ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol., Technol. Aliment. 10(3) 2011, 387-397
ISSN 1644-0730 (print) ISSN 1889-9594 (online)

AN ASSESSMENT OF THE INFLUENCE OF B GROUP VITAMINS ON THE C-REACTIVE PROTEIN CONCENTRATION AND CHOSEN INDICATORS OF PROTEIN METABOLISM IN MALE RATS*

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Introduction. The objective of this project was to examine on an animal model the influence of the diet composition and its supplementation with B group vitamins on the concentration of chosen protein indicators, including C-reactive protein (CRP) as an inflammation marker.
Material and methods. The research was done on 36 male, around 5-months-old Wistar rats. The animals were divided into 3 dietary groups: group I has received the basic food mix, which included, among others, whole grain wheat and maize; group II and III received a modified food mix, in which a part of whole corn wheat from the basic feed was replaced with wheat flour, and 50% of whole corn maize was replaced with saccharose. The animals from groups I and II were given clean, settled water from the water supply system, and group III was given water solution of vitamins B_1, B_2, B_6 and PP.
Results. It has been established that both the change of diet, as well as it supplementation with B group vitamins are conductive to a reduced consumption of feed, and as a result, a reduced consumption of proteins by the animals from these groups. In the muscles and livers of the animals fed modified unsupplemented feed a smaller amount of protein was found in comparison to the animals fed the basic feed. In the blood serum of the animals from this group a significant concentration of albumins and a significant rise in the concentration of creatinine was found in relation to the concentration observed in the animals fed the basic feed and the ones fed the modified supplemented feed. The concentration of CRP in the group of animals fed the modified unsupplemented feed was significantly higher than in the other two groups of animals. In the blood serum of the animals who

* The study was financed by the Ministry of Science and Higher Education under a research Project No 1248/PO1/2010/39.

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received the supplement the concentrations of glucose, staple protein and its fractions: albumins, α_2-globulins, β-globulins and γ-globulins, were significantly higher than in the group fed the basic feed. The concentration of CRP was significantly reduced in relation to the group of animals fed the modified unsupplemented feed.
Conclusions. The analysis of the results allows to conclude that the diet where the whole grain was isocalorically replaced by flour and saccharose was conclusive to a reduced consumption of feed and protein, which could have an impact on the smaller amount of protein in the muscles of the examined animals. The higher concentration of glucose, staple protein and its fractions: albumins, α_2-globulins, β-globulins and γ-globulins, observed in the blood of the examined rats as a result of the change in diet and intensified by B group vitamins supplementation, can be evidence of an ungoing inflammation, which was confirmed in the animals fed modified unsupplemented feed by the rise in CRP concentration. The fall in the CRP concentration in the blood serum of the rats fed the modified supplemented feed can prove that the repair process has already started.

Key words: supplementation, B group vitamins, protein, C-reactive protein (CRP), rats

INTRODUCTION

In the available literature B group vitamins are usually discussed with reference to their deficiency in human organisms [Anuszewska 2001] and different species of animals, with the assessment of their influence on the biochemical parameters of blood, metabolism [Maslovskaya and Lukashik 1990] and tolerated upper levels of intake.

For many years food producers have been using functional food additives (among others, B group vitamins) in such products as juices and soft drinks, soft drink concentrates, baked goods, dairy and foods aimed at particular groups of consumers. The additives in food products are supposed to have a positive impact on consumers' health, and the use of additives should be justified and balanced [Kunachowicz et al. 2004] and it should not lead to negative effects on one's health, which might result from, for example, an excessive supply.

The pharmaceuticals which are advertised in the media as a universal remedy to satisfy deficiency of energy providing and supportive nutrients are also being researched.

