# Blood characteristics and histomorphology of parenchymatous organs in rabbits feeding hempseed cake

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# Abstract

The aim of this study was to evaluate the effect of feeding hempseed cake on blood biochemical and haematological parameters, as well as histological morphology of the livers, kidneys and testes of rabbit males. Rabbits were fed either a standard diet (control group; C; n = 10) or a diet supplemented with hempseed cake (experimental group E5 with 5% hempseed cake; n = 10, and experimental group E10 with 10% hempseed cake; n = 10) per 100 kg of ground complete feed mixture. Based on the biochemical blood analysis, the tested concentrations can be considered to exert at most minimal clinical effects also did not cause serious clinical signs of organ dysfunction or haematological disorders. Most parameters remained within their reference ranges and did not differ significantly between the control and experimental groups. A positive finding is the stability of hepatic enzymes, which rules out hepatotoxic effects of hempseed cake at the administered concentrations. Histological samples of organs were processed using standard procedures and evaluated under a light microscope. Feeding with hempseed cake at both tested concentrations increased vacuole accumulation in hepatocytes, caused occasional incidence of atrophic renal glomeruli, and increased the amount of interstitial connective tissue in the testes, compared to the control group. The occurrence of vacuoles in the liver and atrophic renal glomeruli was comparable to the control group. The increase of interstitial connective tissue in the experimental group E10 were evident compared to the control, without disruption of spermiogenesis. Based on the results of the histomorphology, no dystrophic changes were observed in the examined parenchymatous organs of rabbits after feeding hempseed cake.


# Introduction

Seeds of industrial hemp (*Cannabis sativa* L.) are rich in nutrients and biologically active compounds, and their consumption can be considered beneficial for health. Cultivation of this plant originated in Central Asia (China) and after that expanded across Asia (India, Iran, and Pakistan), South America, Africa, and throughout Europe during medieval times [1] Rupasinghe et al., 2020). Hemp has received attention due to its rapid growth rate and high biomass production ([2] Rehman et al. 2021; [3] Callaway, 2004).

Large quantities of industrial hemp by-products are produced from at least three main hemp uses: fibre, cannabinoid, and oil extraction. The primary byproducts from these processing industries include hempseed meal (a.k.a. hempseed cake), which is produced during the extraction of oil from the seeds, and hemp biomass remaining after extracting cannabinoids, predominantly cannabidiol (a.k.a spent hemp biomass or SHB), both of which can be utilized as animal feed. Hempseed meal is used interchangeably with hempseed cake, as they are technically derived from the same process ([4] Ely and Fike, 2022).

Hemp seed oil has a high percentage of polyunsaturated fatty acids (PUFAs), especially linoleic acid (approx. 56%) and α-linolenic acid (approx. 20%), and it also contains small percentages of γ-linolenic

acid and stearidonic acid, which are rarely seen in vegetable oils ([3] Callaway, 2004; [5] Da Porto et al., 2012).

Hemp seeds are also rich in protein and fibre and contain other biologically active compounds, all of which can contribute to the health effects of hemp seed consumption.

However, notably interesting components of hemp seeds are different types of peptides that have been reported as having antioxidative, antihypertensive and α-glucosidase inhibitory activities ([6] Girgih et al., 2014; [7] Girgih, He, & Aluko, 2014; [8] Ren et al. 2016).

The seeds also contain significant amounts of lignan amides, which are phenolic amides thought to have cytotoxic, anti-inflammatory, antineoplastic and cardioprotective activities ([9] Flores-Sanchez & Verpoorte, 2008; [10] Nigro et al., 2020; [11] Yan et al., 2015).

Industrial hemp in the form of hempseed cake, as a source of essential fatty acids, minerals, vitamins, and fibre, is also used as animal feed. In our previous study ([12] Baláži et al., 2024), feeding hempseed cake to rabbits at two tested concentrations (5% and 10%) showed no negative effects on weekly body weight gain or total average body weight gain compared with the control group. The addition of hempseed cake did not reduce sperm concentration in the ejaculate, sperm motility, or progressive motility. Partial selected haematological and biochemical parameters were evaluated. No adverse effects of hempseed cake feeding on reproduction or health status were observed in male rabbits.

The current study aims to investigate haematological and biochemical parameters at the end of our study and histological appearance of rabbit parenchymatous organs feeding hempseed cake.

# Material and methods

### *Ethical statement*

The treatment of the animals was approved by the Ministry of Agriculture and Rural Development of the Slovak Republic no. SK U 18016 SK P 28004 in accordance with ethical guidelines presented in Slovak Animal Protection Regulation, RD 377/2012, which conforms to European Union Regulation 2010/63.

