flavonoids exhibit a wide range of potential biological efficiency due to their phenolic profile and antioxidant properties **(Montanari et al., 1998)**. Citrus fruits are highly consumed worldwide as fresh produce, juice and the peel is discarded most often as waste which contains a wide variety of secondary ingredients with important antioxidant activity in comparison with other parts of the fruit **(Manthey and Grohmann, 2001)**.

A number of studies confirmed the presence of polyphenols, vitamins, minerals, dietary fibres, essential oils and carotenoids content in citrus fruit. Presence these substances predetermines a citrus fruit prefered product **(Rafiq et al., 2016)**.

Some valuable biologically active substances occur rarely in the citrus fruits. They are oxyprenylated natural products, for example 3,3-dimethylallyloxy-(C5), geranyloxy-(C10), and the farnesyloxy-(C15) related compounds that have been recognized in the last 12 years in citrus varieties **(Munakata et al., 2012)**. Citrus fruits were also seen to be a good source of many natural compounds: prenyloxycoumarins such as auraptene, bergamottin, imperatorin, heraclenin, oxypeucedanin and many others which have been isolated from the citrus juice and peel extracts **(Epifano and Genovese, 2013; Genovese et al., 2014)**. There is a growing acceptance that phenols, amino acids, essential oils, pectin, carotenoids, flavonoids, and vitamin C present in citrus fruits exert beneficial effects **(Wang et al., 2014)**.

The purpose of this study was investigation and a statistically evaluation of the influence of experimental active substances obtained mainly from citrus fruits compared to control coccidiostatats diclazuril and salinomycin sodium in feed mixtures of broiler chickens on their productive efficiency, protein and energy efficiency, protein content and content of selected aminoacids in the breast and thigh muscles.

Scientific hypothesis
Active substances obtained from citrus fruits has an effect on the body weight gain of broiler chickens.

MATERIALS AND METHODS

The object of investigation

The object of the investigation were feed additives, on the basis of active substances obtained mainly from citrus fruits comparised to control coccidiostatats, broiler chickens, productive efficiency of the feed mixtures, protein and energy efficiency and quality of breast and thigh muscles from aspect of the protein, lysine and methionine contents.

Procedure of carrying out an experiment with broiler chickens

In vivo experiment was conducted in practical conditions on poultry experimental station with deep litter breeding system. Experiment included 100 one-day-old broiler chickens of hybride combination Cobb 500 divided into two groups, experimental and control (n = 50).

All conditions were adhered to protection of broiler chickens in the experiment **Council Directive 2007/43/EC**. Spaced deep litter box allowed broiler chickens to perform natural activities. Microclimatic conditions were equal for experimental and control group in accordance with the producent recommendations for the final type broiler chickens Cobb 500 (temperature, relative humidity, air exchange and the light mode). The broiler chickens to the age of 14 days intake a feed from plate feeders and water from the hat drinkers located on the floor. Older chickens intake feed from the tube feeders and drank water from bucket drinkers till the end of the experiment.

Experimental period was divided into four phases during which fed different feed mixtures: starter phase, starter feed mixture, from day 1 to day 10; grower phase, grower feed mixture 1, from 11 to day 20; grower phase, grower feed mixture 2, from 21 to day 35 and final phase, final feed mixture, from 36 to day 42.

Basic feed mixtures were equaled for experimental and control group. Composition of feed mixtures is given in Table 1. Additive substances used in feed mixtures were different for each group. In experimental feed mixtures was used commercial feed additive on the basis of active substances obtained mainly from citrus fruits. Experimental feed mixtures were without coccidiostatas. Control feed mixtures contained commercial feed additive of coccidiostats, which are commonly used in practical conditions. Control feed mixtures were without feed additive on the basis of active substances obtained mainly from citrus fruits.

Basic feed mixtures were a soy cereal type. Base of feed mixtures consisted of corn, wheat and soybean meal during the starter phase 90.33% (starter feed mixture), during the growth phase 90.1% (growth feed mixture 1), 93.05% (growth feed mixture 2) and during final phase of 92.61% (final feed mixture). Meat-and-bone meal it is banned as feed material and deficient amino acids in feed mixtures must be complemented with amino acids pre-mixes. We used pre-mixes L-Lysine HCl and DL-Methionine in our feed mixtures, which were source their active substance, lysine and methionine, in accordance with the physiological needs of broiler chickens.

Composition of L-Lysine HCl:
L-Lysine monohydrochloride, technically pure NH_2-$(CH_2)_4$-$CH(NH_2)$-COOH.HCl,
L-Lysine min 78%.

Composition of DL-Methionine:
DL-Methionine, technically pure $CH_3S(CH_2)_2$-$CH(NH_2)$-COOH,
DL-Methionine min 98%.

We calculated ratio between metabolizable energy and crude protein and between methionine and lysine, which are important for the productive efficiency of feed in broiler chickens, especially in terms of protein intake.

A value of metabolizable energy in $kJ.kg^{-1}$/crude protein in $g.kg^{-1}$ ratio ranged from 55.91 : 1 (starter feed mixture) to 69.95 : 1 (final feed mixture). A value of methionine in $g.kg^{-1}$/lysine in $g.kg^{-1}$ ratio varied from 0.42 : 1 (grower feed mixtures) to 0.43 : 1 (starter or final feed mixture).

Scheme and characteristics of feed additives used in experiment

Scheme of experiment is given in Table 2.