For many years, the Department of Human Nutrition Physiology has been conducting research on animal models concerning the impact of B group vitamin additives (B_1, B_2, B_6 and PP) on the organism's metabolism. The research has demonstrated that a change in diet composition and its supplementation with B group vitamins resulted in a reduced consumption of feed by the rats, which was also accompanied by a weight gain. In terms of carbohydrate and lipid metabolism, in the blood serum of the supplemented animals an increased concentration of glucose was found, as well as a decrease in insulin concentration and an increase of the concentration of VLDL fraction, triacylglycerides, total cholesterol and its fractions LDL, but a decrease of the fraction HDL--cholesterol concentration was confirmed [Friedrich and Goluch-Koniuszy 2007, Friedrich et al. 2005, 2009, Goluch-Koniuszy and Wierzbicka 2011].

What was also observed was a larger concentration of visceral and epicardial fat tissue, an increase of fat content in muscles and liver and a change in the content of fatty acids of the visceral fat tissue, which consists in an increase of concentration saturated and mono-unsaturated fatty acids and a decrease in the contents of the polyunsaturated fatty acids [Friedrich et al. 2005]. An intensification of free radical reactions under the influence of B group vitamin supplementation was also established [Friedrich and Dolot

2009, 2010]. The aforementioned changes may be conductive to local and general inflammation.

C-reactive protein (CRP), synthesised in liver hepatocytes, belongs to acute-phase proteins, which play an important role in nonspecific defence mechanism, that is the local and general inflammation, which is created in response to homeostasis disorder of an organism, which in turn is a result of varied damaging factors, including hyperglycaemia [Buckley et al. 2009].

For this reason, a decision was made to use an animal model to ascertain if a change in diet composition and its supplementation with B group vitamins can change the concentration of chosen protein indicators, including CRP as an inflammation marker.

MATERIAL AND METHODS

After an approval of the Local Bioethics Commission had been granted (permit no. 2/2010), the research was conducted on 36 male Wistar rats, age circa 5 months, of starting body weight 435.2 g ±29.8 g, which were located in separate cages, in an air conditioned vivarium of temperature 21 ±1°C and light/darkness cycle 12 h/12 h.

Following a week long conditioning in the vivarium environment the animals were randomized and sorted into three equinumerous groups of equal body weight, fed *ad libitum* on pelleted feeds composed of the same components, besides those differentiating, produced by the Feeds and Concentrates Plant in Kcynia, Poland, after having implemented the procedure 5.14.5. "Cleaning of machines and devices". Group I received the basic food mix, which included, among others, whole grain wheat and maize; groups II and III received a modified food mix, in which a part of whole corn wheat from the basic mix was replaced with wheat flour, and 50% of whole corn maize was replaced with saccharose; the rest of the ingredients stayed the same as in the basic mix (Table 1). The feeds were isocaloric (Table 2).

Table 1. Percentage of components of fodders

Component	Basic fodder, %	Modified fodder, %
Wheat	36.4	6
Corn grain	20	10
Wheat bran	20	20
Dry whey	3.0	3.0
Fodder salt	0.3	0.3
Soy-bean grain 48%	18	18
Fodder chalk	1.5	1.5
Fosforan 2-CA	0.8	0.8
Wheat flour (type 500)	–	30.4
Saccharose	–	10

Feed and Concentrate Manufactures in Kcynia, Poland, following implementation of Procedure 5.14.5 (Cleaning of Machinery and Equipment).

Table 2. Chemical composition of fodders used in the experiment

Component	Basic fodder	Modified fodder (Mf)
Total protein, %	19.1	18.5
Crude fat, %	2.8	2.3
Carbohydrates, %	63.8	65.5
Dry matter, %	91.8	92.3
Total ash, %	6.1	6.0
Brutto energy		
kcal·g^{-1}	3.99	3.98
kJ·g^{-1}	16.7	16.7
Metabolic energy		
kcal·g^{-1}	3.57	3.57
kJ·g^{-1}	14.9	14.9

The animals from groups I and II drank clean, settled water from the water supply system. The animals in group III, at a time of increased activity, were given 30 ml of water solution of vitamins, in the following amount: B_1 – 0.560 mg, B_2 – 0.130 mg, B_6 – 0.490 mg, PP – 5.25 mg per 100 g of feed. The quantity of given vitamins, calculated in relation to consumed feed, was five times higher than the difference between the amount of these ingredients in the basic food mix and the modified food mix, which in a way imitated human supplementation. After the solution was drunk, the animals were given clean, settled water from the water supply system.

