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Dehulling effect of dietary administered white lupine seeds on the blood biochemistry of broilers

David Zapletal[1], Lenka Kudelkova[2], Petra Jakesova[1], Vlastimil Simek[1], Eva Strakova[1], Pavel Suchy*

[1]*Department of Animal Husbandry, Animal Nutrition and Biochemistry, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic*

[2]*Department of Animal Protection and Welfare and Public Veterinary Medicine, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic*

**Corresponding author: zapletald@vfu.cz*

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Abstract: The aim of the study was to assess the effect of the partial or complete dietary replacement of crude protein (CP) from soybean meal (SBM) with CP from the meal of whole (trial 1) or dehulled (trial 2) white lupine (WL) seeds on the blood plasma biochemical indicators in broilers. Chickens of the control (C) group were fed diets containing the SBM as the main protein source in the feed. It results from the findings that the complete and also partial replacement of CP from the SBM by CP from the meal of whole WL seeds in diets led to a decrease in the plasma cholesterol and triacylglycerides (TAG) levels ($P < 0.001$). Concerning the dietary administration of dehulled WL seeds, only the complete replacement of CP resulted in a decrease in the plasma glucose and TAG levels in 35-day-old broilers ($P < 0.05$). In addition, when including the dehulled WL seeds into the diet, an increase in the plasma aspartate aminotransferase activity was found in both the E1 and E2 groups ($P < 0.01$). Based on the results found, it can be stated that the use of meal from whole WL seeds seems to be a promising protein feed component which has beneficial effects on the health of broilers.

Keywords: feed; lupine kernel; meat-type chickens; plasma biochemical indicators; whole seeds; Zulika variety

Preference for soybean meal (SBM) to produce compound feeds for poultry is influenced by its price, if the SBM price increases, poultry producers seek alternative protein sources showing better economic efficiency for their production. Moreover, with respect to the current increasing demand of consumers not only for high quality poultry products and functional foods, but also for the health of the reared poultry itself (Carmona et al. 2019; Nowak et al. 2019), poultry producers cannot ignore these rising consumers' demands now and they often seek less-conventional feed components to increase the attractiveness of their products on the market.

Recent studies on poultry proved that the dietary inclusion of white lupin (WL) seeds positively affected their meat quality (Tufarelli et al. 2015) and it may also offer certain health benefits for poultry (Viveros et al. 2007; Geigerova et al. 2017). Nevertheless, the dietary administration of a higher proportion of meal from whole WL seeds may sometimes result in the deterioration of economically important traits in poultry fattening (Nalle et al. 2012;

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Geigerova et al. 2017). In this regard, dehulling WL seeds increases the levels of nutrients for broiler chickens to concentrations comparable with SBM (Tufarelli et al. 2015), while the inclusion of dehulled WL seeds had no adverse effects on the growth performance and carcass traits of the broilers. However, the dietary inclusion of WL meal from the dehulled seeds on the blood biochemistry profile of the broiler chickens has not yet been studied in the literature. The objective of the present study was to assess the effect of the partial or complete dietary replacement of CP from SBM with CP from the meal of whole or dehulled WL seeds, the Zulika variety, on selected blood plasma biochemical indicators in broilers undergoing fattening. An integral part of the study was to assess the sex-effect on the observed biochemistry indicators.

MATERIAL AND METHODS

Animals and experimental design

The experimental procedures were approved by the Animal Welfare Committee. Within both trials (trial 1 and trial 2) of the present study, all the broilers (Ross 308) were housed in an accredited experimental stable under controlled housing conditions that fully complied with the standards used for Ross 308 broilers. The birds were housed in floor pens.