Experimental group

Biocitro feed additive is to be used in the drinking water 1.0 mL per 1.0 L.

Composition of Biocitro:

mixture of citrus fruit essential oils obtained by pressing of *Citrus paradisi*, *Citrus reticulata* Blanco, *Citrus aurantium bergamia* ss. and *Citrus sinensis*; the main

Table 1 Content of nutrients and metabolizable energy in basal feed mixtures.

	Feed mixtures			
	Starter (from day 1 to day 10)	**Grower 1 (from day 11 to day 20)**	**Grower 2 (from day 21 to day 35)**	**Final (from day 36 to day 42)**
Crude protein, $g.kg^{-1}$	220.00	207.00	197.00	188.00
ME_N, $kJ.kg^{-1}$	12,300	12,750	13,150	13,150
ME_N/Crude protein	55.91:1	61.59:1	66.75:1	69.95:1
Lysine, $g.kg^{-1}$	14.00	12.50	12.50	11.50
Methionine, $g.kg^{-1}$	6.00	5.20	5.20	5.00
Methionine/Lysine	0.43 :1	0.42:1	0.42:1	0.43:1
Calcium, $g.kg^{-1}$	9.00	8.50	8.50	8.50
Phosphorus (non-phytate), $g.kg^{-1}$	4.20	4.00	4.00	4.00

ME_N = apparent metabolizable energy corrected for nitrogen balance.

Table 2 Scheme of experiment.

Group	Broiler chickens Cobb 500, *n*	Feed additive	Active substances
Experimental	50	Biocitro*	obtained mainly from citrus fruits
Control	50	Clinacox 0.5%**	diclazuril
		Sacox 12%***	salinomycin sodium

*Drinking water; **Starter feed mixture; ***Grower feed mixtures 1, 2.

compounds of citrus biomass are L-ascorbic acid and flavonoids, sucha s naringin, hesperidin, quercetin and rutin, vitamin C – L-ascorbic acid, the organic acids – citric acid and fatty acids, such as α-linolenic acid, linoleic acid, γ-linolenic acid and oleic acid.

Control group

Clinacox and Sacox are premixes used in commercial feed mixtures of the control group. Clinacox 0.5% is premix with the active substance diclazuril 0.5 g per 100 g for the prevention of coccidiosis caused by *Eimeria acervulina*, *E. brunetti, E. maxima, E. mitis, E. necatrix and E. tenella.*

Composition of Clinacox 0.5%:
diclazuril 0.5 g per 100 g,
soybean meal 99.25 g per 100 g,
polyvidon K30 0.2 g per 100 g,
sodium hydroxide 0.0538 g per 100 g.

Characteristics of active substance diklazuril $C_{17}H_9C_{l3}N_4O_2$:
(±)-4-chlorofenyl[2,6-dichloro-4-(2,3,4,5-tetrahydro-3,5-dioxo-1,2,4-triazin-2-yl)fenyl]acetonitril. Number CAS: 101831-37-2.

Related impurities:

degradation compound (R064318) ≤0.1%, other related impurities (T001434, R066891, R068610, R070156, R070016) ≤0.5% individually, total impurities ≤1.5%.

Sacox 12% is premix with active substances salinomycin sodium for the prophylactic control of various kinds of coccidia, such as *Eimeria acervulina*, *E. maxima, E. necatrix*, *E. mivati, E. tenella and E. brunetti*. Its dose is from 50 to 70 mg per 1,000 g of feed mixture.

Composition sacox 12%:

salinomycin sodium: ≥120 *g* per 1,000 g, silicon dioxide from 10 to100 g per 1,000 g and calcium carbonate from 350 to 700 g per 1,000 g.

Characteristics of active substance salinomycin sodium $C_{42}H_{69}O_{11}Na$:
Number CAS: 53003-10-4, sodium salt of a polyether monocarboxylic acid produced by fermentation of *Streptomyces albus* (DSM 12217).

Related impurities:
<42 mg elaiophylin per kg salinomycin sodium, <40 g 17-epi-20-desoxy-salinomycin per kg salinomycin sodium.

Parameters and procedure their an investigation

Productive parameters

Body weight gain per bird

We calculated average body weight gain per bird (BWG) as difference between total final body weight of broiler chickens in group and body weight of one-day-old broiler chickens in group. We divided the result by the number of birds in group.

Each one-day-old broiler chicken under the experiment weighed 42.0 g in the experimental and control group. We weighted their body weight by scale Kern 440-43 N type with a maximum weight of 400.0 g and final body weight by ECE20K20 type with a maximum weight of 20.0 kg.

Feed intake per bird

We calculated an average feed intake per bird (FI) on the basis of total consumed a starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture in group. We divided consumed a quantity feed by number of birds in the group. Average feed intake per bird presents an average feed intake per total body weight gain and bird.

Feed intake per 1 kg of body weight gain

We calculated an average feed intake per 1 kg of body weight gain (FI per 1 kg of BWG) on the basis of total consumed a starter feed mixture, grower feed mixture 1, grower mixture 2 and final feed mixture in group. We divided consumed a quantity feed by total body weight gain of broiler chickens in group.

Protein intake per total body weight gain and bird

We calculated of average protein intake per total body weight gain and bird according to Equation 1.

$$PI = FI \times CP \quad (1)$$

where:
PI = average protein intake per total body weight gain and bird, g;
FI = average intake of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture per bird, kg;
CP = crude protein content per 1 kg of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture, $g.kg^{-1}$.

