Academia Journal of Agricultural Research 3(12): 395-401, December 2015
DOI: 10.15413/ajar.2015.0162
ISSN: 2315-7739


Academia Publishing

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*Research Paper*

# Biochemical and Hematological Response of Rats on Defatted Rape Seeds Addition into the Diet

Jana Tvrdá[1], Nikol Tůmová[1], Alena Fučíková[1], Jarmila Zídková[2], Magdalena Melčová[2], Jiřina Száková1*, Petr Mlejnek[3], Václav Zídek[3], Oto Mestek[4], Antonín Kaňa[4], Pavel Tlustoš[1]

[1]Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences in Prague, Prague, Czech Republic.
[2]Faculty of Food and Biochemical Technology, University of Chemistry and Technology, Prague, Czech Republic.
[3]Institute of Physiology, Academy of Science of the Czech Republic, Prague, Czech Republic.
[4]Faculty of Chemical Engineering, University of Chemistry and Technology, Prague, Czech Republic.

*Corresponding author. E-mail: szakova@af.czu.cz. Tel: +420 224382753. Fax: +420 234381801.

*Accepted 28th August, 2015*

**ABSTRACT**

Defatted meals of oilseeds play an important role as a protein resource in animal nutrition, but differ in their nutritional profiles. In our experiment, the effects of increasing the defatted rape seeds (DRS) rate in the diet of male Wistar Kyoto rats on the main hematological and biochemical parameters were evaluated. Twenty-four animals were randomly divided into four groups per six animals and fed by a semi-synthetic diet according to the experimental design for forty-seven (47) days. The experimental groups were: i) group DRS0 fed with the control diet; ii) group DRS30, where 30% of the soybean meal in the diet was replaced with DRS; iii) group DRS60, where 60% of the soybean meal in the diet was replaced with DRS; and iv) group DRS100, where 100% of the soybean meal in the diet was replaced with DRS. The comparison of the results showed no significant ($P < 0.05$) differences among the values of the animal weight, red blood and white blood pictures, and indicators of the potential hepatotoxicity. In the opposite, the increasing DRS rate in the diet resulted in a decrease of plasmatic cholesterol concentration as well as, an increase of specific activity of the selected antioxidative enzymes such as glutathione reductase (GR), glutathione S-transferase (GST), thioredoxin reductase (TrxR), and catalase (CAT) in the plasma.The experimental results indicated that DRS can be used as an appropriate substitution of soybean meal with no adverse effect on the animals' health. Moreover, the results indicated positive health effects of the DRS leading to a decrease of cholesterol concentration and improvement of the antioxidative activity of the appropriate enzymes.

**Key words:** Rat, defatted rape seeds, hematological parameters, specific enzymatic activity.

## INTRODUCTION

Oilseed rape (*Brassica napus* L.), a member of the mustard family (*Brassicaceae*), is the predominant oil crop in Europe (Wittkop et al., 2009). In recent decades, the increasing interest for vegetable oils as food and/or biofuel has been reported (Abbadi and Leckband, 2011). Defatted rape seeds (DRS) is a protein-rich feedstuff resulting as a by-product during the rapeseed oil production through extraction (Meyer et al., 2010). Hill (1991) and Smithard (1993) suggested DRS as the most important protein-rich feedstuff in the temperate climate as an alternative to soybean meal. For example, Meyer et al. (2010) proved that DRS is suitable to replace soybean meal in diets for growing fattening bulls. The DRS contains between 31 and 37% nitrogen compounds where its quality strongly depends on rapeseed variety (Zeman et al., 2006). The overall digestibility of crude protein and amino acids of DRS were

in general comparable with conventional protein feed ingredients for pig nutrition (Presto et al., 2011). The nitrogen-corrected apparent metabolizable energy value of the extracted DRS vary according to their chemical constituents, especially the fiber components (Toghyani et al., 2014). Moreover, the digestibility of organic matter from DRS can be improved in piglets and adult pigs by approximately 10% by dehulling the seeds (Kracht et al., 2004). On the contrary, heating of the seeds at 130°C can result in decreasing digestibility of DRS amino acids for growing pigs (Almeida et al., 2014).