A 3-phase feeding programme was used in each trial; while the starter, grower, and finisher diets were offered from day 1 to 13, 14 to 28, and 29 to 35, respectively. The compound feeds were composed of the following components: wheat, maize, extracted soybean meal and/or meal of WL seeds, soy oil, animal fat, methionine, lysine, threonine, monocalcium phosphate, NaCl, sodium sulphate, mineral premixes and enzymes. In addition, the starter diets also contained fish meal. In the feeds, the following nutrients were determined: crude protein (CP), crude fibre, acid-detergent fibre (ADF), neutral-detergent fibre (NDF), acid-detergent lignin (ADL), crude fat (CFa), crude starch (CSt), nitrogen-free extractives (NFE), crude ash, calcium (Ca), inorganic phosphate (P_i) and metabolisable energy (ME). The dry matter of the feeds was determined by weight upon drying the sample at 105 °C under the prescribed conditions. The Kjeldahl method using a Buchi analyser (Centec Automatika, Prague, Czech Republic) was used for the CP determination. The crude fibre, ADF, NDF and ADL were determined by an ANKOM 220 Fiber Analyzer (O.K. Servis BioPro, Prague, Czech Republic). The Soxhlet method was used to determine the CFa. The crude starch was determined by an Automatic Digital Polarimeter P3002RS (Krüss, Hamburg, Germany). The crude ash was determined by weighing the sample after incineration at 550 °C under the prescribed conditions. The levels of Ca and P_i were determined through incinerating and leaching the sample by extraction and the subsequent titration according to the Association of Official Agricultural Chemists (AOAC 2001). Thereafter the NFE content was calculated. The values of the ME were obtained by the following equation:

$$ME = [(34.31 \times CFa) + (15.51 \times CP) + (6.69 \times CSt) + (13.01 \times NFE)/1\,000] \quad (1)$$

where:
ME – metabolisable energy;
CFa – crude fat;
CP – crude protein;
CSt – crude starch;
NFE – nitrogen-free extractives.

The feed and water were supplied *ad libitum*. The experimental period of each trial lasted for 35 days.

Trial 1

A total of 240 (120 males and 120 females) 1-day-old broiler chickens were used in the experiment. The chickens were randomly placed into 3 groups (1 control and 2 experimental groups; 80 birds per group). The chicks from each group were housed in 4 pens (20 birds/pen). The chickens of the control (C) group were fed diets containing the SBM as the main protein source in feed. In the experimental groups, the content of the CP from the SBM in the C group was replaced partially or completely by the CP from the meal of whole WL seeds of the Zulika variety, as an alternative source of the dietary proteins. The experimental group E1 was fed a diet in which the CP from the SBM in the C group was replaced by 50% with the CP from the meal of the whole WL seeds. The experi-

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Table 1. The nutrients' composition of the diets in trial 1

Nutrient content (g/kg as fed)	Feed								
	starter			grower			finisher		
	C	E1	E2	C	E1	E2	C	E1	E2
Dry matter	890.5	888.1	890.5	884.1	888.6	886.4	888.4	886.3	890.0
Crude protein	226.6	235.4	221.5	200.6	193.1	197.9	182.2	191.0	181.2
Crude fibre	21.7	31.2	46.5	22.0	43.9	51.0	32.2	31.8	40.2
ADF	35.2	49.0	72.4	35.3	45.0	62.5	38.7	44.1	53.3
NDF	80.0	93.2	117.0	85.7	98.2	112.1	94.7	94.3	116.4
ADL	5.7	5.9	6.2	5.7	3.9	3.8	8.0	4.7	5.0
Crude fat	56.9	51.2	53.9	58.7	60.5	61.9	72.3	58.9	72.0
Crude starch	373.2	359.3	360.0	406.6	404.9	377.7	406.4	404.7	410.2
NFE	530.8	516.8	524.4	557.2	549.9	536.4	558.1	563.3	561.1
Crude ash	54.5	53.5	44.2	45.7	41.2	39.2	43.6	41.2	35.5
Calcium	10.7	11.1	9.5	9.2	8.0	8.0	8.5	7.8	7.1
Inorganic phosphate	6.0	6.2	5.3	5.2	4.7	4.7	4.9	4.8	4.5
ME (MJ/kg)	14.9	14.5	14.5	15.1	14.9	14.7	15.3	15.1	15.3

ADF = acid detergent fibre; ADL = acid detergent lignin; C = control diet; E1 = experimental diet; E2 = experimental diet; ME = metabolisable energy; NDF = neutral detergent fibre; NFE = nitrogen-free extractives

mental group E2 was fed a diet in which the CP content from the SBM in the C group was replaced completely with the CP from the meal of the whole WL seeds. The chemical composition of the diets is outlined in Table 1.

Trial 2

The experimental design of trial 2 was same as trial 1, while the meal from the dehulled WL seeds was used as the source of the alternative vegetable proteins in the experimental diets (E1 and E2 groups). The chemical composition of the diets is outlined in Table 2.

