



## EFFECTS OF PROTEIN INTAKE ON GLUTATHIONE CONCENTRATION AND GLUTATHIONE ENZYME ACTIVITY IN MICE

Danuta Rochon-Szmejchel<sup>1</sup> | Bożena Witek<sup>2</sup> | Grażyna Świderska-Kołacz<sup>2</sup> | Adam Kołataj<sup>3</sup> | Wojciech Hagner<sup>4</sup>

<sup>1</sup> Dandiete” Dietetic Outpatient Clinic, 13-300 Nowe Miasto Lubawskie, Poland (\*Corresponding author)

<sup>2</sup> Department of Animal Physiology, Institute of Biology, Jan Kochanowski University, 25-406 Kielce, Poland

<sup>3</sup> Institute of Genetics and Animal Breeding, Polish Academy of Sciences, 05-552 Jastrzębiec, Poland

<sup>4</sup> Department of Rehabilitation, Nicolaus Copernicus University Collegium Medicum, 85-094 Bydgoszcz, Poland

### ABSTRACT

The aim of the paper was to determine whether multi-generation selection for high body weight gains in experimental mice affects changes in reduced glutathione concentration and glutathione enzyme activity in the liver and kidney of subjects maintained on different protein level in their diets.

The experiment involved 200 mice obtained from the Institute of Genetics and Animal Breeding of the Polish Academy of Sciences in Jastrzębiec (Poland). 100 6-9 week-old Swiss male mice were sampled from a line selected for 24 generations for increased body weight (weights were  $35 \pm 3.2$  g) and 100 control subjects came from a randomly mated, non-selected line (weights were  $25 \pm 2$  g). The selected and non-selected mice received either a standard (16%) or low (10%) protein diet ( $n=10$  animals per each subgroup) for 21 consecutive days. These diets were almost isocaloric (14.04 and 13.47 MJ/kg). The animals were decapitated. In their liver and kidney homogenates, reduced glutathione (GSH) concentration and glutathione transferase, peroxidase and reductase activity were determined.

The reduction of protein level (from 16% to 10%) in diets of animals decreased GSH concentration and glutathione enzyme (transferase and peroxidase) activity in the liver and kidney of selected and non-selected mice. Glutathione reductase revealed a higher activity in the kidney of non-selected mice maintained on the low-protein diet. It may be suggested that GSH concentration and glutathione enzyme activity play an important role in cellular biochemical adaptation, significantly reacting to disturbances of physiological homeostasis. Prolonged selection for increased body weight did not influence the studied parameters.

**Keywords:** Glutathione, Glutathione Enzymes, Protein Intake.

### INTRODUCTION

#### Protein in food intake

One of the factors exerting a significant influence on the metabolic status of animals and humans is the type and amount of food intake, diversified mainly in terms of its energy value and the amount of biologically active protein (Smith et al., 2010; Weigle et al., 2005; Witek et al., 2014). Numerous studies (Witek et al., 2007; Araki et al., 2014; Darling et al., 2009) have proved that a protein-deficient diet disrupts the course of major metabolic pathways and inhibits multidirectional cell functions (Emmans, 1994; Linn et al. 2000).

#### Glutathione

Glutathione has been found to be one of the most interesting forms of molecules playing a key role in the oxidation-reduction system of all tissues and cells present in living organisms, and has thus acquired a voluminous body of literature, currently being addressed in approximately 400 scientific publications a year. The reduced form (GSH) can reversibly convert to its oxidized form (GSSG) and create glutathione disulfide. However, in most cells GSSG constitutes barely 1-3% of total glutathione concentration, whereas 97-99% of their overall content is the GSH form. There has been an extensive literature on this matter (Biswas and Rahman, 2009; Circu and Aw, 2008; Śliwa-Jóźwik et al., 2002; Tormos et al., 2004). The increasingly prevalent interpretations lead to the general acceptance that reduced glutathione might

constitute not only an important indicator, but also regulator of systemic homeostasis.

Glutathione S-transferases (GST, EC 2.5.1.18) demonstrate a high versatility within cells, being present in the cytosol as well as the mitochondria and microsomes. They play a significant role in the detoxification of different harmful xenobiotics, both exogenous and endogenous (Fujii et al., 2011; Morgenstern et al., 2011).