The experiment lasted 6 weeks, in which the amount of consumed feed was being calculated, and in the supplemented group the amount of consumed vitamins was also estimated. The body weight of the animals was controlled once a week.

12 hours before the experiment was completed, the feed had been discontinued. Then the animals were anesthetised with an anaesthetic "Ketanest" and blood from their hearts was drawn. After the coagulum was centrifuged, in the obtained blood serum the following was marked: total protein concentration with application of biuret method, using Marcel Media Bio spectrophotometer; protein fractions (albumins and α_1-, α_2-, β-, γ-globulins) with an application of electrophoresis, in chambers and in Cormay agarose gel, where the reading was done with a use of densitometer DT-93; urea and creatinine and the activity of aspartate aminotransferase (AspAT) by means of the kinetic method with use of BioSystems biotests, on Marcel Media Bio spectrophotometer; CRP using ELISA, by means of the Rat-C-Reactive Protein set from BD^{TM}. In the prepared muscles (*m. quadriceps femoris*, *m. semimembranosus*, *m. adduktor femoris*, *m. superficialis gluteus*) and livers of the animals the amount of the staple protein was marked by means of Kjedahl method (PN-A-04018:1975), on the Kjeltec 2100 Foss Tecator distiller. The obtained results underwent the one-way analysis of variance with a use of statistics computer software Statistica®, using Duncan's new multiple range test.

RESULTS

The analysis of the results showed that both the change in dietary components, as well as its supplementation with B group vitamins were conductive to a decrease in feed consumption as per 100 g of body weight of the animals. As a result, the consumption of protein by the animals from these groups was significantly smaller (Table 3).

Table 3. Effects of diet and vitamin B supplementation on feed consumption, chemical composition of muscles and liver and body weight gain at male rats ($\overline{x}$ ±SD, n = 36)

Trait	Basic fodder (a)	Modified fodder (Mf) (b)	Mf + supplementation (c)	Statistically significant
Feed consumption, g	765 ±62.4	755 ±46.4	738 ±24.1	–
Feed consumption g·100 g^{-1} body weight	164 ±13.1	159 ±15.9	151 ±14.1	a-c**
Protein consumption g·100 g^{-1} body weight	31.3 ±2.5	29.4 ±1.1	27.9 ±2.6	a-b*, a-c**
Protein, %				
Muscle	25.2 ±0.9	23.6 ±0.3	23.3 ±0.3	a-b**, a-c**
Liver	24.4 ±0.4	23.9 ±0.7	24.2 ±0.2	a-b*
Body weight gain g·100 g^{-1} feed	5.1 ±1.0	4.7 ±1.3	5.3 ±1.7	–

*Statistically significant difference $p \leq 0.05$.
**Statistically significant difference $p \leq 0.01$.

In the muscles and livers of the animals fed the modified unsupplemented feed a smaller content of protein was ascertained in relation to the animals fed the basic feed (Table 3). In the blood serum of the animals of this group, in spite of the lack of significant changes in the staple protein concentration, a significantly higher concentration of albumins in relation to the concentration observed in the animals fed the basic feed and modified supplemented feed was observed (Table 4). Among the parameters which illustrated the rhythm of protein metabolism in the organism, a significant increase, in comparison with the group fed the basic feed, was ascertained in the case of creatinine. The concentration of CRP in the animals fed the modified unsupplemented feed was significantly higher, both in relation to the animals fed the basic feed, as well as those fed the modified supplemented feed.

In the blood serum of the animals which received the supplement the concentrations of glucose, stable protein and its fractions: albumins, α_2-globulins, β-globulins and γ-globulins were significantly higher than in the animals fed the basic feed (Table 4). On the other hand, the used B group vitamin supplementation of the modified feed resulted in the decrease in the creatinine concentration in the blood serum in comparison to the animals fed the modified feed. The concentration of CRP was significantly reduced in relation to the animals fed the modified unsupplemented feed. Once supplementation was applied, these amounts were comparable to those in the animals fed the basic feed.