Blood sampling and plasma biochemistry

At the end of the fattening (35 d), 20 chickens (10 males and 10 females) per dietary group were randomly selected (5 chickens/pen) for a biochemical examination within each trial of the present study. The broilers were weighed (KERN TB digital scale with an accuracy of 0.5 g) and, thereafter, their blood was collected by puncturing the *vena basilica*. The blood samples were placed in sample tubes with heparin. In the laboratory, the blood samples were centrifuged for 15 min at 806 × *g*. Subsequently, the biochemical indicators were determined in the blood plasma using a DPC KONELAB 20i Analyzer® (Thermo Fisher Scientific, Uppsala, Finland), while the following plasma indicators were assessed: the total protein (TP), glucose (Glu), total cholesterol (Chol), triacylglycerides (TAG), Ca, P_i and activity of aspartate aminotransferase (AST) and alkaline phosphatase (ALP).

Statistical analysis

The obtained data were statistically evaluated using the STATISTICA CZ v10 software. The arithmetic mean and 95% confidence interval were determined for the body weight (BW) and monitored plasma indicators. The Shapiro-Wilk test was used to test the normal distribution of the data within the respective evaluated groups; normality was found in all the monitored variables. A two-way ANOVA (analysis of variance) was used for the determination of the differences among the evaluated groups in the BW and the monitored biochemical indicators within a trial; the following mathematical model of the two-way ANOVA was used to determine the differences in the assessed indicators among the evaluated groups:

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Table 2. The nutrients' composition of the diets in trial 2

Nutrient content (g/kg as fed)	Feed								
	starter			grower			finisher		
	C	E1	E2	C	E1	E2	C	E1	E2
Dry matter	886.3	892.7	895.2	894.2	894.9	899.2	870.7	890.9	893.8
Crude protein	219.0	211.3	217.7	194.1	188.5	183.5	174.2	163.4	171.2
Crude fibre	23.8	21.1	25.7	23.1	20.9	38.3	21.2	26.6	20.5
ADF	33.8	33.2	38.3	37.0	33.5	45.1	33.9	40.2	35.3
NDF	170.5	103.4	106.6	116.8	92.2	96.7	99.7	97.6	88.2
ADL	4.0	5.3	4.6	6.9	7.3	7.0	7.7	7.9	8.7
Crude fat	48.0	49.6	58.2	58.9	65.5	70.4	54.6	70.5	80.5
Crude starch	379.4	387.4	380.7	397.3	419.7	402.6	429.1	434.1	428.4
NFE	538.6	555.5	542.9	563.4	576.4	563.7	579.4	590.7	582.2
Crude ash	56.9	55.2	50.6	54.6	43.7	43.3	41.3	39.8	39.4
Calcium	8.4	7.9	7.9	8.3	6.1	6.7	4.5	4.3	4.7
Inorganic phosphate	6.5	6.1	6.0	5.6	5.3	5.0	5.0	4.7	4.9
ME (MJ/kg)	14.6	14.8	15.0	15.0	15.5	15.3	15.0	15.5	15.9

ADF = acid detergent fibre; ADL = acid detergent lignin; C = control diet; E1 = experimental diet; E2 = experimental diet; ME = metabolisable energy; NDF = neutral detergent fibre; NFE = nitrogen-free extractives

$$Y_{ijk} = \mu + D_i + S_j + (D \times S)_{ij} + e_{ijk} \quad (2)$$

where:

μ – overall mean;
D_i – fixed effect of the diet (i = C, E1 and E2);
S_j – fixed effect of the sex (j = male and female);
$(D \times S)_{ij}$ – interaction between the diet and the sex;
e_{ijk} – random residual error.

The differences among the groups were tested by post hoc Tukey's test.

RESULTS

Trial 1

The complete and also partial replacement of the CP from the SBM by the CP from the meal of the whole WL seeds in the broiler diets led to a decrease in both the plasma Chol and TAG concentrations ($P < 0.001$; Table 3). In addition, the chickens in the E1 group showed lower plasma Glu concentrations ($P < 0.05$) compared to the chickens in the C and E2 groups. As for the sex, the males showed higher BW ($P < 0.001$) and plasma concentrations of the Chol ($P < 0.001$), TAG ($P < 0.01$), Ca and Glu ($P < 0.05$) and also the plasma activity of the ALP ($P < 0.01$) when compared to the females. By contrast, the females showed a higher plasma AST activity in comparison to the males ($P < 0.01$). The diet × sex interaction (not stated in Table 3) affected the plasma concentrations of the Chol ($P < 0.01$), P_i ($P < 0.05$) and the plasma ALP activity also ($P < 0.05$).