Glutathione peroxidases (GPx, EC 1.11.1.9) protect the cell mainly from free oxygen radicals, exhibit a capacity for the reduction of both organic and inorganic peroxides, contain selenium atoms in their molecules, usually in the form of selenocysteine in the active site. Glutathione peroxidases act in concert with superoxide dismutase (SOD) and catalase (CAT), essentially protecting cellular structures against oxygen radicals (Arthur, 2000; Chabory et al., 2010).

Glutathione reductase (GR, EC 1.6.4.2) catalyzes the reduction of glutathione disulfide (GSSG) into reduced glutathione (GSH) (Bazzichi et al., 2002; Ulus and Tandogan, 2007).

Selection is a method widely used in animal husbandry and has already been addressed by a large number of reports concerned with methods for improving their utility properties. One of the areas of its application is the attempt to raise the rate of body size increase in growing animals (Eisen and Coley, 1990; Moruppa et al., 1990).

## Aim of the study

Glutathione is considered to be a significant indicator of cellular homeostasis. The aim of the study was to observe whether a decrease in the amount of protein in the diet of mice used for the experiment from the standard 16% to the level of 10% as well as selection for improved growth rate considerably affect the concentration of reduced glutathione (GSH) in the liver and kidney of test subjects and alter the activity of glutathione enzymes in the form of glutathione transferase, peroxidase and reductase in the said glands.

## MATERIALS AND METHODS

### Animal test subjects and tissue processing

The study was conducted on 200 male Swiss mice. The first group consisted of 100 subjects aged 8-10 weeks and with a mean body weight of  $25.0 \pm 2$  grams. The mice were selected randomly from the population bred by the Institute of Genetics and Animal Breeding of the Polish Academy of Sciences in Jastrzębiec. The second group consisted of 100 subjects with a mean body weight of  $35.0 \pm 3.2$  grams, which were also obtained from the Institute and picked out from the population of animals bred selectively for improved growth rate by coupling parent subjects of the highest body mass through 24 successive generations. There was a statistically significant difference in body weight between the two animal subject groups ( $p < 0.001$ ). After being taken away from their mothers at the age of 6 weeks, the mice were kept under standard farm conditions. During the first 21 days at the beginning of the experiment, that is between the sixth and ninth month of age, test animals from both groups were fed with diets containing respectively 10% and 16% protein and having comparable energy values. The first group, considered as control, included mice maintained on a standard diet (SD) containing 16% protein. The second group consisted of mice fed with a low-protein diet (LPD) containing 10% protein. Both groups of mice were subjected to the said dietary regimens for the period of 21 days. The animals were healthy and in good physical condition, and were provided with constant professional veterinary care.

After the ninth week of life and third week of the experiment, mice from both groups were subjected to short-term anesthesia induced by diethyl ether, then euthanized by cervical dislocation and decapitated, after which the livers and kidneys were isolated. The livers were perfused with physiological saline solution at  $4^{\circ}\text{C}$  to remove residual blood. Tissues were cleaned, weighted out and cut into pieces on ice. The pieces were homogenized using a low-speed Potter-Elvehjem tissue grinder (smooth-walled tube made of thick Jena glass with a fitted Teflon pestle on a stainless-steel shaft rotated by motor drive) in 0.1M phosphate buffer, pH 7.4, containing 10 mM EDTA, at a ratio of 100 mg tissue to 1 ml buffer. Homogenization was performed at  $5^{\circ}\text{C}$  and 200 rpm of an MR-25 shaft by means of five full up- and downward movements of the pestle (to the bottom of the tube). The obtained homogenates were centrifuged for 10 min at 12 thousand rpm and  $+4^{\circ}\text{C}$ , in a Janetzki K-24 centrifuge.

The experiment was carried out upon permission no. 14/2013 and 15/2013 granted by the Local Ethics Committee for Animal Experimentation in Bydgoszcz, in accordance with the Act of

2005 on animal experimentation (Dz. U. [Journal of Laws] No. 33, item. 289) and the regulation of the Minister of Scientific Research and Information Technology of 2005 on the National Ethics Committee for Animal Experimentation and local ethics committees for animal experimentation (Dz. U. [Journal of Laws] No. 153, item. 1275).

## Analytical methods

### Determination of reduced glutathione concentration

The concentration of reduced glutathione in the analyzed liver and kidney homogenates was determined using the Ellman method (Ellman, 1959).

### Determination of glutathione transferase activity

The activity of glutathione transferase was determined using the method described by Habig et al. (1974).

### Determination of glutathione peroxidase activity

The activity of glutathione peroxidase was determined using the method described by Chiu et al. (1976).