Table 4. Effects of diet and vitamin B supplementation on serum concentration of glucose, chosen indicators of protein transmutation and C-reactive protein at male rats ($\overline{x}$ ±SD, n = 36)

Trait	Basic fodder (a)	Modified fodder (Mf) (b)	Mf + supplementation (c)	Statistically significant
Glucose, mmol·l^{-1}	5.69 ±0.60	5.94 ±0.57	6.34 ±0.75	a-c*
Total protein, g·l^{-1}	57.2 ±1.7	58.8 ±1.4	62.2 ±1.4	a-c**, b-c**
Albumin, g·l^{-1}	26.0 ±1.8	27.6 ±1.1	28.1 ±1.5	a-b*, a-c**
α_1-globulin, g·l^{-1}	14.0 ±1.5	13.8 ±0.9	14.8 ±1.7	–
α_2-globulin, g·l^{-1}	4.1 ±0.5	4.5 ±0.5	4.7 ±0.5	a-c*
β-globulin, g·l^{-1}	8.5 ±0.5	9.1 ±0.5	9.6 ±0.8	a-c**
γ-globulin, g·l^{-1}	3.8 ±0.6	3.8 ±0.7	4.6 ±1.0	a-c*, b-c*
A/G	0.85 ±0.1	0.88 ±0.1	0.84 ±0.1	–
AspAT, U·l^{-1}	48.1 ±13.7	42.7 ±9.9	39.7 ±10.1	–
Urea, mmol·l^{-1}	1.03 ±0.15	1.05 ±0.07	1.06 ±0.10	–
Creatinine, µmol·l^{-1}	4 932 ±406	5 365 ±274	5 047 ±247	a-b*, b-c*
CRP, ng·ml^{-1}	61.2 ±8.6	87.4 ±31.6	57.9 ±22.7	a-b**, b-c**

*Statistically significant difference $p \leq 0.05$.
**Statistically significant difference $p \leq 0.01$.

DISCUSSION

The analysis of the results shows that the change in the dietary components and the application of supplementation result in a significant decrease in the feed consumption among the researched animals. The factors taken into account, due to their influence on the feed consumption, had an impact on the smaller protein consumption. It could have resulted in the observed smaller concentration of protein in the muscles of the animals fed the modified feed, both the supplemented and the unsupplemented one.

Insulin is one of the factors contributing to the biosynthesis of the muscle proteins through the influence on the incorporation of amino acids in tissues. Taking into account the results of the research conducted by Friedrich and Sadowska [2005], who claimed that the change in diet, which took place also in this experiment, is conductive to insulin immunity among male rats, which might be confirmed by a significant increase in glucose concentration in the blood of the examined animals, also observed in this experiment. It can be assumed that a smaller concentration of protein in the muscles of the examined animals may have resulted from the slower protein synthesis, as a result of modified amino acid transport to tissues.

The smaller concentration of protein in the muscles of the animals fed the modified feed, in relation to those fed the control feed, was accompanied by a rise in creatinine concentration in the blood serum, probably as a result of their intensified catabolism. The lack of significant differences in AspAT activity or in urea concentration between

the groups of animals can be explained by the appropriate physiological rhythm of amino acids metabolism in tissue.

As was presented by Visser et al. [2002], a reduced muscle mass is correlated with a higher concentration of pro-inflammatory cytokines (IL-6 and TNF-α), which have an impact on the hepatic synthesis of CRP. Its increased concentration was observed in the presented research in the group of animals fed the modified feed (Table 4).

The concentration of particular protein fractions in the blood serum is a result of the pace of their synthesis and degradation and the division between the serum and interstitial fluid. The change in dietary composition and its supplementation with B group vitamins resulted in significant changes in the concentration of total protein and albumins, α_2-globulins and β-globulins, which indicates an increased activity of the liver in the process of their synthesis.