The excellent nutritional value of DRS is impaired by the presence of glucosinolates (Haddad and Allaf, 2004). Glucosinolates are the substituted esters of thio-amino acids, and their synthesis is based on the corresponding amino acids. The highest glucosinolate synthesis in developing seeds was reported cca. 26 days after flowering (Bhardwaj and Hamama, 2003). Glucosinolates are discussed mostly from the aspect of their anti-nutrition, anti-microbial, anti-fungicidal, and anti-bacterial effects and as natural bio-fumigants (Zukalová and Vašák, 2002).

Concerning the anti-nutrition properties, especially their decomposition products, iso-thio-cyanates can produce substituted 2-oxazolidinethione (goitrine) having a strong goitrogenic property (Zukalová and Vašák, 2002). Decreasing utilization of iodine in the thyroid gland resulting in low levels of triiodthyronine and thyroxine were reported as well as, hepatotoxic effect (Heaney et al., 1994 a,b). Moreover, the DRS also contains relatively high amounts of other anti-nutritive fiber compounds, phenolic acids and phytate etc (Mailer et al., 2008; Wittkop et al., 2009). The role of microflora of the gastrointestinal tract should be taken into account in this case (Bjergegaard et al., 1998; Campbell and Schöne, 1998).

Recently, almost all of the oilseed rape production in Europe is from zero erucic acid and low seed glucosinolate types (so-called 00-quality)(Wittkop et al., 2009). The DRS from these seeds contains glucosinolates in the range from 10 to 30 mmol.kg$^{-1}$as compared to 150 mmol.kg$^{-1}$ of glucosinolates in the 0-quality seeds (Zeman et al., 2006). The glucosinolate contents in DRS can also be affected by the extraction conditions such as temperature and humidity (Kendall et al., 1991).

The adverse effect of glucosinolate contents in DRS on the feed intake and growth rate of rats was reported by Smulikowska et al. (1998), whereas no similar effect was observed for chickens and weaner pigs (Smulikowska et al., 1998; Brand et al., 1999). Different responses of animals on the DRS dietary intake resulted in different recommendations of the DRS percentage as a soybean meal substitute in the diet. Wherea maximum intake level of 15% DRS in diets for chickens and 8% in diets for laying hens can be recommended without limitations, the use of rapeseed cannot be recommended for rabbits during the starter period, and a rapeseed inclusion rate of 5% during the finisher period should not be exceeded (Daenicke et al., 2004; Peter and Daenicke, 2003; Rutkowski et al., 2014).

Among the possible harmful effects of glucosinolates in animal organisms their effect on the activity of thyroid hormones is most widely investigated (Degroot et al., 1991; Trávníček et al., 2001, Woyengo et al., 2011). Although, the nutritional effect of DRS was investigated by using wide scale of animals including rats, the informations concerning the potential changes of biochemical and hematological parameters are relatively rare. However, Collet et al. (2014) and Subuh et al. (1995) reported the possible hepatotoxicity of the hydrolysis products of the various glucosinolate secondary compounds found in high concentrations of turnip and rape in rats and cattle, respectively. In adddition, Degroot et al. (1991) observed decrease of blood hemoglobin levels as well as, the microscopic hepatic changes in rats fed by high doses of Brussels sprouts (*Brassica oleracea*) and Tripathi et al. (2001) showed decreased blood hemoglobin levels in lambs fed by *Brassica juncea*. More recently, Durge et al. (2014) observed decreased blood hemoglobin and hematocrit in goats fed by *B. juncea* meal, and recommended these observations for further investigation.