### *Manipulations with animals*

Clinically healthy rabbit males of the New Zealand White line (National Agricultural and Food Centre - RIAP Nitra, Slovak Republic) were used in this experiment. Rabbit males were fed either a standard diet (control group; C; n=10) or a diet enriched with hempseed cake (experimental group E5 with 5% of a hempseed cake; n=10) and (experimental group E10 with 10% of a hempseed cake; n=10) in 100 kg of the milled complete feed mixture. Animal housing is described in detail in our previous study ([12] Baláži et al., 2024). The males were fed with standard food (C) or food enriched with hempseed cake (E5, E10) for 4 months and they were weighted weekly. Average weekly and total weight gains were recorded, also semen analyses were also recorded. ([12] Baláži et al., 2024). At the end of experiment males were

euthanized to obtain parenchymatous organs (liver, kidney and testis). Blood was collected for haematological and biochemical analysis.

***Histological analysis***

For histological analysis, samples of rabbit liver, kidney and testis were fixed in 10% neutral formalin (Sigma-Aldrich) for 24 h, washed in a flowing water for 2 h, dehydrated in 70, 96 (2h) and 100% ethanol (1h) and embedded into Technovit 7100 (Heraeus Kulzer GmbH&CoKG, Wehrheim/Ts., Germany) according to the manufacturer's instructions. Thereafter, the ovaries were serially sectioned at 5 μm thickness using a standard rotation microtome AC-820 (American Corporation, USA). Sections were stained with a buffered solution of basic fuchsin and toluidine blue, diluted in a ratio of 3:2 ([13] Bourne and St. John, 1978) and embedded into Entellan (Merck, GmbH, Ltd, Darmstadt, Germany) according to [14] Hayat (1993), subsequently analysed with a light microscope.

***Biochemical and haematological analyses***

After the experiment, blood samples were collected from two individuals from each group (C, E5, E10) from the central auricular vein. For biochemical analyses, blood was collected in a tube without additive, for haematological analyses the tube contained an anticoagulant agent, namely EDTA ([12] Baláži et al., 2024).

Unclothed blood samples were analysed with an Abacus VET 5 haematology analyser (Diatron, Budapest, Hungary) ([15] Massányi et al, 2020). Following haematological parameters were analysed: WBC—total white blood cell count, LYM—lymphocytes count, MID—cell population of middle dimensions including monocytes and eosinophils, GRA—granulocytes count, LYM %—lymphocyte percentage, MID %—cell population of middle dimensions including monocytes and eosinophils percentage, GRA %—granulocytes percentage, RBC—red blood cell count, HGB—haemoglobin, HCT—haematocrit, MCV—mean corpuscular volume, MCH—mean corpuscular haemoglobin, MCHC—mean corpuscular haemoglobin concentration, RDWc—red cell distribution width calculated, RDWs—red cell distribution width standard, PLT—platelet count, PCT %—platelet percentage, MPV—mean platelet volume, PDWc—platelet distribution width calculated, and PDWs—standard.

Clotted blood was further processed by centrifugation (1006 x *g* for 20 minutes) and the obtained serum was immediately analysed. Sodium (Na) and potassium (K) concentrations were analysed using a semi-automatic ion-selective analyser EasyLyte (Medica, Bedford, MA, USA) ([16] Kovacik et al., 2020). Other blood parameters (Ca—calcium, P—phosphorus, Mg—magnesium, TP—total proteins, AST—aspartate aminotransferase, ALT—alanine aminotransferase, ALP—alkaline phosphatase, Bili—direct bilirubin, Creat—creatinine, Glu—glucose, Chol—cholesterol, TG—triglycerides, UA - uric acid) were analysed with commercial Randox kits, using a fully-automatic biochemical analyser Randox RX Monaco (Randox Laboratories, Crumlin, UK) ([17] Dupak et al., 2020; [12] Baláži et al., 2024).

***Statistical analysis***

For statistical evaluation was used STATGRAPHICS Centurion© (StatPoint Technologies, Inc., Warrenton, VA, USA). After basic statistical evaluation (results are presented as mean ± standard deviation), biochemical and haematological parameters were subjected to One-way ANOVA to determine statistical significance between control group (C) and experimental groups (E5, E10). *P*-value levels less than 0.05 were considered statistically significant.