A significant increase in the concentration of fraction of γ-globulins, which includes immoglobulins and acute-phase protein CRP in animals fed the modified supplemented feed, in comparison to the animals fed the basic feed, can lead to an assumption that hepatocytes and the immune system were stimulated to defend the organism in reply to an increase in the glucose concentration in the blood [Ross 1999]. The increase in the glucose concentration, observed in both experimental groups, may be the factor which induces chronic inflammation, as it leads to glycation, activation of endothelium tissue, neutrophil granulocytes, monocytes, lymphocytes and trombocytes, initiating local inflammation [Festa et al. 2003, Haffner 2003].

The increase in CRP concentration in animals fed modified feed, as an independent factor in causing insulin immunity [Nakanishi et al. 2003, Yudkin et al. 1999], could also result from a significant increase, in the same rats, in triglycerides, total cholesterol and fraction of LDL cholesterol in the blood serum under the influence of the change in dietary components, as it has been already established in our research [Goluch-Koniuszy and Wierzbicka 2011]. This confirms the aforementioned positive correlation between the concentration of these blood parameters and CRP, and, as it has been ascertained in our previous research [Goluch-Koniuszy and Wierzbicka 2011], negative correlation between the low concentration of fraction of HDL cholesterol [Fröhlich et al. 2000, Nakano et al. 2010, Ridker et al. 2002, Streja et al. 2003]. During the inflammation process larger amounts of B_6 are used, which might deepen the already existing deficiencies resulting from the supply of this vitamin with the modified feed [Rall and Meydani 1993].

The observed increase in the glucose concentration in the blood of animals fed the modified supplemented feed, in comparison to the animals fed the basic feed, is puzzling in the context of a positive influence of thiamine in this respect. However, in the presented research the animals were fed a complex of vitamins, and it was established by Rabbani et al. [2009] that even though supplementing with thiamine lowers the glucose concentration in the blood of diabetics, administering it with other B group vitamins does not produce the same effect.

The applied supplementation with B group vitamins, in spite of a significant increase in glucose concentration in the blood of the examined animals, resulted in the lower concentration of CRP in the blood serum of examined animals in comparison to the group fed modified unsupplemented feed. Perhaps vitamin B_6 is responsible for this phenomenon, as its supplementation is negatively correlated with CR concentration [Friso et al. 2001, 2004]. In another research it has been also ascertained that supplementation with B_1 [Chang et al. 2007, Church et al. 2003], or B-complex or multivita-

mins [González-Ortiz et al. 2010] in different diseases resulted in a significant decrease of CRP concentration.

On the basis of the conducted research it cannot be explicitly ascertained if the used supplementation with B group vitamins of the modified diet, rich in monosaccarides, in the 6-week long experiment, managed to inhibit or initiate inflammations. It is necessary to conduct expanded research, which would include indicating local markers and general pro-inflammatory processes (e.g. IL1, IL6, TNF-α, ICAM-1; VCAM-1). As the research conducted by Laskowska-Klita and Czerwińska [2002] established, the increase of CRP concentration can be delayed in relation to the concentration of other inflammation markers, which influence the hepatic release of CRP [Libby and Ridker 1999]. Furthermore, the inflammatory response has several stages, spread in time, characterised by a varied kinetics of acute-phase proteins and may depend on individual qualities of the organism [Kastro et al. 1996]. Moreover, it cannot be ruled out that during 6 weeks of experimentation some phenomena might have already occurred in the organisms of the animals and they limited the destructive processes and stimulated the reproductive and repair processes to restore the homeostasis of the organism.

CONCLUSIONS

The analysis of the results allows to conclude that:

1. The diet in which whole grain was replaced isocalorically with flour and saccarose is conductive to a reduced consumption of feed and protein, which could have an impact on a smaller amount of protein in the muscles of the examined animals.

2. The increase in the concentration of glucose, total protein and its fractions: albumins, α_2-globulins, β-globulins and γ-globulins in the blood of the examined rats, observed under the influence of the change in dietary components, and increased with its supplementation with chosen vitamins from B group may be evidence of the ongoing inflammation, which in the animals fed the modified unsupplemented feed was confirmed with the increase of CRP concentration.

3. The observed decrease of CRP concentration in the blood serum of rats which were fed modified supplemented feed may prove that the repair processes were implemented; however, it requires further research, which will include local markers and general inflammation.