Moreover, increasing activities of detoxification enzymes such as glutathione S-transferase (GST) in a variety tissues of rats exposed to glucosinolates were observed by Munday and Munday (2004) and Razis et al. (2013). On the contrary, Castro-Torres et al. (2014) established that the juice of black radish decreases cholesterol levels in plasma and dissolves gallstones in mice. They explained these findings by the therapeutic effect of isothiocyanates, the hydrolysis products of glucosinolates. These compounds demonstrated antioxidant properties as well as, their ability to diminish hepatic cholesterol levels.

Therefore, our experiment was concerned more likely on the potential changes in hematological parameters as well as, on the selected detoxification mechanisms than on the well described animal performance or activity of thyroid hormones.The main objective of our experiment was i) to describe the biochemical and hematological parameters of rats as affected by increasing DRS rate in the diet, and ii) to optimize the potential DRS rate in the diet without any adverse effect on selected health indicators of the animals. Hypothesis: the optimized rate of 00-quality DRS does not have any adverse effect on the biochemical and hematological response of rat organisms. Therefore, DRS can effectively replace soybean meal in the rat diet.

## MATERIALS AND METHODS

### Experimental design

Twenty-four male Wistar Kyoto rats (average body weight of approximately, 200 g) were obtained from the breeder (Velaz, Prague, Czech Republic) at 30 days of age and housed in cages (one animal per cage) in a room with a

controlled temperature (varying from 23 to 25°C respectively) under natural light conditions. The animals were randomly divided into four groups of six animals each and fed with a semi-synthetic diet according to the experimental design for forty-seven (47) days. Feed and water were supplied to the animals *ad libitum*. Feed consumption and body weight of the animals were monitored weekly. The control group was fed with untreated semi-synthetic diet consisting of 50% wheat coarse meal, 13% fish meal, 14% soybean meal, 0.28% $CaHPO_4$, 1.12% limestone, 4% alfalfa hay, 1% mineral additives (AMINOVITAN STER PLUS, Biofaktory Ltd., Czech Republic), 7.5% feeding yeast, 4.5% wheat germs, and 10% oat meal. In the case of treated groups, defined portions of soybean meal were replaced by the defatted DRS prepared from the oilseed rape seeds of the 00-quality variety NK Oktans as follows: the seeds were milled and defatted in the Soxhlet apparatus by using hexane as the extraction agent for four hours. Subsequently, the meal was dried at 105°C for two hours and homogenized. The experimental diets for the individual experimental groups were prepared as follows: i) group DRS0 fed the control diet; ii) group DRS30, where 30% of the soybean meal in the diet was replaced with DRS; iii) group DRS60, where 60% of the soybean meal in the diet was replaced with DRS; and iv) group DRS100, where 100% of the soybean meal in the diet was replaced with DRS.

After the termination of the experiment, the animals were euthanized by exsanguination after being anesthetized with Xylapan (xylasin) and Narketan (ketamin), and whole blood and liver were sampled. The sampled tissues were kept at -18°C; aliquots of blood samples were taken into the heparinized tubes. Blood plasma was obtained by centrifugation at 2500 × g for 10 min at 4°C.

## Analytical methods

Among the hematological parameters, total number of erythrocytes (Er, $T.L^{-1}$), hemoglobin (Hb, $g.100\ mL^{-1}$), hematocrit value (Hct, %), mean cell volume (MCV, fL), and total number of leukocytes (Le, $G.L^{-1}$) were determined in the whole blood stabilized by $K_2EDTA$. All parameters were determined on computerized analyzer NIHON KOHDEN MEK 5208. Blood hemolysis solution ISOTONAC 3 MEK 640 was used for the determination of the number of leukocytes and hemoglobin values. For an evaluation of the distribution of individual subpopulations of the white blood cells in the blood film, the microscopic assessment was applied using Nikon YS 100.