# Results

### *Serum biochemistry evaluation*

Mineral profile parameters (K, Cl, Ca, P, and Mg) showed stable values with no statistically significant differences between groups, remaining within normal physiological ranges. All groups showed low bilirubin values within reference value without statistically significant differences. Liver enzymes (ALP, ALT, and AST) showed no significant changes, with all groups within reference ranges, excluding hepatotoxic effects of hempseed. Creatinine showed a statistically significant decrease in the experimental groups (E5: 56.21 $\mu M.l^{-1}$; E10: 41.61 $\mu M.l^{-1}$) compared to the control (80.18 $\mu M.l^{-1}$). Serum glucose concentrations remained stable in all groups (C: 7.59 $mM.l^{-1}$; E5: 8.30 $mM.l^{-1}$; E10: 7.87 $mM.l^{-1}$) within reference values. Serum cholesterol was statistically significantly lower in the E5 group (0.43 $mM.l^{-1}$) compared to the control (0.97 $mM.l^{-1}$), indicating a effect on lipid metabolism. Total protein values showed a slight decrease in the experimental groups compared to the control, but without statistical significance. Triglycerides (TG) and uric acid (UA) did not show statistically significant differences between the groups. Overall, we can conclude that most of the monitored biochemical parameters (Table 1) were within the reference values and without significant negative changes, while statistically significant differences in creatinine and cholesterol may indicate specific metabolic adaptations to the presence of hempseed meal in the feed.

**Table 1.** Serum chemistry evaluation of animals after experimental diet (results are presented as mean ± SD).

| Biochemical parameter | C | E5 | E10 | *p*-value | Reference range |
|---|---|---|---|---|---|
| K ($mM.l^{-1}$) | 4.36 ± 0.12 | 4.35 ± 0.01 | 4.16 ± 0.09 | 0.1896 | 3.5-6.9 |
| Cl ($mM.l^{-1}$) | 104.7 ± 1.84 | 103.1 ± 1.13 | 102.4 ± 0.42 | 0.3175 | - |
| Ca ($mM.l^{-1}$) | 3.83 ± 0.22 | 3.88 ± 0.17 | 3.85 ± 0.13 | 0.9726 | 2.7-3.5 |
| P ($mM.l^{-1}$) | 1.26 ± 0.19 | 1.17 ± 0.03 | 1.23 ± 0.02 | 0.7114 | 1.3-2.1 |
| Mg ($mM.l^{-1}$) | 1.18 ± 0.10 | 1.25 ± 0.07 | 1.09 ± 0.09 | 0.3129 | - |
| ALP ($U.l^{-1}$) | 21.5 ± 6.36 | 34.5 ± 4.95 | 46.0 ± 8.49 | 0.0800 | 12-96 |
| ALT ($U.l^{-1}$) | 46.6 ± 6.51 | 29.95 ± 6.86 | 52.75 ± 10.82 | 0.1407 | 45-80 |
| AST ($U.l^{-1}$) | 42.3 ± 0.0 | 30.55 ± 9.97 | 35.5 ± 14.00 | 0.5604 | 35-130 |
| Bili ($\mu M.l^{-1}$) | 1.35 ± 0.21 | 0.8 ± 0.14 | 1.2 ± 0.0 | 0.0685 | 0-12 |
| Creat ($\mu M.l^{-1}$) | **80.18 ± 0.78$^{a}$** | **56.21 ± 10.52$^{b}$** | **41.61 ± 2.34$^{b}$** | **0.0191** | 44.2-221 |
| Glu ($mM.l^{-1}$) | 7.59 ± 0.72 | 8.30 ± 0.76 | 7.87 ± 0.60 | 0.6394 | 4.1-8.6 |
| Chol ($mM.l^{-1}$) | **0.97 ± 0.13$^{a}$** | **0.43 ± 0.04$^{b}$** | 0.63 ± 0.14 | **0.0380** | 0.3-2.1 |
| TP ($g.l^{-1}$) | 59.36 ± 0.16 | 53.02 ± 6.88 | 50.82 ± 0.57 | 0.2317 | 54-75 |
| TG ($mM.l^{-1}$) | 1.65 ± 1.49 | 0.99 ± 0.07 | 2.10 ± 0.33 | 0.5308 | - |
| UA ($\mu M.l^{-1}$) | 3.01 ± 2.24 | 5.39 ± 0.22 | 3.81 ± 2.02 | 0.4752 | - |

*ns* – not significant; values with different superscript letters in a same row are significantly different; [1]MSD Merck Veterinary Manual (*Modified Sept 2024*)- Serum Biochemical Analysis Reference Ranges.