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OCENA WPŁYWU SUPLEMENTACJI DIETY WITAMINAMI Z GRUPY B NA STĘŻENIE BIAŁKA C-REAKTYWNEGO I WYBRANE WSKAŹNIKI PRZEMIAN BIAŁKOWYCH U SAMCÓW SZCZURA

Wstęp. Celem pracy było zbadanie na modelu zwierzęcym, jaki wpływ wywiera zmiana składu diety i jej suplementacja witaminami z grupy B na stężenia wybranych wskaźników białkowych, w tym białka C-reaktywnego jako markera procesów zapalnych.
Materiał i metody. Badania przeprowadzono na 36 samcach szczura rasy Wistar, w wieku około 5 miesięcy. Zwierzęta podzielono na trzy grupy żywieniowe: I otrzymywała mieszankę podstawową zawierającą m.in. pełne ziarna pszenicy i kukurydzy, II i III – mieszankę zmodyfikowaną, w której, w stosunku do podstawowej, część pełnych ziaren

pszenicy zastąpiono mąką pszenną, a 50% kukurydzy – sacharozą. Do picia zwierzęta grupy I i II otrzymywały czystą, odstaną wodę wodociągową, a grupy III – wodny roztwór witamin: B_1, B_2, B_6 i PP.

Wyniki. Stwierdzono, że zarówno zmiana składu diety, jak i jej suplementacja witaminami z grupy B sprzyjały zmniejszonemu spożyciu paszy, a w konsekwencji białka. W mięśniach i wątrobach szczurów żywionych paszą zmodyfikowaną niesuplementowaną wykazano mniejszą zawartość białka w stosunku do oznaczonej u zwierząt karmionych paszą podstawową. W surowicy krwi tej grupy zaobserwowano istotnie większe stężenie albumin oraz istotny wzrost stężenia kreatyniny w porównaniu z obserwowanym u zwierząt żywionych paszą podstawową oraz zmodyfikowaną suplementowaną. Stężenie białka C-reaktywnego u szczurów żywionych paszą zmodyfikowaną niesuplementowaną było istotnie większe niż w pozostałych. W surowicy krwi zwierząt suplementowanych, w stosunku do żywionych paszą podstawową oznaczono istotnie wyższe stężenia: glukozy, białka ogólnego i jego frakcji: albumin, α_2-globulin, β-globulin, γ-globulin. Stężenie białka C-reaktywnego zmniejszyło się istotnie w porównaniu z grupą zwierząt żywioną paszą zmodyfikowaną niesuplementowaną.

Wnioski. Analiza uzyskanych wyników pozwoliła na stwierdzenie, że dieta z pełnymi ziarnami zbóż izokalorycznie zastąpionymi mąką i sacharozą sprzyjała zmniejszonemu spożyciu paszy oraz białka, co mogło wpływać na mniejszą zawartość białka w mięśniach badanych zwierząt. Pod wpływem zmiany składu diety obserwowano – nasilany po zastosowaniu jej suplementacji wybranymi witaminami z grupy B – wzrost stężenia glukozy, białka całkowitego oraz jego frakcji: albumin, α_2-globulin, β-globulin i γ-globulin. Może on świadczyć o toczącym się procesie zapalnym, który u zwierząt żywionych paszą zmodyfikowaną niesuplementowaną został potwierdzony wzrostem stężenia białka C-reaktywnego. O uruchomieniu procesów naprawczych może świadczyć spadek stężenia CRP w surowicy szczurów żywionych paszą zmodyfikowaną suplementowaną.

Słowa kluczowe: suplementacja, witaminy z grupy B, białko, białko C-reaktywne (CRP), szczury

Received – Przyjęto: 23.12.2010

Accepted for print – Zaakceptowano do druku: 20.03.2011

For citation – Do cytowania: Goluch-Koniuszy Z., Sadowska J., Wierzbicka A., 2011. An assessment of the influence of B group vitamins on the C-reactive protein concentration and chosen indicators of protein metabolism in male rats. Acta Sci. Pol., Technol. Aliment. 10(3), 387-397.