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Effect of L-carnitine on the level of biochemical and antioxidant indices of blood of turkey hens

Wpływ L-karnityny na poziom wskaźników biochemicznych
i antyoksydacyjnych krwi indyczek

Summary. This study was aimed at determining the effect of L-carnitine addition to drinking water for turkey hens on the level of biochemical and antioxidative indices in their blood. Experiments were conducted with heavy Big-6 turkey hens reared since the 6^{th} till the 16^{th} week of life. Group I included control birds that received pure water for drinking. Turkey hens from group II received a liquid preparation of L-carnitine to drinking water in a dose of 0.83 ml/ l water, which was administered four times, i.e. in weeks 5, 8, 11 and 14 of life for the period of 5 days. Ate the end of the 9^{th} and 15^{th} weeks of birds' life, blood was sampled for analyses from the brachial vein of 15 birds from each group. Samples of blood plasma were determined for the content of biochemical markers, including: uric acid (UA), urea (UREA), bilirubin (BIL), creatinine (CREAT), albumin (ALBUMIN), iron (Fe), copper (Cu), and zinc (Zn). In terms of antioxidative markers, samples of blood serum were analyzed for the levels of superoxide dismutase (SOD), catalase (CAT), ferric-reducing ability of plasma (FRAP), and vitamin C. Further analyses were carried out to determine levels of lipid peroxidation products, i.e.: peroxides (H_2O_2) and malondialdehyde (MDA). The application of L-carnitine addition to drinking water for turkey hens was found to contribute to increased levels of low-molecular antioxidants, i.e. vitamin C, iron, and zinc, and to a reduced level of catalase in their blood plasma. The results obtained point to the feasibility of applying L-carnitine as an additive maintaining the oxidative-antioxidative balance in turkey hens, yet they need to be verified in further studies involving different modes of dosage.

Key words: turkeys, L-carnitine, blood, antioxidative and biochemical indices

INTRODUCTION

Carnitine, as an organic chemical compound, plays a key role in transporting long-chain fatty acids being acyl derivates of L-carnitine into the mitochondria, which enables their β-oxidation (oxidation), which is a source of energy (ATP) to the body. Apart from being a carrier of fatty acids, L-carnitine play also a detoxifying function [Matsuoka and Igisu 1993]. It occurs on the pathway of metabolites burning, and free fatty acids as esters of L-carnitine are transported outside mitochondria, which prevents the formation of their toxic compounds [Kalaiselvi *et al.* 1998]. It additionally prevents the consumption of amino acids in order to meet energy demands, and thereby exhibits the protective activity against proteins [Urbaityte *et al*. 2006]. Literature data indicate that carnitine is capable of stimulating the immune system [Daskiran, 2009, Buysea *et al.* 2007]. In addition, it displays capability for trace elements binding and hence is acknowledged as one of the most valuable antioxidants [Thangasamy *et al.* 2008, Gulcin 2006].

The main dietary source of carnitine are products of animal origin, red meat and dairy products in particular. Along with withdrawing animal meals from animal feeding, a problem of L-carnitine deficit has emerged. It occurs most often in intensively-growing animals, in the case of diet very rich in fats, in periods of stress (transport, antibiotic therapy, vaccination), at low ambient temperature, and during bacterial infections [Daskiran 2009]. Therefore the application of L-carnitine as an additive in animal feeding may be of very great significance.

Taking the above into account, the reported study was aimed at determining the effect of L-carnitine addition to drinking water for turkey hens on levels of biochemical and antioxidative indices in their blood.

MATERIAL AND METHODS

The experiment was conducted with Big-6 heavy turkey hens reared from the 6^{th} till the 16^{th} week of life. The birds were kept under zootechnical conditions appropriate for turkeys fattening [Faruga and Jankowski 2000]. During the experiment, the turkey hens of both groups were fed *ad libitum* full-dose feed mixtures (Tab. 1) following recommendations of the Poultry Feeding Standards [Normy żywienia drobiu 2005] and had free access to drinking water. Group I included control birds (n = 60) that were receiving pure water for drinking. Turkey hens from group II (n = 60) received a liquid preparation of L-carnitine to drinking water in a dose of 83 ml/l, that was administered four times, i.e. in week 5, 8, 11 and 14 of life for the period of 5 days.