Trial 2

Only the complete dietary replacement of the CP from the SBM by the CP from the meal of the dehulled WL seeds led to a decrease in the plasma Glu and TAG concentrations ($P < 0.05$) in the fattened broilers (Table 4); the E1 group did not show any differences in these blood indicators in comparison to the C and E2 groups ($P > 0.05$). Furthermore, both the partial and complete replacement of the CP from the SBM by the CP from the meal of the dehulled WL seeds resulted in an increase in the plasma AST activity of the chickens ($P < 0.01$). In addition, the chickens in the E1 group displayed a lower plasma TP concentration compared to the chickens in the E2 group ($P < 0.05$). Concerning the sex, the males showed higher BW ($P < 0.001$) and plasma concentrations of the Glu and P_i ($P < 0.05$) and also the plasma activity of the ALP ($P < 0.01$) when

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Table 3. The blood plasma indicators of the 35-day-old chickens in relation to the diet and sex in trial 1

Item	Diet						Sex				Significance	
	control		E1		E2		male		female		diet	sex
	mean	CI	mean	CI	mean	CI	mean	CI	mean	CI		
Body weight (kg)	2.7	2.55–2.74	2.6	2.49–2.66	2.6	2.46–2.67	2.7	2.65–2.77	2.5	2.42–2.53	NS	***
Total protein (g/l)	33.1	31.74–34.46	30.9	29.43–32.39	32.5	30.60–34.47	32.1	30.70–33.44	32.3	31.02–33.56	NS	NS
Glucose (mmol/l)	14.0^{a}	13.68–14.26	13.2^{b}	12.53–13.91	14.0^{a}	13.65–14.34	14.0	13.81–14.24	13.4	12.93–13.94	*	*
Cholesterol (mmol/l)	4.0^{A}	3.88–4.16	3.4^{B}	3.23–3.63	3.5^{B}	3.26–3.66	3.9	3.74–3.98	3.4	3.23–3.59	***	***
TAG (mmol/l)	1.3^{Aa}	1.10–1.40	0.9^{ABb}	0.68–1.18	0.8^{B}	0.70–0.91	1.1	0.95–1.32	0.9	0.75–0.95	***	**
AST (μkat/l)	8.5	6.97–10.06	7.6	6.69–8.57	7.6	6.74–8.40	7.0	6.42–7.51	8.8	7.76–9.92	NS	**
ALP (μkat/l)	60.2	42.86–77.64	51.0	42.90–59.03	55.5	45.03–65.92	64.6	53.50–75.66	46.5	39.02–54.07	NS	**
Ca (mmol/l)	3.2	3.07–3.28	3.0	2.79–3.15	3.1	2.97–3.17	3.2	3.07–3.23	3.0	2.87–3.12	NS	*
P_i (mmol/l)	2.8	2.67–2.89	2.7	2.59–2.79	2.8	2.76–2.97	2.8	2.69–2.83	2.8	2.69–2.90	NS	NS

Ca = calcium; CI = 95% confidence interval; E1 = experimental diet; E2 = experimental diet; NS = not significant; P_i = inorganic phosphate; TAG = triacylglycerides
$^{a–b}$The means within a row with different superscript letters differ at $P < 0.05$ (*); $^{A–B}$The means within a row with different superscript letters differ at $P < 0.01$ (**); ***$P < 0.001$

Table 4. The blood plasma indicators of the 35-day-old chickens in relation to the diet and sex in trial 2