Protein intake per 1 kg of body weight gain

We calculated of average protein intake per 1 kg of body weight gain according to Equation 2.

$$PI \text{ per 1 kg of } BWG = FI \times CP/BWG \quad (2)$$

where:
PI per 1 kg of BWG = average protein intake per 1 kg of body weight gain, g;
FI = average intake of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture per bird, kg;
CP = crude protein content per 1 kg of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture, $g.kg^{-1}$;
BWG = average body weight gain per bird, kg.

Energy intake per total body weight gain per bird

We calculated of average energy intake per total body weight gain and bird according to Equation 3.

$EI = FI \times ME_N$ (3)

where:

EI = average energy intake per total body weight gain per bird, g;

FI = average intake of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture per bird, kg;

ME_N = metabolizable energy content per 1 kg starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture, $kJ.kg^{-1}$.

Energy intake per 1 kg body weight gain

We calculated of average energy intake per 1 kg of body weight gain according to Equation 4.

$EI\ per\ 1\ kg\ of\ BWG = FI \times ME_N/BWG$ (4)

where:

EI per 1 kg of BWG = average energy intake per 1 kg of body weight gain, g;

FI = average intake of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture per bird, kg;

ME_N = metabolizable energy content per 1 kg of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture, $g.kg^{-1}$;

BWG = average body weight gain per bird, kg.

Lysine intake per total body weight gain per bird

We calculated of average lysine intake per total body weight gain and bird according to Equation 5.

$LysI = FI \times Lys$ (5)

where:

LysI = average lysine intake per total body weight gain and bird, g;

FI = average intake of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture per bird, kg;

Lys = lysine content per 1 kg of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture, $g.kg^{-1}$.

Lysine intake per 1 kg body weight gain

We calculated of average lysine intake per 1 kg of body weight gain according to Equation 6.

$LysI\ per\ 1\ kg\ of\ BWG = FI \times Lys/BWG$ (6)

where:

LysI per 1 kg of BWG = average lysine intake per 1 kg of body weight gain, g;

FI = average intake of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture per bird, kg;

Lys = lysine content per 1 kg of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture, $g.kg^{-1}$;

BWG = average body weight gain per bird, kg.

Methionine intake per total body weight gain per bird

We calculated of average methionine intake per total body weight gain and bird according to Equation 7.

$MetI = FI \times CP$ (7)

where:

MetI = average methionine intake per total body weight gain and bird, g;

FI = average intake of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture per bird, kg;

Met = methionine content per 1 kg of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture, $g.kg^{-1}$.

Methionine intake per 1 kg body weight gain

We calculated of average protein intake per 1 kg of body weight gain according to Equation 8.

$MetI\ per\ 1\ kg\ of\ BWG = FI \times CP/BWG$ (8)

where:

MetI per 1 kg of BWG = average methionine intake per 1 kg of body weight gain, g;

FI = average intake of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture per bird, kg;

Met = crude protein content per 1 kg of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture, $g.kg^{-1}$;

BWG = average body weight gain per bird, kg.

Protein and energy efficiency ratio

Protein efficiency ratio

We calculated a protein efficiency ratio (PER) according to Equation 9:

$PER = BWG/PI$ (9)

where:

PER = protein efficiency ratio;

BWG = average body weight gain per bird, g;

PI = average protein intake per total body weight gain and bird, g.

Energy eficiency ratio

We calculated a energy efficiency ratio (EER) according to Equation 10:

$EER = BWG/EI \times 100$ (10)

where:

EER = energy efficiency ratio, %

BWG = average body weight gain per bird, g

EI = average energy intake per total body weight gain and bird, kJ.

Breast and thigh muscle quality from aspect of the protein, lysine and methionine contents

Sample analyses

We carried out a sampling and chemical analysis of breast and thigh muscles according to **Angelovičová et al. (2016)**. Broiler chickens in the number of 6 pcs from experimental and control group, above average body (live) weight of 1800.0 g, were randomly selected at the end of the experiment (day 42). A slaughtering of broiler chickens by human rapid cut of the carotid artery (*Ateria carotis communis*) was realized. Subsequently, feathers as well as internal parts of chickens were mechanically removed. Breast and thigh muscles, which have been used for chemical analysis, were separated from the carcass as well. A sample (50.0 g) of breast and thigh muscles without a skin of every chicken was measured by the method of FT IR Nicolet 6700 (the program OMNIC™

Series Software, producer Thermo Fisher Scientific, USA). A molecular spectroscopy was performed for infrared spectrum of muscle homogenates analyses. The principle of this method is infrared absorption spectrum of the sample passes and there is a change from the rotary vibrating energy conditions of the molecule depending on the changes of the dipole moment of the molecule. Analytical output is the infrared spectrum, which is a graphical representation of the function of the energy dependence, mostly mentioned as a percentage of transmittance (T) or in units of absorbance (A) at a wavelength of the incident radiation. Permeability is defined as ratio of the radiation intensity which has passed through the sample (I) and of the emission intensity of emitted source (Io). Absorbance is defined as a decimal logarithm of 1/T. Samples of breast and thigh muscles were measured for protein content, lysine content and methionine content.

Statistical analyses

The results of productive efficiency of feed mixtures and protein and energy efficiency are presented as average values. The values of these parameters were investigated jointly by groups. The results of total body weight gain, breast and thigh muscle quality from aspect protein, lysine and methionine contents were investigated individually and statistically evaluated for group as average values, standard deviation, coefficient of variation and variation range as the difference between the minimum and maximum value of the data distribution.