At the end of the 9^{th} and 15^{th} of turkey hens life, blood was sampled for analyses from the brachial vein of each time the same 15 birds from each experimental group. With the use of monotests by Cormay company, blood plasma was determined spectrophotometrically for contents of selected biochemical indices: uric acid (UA), urea (UREA), bilirubin (BIL), creatinine (CREAT), and albumin (ALBUMIN). Samples of blood plasma were also analyzed for contents of iron (Fe), zinc (Zn) and copper (Cu). Plasma levels of microelements were determined at the Central Apparatus Laboratory, University of Life Science, using AAS technique on a UniCam 939 spectrophotometer.

Copper was determined at λ = 324.8 nm, zinc at λ = 213.9 nm and iron at λ = 2487.3 nm. Analytical ranges for the elements assayed were as follows: 0–1 mg l^{-1}, 0–2 mg l^{-1} and 0-10 mg l^{-1}, respectively.

Table 1. Nutrient content of the standard diets
Tabela 1. Zawartość pokarmowa standardowych mieszanek

Ingredients Składnik	Grower 1 (6–9 week)	Grower 2 (10–13 week)	Finisher 1 (14–17 week)
Corn, % Kukurydza, %	25.0	25.0	20.0
Wheat, % Pszenica, %	30.6	36.8	56.6
Soybean, % Soja, %	33.5	28.0	15.0
Meat and bone meal, % Mączka mięsno-kostna, %	5.00	5.00	4.0
Soya oil, % Olej sojowy, %	2.00	2.00	1.2
Fodder chalk, % Kreda pastewna, %	0.70	0.50	0.50
Cytromix Plus, %	0.20	0.20	0.20
Farmix*, %	3.00	2.50	2.50
Nutrient composition – Składniki odżywcze			
Crude protein, (CP), % Białko surowe, %	23.0	19.5	17.0
ME, kcal kg^{-1}	2900	2950	3000
Lysine, % Lizyna, %	1.45	1.25	1.05
Methionine + cysteine, % Metionina + cysteina, %	0.95	0.85	0.75
Tryptophan, % Tryptofan, %	0.25	0.21	0.18
Threonine, % Treonina, %	0.92	0.79	0.67
Calcium, % Wapń, %	1.20	1.15	1.10
Phosphorus, % Fosfor, %	0.65	0.55	0.50
Sodium, % Sód, %	0.15	0.15	0.15

*Farmix – the mineral and vitamin premix provided the following per kilogram of diet – 3 000 000 IU of vitamin A; 900 000 IU of vitamin D_3; 10 000 mg of vitamin E; 500 mg of vitamin K_3; 700 mg of vitamin B_1; 2 000 mg of riboflavin; 1 200 mg of vitamin B_6; 6 mg of vitamin B_{12}; 400 mg of folic acid; 72 mg of biotin; 15 000 mg of niacin; 120 000 mg of choline; 4 200 mg of calcium pantothenicum; 30 000 mg of Mn; 18 000 mg of Zn; 12 000 mg of Fe ; 3 000 mg of Cu ; 200 mg of I; 60 mg of Se; 40 mg of Co; 15 g of Ca.

Furthermore, spectrophotometric analyses were made in blood plasma to determine the level of superoxide dismutase (SOD) – with the adrenaline method [accorging to Misra] [Greenwald 1985] modified in respect of the wavelength, i.e. $\lambda = 320$ nm. The method was modified in order to achieve higher selectivity of transitional reaction products at the same length of light [Bartosz 1995]. The activity of catalase (CAT) enzyme was determined according to Bartosz [1995]. Analyses conducted in blood plasma for parameters of the antioxidative system included assays of: ferric-reducing ability of plasma (FRAP) – according to Benzie and Strain [1996], and vitamin C – according to Omaye *et al.* [1979]. In addition, the above-mentioned biological material was analyzed for levels of lipid peroxidation products, i.e.: peroxides (H_2O_2) – according to Gay and Gębicki [2002], and malondialdehyde (MDA) as the end product of tissue lipids oxidation – according to Salih *et al.* [1987].

Numerical data obtained were subjected to a one-way analysis of variance ANOVA, assuming the significance level of 0.05, using Statistica ver. 6.1 software.