In the case of biochemical parameters, alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, urea, cholesterol, triacylglycerole, and glucose contents in blood plasma were determined using computerized analyzer Cobas 6000 (Roche, Switzerland). The activities of selected antioxidant enzymes such as glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), thioredoxin reductase (TrxR), and catalase (CAT) were measured in plasma, liver extracts, and erythrocyte lysates of rats under the diet. The liver extracts were prepared by homogenization of 0.08 g of liver with 1 mL of the buffer (0.01 $mol.L^{-1}$ buffered saline solution at pH 7.4, 0.8 g of 0.14 $mol.L^{-1}$ NaCl, 0.29 g of 8 $mmol.L^{-1}$ $Na_2HPO_4.12H_2O$, 0.02 g of 1.5 $mmol.L^{-1}$ $KH_2PO_4$, 0.02 g of 2.7 $mmol.l^{-1}$ KCl per 100 mL of the solution). The homogenates were then centrifuged (Jouan, France) for 10 min at 6700 × g and 4°C and the supernatants kept until measurement at -20°C. For preparation of erythrocyte lysates, the following procedure was applied: the heparinized whole blood was centrifuged for 15 min at 250 × g and 25°C, the erythrocytes were triple washed with saline solution and incubated with distilled water for 15 min at 4°C. Subsequently, the samples were centrifuged for 10 min at 2880 × g and 4°C and the supernatant kept at -20°C until analysis. Determination of specific enzymatic activity of the followed enzymes in plasma, liver extracts, and erythrocyte lysates were performed spectrophotometrically using Libra S22 (Biochrom, UK) and PowerWave XS (BioTek, USA) devices. Catalase was determined at λ=240 nm, glutathionperoxidase at λ= 340 nm, glutathionreductase in plasma and liver extracts at λ= 412 nm and in the lysates of erythrocytes at λ= 340 nm, thioredoxin reductase at λ= 412 nm, and glutathion-S-transferase at λ= 340 nm. All the analyses were performed at 25°C.

## Statistics

The data obtained for individual hematological and biochemical analyses in whole blood, plasma, liver extracts, and erythrocyte lysates were subjected to Dixon's test for identification of outliers (significance level $\alpha = 0.05$) using Microsoft Excel 2007 (Microsoft Corporation, USA). Subsequently, one-way analysis of variance was used at the significance level $\alpha = 0.05$ by using the Statistica 2011 version 9.1 program (StatSoft, USA).

## RESULTS AND DISCUSSION

The finall animal weight in the end of the experiment varied between 463±28g for group DRS30 and 500±46g for DRS100 without any significant difference ($P < 0.05$), where no relation to the changing DRS content in the diet was reported. The effect of DRS on the rat weight and/or weight gain previously published in the scientific literature is ambiguous.

The results of the main haematological parameters are summarized in Table 1. The total erythrocyte count varied between 6.94 and 7.65 $T.L^{-1}$, whereas the physiological range for this value varied between 5 and 10 $T.L^{-1}$,

**Table 1.** Average contents of main hematological parameters. The averages marked by the same letter did not significantly differ at P < 0.05 within individual columns, n=6; data **are** presented as mean ± standard deviation.

| Treatment | Er (T.L¹) | Hct (%) | MCV (fL) | Hb (g.100 mL$^{-1}$) |
|---|---|---|---|---|
| DRS0 | 7.65±0.66$^{a}$ | 44.9±4.4$^{a}$ | 63.6 ±1.5$^{a}$ | 13.2± 0.7$^{a}$ |
| DRS30 | 6.94±0.31$^{a}$ | 43.0±1.7$^{a}$ | 62.0±3.8$^{a}$ | 13.1± 0.2$^{a}$ |
| DRS60 | 7.39±0.53$^{a}$ | 46.9±2.6$^{a}$ | 63.3±1.5$^{a}$ | 13.4± 0.8$^{a}$ |
| DRS100 | 6.85±0.61$^{a}$ | 44.6±4.0$^{a}$ | 65.2±3.3$^{a}$ | 12.9± 0.6$^{a}$ |

Er - total erythrocyte count, Hct - hematocrit, MCV- mean cell volume, Hb - hemoglobin