T-test was ued at the significance level α = 0.05 to compare a difference between experimental and control groups. A Pearson´s correlation coefficient (r_{xy}) reflects a degree of relation between two variables of the indicators of chicken breast muscle and thigh muscle. Pearson´s correlation coefficient (r_{xy}) reflects the degree of linear relation between the two data sets. Its value is between -1 and +1. A value of +1 means, that there is a perfect positive linear relation between two data sets. A value of –1 means that there is a perfect negative linear relation and a value of 0 means, that there is no linear relation at all between data sets. The results of correlation coefficient are complemented by statistical significance at the significance level of α = 0.05, α = 0.01 and α = 0.001. SAS statistical package (5) was used to perform statistical analyses.

RESULTS AND DISCUSSION

Productive parameters

Body weight gain per bird

Average total body weight gain per bird is given in Table 3.

Obtained the active substances from vegetable products confirmed in experiments *in vivo* effects for the secretion of gastric juice, which led to the increase of body weight of the broiler chickens. Mentioned an effect is associated with improved digestibility and absorption of nutrients from feed **Močár et al. (2012)**. A similar conclusion is shown in otther study. Important propriety which has also been observed recently in chickens is the benefit of some natural substances on the gastrointestinal enzymatic activity, most likely improving nutrient digestibility **(Jang et al., 2006)**.

Average total body weight gain of broiler chickens was found 2036.09 g in our experiment by effect of active substances obtained mainly from citrus fruits compared to average total body weight gain 1771.27 g in control group with diclazuril and salinomycin sodium. The results of SD and %CV for total body weight gain per bird by effect of active substances obtained mainly from citrus fruits varied more compared to calculated results of SD and %CV for total body weight gain per bird of control group with diclazuril and salinomycin sodium. Many research institutions focused their attention on the development of alternatives to feed antibiotics, including a control of coccidiosis, with respect to recent global trend to ensure poultry production without them **(Lillehoj and Lee, 2012)**. According to some authors **(Langhout, 2000; Manzanilla et al., 2004)**, positive results may be obtained, when essential oils extracted from different plants are enriched by the addition of the more relevant active substances. Investigated active substances obtained mainly from citrus fruits, according to our experimental results clearly demonstrate significant positive effects on total body weight gain of broiler chickens, which were confirmed by increased values compared to control group with coccidiostatats diclazuril and salinomycin sodium.

Feed intake per bird, and 1 kilogram of body weight gain, protein, energy, lysine and methionine intake per total body weight gain and bird, and 1 kg of body weight gain

Average feed intake per bird, and 1 kg of body weight gain, protein, energy, lysine and methionine intake per total body weight gain and bird, and 1 kg of body weight gain is given Table 4.

An effect of active substances obtained mainly of citrus fruits tended to slightly increase feed intake per bird in our experiment in contrast to feed intake of control group with diclazuril and salinomycin sodium (4340.0 g). Feed supplemented with active substances obtained mainly from citrus fruits is probably tastier for broiler chickens. Our results of an average feed intake per 1 kg of body weight gain had the opposite tendency as average feed intake expressed per bird. Average feed intake per 1 kg of body weight gain showed slightly lower average value 2,161.0 g in the experimental group, in which the broiler chickens consumed of feed mixtures with active substances

Table 3 Average total body weight gain per bird in g.

Group	n	M	SD	%CV	R	t-test
Control	50	1771.27	161.30	9.11	1378 – 2178	p <0.05
Experimental	50	2036.09	325.16	15.97	1178 – 2678	

Note: n = number of samples; M = mean; SD = standard deviation; %CV = coefficient of variation; R = variation range as the difference between the minimum and maximum value of the data distribution; Control = diclazuril and salinomycin sodium; Experimental = active substances obtained mainly from citrus fruits; p <0.05 = statistically significant difference between groups.

Table 4 Average feed intake per bird, and 1 kg of body weight gain, protein, lysine and methionine intake per total body weight gain per bird, and 1 kg of body weight gain.

	Group	
	Control	**Experimental**
Feed intake per bird*, g	4,340.00	4,400.00
Feed intake per 1 kg of body weight gain, g.kg^{-1}	2,450.22	2,161.00
Protein intake per total body weight gain and bird, g	864.42	875.70
Protein intake per 1 kg of body weight gain, g.kg^{-1}	488.20	430.09
Energy intake per total body weight gain and bird, g	56,246.00	57,035.00
Energy intake per 1 kg of body weight gain, g.kg^{-1}	31,754.62	28,012.02
Lysine intake per total body weight gain and bird, g	53.66	54.35
Lysine intake per 1 kg of body weight gain, g.kg^{-1}	30.29	26.69
Methionine intake per total body weight gain and bird, g	22.70	23.00
Methionine intake per 1 kg of body weight gain, g.kg^{-1}	12.82	11.30

Note: Feed intake per bird = feed intake per total body weight and bird; Control = diclazuril and salinomycin sodium; Experimental = active substances obtained mainly from citrus fruits.