RESULTS AND DISCUSSION

Results of analyses referring to levels of selected biochemical indices in blood plasma of the turkey hens examined were presented in Table 2. In the case of those indices, over the entire fattening period, statistically significantly ($p \leq 0.05$) lower values were noted for uric acid and urea in the group receiving L-carnitine addition, compared to the control group. The turkey hens administered L-carnitine with drinking water were also characterized by lower ($p \leq 0.05$) levels of creatinine, bilirubin and albumin, yet already at the end of rearing (15^{th} week of life). Uric acid is the end product of purine bases metabolism in the body. It additionally displays significant antioxidative effects in body fluids. It is an inhibitor of lipids peroxidation, enters into reactions with oxidants, binds iron ions, and is capable of capturing a hydroxyl radical. An increased content of uric acid in blood plasma may be due to adaptation response of the body to oxidative stress induced by the impairment of antioxidative mechanisms or/and increased rate of the generation of reactive oxygen species [Augustyniak and Skrzydlewska 2009]. An increased plasma level of urea (the end product of nitrogen metabolism of proteins), likewise that of uric acid, may be an indicator of oxidative stress and an outcome of body adaptation to enhanced generation of reactive oxygen species. This has been confirmed by results of experiments conducted on rats with acute pancreatitis [Barham *et al.* 2006]. Therefore, the significant decrease in plasma levels of uric acid and urea in the turkey hens receiving L-carnitine may indicate that their bodies were free of oxidative stress. As reported by Dayanandan *et al.* [2001], Gulcin [2006] and Urbaityte *et al.* [2006], L-carnitine is capable of eliminating reactive oxygen species. Creatinine is the key metabolite of skeletal muscles and originates from metabolic transformations of creatine. The latter serves to accumulate and release energy indispensable for the course of many chemical processes ongoing in cells [Pawłowska-Góral *et al.* 2003]. The enhanced production of creatinine is due to physical effort which is linked with increased demands of cells for oxygen and with higher quantities of reactive oxygen species generated in the body [Moffarts *et al.* 2005]. The application of L-carnitine in turkey hens diet, however, caused a decrease in creatinine level in blood plasma of the birds at the

terminal period of fattening, which also points to a low level of reactive oxygen species and, thus, to the balance of pro- and antioxidative processes. Bilirubin belongs to low-molecular intervention antioxidants, acting in the water phase, that interrupt free-radical processes by entering into reactions with reactive oxygen species and neutralizing them [Arivazhagan *et al.* 2000]. Taking onto account the fact that the increased blood level of bilirubin may induce toxic damage, inflammation, diseases and cirrhosis of liver, which are accompanied by excessive production of reactive oxygen species and insufficient antioxidative defense, it may be speculated that the application of L-carnitine supplement that reduced plasma level of bilirubin may prevent those ailments. A diminished level of bilirubin was also noted in blood plasma of turkey hens receiving a mixture of synthetic antioxidants [Czech and Ognik 2010].

Table 2. Level of biochemical indices in blood of turkey hens
Tabela 2. Poziom wskaźników biochemicznych we krwi indyczek

Parameter Cecha	Week of life Tydzień życia	Feeding groups Grupy doświadczalne		SEM
		control kontrola	L-carnitine L-karnityna	
UA µmol l^{-1}	9	280.4[a] ± 23.1	207.7[b] ± 28.6	3.24
	15	266.5[a] ± 22.6	179.3[b] ± 28.1	3.18
	$\bar{x}$	273.4[a] ± 22.85	193.5[b] ± 28.35	3.14
UREA mmol l^{-1}	9	0.77[a] ± 0.11	0.46[b] ± 0.06	0.021
	15	0.68[a] ± 0.13	0.43[b] ± 0.07	0.035
	$\bar{x}$	0.725[a] ± 0.12	0.445[b] ± 0.065	0.024
CREAT µmol l^{-1}	9	16.08 ± 3.51	16.0 ± 3.06	0.102
	15	37.2[a] ± 9.65	27.2[b] ± 7.58	0.245
	$\bar{x}$	26.63 ± 6.58	21.59 ± 5.32	0.321
BIL µmol l^{-1}	9	3.85 ± 0.77	3.49 ± 0.50	0.035
	15	4.63[a] ± 0.49	3.54[b] ± 0.36	0.028
	$\bar{x}$	4.24 ± 0.63	3.51 ± 0.43	0.034
ALBUMIN g l^{-1}	9	13.52 ± 1.40	13.36 ± 1.11	0.232
	15	15.64[a] ± 2.27	12.91[b] ± 0.89	0.654
	$\bar{x}$	14.58 ± 1.835	13.13 ± 1.0	0.423