***Haematological evaluation***

Haematological parameters showed minimal changes in rabbits fed hempseed compared to the control group (Table 2). White blood cell (WBC) counts remained within the reference range in all groups (C: 9.95 ± 0.21 x $10^{9}\cdot L^{-1}$; E5: 9.83 ± 1.30 x $10^{9}\cdot L^{-1}$; E10: 8.86 ± 0.30 x $10^{9}\cdot L^{-1}$) with no statistically significant differences. Lymphocytes showed stable values without significant differences between groups (C: 6.05 ± 0.35; E5: 5.07 ± 0.41; E10: 5.27 ± 0.65 x $10^{9}\cdot L^{-1}$). Monocytes and neutrophils were within the reference range in all groups with no statistically significant differences. Red blood cell (RBC) counts were within the reference range (C: 6.86 ± 0.21; E5: 6.54 ± 0.51; E10: 7.12 ± 0.21 x $10^{12}\cdot L^{-1}$) with no statistically significant differences. Haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean

corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) showed stable values without significant changes between groups. Platelet (PLT) count showed a statistically significant decrease in experimental group E5 (171.0 ± 12.73 x $10^9 \cdot L^{-1}$) compared to the control group (255.5 ± 7.78 x $10^9 \cdot L^{-1}$) and group E10 (241.5 ± 21.92 x $10^9 \cdot L^{-1}$), which may indicate mild thrombocytopenia. PCT was also statistically significantly lower in group E5 (0.100 ± 0.00%), with a significant difference compared to groups C (0.155 ± 0.01%) and E10 (0.155 ± 0.01%), which correlates with the observed decrease in platelet count. Mean platelet volume (MPV) and platelet distribution width (PDW) showed only minimal changes with no statistically significant differences.

**Table 2.** Haematological evaluation of animals after experimental diet (results are presented as mean ± SD).

| Haematological parameter | C | E5 | E10 | *p*-value | Reference ranges[1] |
|---|---|---|---|---|---|
| WBC ($10^9.l^{-1}$) | 9.95 ± 0.21 | 9.83 ± 1.30 | 8.86 ± 0.30 | 0.4202 | 6-12 |
| LYM ($10^9.l^{-1}$) | 6.05 ± 0.35 | 5.07 ± 0.41 | 5.27 ± 0.65 | 0.2534 | 1.6-10.6 |
| MON ($10^9.l^{-1}$) | 0.415 ± 0.02 | 0.295 ± 0.09 | 0.545 ± 0.23 | 0.3570 | 0.05-0.5 |
| NEU ($10^9.l^{-1}$) | 3.34 ± 0.05 | 4.47 ± 1.63 | 3.05 ± 0.13 | 0.3982 | 1-9.4 |
| LYM (%) | 61.2 ± 1.70 | 52.25 ± 11.10 | 59.4 ± 5.37 | 0.5043 | 30-85 |
| MON (%) | 4.15 ± 0.21 | 2.95 ± 0.50 | 6.20 ± 2.83 | 0.2861 | 1-4 |
| NEU (%) | 31.65 ± 2.34 | 44.8 ± 10.60 | 34.4 ± 2.55 | 0.2461 | 20-60 |
| RBC ($10^{12}.l^{-1}$) | 6.86 ± 0.21 | 6.54 ± 0.51 | 7.12 ± 0.21 | 0.3606 | 4-7 |
| HGB ($g.dl^{-1}$) | 13.25 ± 0.35 | 12.75 ± 0.07 | 13.75 ± 0.64 | 0.2058 | 8-15 |
| HCT (%) | 42.38 ± 0.53 | 41.19 ± 0.22 | 42.84 ± 2.26 | 0.5257 | - |
| MCV (fl) | 62.0 ± 2.83 | 63.0 ± 4.24 | 60.0 ± 1.41 | 0.6495 | 58-67 |
| MCH (pg) | 20.1 ± 0.14 | 19.5 ± 1.41 | 19.25 ± 0.35 | 0.6334 | 17.1-23.5 |
| MCHC ($g.dl^{-1}$) | 31.35 ± 0.50 | 30.9 ± 0.0 | 32.1 ± 0.14 | 0.0597 | 29-37 |
| RDWc (%) | 15.9 ± 0.14 | 15.9 ± 0.42 | 15.4 ± 0.28 | 0.3085 | - |
| RDWs (fl) | 41.2 ± 0.28 | 41.4 ± 1.13 | 41.2 ± 0.28 | 0.1261 | - |
| PLT ($10^9.l^{-1}$) | **255.5 ± 7.78$^a$** | **171.0 ± 12.73$^b$** | **241.5 ± 21.92$^a$** | **0.0222** | 250-650 |
| MPV (fl) | 6.05 ± 0.07 | 5.85 ± 0,21 | 6.50 ± 0.14 | 0.0504 | - |
| PCT (%) | **0.155 ± 0.01$^a$** | **0.100 ± 0.0$^b$** | **0.155 ± 0.01$^a$** | **0.0038** | - |
| PDWc (%) | 29.5 ± 0.70 | 29.2 ± 1.84 | 31.85 ± 0.64 | 0.1958 | - |
| PDWS (fl) | 7.25 ± 0.35 | 7.15 ± 0.92 | 8.65 ± 0.35 | 0.1483 | - |

*ns* – not significant; values with different superscript letters in a same row are significantly different; [1]MSD Merck Veterinary Manual (*Modified Sept 2024*) - Haematology Reference Ranges.