**Table 2.** Average contents of selected white blood cells; total leukocyte count and distribution of individual populations. The averages marked by the same letter did not significantly differ at P < 0.05within individual columns, n=6; data are presented as mean ± standard deviation.

| Treatment | Le (G.L$^{-1}$) | NeG (%) | EoG (%) | BaG (%) | Lym (%) |
|---|---|---|---|---|---|
| DRS0 | 10.4± 3.1$^{a}$ | 13.5± 2.6$^{a}$ | 0.67±0.21$^{a}$ | 3.17± 0.67$^{a}$ | 80.0± 10.1$^{a}$ |
| DRS30 | 11.3±2.4$^{a}$ | 13.8± 4.3$^{a}$ | 1.40±0.67$^{a}$ | 3.60±1.21$^{a}$ | 79.0± 8.2$^{a}$ |
| DRS60 | 9.8± 2.1$^{a}$ | 15.5± 4.8$^{a}$ | 1.83±0.60$^{a}$ | 4.67±1.83$^{a}$ | 77.2±8.6$^{a}$ |
| DRS100 | 10.0± 2.9$^{a}$ | 16.8± 6.0$^{a}$ | 1.00± 0.63$^{a}$ | 1.00± 0.49$^{a}$ | 79.5± 5.1$^{a}$ |

Le -total leukocyte count, NeG-neutrophil granulocytes, EoG-eosinophil granulocytes, BaG-basophil granulocytes, Lym-lymfocytes.

**Table 3.** Average contents of main biochemical parameters determined in blood plasma of the animals; The averages marked by the same letter did not significantly differ at P < 0.05 within individual columns, n=6; data are presented as mean ± standard deviation.

| Treatment | ALT (µkat.L$^{-1}$) | ALP (µkat.L$^{-1}$) | total protein (g.L$^{-1}$) | Glucose (mmol.L$^{-1}$) | Urea (mmol.L$^{-1}$) | Chol (mmol.L$^{-1}$) | Tri\ (mmol.L$^{-1}$) |
|---|---|---|---|---|---|---|---|
| DRS0 | 1.00± 0.19$^{a}$ | 3.1± 1.3$^{a}$ | 59.4±6.5$^{a}$ | 9.7± 1.2$^{a}$ | 8.6± 2.1$^{a}$ | 1.08±0.16$^{a}$ | 1.37± 0.75$^{a}$ |
| DRS30 | 0.99± 0.12$^{a}$ | 4.5± 2.0$^{a}$ | 55.9±12.4$^{a}$ | 8.6± 0.7$^{a}$ | 8.2± 0.7$^{a}$ | 1.35±0.17$^{a}$ | 1.69±0.95$^{a}$ |
| DRS60 | 1.20± 0.21$^{a}$ | 4.7± 1.5$^{a}$ | 54.6±9.1$^{a}$ | 9.8± 0.9$^{a}$ | 7.1±1.0$^{a}$ | 0.91±0.29$^{b}$ | 1.69± 1.27$^{a}$ |
| DRS100 | 1.23±0.25$^{a}$ | 4.5± 0.6$^{a}$ | 54.6± 6.6$^{a}$ | 10.1± 1.9$^{a}$ | 8.3± 1.1$^{a}$ | 0.63±0.17$^{bc}$ | 2.16± 0.67$^{a}$ |

ALT-alanin aminotransferase, ALP-alkaline phosphatase, Chol-cholesterole, Tri-triacylglycerole.

indicating no adverse effect of DRS addition on this parameter (Sharp and Villano, 2013; Suckow et al., 2006). Similarly, the hematocrit values varying between 43 and 45%, and hemoglobin varying between 12.9 and 13.2 g.100 mL$^{-1}$ fell within the physiological values (35 to 57% and 11 to 19 g.100mL$^{-1}$, respectively, (Sharp and Villano, 2013; Suckow et al., 2006). The values of mean cell volume varied between 62 and 65.2 fL, and were slightly higher as compared to the physiological range (46 to 65 fL) given by Sharp and Villano (2013) and Suckow et al. (2006) but fell within the range recommended by Feldman et al. (2000), that is, from 55 to 71 fL. No significant differences (P < 0.05) were reported for the parameters of the red blood picture. The results of the white blood picture (Table 2) showed no differences (P < 0.05) for either total leukocyte count or distribution of individual subpopulations of the white blood cells.