a b – values in rows denoted with various letter differ statistically significantly, $p \leq 0.05$
a, b – wartości w wierszach oznaczone różnymi literami różnią się istotnie przy $p \leq 0,05$
UA – uric acid – kwas moczowy, UREA – urea – mocznik, CREAT – creatinine – kreatynina, BIL – bilirubin – bilirubina, ALBUMIN – albumin – albumina

Albumins constitute ca. 60% of total protein. They are produced in liver and are responsible for maintaining blood volume and binding hormones, drugs and amino acids. Together with albumin a numerous group of various substances are being transported in blood. By binding e.g. ions of transitory metals, those proteins attenuate the enhanced free-radical reactions [Dobicki *et al.* 2007]. They are included amongst preventing antioxidants, because they prevent the generation of reactive oxygen species, hydroxyl radi-

cal in particular [Pawłowska-Góral *et al.* 2003]. The reduced level of albumins in blood plasma of the turkey hens administered L-carnitine may point to the positive effect of this additive on reduced quantities of products of partial oxygen reduction formed in the body, which in turn does not require the increased engagement of preventing antioxidants in body defense against free oxygen radicals. However, experiments with ewes receiving L-carnitine addition demonstrated an insignificant increase of albumins in blood plasma [Citil *et al.* 2009].

Table 3. Levels of pro- and antioxidative markers in blood of turkey hens
Tabela 3. Poziomy markerów pro-i antyoksydacyjne we krwi indyczek

Parameter Cecha	Week of life Tydzień życia	Feeding groups Grupy doświadczalne		SEM
		control kontrola	L-carnitine L-karnityna	
H_2O_2 µmol l^{-1}	9	$4.39^{a} \pm 1.65$	$2.46^{b} \pm 1.08$	0.183
	15	4.01 ± 1.36	5.05 ± 1.78	0.172
	$\bar{x}$	4.20 ± 1.505	3.75 ± 1.43	0.121
MDA µmol l^{-1}	9	0.22 ± 0.08	0.25 ± 0.11	0.035
	15	0.40 ± 0.11	0.34 ± 0.09	0.038
	$\bar{x}$	0.31 ± 0.095	0.29 ± 0.10	0.025
SOD U ml^{-1}	9	23.9 ± 2.02	24.3 ± 1.65	0.725
	15	15.85 ± 1.65	15.60 ± 3.71	0.832
	$\bar{x}$	19.8 ± 1.83	19.95 ± 2.68	0.625
CAT U ml^{-1}	9	$7.1^{a} \pm 1.16$	$2.73^{b} \pm 0.72$	0.827
	15	6.81 ± 6.35	6.02 ± 1.93	1.110
	$\bar{x}$	$6.95^{a} \pm 3.75$	$4.37^{b} \pm 1.325$	1.023
FRAP µmol l^{-1}	9	$64.48^{a} \pm 4.4$	$49.59^{b} \pm 8.9$	1.247
	15	78.42 ± 17.4	78.34 ± 20.0	1.112
	$\bar{x}$	71.4 ± 10.9	63.9 ± 14.45	0.012
VIT C mg l^{-1}	9	$0.056^{b} \pm 0.01$	$0.154^{a} \pm 0.03$	0.012
	15	$0.058^{b} \pm 0.01$	$0.152^{a} \pm 0.02$	0.010
	$\bar{x}$	$0.057^{b} \pm 0.01$	$0.153^{a} \pm 0.025$	0.018

a, b – values in rows denoted with various letter differ statistically significantly, $p \leq 0.05$
a, b – wartości w wierszach oznaczone różnymi literami różnią się istotnie przy $p \leq 0,05$
H_2O_2 – hydrogen peroxide – nadtlenek wodoru, MDA – malondialdehyde – dialdehyd malonowy, SOD – superoxide dismutase – dysmutaza ponadtlenkowa, CAT – catalase – katalaza, FRAP – ferric-reducing ability of plasma – edukcja jonu żelaza, VIT C – vitamin C – witamina C