obtained mainly from citrus fruits compared to 2,450.0 g in the control group with diclazuril and salinomycin sodium. Active substances obtained from citrus fruits can affect the endogenous secretion of broiler chickens **(Cross et al., 2007)**. Feed conversion ratio was found significantly better after feeding diet supplemented with 1.0% and 2.0% dried sweet orange (*Citrus sinensis*) pulp than in control group without this feed additive. Based on some observed data, dried or pelleted citrus pulp is one of the most desirable energy feeds and can be considered in feeding programs as a feed with high digestible nutrient content. It includes a mixture of citrus peel, pulp, and seed as by-product energy concentrate feed for domestic animals **(Arthington et al., 2002)**. The consumption of feed mixtures with supplements on the natural base has been investigated in other *in vivo* experiment with broiler chickens. Average consumption of feed mixtures with supplements on a natural base in other experiment, expressed as consumption of feed per 1 kg of body weight gain, was found slightly lower opposite our results, i.e. 1807.0 g, 1822.0 g and 1835.0 g and of commercial feed mixtures 2013.0 g **(Azza a Naela (2014)**. These values of feed intake per 1 kg of body weight gain are lower compared to the average feed intake per kg body weight gain of broiler chickens in our experiment. This difference in feed intake per 1 kg of body weight gain can be related to different final hybrid of broiler chickens, which was used in the experiment. These authors used the final hybrid Arbor Acres and we used in our experiment a final hybrid Cobb 500. Digestive system development of broiler chickens is faster shortly after hatching than the body development. Gastrointestinal tract begins to form already during the embryonic stage **(Moran, 1985)** and it becomes fully functional within a few days after hatching **(Iji et al., 2001a, 2001b)**. The development of the gastrointestinal tract after hatching is very important for the growth chickens and future performance through effective utilization of dietary nutrients **(Faria et al., 2005)**. Submission of high quality nutrition to young chickens at an early stage of development is very important to ensure the rapid development and growth of the gastrointestinal tract, as well as the body. Proteins in this respect the most essential nutrients, which determine the initial development of the gastrointestinal tract and muscle mass in the later stages of broiler chicken growth **(Hargis a Creger, 1980)**.

Our results of average dietary protein intake per total body weight gain of broiler chickens were found 875.70 g by effect of active substances obtained mainly from citrus fruits and slightly lower 864.42 g in control group with dikluzaril and salinomycin sodium. Average dietary protein intake per 1 kg body weight gain of broiler chickens was achieved 430.09 g by active substances obtained mainly citrus fruits opposite slightly higher protein consumption 488.02 g in control group with diclazuril and salinomycin sodium. Protein source and protein quality are very important for growth and development of broiler chickens. This fact was confirmed in different growth intensity of broiler chickens that were fed with different proportion of vegetable and animal proteins in the feed mixtures **(Hossain et al., 2012, 2013a)**. Dietary protein is the most expensive component of a broiler diet. Methionine is an amino acid of critical importance in commercial poultry diets, because it is typically the first-limiting amino acid **(Adbalqdadir and Arabi, 2014)**. One of the most expensive components of broiler chickens diets is protein or more specifically amino acids **(Dozier et al., 2009)**. A need for dietary lysine is greater for breast meat yield than for growth rate **(Sterling et al., 2006; Dozier et al., 2006)**.

Birds regulate their energy need by feed intake. If energy level increases in feed mixtures, birds satisfy their energy needs by decreasing feed intake **(Nahashon et al., 2005; Veldkamp et al., 2005)**. Our results of average dietary energy intake per bird were found slightly higher (57,035.00 kJ) in the experimental group with active substances obtained mainly from citrus fruits in contrast to the control group with diclazuril and salinomycin sodium (56,246.0 kJ). Average dietary energy intake per 1 kg of body weight gain was found 28,012.02 kJ.kg^{-1} in the experimental group by effect of active substances obtained mainly from citrus fruits, and 2,450.0 kJ.kg^{-1} in the control group with diclazuril and salinomycin sodium. According to literary knowledge is know that modern broiler chickens selected for rapid growth do not regulate voluntary feed intake to achieve energy balance. This altered ability of broiler chickens to adjust feed intake due to differences in content of dietary metabolizable energy was most likely to result from continued selection for rapid juvenile growth rates. These animas may have altered hypothalamic mechanisms that regulate feed intake **(Bokkers and Koene, 2003)**. Other studies shown no effect of dietary metabolizable energy content on feed intake between broiler chickens fed *ad libitum* feed mixtures containing two energy levels of 13,380 and 15,000 kJ.kg^{-1} **(Mbajiorgu et al., 2011)**. Feed intake may be affected by flavoring substances which today can be used in feed mixtures. We can include our investigated feed additive obtained mainly from citrus fruits these gustatory substances. Metabolizable energy intake is distributed in body into energy requirement for maintenance and energy retained in the body in the form of protein and fat **(Lopez and Leeson, 2005)**.

Because body proteins are in a dynamic state, with synthesis and degradation occur ring continuously, an adequate intake of dietary amino acid is required **(NRC, 1994)**. Broiler chickens are characterized by good storage capabilities muscle. Lysine provides the basic functions to create muscle mass. Lysine is considered to be the second most limiting amino acid after the sulfur-containing amino acids for broiler chickens fed feed mixtures based on corn and soybean meal. For these reasons lysine was chosen as the reference amino acid as the ideal protein concept, in which all other essential amino acids are formulated into the feed mixtures as a ratio to lysine **(Emmert and Baker, 1997)**. Our results of average dietary lysine intake per bird were found 54.35 g in experimental group with active substances obtained mainly from citrus fruits and slightly lower 53.66 g in control group with diclazuril and salinomycin sodium. Values of average lysine intake per 1 kg body weight gain have been demonstrated reduction in experimental group with active substances obtained mainly from citrus fruits (26.69 g) compared to control group with diclazuril and salinomycin sodium (30.29 g). In general, amino acid sufficient need for broiler chickens was determined depending on many factors, including genotype, age and sex. This potential was determined based on nitrogen balance **(Samadi and Liebert, 2007)**.