Item	Diet						Sex				Significance	
	control		E1		E2		male		female		diet	sex
	mean	CI	mean	CI	mean	CI	mean	CI	mean	CI		
Body weight (kg)	2.5	2.37–2.67	2.6	2.50–2.76	2.6	2.51–2.68	2.8	2.72–2.85	2.4	2.32–2.44	NS	***
Total protein (g/l)	28.5^{ab}	27.63–29.39	28.0^{b}	27.13–28.91	29.7^{a}	28.54–30.94	28.5	27.63–29.26	29.1	28.22–29.91	*	NS
Glucose (mmol/l)	15.0^{a}	14.43–15.52	14.4^{ab}	13.99–14.71	14.3^{b}	13.98–14.53	14.8	14.35–15.14	14.3	14.05–14.57	*	*
Cholesterol (mmol/l)	3.5	3.26–3.66	3.5	3.33–3.63	3.6	3.43–3.83	3.6	3.42–3.74	3.5	3.33–3.59	NS	NS
TAG (mmol/l)	0.7^{a}	0.55–0.74	0.6^{ab}	0.48–0.67	0.5^{b}	0.41–0.55	0.5	0.45–0.62	0.6	0.53–0.65	*	NS
AST (μkat/l)	7.6^{Bb}	6.77–8.47	9.6^{Aab}	8.57–10.65	9.3^{ABa}	8.32–10.20	9.1	8.32–9.87	8.6	7.72–9.41	**	NS
ALP (μkat/l)	41.0	32.80–49.16	44.6	33.62–55.51	43.6	34.79–52.40	49.7	41.44–57.86	36.4	30.88–41.99	NS	**
Ca (mmol/l)	2.8	2.76–2.90	2.9	2.80–2.98	2.8	2.78–2.90	2.9	2.81–2.93	2.8	2.78–2.89	NS	NS
P_i(mmol/l)	2.2	2.09–2.27	2.2	2.11–2.33	2.2	2.17–2.28	2.3	2.19–2.34	2.2	2.09–2.21	NS	*

Ca = calcium; CI = 95% confidence interval; E1 = experimental diet; E2 = experimental diet; NS = not significant; P_i = inorganic phosphate; TAG = triacylglycerides
$^{a–b}$The means within a row with different superscript letters differ at $P < 0.05$ (*); $^{A–B}$The means within a row with different superscript letters differ at $P < 0.01$ (**); ***$P < 0.001$

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compared to the females. The diet × sex interaction (not shown in Table 4) did not affect the BW or the monitored blood indicators ($P > 0.05$).

DISCUSSION

Both in trial 1 and in the trial 2, the BW of the 35-day-old broilers was not influenced by the diet in the present study, while, as expected, a considerably higher BW was found in the males than the females in both trials. In addition, Ross 308 broilers of both sexes showed a noticeably higher BW in the present study than those fattened in the study of Tumova and Chodova (2018). As for the plasma TP concentration of chickens, its level was not influenced by the diet used in trial 1. In the case of the dietary administration of the meal from the dehulled WL seeds in trial 2, the E1 group displayed a lower TP concentration when compared to the E2 group, which should rather be related to the generally lower CP concentration in the finisher feed of the E1 group than to the dietary dehulled lupin administration. Moreover, a sex-related effect on the plasma TP concentration of the 35-day-old chickens was not found in the present study, which is in agreement with results of the study conducted by Strakova et al. (2008) in 42-day-old broiler chickens fed a diet containing 10% of the meal from the dehulled WL seeds.

The dietary hypoglycaemic effect of the WL seeds found in the chickens of the present study is in agreement with the findings observed in a previous study in an animal model, in which a more noticeable reduction of the serum Glu concentrations in broilers was found with an increasing concentration of the dietary WL seeds (Viveros et al. 2007). Lupin seed proteins, mainly gamma-conglutin, is unique among legume proteins, because it has been shown to be promising in glycaemic control (Agrawal et al. 2015). A noticeable plasma Glu lowering effect was found in the present study when the meal from the dehulled WL seeds was used in the diets; while the complete replacement of the CP from the SBM by the CP from the meal of the dehulled WL seeds led to a considerable reduction of the plasma Glu, which was likely associated with the higher content of the compounds showing hypoglycaemic effect. Besides that, within both trials of the present study, the males showed a higher plasma Glu concentration compared to the females which is in agreement with the finding of Strakova et al. (2008).