Data concerning changes in parameters of the redox status of blood plasma of the turkey hens receiving L-carnitine were collated in Table 3. The addition of L-carnitine was found to contribute significantly ($p \leq 0.05$) to diminished levels of hydrogen peroxide, catalase and total antioxidative potential (FRAP) in the early stage of fattening (9^{th} week of life). The administration of L-carnitine in drinking water for turkey hens caused also a decrease in plasma level of vitamin C noted throughout the whole fattening

period of the birds. Hydrogen peroxide is formed as a result of two electrons attachment to an oxygen molecule catalyzed by metal-flavin enzymes (e.g. xanthine oxidase oxidizing xanthine to uric acid) or as a result of an electron attachment to superoxide anion-radical in the reaction of dismutation catalyzed by superoxide dismutase. It is an exceptionally strong inhibitor of enzymatic systems [Bartosz 1995]. The lower level of hydrogen peroxide achieved in the reported study in blood plasma of turkey hens receiving L-carnitine points to a lack of the reaction of lipids peroxidation. It is likely to be due to the capability of L-carnitine for binding metal ions and capturing reactive oxygen species, including superoxide anion-radicals from which as a result of an electron attachment hydrogen peroxide is being formed that is toxic to cells [Citil *et al.* 2009]. The capability of L-carnitine for inhibiting lipids peroxidation has been demonstrated in a study conducted with rats that were characterized by a reduced level of malondialdehyde in tissues [Augustyniak *et al.* 2009]. The reduced plasma level of hydrogen peroxide noted in the turkey hens receiving L-carnitine was correlated with a significant decrease in the activity of catalase in their plasma. The enhanced activity of catalase, breaking down the accumulated hydrogen peroxide, is usually observed under stress and disease conditions [Baham *et al.* 2006]. An increased FRAP value in plasma is usually a desirable phenomenon as it proves better protection of cells and tissues against toxic effects of reactive oxygen species. The higher total antioxidant potential (FRAP) in the serum of control birds may, however, reflect organism's adaptation at the early stage of oxidative stress [Pawłowska-Góral *et al.* 2003]. For the highest number of health-related problems, often induced by stressful conditions of rearing, occur in turkeys, especially at the beginning of rearing. Therefore, the lower values of FRAP noted in plasma of the turkey hens receiving L-carnitine may point to reduced quantities of reactive oxygen species generated in the body and to adaptation of birds' bodies to the stress as a result of additive administration. The decreased ferric-reducing ability of plasma was determined in turkeys fed diets with the addition of a mixture of synthetic antioxidants [Czech and Ognik 2010]. Turkeys are capable of synthesizing ascorbic acid, yet in the intensive rearing their own synthesis often proves insufficient to cover metabolic demands. It is a fact that the appropriate supply of L-carnitine in diet exerts an economic effect on vitamin C, because the ascorbic acid is one of the co-factors in the biosynthesis of carnitine [Citil *et al.* 2009]. Its deficiency is noted the most frequently in animals from commercially-bred herds where animals are expected to reaching high production performance, therefore the administration of L-carnitine to turkey hens during intensive rearing seems to be advisable [Daskiran 2009]. By affecting an increase in the level of vitamin C in blood plasma and tissues, carnitine facilitates the functioning of the immune system, increases the antioxidative potential of the body and birds' resistance to environmental stress. Owing to the fact that most of the changes in antioxidative stress parameters were observed at the early stage of rearing (9^{th} week of birds life), it may be speculated that along with turkey hens body weight increasing the supplementation of L-carnitine in the dose of 0.83 ml/l exerted a less effective influence on the status of antioxidative defense.