### Determination of glutathione reductase activity

The activity of glutathione reductase was determined using the method described by Szczeklik (1974).

### Determination of total protein

Protein concentration was determined using the method of Lowry et al. (1951), as modified by Kirschke and Wiederaenders (1984).

## Statistical analysis of results

The obtained results were analyzed statistically by means of the Student's t-test and multifactorial analysis of variance using the SAS/STAT software [1999-2001].

## RESULTS

Results obtained in the study are presented in Table 1.

**Table 1.** Changes in the concentration of reduced glutathione and the activity of glutathione transferase, peroxidase and reductase in the liver and kidney of experimental mice (n per each subgroup = 10).

Diet	Liver		Kidney	
	Non-selected mice	Selected mice	Non-selected mice	Selected mice
<b>Glutathione concentration</b> [mmol/g protein/hour]				
16%	$5.54 \pm 0.61^{1a}$	$5.94 \pm 0.92^{2b}$	$2.27 \pm 0.28^{3a}$	$2.43 \pm 0.29^{4b}$
10%	$4.06 \pm 0.36^{1c}$	$4.13 \pm 0.49^{2d}$	$2.03 \pm 0.22^{3c}$	$1.93 \pm 0.26^{4d}$
<b>Glutathione transferase activity</b> [mmol/g protein/hour]				
16%	$327.14 \pm 29.44^5$	$317.06 \pm 34.88^6$	$132.15 \pm 22.46^7$	$141.16 \pm 21.87^8$

10%	261.34 ± 29.68 <sup>5</sup>	273.18 ± 24.58 <sup>6</sup>	73.49 ± 10.55 <sup>7</sup>	80.36 ± 9.13 <sup>8</sup>
<b>Glutathione peroxidase activity</b> [mmol/g protein/hour]				
16%	39.55 ± 1.38 <sup>9</sup>	43.29 ± 5.39 <sup>10</sup>	12.41 ± 1.12 <sup>11</sup>	12.38 ± 1.54 <sup>12</sup>
10%	30.22 ± 4.22 <sup>9</sup>	34.28 ± 4.78 <sup>10</sup>	11.02 ± 0.88 <sup>11</sup>	10.22 ± 1.34 <sup>12</sup>
<b>Glutathione reductase activity</b> [mmol/g protein/hour]				
16%	16.99 ± 2.21 <sup>13</sup>	15.28 ± 2.14 <sup>14</sup>	3.68 ± 0.51 <sup>15,e</sup>	2.89 ± 0.26 <sup>e</sup>
10%	14.21 ± 1.85 <sup>13</sup>	12.34 ± 0.98 <sup>14</sup>	4.35 ± 0.56 <sup>15,f</sup>	2.99 ± 0.33 <sup>f</sup>

1-1;... 15-15; a-a; ... f-f - p<0.01;

The table shows that there was no significant difference in the values of glutathione concentration in the liver between the groups of non-selected and selected mice on the standard diet (5.54 against 5.94 mmol/g protein) and on the low protein diet (4.06 against 4.13). However, test subjects maintained on the diet with standard protein levels demonstrated a significantly higher amount of glutathione in the liver as compared to animals fed with the low-protein diet (5.54 against 4.06 mmol/g at p<0.01 and 5.94 against 4.13 mmol/g at p<0.01).

Similarly, no differences were found in the kidney between test subjects from the non-selected and selected group (2.27 against 2.43 and 2.03 against 1.93). However, animals fed with a higher (standard) amount of protein in their diet demonstrated a higher level of glutathione as compared to subjects receiving the low-protein diet (2.27 against 2.03 at p<0.05 and 2.43 against 1.93 at p<0.01).

The liver was found to have higher levels of glutathione than the kidney (5.54 against 2.27 and 5.94 against 2.43 for the standard diet, and 4.06 against 2.03 and 4.13 against 1.93 for the low-protein diet at p<0.01).

Glutathione transferase did not reveal any significant differences in the activity of the analyzed enzyme between the selected and non-selected control animals (327.14 against 317.06 and 261.34 against 273.18). However, animals fed with low-protein diet demonstrated a significantly lower activity of glutathione transferase as compared to animals fed with the diet containing normal protein levels (261.34 against 327.14 and 273.18 against 317.06 at p<0.01).