Ćurić et al. (2003) in their research observed no changes in the hematological parameters of pigs fed with diet containing up to 10% DRS. Similar findings were published by Trávníček et al. (1995) in the case of sheep. Therefore, no adverse effect of DRS addition to the rat diet was found on the main hematological parameters. Recent investigations suggest an improvement of the cardiovascular system where the alcalase and pepsin hydrolysates of DRS proteins showed more appreciable anti-hypertensive effects when compared to other plants such as the pea and hemp seed proteins. Therefore, these hydrolysates may serve as useful ingredients for anti-hypertensive functional foods formulas (Alashi et al., 2014).

The results of the main biochemical parameters in the blood plasma are summarized in Table 3, and the specific activities of the antioxidative enzymes presented in Table 4. Among the enzymes indicating potential adverse effect of DRS in feed on liver function, ALT and ALP activity were assessed, and no significant changes (P < 0.05) among the experimental groups were observed. Although, Czech and Grela (2004) observed the effect of phytate contents on ALT and ALP activity, no similar pattern was reported in our case where phytate-rich DRS (Wittkop et al., 2009) was applied. Similarly, no potential hepatotoxic effect of glucosinolates (Heaney et al., 1994b) was indicated by our experiment.

The total protein content varied between 54.6 and 59.4

**Table 4.** Average contents of specific activities of antioxidative enzymes determined in animal tissues; The averages marked by the same letter did not significantly differ at P < 0.05within individual columns, n=6; data are presented as mean ± standard deviation.

| Treatment | GPx (U.mg$^{-1}$ protein) | G (U.mg$^{-1}$ protein) | GST (U.mg$^{-1}$ protein) | TrxR (U.mg$^{-1}$ protein) | CAT (U.mg$^{-1}$ protein) |
|---|---|---|---|---|---|
| **Plasma** | | | | | |
| DRS0 | 9.33±0.70$^{a}$ | 15.4±5.3$^{a}$ | 1.51±0.51$^{a}$ | 3.91±0.33$^{a}$ | 1.53±0.40$^{a}$ |
| DRS30 | 7.55±1.00$^{b}$ | 22.0±6.7$^{a}$ | 2.43±0.41$^{a}$ | 4.76±0.29$^{a}$ | 2.11±0.65$^{a}$ |
| DRS60 | 7.84±0.99$^{a}$ | 28.1±6.9$^{b}$ | 3.23±0.88$^{b}$ | 5.04±0.42$^{b}$ | 3.38±1.41$^{b}$ |
| DRS100 | 6.90±0.91$^{b}$ | 34.7±9.4$^{b}$ | 3.63±0.51$^{b}$ | 5.05±0.77$^{b}$ | 3.37±0.98$^{b}$ |
| **Liver extracts** | | | | | |
| DRS0 | 31.5±4.0$^{a}$ | 10.79±1.04$^{a}$ | 3.59±0.40$^{a}$ | 7.41±0.73$^{a}$ | 51.3±6.6$^{a}$ |
| DRS30 | 36.4±4.3$^{a}$ | 11.62±1.39a | 3.69±0.50a | 7.33±0.79a | 50.5±4.9a |
| DRS60 | 38.3±3.9$^{a}$ | 12.84±0.77$^{b}$ | 3.96±0.37$^{a}$ | 7.83±0.82$^{a}$ | 54. 9±7.2$^{a}$ |
| DRS100 | 32.9±2.3$^{a}$ | 11.18±0.86$^{a}$ | 3.56±0.50$^{a}$ | 6.37±1.17$^{a}$ | 47.8±2.9$^{a}$ |
| **Erythrocyte lysates** | | | | | |
| DRS0 | 22.3±6.8$^{a}$ | 3.41±1.13$^{a}$ | 4.84±1.71$^{a}$ | 4.42±1.83$^{a}$ | 53.8±8.7$^{b}$ |
| DRS30 | 21.1±5.6$^{a}$ | 3.12±0.66a | 5.72±1.55a | 7.78±1.44$^{b}$ | 48.5±6.2$^{b}$ |
| DRS60 | 20.8±3.9$^{a}$ | 2.84±0.73$^{a}$ | 8.33±1.50$^{b}$ | 13.3±2.6$^{b}$ | 36.5±6.8$^{a}$ |
| DRS100 | 20.4±4.4$^{a}$ | 2.84±1.01$^{a}$ | 10.2±1.8$^{b}$ | 11.5±2.5$^{b}$ | 43.5±11.7$^{b}$ |