Methionine was set in the first position among amino acids limiting chicken growth **(Fancher and Jensen, 1989)**. Methionine is very important for poultry as an essential amino acid for protein synthesis, a methyl donor group for normal cellular metabolism and for normal formation of coenzyme S-adenosylmethionine, a precursor of important intermediates in metabolic pathways such as cystine or carnitine, an amino acid involved in polyamine synthesis, and also as a sulfur donor **(Bunchasak, 2009)**. Average dietary methionine intake per bird was found 23.0 g in our experiment by effect of active substances obtained mainly from citrus fruits and 22.70 g, i.e. less in control group with diclazuril and salinomycin sodium. Values of average methionine intake per 1 kg body weight gain have been demonstrated reduction in experimental group with active substances obtained mainly from citrus fruits (11.30 g) in contrast to control group with diclazuril and salinomycin sodium (12.82 g). The methionine addition in the poultry diet demonstrated a tendency to less total body fat **(Rostagno et al., 1995)**, to support growth performance **(Chavez et al., 2004)**. Feed with dietary methionine excess has been reported to impair body weight gain **(Harper et al., 1970)**.

Feed mixtures are submitted to broiler chickens with nutrient balances, including amino acids and energy. Broiler chickens have feed available *ad libitum*. They consume sufficient and balanced amount of amino acids. A proper balance feed mixture provides enough amino acids in a balanced ratio, thereby preventing a lack of amino acids or amino acid toxicity **(Zulkifli et al., 2001)**. The essential amino acid requirements for broiler chickens meet the formulation of feed mixture with addition of synthetic amino acids. Such feed mixture compositium improves the overall balance of amino acids and maintains overall performance **(Zarate et al., 2003)**. It is not easy to obtain accurate dietary amino acid needs for broiler chickens. Numerous researchs have been conducted to determine the essential amino acid requirements. These needs are influenced by factors such as growth in response to changing levels of dietary amino acids is not linear; antagonism or toxicity may occurr between two or more amino acids or anti-nutritional factors or interactions between certain amino acids and other nutrients; metabolizable variations under physiological and environmental conditions **(Oviedo-Rondon and Waldroup, 2002)**. Precise estimation of the lysine requirement is of the importance to defining the ideal ratios of other essential amino acids **(Baker, 2009)**. Lysine deficiency or fasting posthatch may decrease protein synthesis. It concerns mainly of the breast muscle **(Bigot et al., 2003)**. The lack of lysine increases proteolysis in breast muscle **(Tesseraud et al., 2009)**. Lysine and methionine are precursors of L-carnitine and assotiated to energy and lipid metabolism in broiler chickens **(Borum, 1983)**.

Protein and energy efficiency ratio

Protein and energy efficiency ratio is given in table 5.

In general, the nutritional quality of proteins depends on the balancing of amino acids that contribute to body weight gain of broiler chickens. The amount of body weight gain is very important per unit of protein intake by feed. The content of amino acids and proteins in the feeding of the feed is determined by analytical methods but information on their bioavailability is insufficient. For this reason, growth test used to compare the relative value of different proteins on the basis of qualitative growth of broiler chickens **(Rezaeipour et al., 2014)**. We used an indicator of protein efficiency ratio (PER) to evaluation the protein quality of the feed mixture quality in our experiment.Average protein efficiency ratio was found 2.36 in the broiler chickens in the broiler chickens that fed feed mixtures with active substances obtained mainly from

Table 5 Protein and energy efficiency ratio.

Group	Control	Experimental
PER	2.05	2.36
EER	3.15	3.57

Note: PER = protein efficiency ratio; EER = energy efficiency ratio; Control = diclazuril and salinomycin sodium; Experimental = active substances obtained mainly from citrus fruits.

citrus fruits compared to slightly lower value of average protein efficiency ratio 2.05 in broiler chickens of control group with diclazuril and salinomycin sodium. Tendency of the higher protein efficiency ratio by effect of active substances obtained mainly from citrus fruits is related to the higher body weight gain of broiler chickens compared to protein efficiency ratio by effect of diclazuril and salinomycin sodium.

Energy efficiencies can be significantly affected by composition of feed mixtures and the purpose for which the energy is used **(Lopez and Leeson, 2008)**. Dietary energy is utilised more efficiently for body fat deposition than for body protein deposition. This variation prevents accurate prediction of nutrient requirements for broiler chickens **(Lopez and Leeson, 2005; Shantawi, 2014)**. Energy efficiencies of protein deposition can range from 0.36 to 0.70 and the energy efficiencies of fat deposition from 0.55 to 0.92 **(Sakomura et al., 2005)**. These variations may have resulted from differences in the genetic factor, feed mixture composition and environment **(Shantawi, 2014)**. The conditions in which was conducted our experiment were the same for experimental and control group except that groups differed according to each feed additive. We found higher energy efficiency ratio in these defined experimental conditions (see section Materials and methods) by effect of active substances obtained mainly from citrus fruits n contrast to the effect of control group coccidiostatats diclazuril and salinomycin natrium. **Angelovičová et al. (2016)** investigated the same feed additive Biocitro in relation to the fatty acid profile in the chicken meat. The effect this active substance tendentiously reduced fat content in breast and thigh muscles compared to control feed with mixtures coccidiostatats narasin, salinomycin sodium and nicarbasin.