Animals fed with the standard diet were found to have significantly higher levels of transferase activity in the kidney than those maintained on the low-protein diet (132.15 against 73.49 and, respectively, 141.16 against 80.36 at p<0.01). No differences were found between the groups of selected and non-selected animals (132.15 against 141.16 and 73.49 against 80.36).

The liver of animals fed with the normal-protein diet demonstrated a higher level of glutathione peroxidase activity as compared to those receiving diet which was low in protein (39.55 against 30.22 and 43.29 against 34.28 at p<0.01). No

statistically significant differences were observed with respect to the levels of glutathione peroxidase activity between the groups of selected and non-selected animals (39.55 against 43.29 and 30.22 against 34.28).

The kidney of animals fed with normal levels of protein demonstrated significantly higher levels of glutathione peroxidase activity (12.41 against 11.02 and 12.38 against 10.22, respectively), while no such differences were found between the genetic groups (12.41 against 12.38 and 11.02 against 10.22).

Glutathione reductase activity was higher in the liver of mice fed with the standard diet (16.99 against 14.21 and 15.28 against 12.34 at p<0.01). No such differences were found between the genetic groups (16.99 against 15.28 and 14.21 against 12.34). As regards the kidney, non-selected animals receiving low-protein diet had a higher level of glutathione reductase activity as compared to the group receiving standard diet (4.35 against 3.68 at p<0.01). Statistically significant differences were also found between the genetic groups (3.68 against 2.89 and 4.35 against 2.99 at p<0.01).

## DISCUSSION

Diets containing different levels of protein induce observable changes in the metabolism of both humans and laboratory animals. Data obtained in the experiment indicate that reducing the level of protein in the diet of the test mice was connected with a significant decrease in the concentration of reduced glutathione in the liver and kidney of the animals. Similarly, there was a significant decrease in the activity of glutathione transferase and glutathione peroxidase. The activity of glutathione reductase was also decreased in the liver, whereas in the kidney of non-selected mice it was increased.

The revealed changes, except for the last case, can lead to the conclusion that there exists a certain regularity whereby a diet deficient in protein may trigger unfavorable changes in the profile of the biochemical homeostasis of the organism through decreasing, among other things, the level of reduced glutathione serving as an antioxidant essential for the reduction of free oxygen radicals in the cells in the course of energetic reactions occurring within.

A decrease in the level of protein in the diet of the test animals also translated into changes in the activity of the analyzed enzymes, which validates the above assumptions.

## Selection

It is worth noting that selection was found not to contribute to significant differences with respect to the analyzed parameters since no such differences were revealed when comparing the group of selected animals against the group of non-selected ones. The only exception was the activity of glutathione reductase in the kidney, whose levels were found to be significantly lower in mice selected for higher body weight gain than in non-selected mice. Also, the kidneys of selected animals demonstrated no statistically detectable differences in glutathione reductase activity between the group of mice fed with low-protein chow and the control group fed with a standard amount of protein. This was the only case which was unlike all other comparisons where the revealed differences were always to the benefit of animals fed with a normal amount

of protein. The exception seemed not to disrupt the observed regularity whereby the level of glutathione and the activity of the analyzed glutathione enzymes were significantly lower in animals fed with low-protein diet than in those fed with optimal levels of protein.

### General considerations

Results obtained suggest that through decreasing both the concentration of glutathione and the activity of the analyzed enzymes, both the liver and the kidney exhibited great flexibility and adaptivity to the modified intake of protein in food. The present study, based on the proposed experimental model, might contribute to the general interpretation of the nature of homeostasis in tissues of animals and humans. These conclusions may suggest the need for further experiments connected with some processes of aging. Some researchers claim that a possible method is to reduce the level of food intake to 30%, or even to 50%, particularly in rodents. The source of such a correlation is supposed to lie in lowering the rate of tissue metabolism, thus decreasing the amount of free radicals and consequently the extent of damage that they cause. Glutathione concentration in tissues of subjects maintained on a diet deficient in protein might constitute a good indicator of such correlations, which was also validated by the presented experiment (Garlick et al., 1999; Hum et al., 1992; Uhlig and Wendel, 1992; Świdarska-Kolacz, 2007).

A reduction in the level of glutathione in cells, e.g. in the case of protein intake deficiency, might be assumed to correlate with a lack of amino acids necessary for its synthesis, particularly methionine. A likely result is that the organism might change the tempo and directions of the use of amino acids depending on the compromised metabolic process and direct the quantitatively deficient amino acid to, for instance, the resynthesis of an enzyme which is needed urgently in a given metabolic situation.