The well-known cholesterol-lowering effect of dietary administered lupin seeds in poultry (Viveros et al. 2007; Jerabek et al. 2018) was confirmed in the present study only when the meal from the whole WL seeds was used. The dietary administration of the dehulled WL seeds did not affect the total plasma Chol concentration in the chickens of the present study. This finding is not in agreement with the results of the study conducted by Tufarelli et al. (2015) who proved the cholesterol-lowering effect of the dietary administered micronised-dehulled WL seeds in the breast meat of meat-type guinea fowls. Recently, in human studies, it was found that the reduction in both the total and LDL (low-density lipoproteins) Chol blood concentrations were related to the dietary exposure of the lupin protein isolates and also to the lupin fibre isolate (Hall et al. 2005; Pavanello et al. 2017). It may be concluded that mainly the lupin hulls, normally containing about 52–54% of the total fibre content containing biologically active compounds which, when administered in a diet, may reduce the total Chol blood concentration in chickens. As for the sex-related effect on the plasma Chol level in the 35-day-old chickens of the present study, a higher Chol concentration was proved in the males when compared to the females in the trial 1 and a similar, however, not a significant trend was found in trial 2. This finding is not in agreement with study of Strakova et al. (2008) who observed a higher Chol plasma concentration in the females compared to the males.

Both the partial and complete replacement of the CP from the SBM by the CP from the meal of the WL seeds led to a decrease in the plasma TAG concentration in the chickens of both trials performed in the present study. Unlike the Chol, a similar TAG-lowering effect was observed in the chickens when the diet contained the meal from both the whole WL seeds and the dehulled WL seeds. Generally, the decreasing TAG plasma concentration with the increasing dietary proportion of the WL meal in the present study is in agreement with the findings found by Viveros et al. (2007) and Jerabek et al. (2018) who used the meal from whole WL seeds in poultry diets. In addition, the values of the Chol concentration in the chickens within the respective dietary groups of the present study are similar to those found by Wen et al.

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(2019) in broilers and also by Jerabek et al. (2018) in fattened meat-type mallard ducks, when likewise the partial or complete dietary replacement of the SBM with the meal from whole WL seeds was used. Simek et al. (2018) found that partial dietary inclusion of WL seeds also showed a TAG-lowering effect in pet rabbits. Recently, Bettzieche et al. (2008) revealed that the TAG-lowering effect of lupin seeds is caused by the downregulation of the fatty acid synthesis genes and the upregulation of the genes involved in the TAG hydrolysis. Besides that, male chickens showed a higher plasma TAG concentration when compared to females in trial 1 of the present study.

In the chickens of the present study, the dietary administration of the meal from whole WL seeds caused a slight, but not significant, decrease in the activities of the AST and ALP in their plasma, which can be considered as positive in regard to their health status. By contrast, the dietary administration of the meal from the dehulled WL seeds significantly elevated the plasmatic AST activity compared to the C group, which is considered undesirable, since the increased AST enzyme activity is usually related to the impairment of hepatic cells.

In addition, a lower plasmatic AST activity was shown in the males when compared to the females in trial 1, which is in agreement with results of the study conducted by Strakova et al. (2008); however, this trend was not confirmed in trial 2 of the present study. As for the plasmatic ALP activity in the chickens of the present study, the males showed a higher activity compared to the females in both trials performed.

Neither the plasma Ca or P_i concentration in the chickens was influenced by the dietary treatments in both trials of the present study. Although it is known that WL seeds are a considerable source of phosphorus and their dietary administration, mainly with their hulls, may elevate the plasma P_i in poultry (Jerabek et al. 2018), this did not happen in the present study, since the total P_i contents in the E1 and E2 grower and finisher feeds were intentionally lowered in comparison with the C diets. Moreover, the males in the present study showed a slightly higher plasma Ca concentration when compared to the females. Furthermore, the males of trial 2 showed a slightly higher plasma P_i than the females, which is in agreement with the findings of Strakova et al. (2008).

In conclusion, the dietary replacement of the CP from the SBM by the CP from the meal from the whole WL seeds led to a decrease in the plasma Chol and TAG concentrations of the chickens. When the CP from the SBM was replaced by the meal from the dehulled WL seeds, on one hand, hypoglycaemic and TAG-lowering effects were found, and, on the other hand, the elevating activity of the AST in the broiler plasma was observed. In this regard, the use of meal from the whole WL seeds seems to be a more promising protein feed component for broilers. In addition, the sex of the chickens had a major effect on the BW and majority of the monitored biochemistry indicators.

Conflict of interest

The authors declare no conflict of interest.

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