Breast and thigh muscles quality from aspect of protein, lysine and methionine contents

Protein content in the breast and thigh muscles

Average protein content in the breast and thigh muscles is given in Table 6.

Chemical composition of chicken meat is affected by nutrition, age, animal genotype and other factors of environment **(Haščík et al., 2009)**. Energy value of meat is relatively low, but the protein content is relatively high compared to other types of meat **(Medveď and Angelovičová, 2010)**. The most abundant muscle protein fractions are structural proteins that participate 54-70% of total muscle protein. Myofibrillar proteins contribute to meat tenderness, determine the capacity of water retention and meat hydration, fat emulsifying and gelling capacity. They are important for technological quality, as well as the nutritional value of meat. Myofibrillar proteins contribute about 70% to the nutritional value of meat by essential amino acids contents **(Ionescu et al., 2009)**. Chicken meat contains about 16.44-3.31% protein **(Chuaynukool et al., 2007).** Our results of average protein content in breast muscle was found 24.13 g.100 g^{-1} by effect of active substances obtained mainly from citrus fruits and compared to slightly less protein content 23.87 g.100 g^{-1} in control group with diclazuril and salinomycin sodium.

The results of SD and %CV for protein content in the breast muscle by effect of active substances obtained mainly from citrus fruits varied more compared to calculated results of SD and %CV for protein content in the breast muscle of control group with diclazuril and salinomycin sodium. Feed additives used in our experiment, such as active substances obtained mainly from citrus fruits in experimental group, and coccidiostatats diclazuril and salinomycin sodium in control feed mixtures, did not have a statistically significant (p >0.05) effect on protein content in the breast

Table 6 Average protein content in the breast and thigh muscles, g per 100 g.

	n	M	SD	%CV	R	t-test
Group			**Breast muscles**			
Control	50	23.87	0.28	1.17	23.50 – 24.52	p >0.05
Experimental	50	24.13	0.35	1.45	23.56 – 24.61	
			Thigh muscles			
Control	50	22.44	0.49	2.18	22.20 – 22.83	p >0.05
Experimental	50	22.10	0.51	2.31	21.56 – 22.90	

Note: n = number of samples; M= average; SD = standard deviation; %CV = coefficient of variation; R = variation range as the difference between the minimum and maximum value of the data distribution; Control = diclazuril and salinomycin sodium; Experimental = active substances obtained mainly from citrus fruits; p >0.05 = no statistically significant difference between groups.

Table 7 Average lysine content in the breast and thigh muscles, g per 100 g.

	n	M	SD	%CV	R	t- test
Group			**Breast muscles**			
Control	50	2.16	0.18	8.33	1.96 – 2.44	p >0.05
Experimental	50	2.42	0.25	10.33	2.21 – 2.81	
			Thigh muscles			
Control	50	2.11	0.15	7.11	1.92 – 2.30	p >0.05
Experimental	50	2.24	0.19	8.48	2.03 – 2.47	

Note: n = number of samples; M = average; SD = standard deviation; %CV = coefficient of variation; R = variation range as the difference between the minimum and maximum value of the data distribution; Control = diclazuril and salinomycin sodium; Experimental = active substances obtained mainly from citrus fruits; p >0.05 = no statistically significant difference between groups.

Table 8 Average methionine content in the breast and thigh muscles, g per 100 g.

	n	M	SD	%CV	R	t- test
Group			**Breast muscles**			
Control	50	0.79	0.11	13.92	0.71 – 0.90	p >0.05
Experimental	50	0.89	0.11	12.36	0.79 – 0.98	
			Thigh muscles			
Control	50	0.76	0.06	7.89	0.68 – 0.82	p >0.05
Experimental	50	0.84	0.07	8.33	0.73 – 0.90	

Note: n = number of samples; M = average; SD = standard deviation; %CV = coefficient of variation; R = variation range as the difference between the minimum and maximum value of the data distribution; Control = diclazuril and salinomycin sodium; Experimental = active substances obtained mainly from citrus fruits; p >0.05 = no statistically significant difference between groups.

Table 9 Korelation relation (r_{xy}) between lysine and methionine in breast and thigh muscles of broiler chickens.

		Breast muscle	Thigh muscle
Group		**Lysine**	
Control	**Methionine**	0.98^{+++}	0.93^{++}
Experimental		0.95^{+}	0.82^{++}

Note: numerical value is result of correlation coefficients (rxy) between two variables;
Control = diclazuril and salinomycin sodium; Experimental = active substances obtained mainly from citrus fruits;
$^{+++}$, $^{++}$, $^{+}$ = Difference between groups means is statistically significant (p <0.001, p <0.01, p <0.05).

muscle.

Average protein content in thigh muscle was found 22.10 g.100 g^{-1} by effect of active substances obtained mainly from citrus fruits and compared to slightly higher protein content 22.44 g.100 g^{-1} in control group with diclazuril and salinomycin sodium. The results of SD and %CV for protein content in the thigh muscle by effect of active substances obtained mainly from citrus fruits varied more compared to calculated results of SD and %CV for protein content in the thigh muscle of control group with diclazuril and salinomycin sodium.