A decrease in the activity of glutathione transferase in the liver and kidney of animals with a lower intake of protein might also contribute to weakening the intracellular antioxidant protection, particularly in conjunction with the observed parallel signs of a decrease in the level of glutathione. This enzyme remains interconnected with glutathione reductase, as a decrease in GSH concentration correlates with an increase in GSSG, which is then reduced back to the GSH form through the agency of glutathione reductase. An increase in the concentration of GSSG is harmful for the cells, especially above a certain physiological threshold, as is in the case of oxidative stress. Therefore, due to its capacity to reduce GSSG levels, glutathione reductase is then especially needed by the organism.

It may be suggested that not only a complete lack of protein in food intake or a total starvation regimen, but so much as a certain degree of protein deficiency might affect the level of the most important tissue antioxidant, that is glutathione, and modify the oxidation-reduction system based on its enzymes, thus also modulating their activity.

### Conclusions

1. A decrease in the level of protein from 16% to 10% in the diet of the test animals correlated with a significant decrease in the concentration of reduced glutathione in

the liver and kidney of both non-selected experimental mice and mice selected for increased body weight.

2. Activity of glutathione transferase and glutathione peroxidase in the liver and kidney of the test animals was found to be significantly lower in the group of animals receiving low-protein diet.
3. Activity of glutathione reductase in the kidney of non-selected animals maintained on a low-protein diet (10%) was found to exhibit higher values than in animals maintained on a diet containing an optimal amount of protein (16%).
4. Selection for increased body weight gain of test animals was revealed to have no significant influence on the level of glutathione in their liver and kidney and on changes in the activity of the analyzed glutathione enzymes in the said glands.
5. It might be suggested that the clearly observable reactions of the analyzed parameters to the effects of reduced protein intake constitute a reflection of disturbances in the existing normal, that is physiological, homeostasis of the oxidation-reduction potential of hepatic and renal cells. Thus, the above analyzed reactions might be regarded as indicators of homeostasis and systemic adaptation applied for the purposes of the analysis of the dietetic nutrition of animals and humans.

### Declaration of Conflicting Interest

The Authors declare that there is no conflict of interest.

### REFERENCES

1. Araki S., Okazaki M. and Goto S. (2014). Impaired lipid metabolism in aged mice as revealed by fasting-induced expression of apolipoprotein mRNAs in the liver and changes in serum lipids. *Gerontology*, 50, p. 206-215.
2. Arthur J.R. (2000). The glutathione peroxidases. *Cellular and Molecular Life Sciences*, 57, p. 1825-1835.
3. Bazzichi L., Ciompi M.L., Betti L., Rossi A., Melchiorre D., Fiorini M., Giannaccini G. and Lucacchini A. (2002). Impaired glutathione reductase activity and levels of collagenase and elastase in synovial fluid in rheumatoid arthritis. *Clinical and Experimental Rheumatology*, 20, p. 761-766.
4. Biswas S. and Rahman I. (2009). Environmental toxicity, redox signaling and lung inflammation: the role of glutathione. *Molecular Aspects of Medicine*, 30, p. 60-76.
5. Chabory E., Damon C., Lenoir A., Henry-Berger J.,

Vernet P., Cadet R., Saez F. and Drevet J.R. (2010). Mammalian glutathione peroxidases control acquisition and maintenance of spermatozoa integrity. *Journal of Animal Science*, 88, p. 1321-1331.

6. Chiu D.T.Y., Stults F.H. and Tappel A.L. (1976). Purification and properties of rat lung soluble glutathione peroxidase. *Biochimica et Biophysica Acta*, 445, p. 558-566.

7. Circu M.L. and Aw T.Y. (2008). Glutathione and apoptosis. *Free Radical Research*, 42, p. 689-706.

8. Darling A.L., Millward D.J., Torgerson D.J., Hewitt C.E. and Lanham-New S.A. (2009). Dietary protein and bone health: a systematic review and meta-analysis. *The American Journal of Clinical Nutrition*, 90, p. 1674-1692.

9. Eisen E.J. and Coley M.T. (1990). Correlated responses in body composition based on selection for different indicator traits in mice. *Journal of Animal Science*, 68, p. 3557-3562.

10. Ellman G.L. (1959). Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*, 82, p. 70-77.

11. Emmans G.C. Effective energy: a concept of energy utilization applied across species. *British Journal of Nutrition*, 71, p. 801-821.