Table 4. Level of microelements in blood of turkey hens
Table 4. Poziom mikroelementów we krwi indyczek

Parameter Cecha	Week of life Tydzień życia	Feeding groups Grupy żywieniowe		SEM
		control kontrola	L-carnitine L-karnityna	
Fe µmol l^{-1}	9	47.73 ± 6.1	49.03 ± 5.9	0.948
	15	27.30^{b} ± 4.3	43.73^{a} ± 3.6	0.752
	$\bar{x}$	42.51 ± 5.2	46.38 ± 4.75	0.781
Zn µmol l^{-1}	9	18.5^{b} ± 1.02	27.4^{a} ± 2.5	0.448
	15	31.8 ± 1.2	35.0 ± 1.7	0.335
	$\bar{x}$	25.2^{b} ± 1.11	31.2^{a} ± 2.1	0.426
Cu µmol l^{-1}	9	3.24 ± 1.09	3.89 ± 1.03	0.212
	15	4.98 ± 0.38	5.68 ± 0.47	0.318
	$\bar{x}$	4.11 ± 0.735	4.78 ± 0.75	0.131

a, b – values in rows denoted with various letter differ statistically significantly, $p \leq 0.05$
a, b –wartości w wierszach oznaczone różnymi literami różnią się istotnie przy $p \leq 0,05$

Results of analyses referring to the levels of microelements in blood of the turkey hens examined were presented in Table 4. The addition of L-carnitine ($p \leq 0.05$) affected an increased level of iron (16th week of life) and zinc (9th week of life). Ions of transitory metals: copper, zinc and iron, deserve special attention as they are constituents of catalytic centers of enzymes. Their optimal concentration in blood plasma enables the proper functioning of enzymatic antioxidative systems, and any deficiencies lead to their suppressed activity. Zinc belongs to low-molecular antioxidants, and is a co-factor of superoxide dismutase. Iron also belongs to low-molecular antioxidants and is a co-factor of catalase, as it imparts antioxidative properties to this enzyme. Nevertheless, the excess of Fe^{2+} contributes to the generation of a hydroxyl radical from hydrogen peroxide and superoxide anion-radical in the so-called Haber-Weiss' reaction, which may lead to the stimulation of the lipids peroxidation process [Kleczkowski *et al.* 2004].

CONCLUSIONS

1. The application of L-carnitine addition to drinking water for turkey hens caused a decrease in catalase activity, uric acid and urea thus contributing to increased levels of low-molecular antioxidants (vitamin C, iron, and zinc) in blood plasma.

2. The results obtained point to the feasibility of applying L-carnitine as an additive maintaining the oxidative-antioxidative balance in turkey hens. They, however, need to be verified in experiments with different modes of dosage.

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Streszczenie. Celem badań było określenie wpływu dodatku L-karnityny do wody dla indyczek na poziom wskaźników biochemicznych oraz antyoksydacyjnych krwi. Badania przeprowadzono na indyczkach typu ciężkiego Big-6, utrzymywanych od 6 do 16 tygodnia życia. Grupa I stanowiła grupę kontrolną, otrzymującą do picia czystą wodę. Indyczki z grupy II czterokrotnie, tj. w 5, 8, 11 i 14 tygodniu życia przez okres 5 dni otrzymywały wodę z dodatkiem płynnego preparatu L-karnityny w ilości 0,83 ml/l wody. Pod koniec 9 i 15 tygodnia życia indyczek pobrano krew do analiz z żyły skrzydłowej od 15 ptaków z każdej grupy doświadczalnej. W osoczu krwi oznaczono zawartość wskaźników biochemicznych: kwasu moczowego (UA), mocznika (UREA), bilirubiny (BIL), kreatyniny (CREAT), albuminy (ALBUMIN), żelazo (Fe), miedzi (Cu), cynku (Zn). W zakresie parametrów antyoksydacyjnego w osoczu krwi oznaczono dysmutazę ponadtlenkową (SOD), katalazę (CAT), całkowity potencjał antyoksydacyjny osocza (FRAP), witaminę C. Ponadto oznaczono również poziom produktów peroksydacji lipidów: nadtlenki (H_2O_2) oraz dialdehyd malonowy (MDA). Stwierdzono, że zastosowanie dodatku L-karnityny do wody pitnej dla indyczek przyczyniło się do wzrostu antyoksydantów niskocząsteczkowych – witaminy C, żelaza, cynku oraz obniżenia poziomu katalazy w osoczu krwi. Uzyskane wyniki wskazują na możliwość stosowania L-karnityny jako dodatku utrzymującego równowagę oksydacyjno-antyoksydacyjną indyczek, wymagają one jednak weryfikacji w dalszych badaniach obejmujących inny sposób dawkowania.

Słowa kluczowe: indyki, L-karnityna, krew, wskaźniki antyoksydacyjne i biochemiczne