GPx- glutathione peroxidase, GR- glutathione reductase, GST-glutathione S-transferase, TrxR - thioredoxin reductase, and CAT- catalase.

g/Land fell within the physiological range 45 to 84 g/L (Sharp and Villano, 2013; Suckow et al., 2006). The plasma glucose concentration varied between 8.6 and 10.1 mmol/Land fell within the physiological range of 4.5 to 16.6 mmol.L$^{-1}$ (Sharp and Villano, 2013). The urea concentrations in the rat plasma (7.1 to 8.6 mmol/L) corresponded to the physiological range measured by Moustafa (1997), where the values varied between 8.8 and 13.0 mmol/L. The triacylglycerole concentrations varied between 1.37 and 2.16 mmol/L, corresponding to the results of Santos et al. (2000), where the results fell within the interval 0.77 to 2.60 mmol/L. No significant differences were observed among the individual animal groups (P < 0.05) for all these parameters, indicating no changes among the potential indicators of hepatotoxicity.Thus, the potential hepatotoxic effects of glucosinolates as reported for instance by Collet et al. (2014) were not confirmed.A different situation occurred in the case of cholesterol concentrations in the plasma. The values varied between 0.63 and 1.35 mmol/L, where a significant decrease of this parameter (P < 0.05) was observed with increasing DRS rate (Table 3) in accordance with the report of Castro-Torres et al. (2014).Ikeda et al. (1989) observed the hypocholesterolomic effect of fiber compounds in the diet. It should be taken into account because the fiber content in the DRS should be two-fold higher as compared to soybean meal (Zeman et al., 2006). The increased content of fiber compounds can result in limited absorption of cholesterol in the intestines (Kritchevsky, 1978). Solomon et al. (1991) in their study observed lower cholesterol concentrations in lambs fed with a diet containing DRS.

The interrelationships between fat concentration and/or composition in the diet and cholesterol absorption have been known for a long time, as described by Vahouny et al. (1978). Phytosteroles and phytostanoles contained in vegetable oils are especially responsible for decreasing cholesterol absorption (Peterson, 1951) by 10 to 15%, where the stanoles are more effective in this context as compared to phytosteroles (O'Neill, 2005). The rapeseed oil contains 450 to 780 mg steroles per 100 g, whereas the soybean oil contains only 180 to 410 mg steroles per 100 g (Baranyk et al., 2007).

Other important factors affecting the levels of plasmatic cholesterol are the plasmatic unsaturated fatty acids (Cleghorn et al. 2003; Grundy and Denke 1990). Baranyk et al. (2007) reported up to 64% of unsaturated fatty acids in DRS compared to only 23% in soybean meal. Therefore, the substitution of soybean meal by DRS seems to be a reasonable measure for efficient decrease of cholesterol concentration in rat plasma.