***Histological findings of the liver, kidneys, and testes***

In liver samples from the experimental groups (E5 and E10), vacuolization of the hepatocyte cytoplasm was observed, with the cells appearing transparent. The cell nuclei retained their normal appearance, and the occurrence of hyperchromatic nuclei was sporadic in all groups. Hepatocyte vacuolization

corresponded to early-stage fatty infiltration, and the presence of larger vacuoles in the cytoplasm of liver cells was more pronounced in group E10 (Fig. 1B). In the experimental groups, hepatic sinusoids around the central vein (*v. centralis*) were sporadically dilated and filled with connective tissue. In samples from the control group (C), an increased occurrence of large lipid droplets in hepatocytes was observed only sporadically (Fig. 1A). The presence of smaller or larger vacuoles in the hepatocyte cytoplasm, probably lipids (lipids are removed during sample processing), varied among the groups. Compared with the control group, the intensity of vacuolization was comparable in the experimental groups.

In kidney samples from the experimental groups (E5 and E10), sporadic incidence of atrophic glomeruli was observed. The parietal layer of the glomerular capsule was comparable with the control group (Fig. 1D). The tubular apparatus was most frequently affected by vacuolization of the proximal tubules. The occurrence of vacuolization and tubular parenchymal atrophy was more or less comparable among the groups. In samples from the control group (C), these changes were observed only sporadically (Fig. 1C).

In testicular samples from the experimental groups (E5 and E10), slight changes in the seminiferous epithelium were observed compared with the control group (C). These changes were most commonly manifested by an increase in interstitial connective tissue between the seminiferous tubules (Fig. 1F). The presence of spermatids in the cells of the seminiferous epithelium was similar across groups. The intensity of interstitial Leydig cell occurrence was comparable between the experimental and control samples (Fig. 1E).

# Discussion

### Biochemical and haematological evaluation

Hempseed cake is obtained by cold pressing hemp seeds during oil extraction for human consumption and may be subsequently incorporated into animal nutrition. Based on the biochemical blood analysis, the tested concentrations can be considered to exert at most minimal clinical effects. Most parameters remained within their reference ranges and did not differ significantly between the control and experimental groups. A positive finding is the stability of hepatic enzymes, which rules out hepatotoxic effects of hempseed cake at the administered concentrations. The mineral profile, bilirubin, glucose, total proteins, triglycerides and uric acid showed stable values, further supporting the safety of dietary supplementation with hempseed cake ([18] Addeo et al., 2025; [19] Cozma et al, 2015; [20] Fallahi et al., 2022; [21] Parker et al., 2022; [22] [18–22] Rbah et al., 2024). A significant decrease in cholesterol levels in the experimental group E5 suggests an effect on lipid metabolism ([23] Jurgoński et al., 2020). In association with the hepatic histological findings (increased fat deposition in hepatocytes), this may indicate impaired export of lipoproteins from the liver ([24] Chen et al., 2025; [25] Marigorta et al., 2024). Under normal conditions, excess lipids are exported into the bloodstream. A change in diet could lead to lipid retention in hepatocytes and a subsequent decrease in circulating lipids in the blood ([22] Rbah et al., 2024). In group E10, cholesterol levels were normal, indicating normalization of cholesterol levels,

which may be the result of dynamic adaptation of the liver to diet, which is essentially a time-dependent adaptation mechanism ([26] Robins et al., 1995; [27] Han et al., 2012). An interesting finding is the decrease in creatinine, an important indicator of kidney function. It is known that elevated serum creatinine concentrations are likely to signal kidney damage or disease ([28] Perrone et al., 1992). On the other hand, the decrease itself is rarely associated with acute kidney problems. Most often, the decrease is associated with muscle loss, which we have not observed. Any decrease could be related to excessive hydration caused by a change in diet. This leads us to the likely possibility of a change in nutrition/feed composition, which could affect protein metabolism and thus creatinine production ([29] Brosnan and Brosnan, 2010). This may be due to different amino acid content and the associated differences in protein utilization without direct association with kidney damage ([19] Cozma et al., 2015; [30] Vastolo et al., 2021; [31] Nedeljkovic et al., 2025). However, changes in the histological appearance of the kidneys-specifically enlarged glomeruli and thickened proximal tubules are associated with elevated creatinine concentrations. These structural alterations may represent a compensatory mechanism to maintain renal filtration capacity ([32] Fong et al., 2014; [33] McArdle et al., 2020). Thus, these findings suggest an adaptive response, or initial changes in the kidneys following hempseed cake administration, which at the tested concentrations and duration do not yet indicate clinical renal dysfunction. This is supported by the observed decrease in serum creatinine concentrations (group E5) followed by an increase (group E10).