12. Fujii J., Ito J., Zhang X. and Kurahash T. (2011). Unveiling the roles of the glutathione redox system in vivo by analyzing genetically modified mice. *Journal of Clinical Biochemistry and Nutrition*, 49, p. 70-78.

13. Garlick P.J., McNurlan M.A. and Patek C.S. (1999). Adaptation of protein metabolism in relation to limits to high dietary protein intake. *European Journal of Clinical Nutrition*, 53, S34-S43.

14. Habig W.H., Pabst M.J. and Jakoby W.B. (1974). Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *The Journal of Biological Chemistry*, 249, p. 7140-7147.

15. Hum S., Koski K.G. and Hoffer L.J. (1992). Varied protein intake alters glutathione metabolism in rats.

*Journal of Nutrition*, 122, p. 2010-2018.

16. Kirschke H. and Wiederanders B. (1984): Methodenzur Aktivitätsbestimmung von Proteinase [Methods for determining proteinase activity], in: Kongress und Tagungsberichte der Martin-Luther-Universität Halle-Wittenberg. Halle (Saale). pp. 11-17.

17. Linn T., Santosa B., Gronemeyer D., Aygen S., Scholz N., Busch M. and Bretzel R.G. (2000). Effect of long-term dietary protein intake on glucose metabolism in humans. *Diabetologia*, 43, p. 1257-1265.

18. Lowry O.H., Rosebrough N.J., Farr A.L. and Randall R.J. (1951). Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry*, 193, p. 265-267.

19. Morgenstern R., Zhang J. and Johansson K. (2011). Microsomal glutathione transferase 1: mechanism and functional roles. *Drug Metabolism Reviews*, 43, p. 300-306.

20. Moruppa S.M., Hill W.G. and Sinnott-Smith P.A. (1990). Effect of selection for growth, body composition and food intake by feeding. *Livestock Production Science*, 24, p. 259-271.

21. Smith D.L. Jr., Nagy T.R. and Allison D.B. (2010). Caloric restriction: what recent results suggest for the future of ageing research. *European Journal of Clinical Investigation*, 40, p. 440-450.

22. Szczeklik E. (1974). *Enzymologia Kliniczna* [Clinical enzymology], Państwowy Zakład Wydawnictw Lekarskich, Warszawa, pp.221-223.

23. Śliwa-Jóźwik A., Jóźwik A. and Kołataj A. (2002). Influence of exogenous glutathione (GSH) as stress factor on the activity of lysosome enzymes in some organs of mice. *ArchivTierzucht*, 45, p. 307-314.

24. Świdarska-Kolacz G., Klusek J. and Kołataj A. (2007). The effect of exogenous GSH, GSSG and GST-E on glutathione concentration and activity of selected glutathione enzymes in the liver, kidney and muscle of mice. *Animal Science Papers and Reports*, 2, p. 111-117.

25. Tormos C., Javier Chaves F., Garcia M.J., Garrido F., Jover R., O'Connor J.E., Iradi A., Oltra A., Oliva M.R. and Sáez G.T. (2004). Role of glutathione in the induction of apoptosis and c-fos and c-jun mRNAs by oxidative stress in tumor cells. *Cancer Letters*, 10, p. 103-113.

26. Uhlig S. and Wendel A. (1992). The physiological consequences of glutathione variations. *Life Sciences*, 51, p. 1083-1094.

27. Ulus N.N. and Tandogan B. (2007). Purification and kinetic properties of glutathione reductase from bovine liver. *Molecular and Cellular Biochemistry*, 303, p. 45-51.

28. Weigle D.S., Breen P.A., Matthys C.C., Callahan H.S., Meeuws K.E., Burden U.R. and Purnell J.Q. (2005).

A high-protein diet induces sustained reductions in appetite, ad libitum caloric intake, and body weight despite compensatory changes in diurnal plasma leptin and ghrelin concentrations. *The American Journal of Clinical Nutrition*, 82, p. 41-48.

29. Witek B., Ochwanowska E., Baranowska D. and Kołataj A. (2007). The effect of selection for body weight on the activity of lysosomal enzymes in rat liver and kidney of mice. *Animal Science Papers and Reports*, 25, p.119-125.

30. Witek B., Fronczyk W., Jóźwik A., Walczak M., Kamińska A. and Kołataj A. (2014). The effect of dietary protein level on selected degradative enzyme activity of hepatocyte cellular subfractions in experimental mice. *Animal Science Papers and Reports*, 32, p. 269-277.