The specific activities of the major antioxidative enzymes are summarized in Table 4. Where the GPx levels in plasma decreased with increasing DRS rate in the diet, the specific activities of plasmatic GR, GST, TrxR, and CAT increased (P < 0.05). The increasing GST specific activity could be related to the decreasing plasmatic GPx because GST is able to reduce some substrates for GPx synthesis (Coles and Ketterer, 1990). The increasing plasmatic GR level is related to the increasing GST activity as well, because GST requires the presence of reduced glutathione for the optimum activity. In the liver extracts, however, the specific activities of the enzymes remained unchanged except for an increased (P < 0.05) level of GR in the RM60 group.

Cichon et al. (1982) proved lower dietary intake of DRS compared to the diet containing soybean meal. They observed lower digestibility of the DRS (up to 87.3%) as

compared to the soybean meal (up to 90.4%). Paradoxically, rats fed withDRS showed higher weight gain, most probably due to higher biological value of the DRS because of a higher content of sulfur-containing amino acids, which are important for rat nutrition. Presto et al. (2011) also reported no difference in the digestibility of the DRS proteins in pig nutrition as compared to the conventional diets. Moreover, Bos et al. (2007) discussed the high nutritional potential of rapeseed proteins for human nutrition characterized by the high postprandial biological value.

Ćurić et al. (2003) observed higher weight gain of pigs after the substitution of sunflower meal with DRS in the diet. Figat et al. (2010) found no changes in weight gain of pigs fed with a diet containing 20% DRS. On the contrary, Smith and Bray (1992) observed decreased weight gain of rats fed by a diet containing DRS. Therefore, the results suggest that the effect of DRS on animal growth will depend on the individual DRS rates as well as, on the composition of the particular RMs applied to the diet.

Smith and Bray (1992) observed increasing hepatic glutathione concentration and GST activity in rats fed with a diet containing DRS, which was not unambiguously verified by our experiment. In the erythrocyte lysates, increased activity of GST and TrxR with an increasing DRS rate ($P < 0.05$) was observed, whereas, the response of CAT activity was ambiguous and GPx and GR activities remained unchanged. The antioxidative response of the rat organism can be affected by the content of antioxidative compounds in the diet. Abdullah et al. (2011) in their study compared the antioxidative activities of polyphenolic compounds and flavonoids in rapeseed, cotton, and soybean meals, where apparently higher contents of these compou0nds were found in the DRS (5.3 mg/gphenolic compounds and 2.3 mg/gflavonoids) compared to soybean (0.9 mg/gphenolic compounds and 0.8 mg/gflavonoids), and the antioxidative activity of DRS was significantly higher as compared to the other meal samples. Similar results were published by Naczk et al. (2002). Attorri et al. (2011) described a positive effect of rapeseed against worsening of the cognitive functions of Sprague-Dawley rats because of the antioxidative activity of tocoferoles. The antioxidative effect of phytic acid was also reported (Eaton and Graf, 1990).

## Conclusion

Summarizing the results, our experiment proved that the substitution of soybean meal with 00-quality DRS (that is, 15% in our case) in the rat diet did not show any harmful effect on the biochemical and hematological response of the rat organisms. Moreover, the results partially elucidated the response of animal biochemical and hematological processes on DRS addition to the diet. In fact, a decrease of plasma cholesterol concentrations and an increase of antioxidative response of the rat organism suggested a potential beneficial effect of DRS for rats.However, the more detailed investigation is necessary to elucidate the role of the DRS components on the physiological parameters of the animals. Subsequently, the effective substitution of the soybean meal with DRS in the diet of other animal species should be discussed.

## ACKNOWLEDGEMENTS

Authors wish to thank the GAČR project No. 13-04580S for their financial support and Proof-Reading-Service.com Ltd., Devonshire Business Centre, Works Road, Letchworth Garden City SG6 1GJ, United Kingdom for correction and improvement of language.

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**Cite this article as:**

Tvrdá J, Tůmová N, Fučíková A, Zídková J, Melčová M, Záková J, Mlejnek P, Zídek V, Mestek O, Kaňa A, Tlustoš P (2015). Biochemical and Hematological Response of Rats on Defatted Rape Seeds Addition into the Diet. Acad. J. Agric. Res. 3(12): 395-401.

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