The total count of white blood cells (WBC), lymphocytes, monocytes, and neutrophils, as well as their percentage distributions, remained stable with no statistically significant differences between groups. These findings rule out substantial activation of the immune system. ([34] Costa et al., 2025; [35] Mujahid et al., 2025). No significant differences were observed in red blood cell counts (RBC), haemoglobin (HGB), or haematocrit (HCT) levels between groups. These results indicate no signs of anaemia or impaired erythropoiesis in the organism. ([36] Abdelnour et al., 2018; [37] Osman et al., 2021; [38] Özkan et al., 2012). These key parameters for oxygen transport indicate that, despite mild histological changes in organs, the blood's ability to effectively carry oxygen remained unimpaired. On the other hand, we observed a significant decrease in platelet count (PLT) and plateletcrit (PCT) in group E5 compared to both the control (C) and E10 groups, suggesting transient thrombocytopenia likely triggered by the organism's rapid response to the dietary change ([39] Agiang et al., 2017; [40] Erkurt et al., 2012; [41] Karimi et al., 2007; [42] Royer et al., 2010). Such a temporary decrease in platelet count and plateletcrit may indicate that at a lower dose (E5) of cannabis extracts, an acute phase of decreased platelet production and increased platelet degradation occurred, while at a higher dose (E10) led to adaptation of the organism or compensatory mechanisms of platelet production ([43] Kaçar et al., 2025; [44] Leyva et al., 2011; [45] Roy and Paul, 2024; [46] Scharf, 2021). The transient decrease in platelets may also represent a response to the described histological changes, which did not progress to pathological conditions, as platelet levels normalized in the experimental group E10. This could be associated with the organism's enhanced ability to adapt at higher doses of hempseed cake in the feed, linked to more stable renal function and thrombopoiesis ([47] Arnoldussen and Witkamp, 2021; [48] Câmara et al., 2016; [42] Royer et al., 2010).

Overall, we can conclude that the decrease in serum creatinine and changes in platelet count correspond to structural changes in the kidneys and are likely part of a complex adaptation to dietary change. The decrease in cholesterol levels with histological findings of increased fat deposition in hepatocytes suggests the influence of diet on lipoprotein export, with subsequent normalization of cholesterol, probably through dynamic adaptation of the liver. Thus, hempseed cake at the tested concentrations did not induce severe clinical signs of organ dysfunction or haematological disorders. Such findings warrant further verification and testing, as similar conclusions are common in *in vivo* conditions where the organism or organ strives to maintain functional capacity despite mild stressors from the environment, dietary changes, or various forms of bioactive substance supplementation ([48] Câmara et al., 2016; [49] Georganas et al., 2020; [50] Juráček et al., 2021; [51] Kopčeková et al., 2018; [52] Kovacik et al., 2020; [16] Kovacikova et al., 2019; [53] Tathong et al., 2024; [54] Tripathi et al., 2008; [55] Vlaicu et al., 2023).

**Histomorphology**

The effects of hempseed cake on reproductive and selected non-reproductive parameters in male rabbits were investigated by Baláži et al. (2024) [12]. Rabbits males were fed either a standard diet (C) or a diet supplemented with hempseed cake (5% and 10% per 100 kg of ground complete feed mixture; E5 and E10). Rabbit body weight gain, sperm concentration, motility, progressive motility, and sperm quality were evaluated using computer-assisted sperm analysis (CASA) and flow cytometry. They found that feeding hempseed cake at both tested concentrations had no effect on weekly body weight gain or total average body weight gain compared with the control group ($p > 0.05$). The addition of hempseed cake from industrial hemp (*Cannabis sativa* L., cultivar Finola) as a protein feed for rabbits did not affect sperm concentration in the ejaculate, sperm motility, or progressive motility ($p > 0.05$). Selected haematological and biochemical parameters were also examined. The E5 group showed positive trends in liver profile parameters, whereas the E10 group exhibited opposite trends, although all values remained within the reference range. No adverse effects of hempseed cake feeding on reproduction or health status of rabbits were detected, and therefore the authors recommend the use of hempseed cake at inclusion levels up to 10% in rabbit nutrition.