Difference between groups was not statistically significant (p >0.05). Proteins are a major component of muscle tissue, which affects their nutritional value, functional properties and sensory properties **(Lawrie, 1998)**.

Lysine content in the breast and thigh muscles

Average lysine content in the breast and thigh muscles is given in Table 7.

Average lysine content in breast muscle was found 2.42 g.100 g^{-1} by effect of active substances obtained mainly from citrus fruits and compared to slightly lower fat content 2.16 g.100 g^{-1} in control group with diclazuril and salinomycin sodium. The values of SD and %CV for lysine content in the breast muscle by effect of active substances obtained mainly from citrus fruits varied more compared to calculated results of SD and %CV for lysine content in the breast muscle of control group with diclazuril and salinomycin sodium.

Difference between groups was not statistically significant (p >0.05).

The measured lysine content in the thigh muscle was tendentiously higher 2.24 g.100 g^{-1} by effect of active substances obtained from citrus fruits in the feed mixtures versus lower lysine content 2.16 g.100 g^{-1} in the thigh musle by effect of control feed mixtures with diclazuril and salinomycin sodium.

The values of SD and %CV for lysine content in the thigh muscle by effect of active substances obtained mainly from citrus fruits varied more compared to calculated results of SD and %CV for lysine content in the thigh muscle of control group with diclazuril and salinomycin sodium.

Difference between groups was not statistically significant (p >0.05).

Protein rich in essential amino acids are the most important component of poultry meat **(Straková et al., 2002)**.

Methionine content in the breast and thigh muscles

Average methionine content in the breast and thigh muscles is given in Table 8.

Methionine has important function in the broiler chickens. It participates in the depositing of breast muscles of broiler chickens. This knowledge was confirmed in an experiment. Studied object was methionine and its impact on the improvement of the production and deposition breast muscle in broiler chickens hatched with lower body weight **(Zhai et al., 2012)**. The average methionine content in the breast and thigh muscles were 0.89 g.100 g^{-1} in the breast muscle and 0.84 g.100 g^{-1} in the thigh muscle by effect of active substances obtained from citrus fruits in the feed mixtures compared to methionine content 0.79 g.100 g^{-1} in the breast musle and 0.74 g.100 g^{-1} in the thigh muscle by effect of control feed mixtures with diclazuril and salinomycin sodium.

The values of SD and %CV for methionine content in the breast and thigh muscles varied nearly the same by effect of active substances obtained mainly from citrus fruits versus diclazuril and salinomycin sodium.

Difference between groups was not statistically significant (p >0.05). Chemical composition of breast musle differs from the chemical composition of thigh muscle **(Straková et al., 2002)**.

Correlation between lysine and methionine in breast and thigh muscles of broiler chickens

Correlation between lysine and methionine in breast and thigh muscles of broiler chickens is given in Table 9.

Methionine and lysine are probably the most studied amino acids in nutrition of broiler chickens. However, the relation between methionine and lysine has not been extensively investigated **(Cafe and Waldroup, 2006)**. The results investigation a methionine and lysine showed no interactions between these amino acids **Si et al. (2004)**. The increase of dietary lysine and methionine can reduce abdominal fat, improve feed conversion ratio, breast muscle yield, carcass efficiency (economical traits) in broiler chickens. It is suggested that amount of lysine and methionine in higher levels of NRC (1994) recommendations may result an increase of economical trait, performance in the broiler chickens **(Bouyeh and Gevorgyan, 2016)**.

In our experiment were composed the feed mixtures with same nutrient and energy contents for experimental and control group. Lysine and methionine contents as well as methionine/lysine ratio were balanced in dependence to growth phase, i.e. Starter, Grower 1, Grower 2 and Final. We focused on an investigation of correlation between lysine and methionine in the breast and thigh muscles in dependence on feed experimental active substances obtained mainly from citrus fruits and control diclazuril and salinomycin sodium. Strong positive dependence, statistically significant (p <0.001, p <0.01, p <0.05), was found between lysine and methionine in the breast muscle as well as in the thigh muscle.

CONCLUSION

Based on achieved results of our experiment with broiler chickens Cobb 500, it can be alleged that experimental active substances obtained mainly from citrus fruits and control diclazuril and salinomycin sodium had comparable influence on protein guality of chicken meat. Differences were reflected on the productive efficiency of feed and performance of broiler chickens.

Active substances obtained mainly from citrus fruits confirmed a statistically significant (p <0.05) effect on the body weight gain of broiler chickens, compared with effect of diclasuril and salinomycin sodium in control group.

An effect of active substances obtained mainly from citrus fruits tended to:

a) slightly increase feed intake per bird, protein, energy, lysine and methionine intake per total body weight gain and bird,

b) slightly decrease feed intake per 1 kg of body weight gain, protein, energy, lysine and methionine intake per 1 kg of body weight gain,

c) slightly increase protein efficiency ratio and energy efficiency ratio.

Active substances obtained mainly from citrus fruits in the feed mixtures did not have a statistically significant effect on protein, lysine and methionine contents in the breast and thigh muscles.

Active substances obtained mainly from citrus fruits and likewise also coccidiostatats diclazuril and salinomycin sodium displayed a strong positive, statistically significant relation between lysine and methionine in the breast and thigh muscles.

We can allege in conclusion that active substances obtained mainly from citrus fruits have shown a tendency to increase productive efficiency of feed mixtures in the broiler chickens, which was reflected in an increase in total body weight gain in the unaltered protein, lysine and methionine contents in breast and thigh muscles.

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