Histological examination of rabbit livers revealed increased accumulation of lipid droplets in the experimental groups compared with the control group. Hepatocellular morphological changes may induce latent metabolic disorders, most frequently manifested by fat accumulation in hepatocytes ([56] Kuntz and Kuntz, 2002). Lipid accumulation (steatosis) in liver cells can occur in the form of small or large droplets, which can be visualized using histological techniques. However, finely dispersed lipids within hepatocytes cannot be visualized microscopically. The causes of hepatic steatosis are diverse and may include phytotoxins ([56] Kuntz and Kuntz, 2002). It is likely that higher doses of hempseed cake, administered in the experimental rabbit group (E10), contributed to increased vacuolisation of hepatocytes compared with the control group.

In the present study, we describe the effects of feeding hempseed cake derived from hemp seeds with low cannabinol content. Industrial hemp contains the compound delta-9-tetrahydrocannabinol (THC), with levels below 0.2%, considering the content in the entire aboveground biomass at any phenophase. Through modern breeding, THC content in certain industrial hemp cultivars has been reduced to below 0.05%. Conversely, higher-quality fiber cultivars are characterized by increased cannabidiol (CBD) content.

Delta-9-tetrahydrocannabinol (THC) is a phytocannabinoid responsible for the psychoactive effects of cannabis. The mechanisms of cannabinoid metabolism and renal excretion and their potential effects on kidney function were investigated by [57] Mlynarska et al. (2024) and [58,59] Rein et al. (2020; 2024). THC is primarily metabolized in the liver, while approximately 20% of its metabolites are excreted in the urine [60] (Musshoff et al., 2006). It has been demonstrated that the highest concentration of synthetic cannabinoids is found in the kidneys ([61] Schaefer et al., 2019). Histological examination following renal biopsy most frequently reveals acute tubular necrosis, with some cases of acute interstitial nephritis ([62] Pendergraft et al., 2014; [63] Alipour et al., 2019). In our study, such alterations were not detected, except for the presence of atrophic glomeruli in group E10.

The cannabinoid delta-9-tetrahydrocannabinol (THC) has been studied by [64] Fonseca and Rebelo (2021), who reported that THC indirectly reduces gonadotropin release, suppresses hypothalamic gonadotropin-releasing hormone (GnRH) secretion, and disrupts folliculogenesis, ovulation, and sperm maturation and function. An association between cannabis exposure and testicular atrophy has been identified, although much of the evidence relies on animal models ([65] Payne et al., 2019). A study in dogs administered daily cannabis extract (12.5 mg/kg body weight) reported testicular degeneration and necrosis after only 30 days of exposure ([66] Dixit et al., 1977). Histological examination of testes in cannabis-exposed mice by [67] Mandal and Das (2010) revealed reduced spermatogonia counts, damage to the basal membrane, and decreased seminiferous tubule diameter. Restoration of spermatogenesis and testicular cell function was observed 45 days after of cannabis cessation exposure; however, testicular weight and seminiferous tubule size were not fully restored. Increase in interstitial connective tissue between the seminiferous tubules was observed in the experimental rabbit group (E10) in our study. Mandal and Das (2010) suggested that testicular damage may result from oxidative stress, supported by a significant decrease in antioxidant enzyme levels in affected testes. This hypothesis was further supported by [68] Alagbonsi et al. (2016), who demonstrated that testicular damage induced by *Cannabis sativa* in rats was ameliorated by administration of a combined antioxidant treatment of melatonin and vitamin C.

## Conclusion

In conclusion, we found that hempseed cake at the tested concentrations did not induce severe clinical signs of organ dysfunction or haematological and biochemical disorders. Most parameters remained within their reference ranges and did not differ significantly between the control and experimental groups. We found that the histological appearance of the liver, kidneys, and testes in the experimental

groups of rabbits fed diets containing 5% and 10% hempseed cake was essentially similar. The observed changes included varying degrees of hepatic vacuolization, representing the lowest degree of regressive damage to hepatic parenchymal cells. In the renal parenchyma, atrophic renal glomeruli occurred sporadically. A higher incidence of the interstitial connective tissue was observed in the experimental group of rabbits fed the higher inclusion level of hempseed cake. The mechanisms by which hemp press cake feeding contributes to these histopathological changes require further investigation in future experiments.

# Declarations

### Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Perplexity Pro (© 2025 Perplexity AI) and DeepL translator (DeepL SE) to translate or to check the grammar of the text and to summarize extensive texts prepared by the authors to improve the readability and the academic quality of the processed text. After using this tool, the authors checked and edited the content and take full responsibility for the content of the publication.

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### Author Contributions

Andrej Baláži**:** Investigation, Visualization, Conceptualization, Data curation, Writing - original draft. Anton Kováčik: Visualization, Software, Data curation, Writing - original draft. Juraj Pivko: Investigation, Data curation, Software, Writing - original draft. Alexander Makarevich: Language correction, Writing - original draft. Peter Chrenek: Supervision, Funding acquisition and project administration, Writing - review & editing. All authors have read and approved the manuscript.

### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

The data presented in this study are available in article.

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# Figures

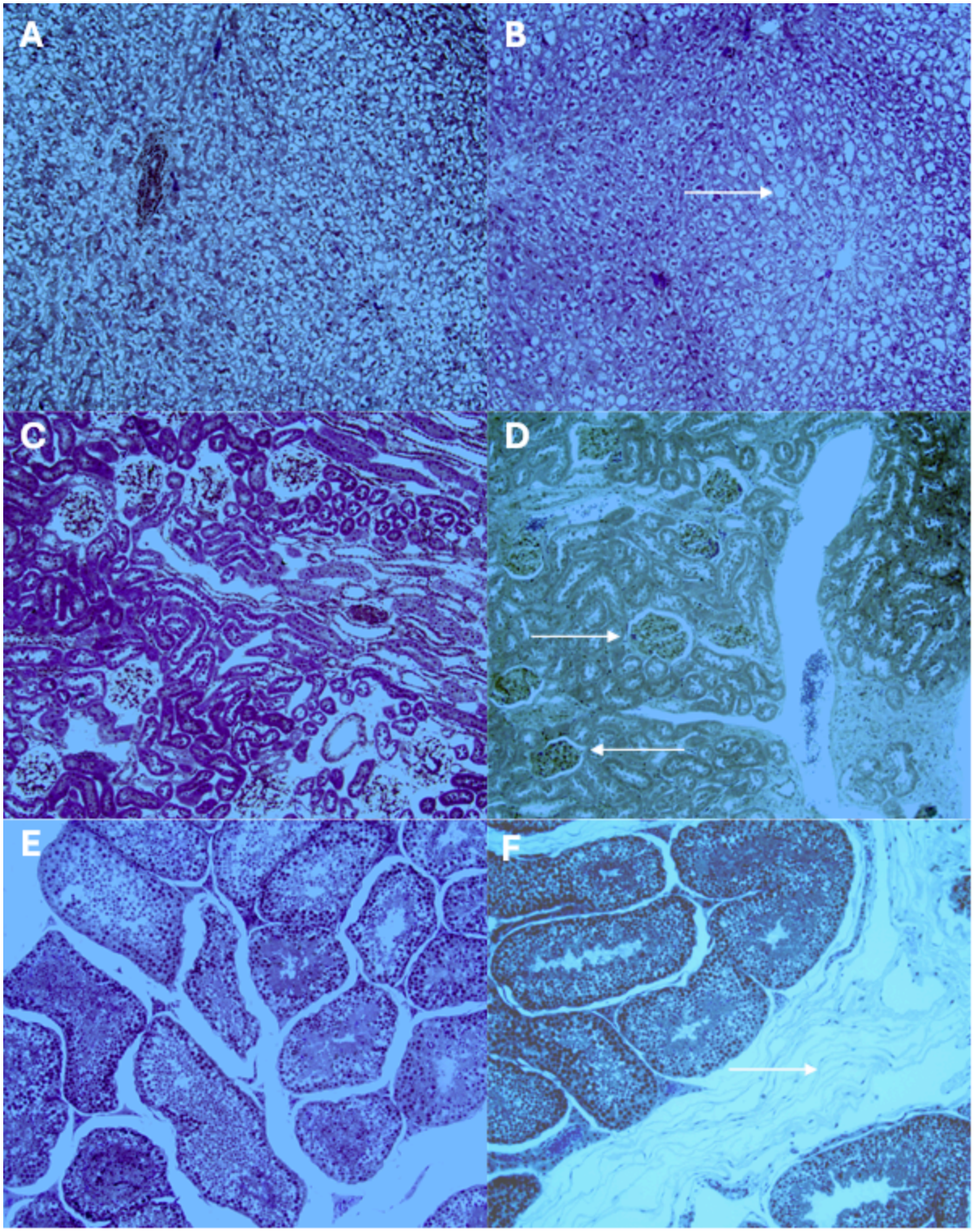


**Figure 1**

Histological appearance of the liver, kidney, and testis in experimental and control groups.
(A) Liver, control group; (B) liver, experimental group E10, the occurrence of fat droplets in the cytoplasm of liver cells (arrow); (C) kidney, control group; (D) kidney, experimental group E10, the sporadic occurrence of atrophic glomeruli (arrows) in the kidney; (E) testis, control group (F) testis, experimental group E10, the occurrence of seminiferous tubules and the proliferation of connective tissue between

them (arrow). Representative micrographs showing histological changes in the examined organs. Staining: basic fuchsin and toluidine blue. Magnification x400.