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RESEARCH ARTICLE

Preclinical evaluation of an innovative dietary intervention for non-alcoholic hepatic steatosis in Sprague-Dawley rats

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Objective: We developed an innovative food designed for special nutritional needs, intended as an adjuvant in the prevention or treatment of non-alcoholic hepatic steatosis. This study evaluates its preclinical effectiveness, with results aimed to inform future clinical trial design in more homogeneous patient populations.

Methods: This preclinical experimental study involved 32 Sprague-Dawley rats divided into four groups: a Control group (standard diet), a High Fat Diet group (30% and 60% fat), Experimental group 1 (high fat diet followed by innovative food), and Experimental group 2 (high fat diet and innovative food administered concurrently). Body weight, urine, blood glucose, and 11 hepatic parameters were measured at the end of the induction and intervention phases.

Results: High fat feeding increased energy intake, weight gain, and fat mass, particularly in males. A decrease in food and water intake was noted during the induction phase in high fat feeding groups. The High Fat Died group showed persistent signs of liver stress. Experimental group 1 showed consistent improvements, with individual variability in response to innovative food intervention. Experimental group 2 showed significant results during induction stages, indicating a stronger protective effect.

Conclusions: A high fat feeding with 30% fat over 10 weeks was insufficient to induce hepatic steatosis, while a 60% fat feeding for additional 5 weeks successfully induced obesity and liver pathology. Post-induction innovative food intervention reduced weight gain and improved liver biomarkers. Blood glucose, transaminases, alkaline phosphatase, and total cholesterol levels suggest that innovative food has protective effects, supporting its potential use in preventing and managing non-alcoholic fatty liver disease.

Keywords: innovative food, Sprague-Dawley rats, hepatic steatosis, high-fat diet

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Introduction

Metabolic dysfunction-associated fatty liver disease (MAFLD), previously termed non-alcoholic fatty liver disease (NAFLD), is the most prevalent chronic liver disorder globally [1]. Its prevalence varies across the globe, affecting an estimated 25% of the population globally [2]. The overall prevalence increased significantly over time to 38%, and was significantly higher in men than in women (40% vs 26%) [3]. Its overall incidence continues to rise alongside obesity and type 2 diabetes, and was estimated to be 47 cases per 1000 person/year rates [3, 4].

MAFLD is defined by hepatic steatosis in association with metabolic risk factors such as insulin resistance, hypertension, and dyslipidemia [4]. It is now recognized as a multisystem disease contributing significantly to cardiovascular and liver-related morbidity and mortality [5].

Lifestyle and diet are key contributors to MAFLD onset and progression [6]. Excessive consumption of saturated fats, refined sugars, and ultra-processed foods promotes liver fat accumulation, inflammation, and fibrosis [7]. High-fat diet consumption has been associated with multiple health complications, especially adverse changes in mitochondrial activity [8] and thereby affecting cognitive functions [9]. Recent work shows the intestinal microbiota and ferroptosis have a key role in the pathological progression of MAFLD, and inhibition of ferroptosis may become a novel therapeutic approach for the treatment [10]. Hence, diet-induced rodent models, particularly those fed HFD, are widely used in preclinical research to mimic human MAFLD and test interventions [11].

Sprague-Dawley rats are commonly used due to their physiological similarities to humans in metabolism and liver function [12]. However, inconsistencies in HFD composition (fat content, type, and source), exposure duration, and strain sensitivity limit cross-study comparability [13, 14]. These variables affect steatosis severity, inflammation, and the transition to steatohepatitis or fibrosis [15].

This study aims to evaluate the effectiveness of an innovative food (IF) intervention in high fat diet (HFD)-induced MAFLD rat models. By assessing liver morphology and function under different dietary conditions, we aim to clarify IF's protective role, promote preclinical insights, and support translatable dietary strategies for MAFLD prevention and treatment.

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Methods

The current study is an experimental preclinical research, conducted as part of the bachelor's research project, performed between January and July 2024.

Animals and housing: thirty-two healthy Sprague–Dawley rats (males and females), six months age (from INCDMM Cantacuzino, Bucharest, Romania), weighing between 241 g - 292 g (females) and 327 g – 606 g (males), were housed under controlled conditions of temperature (23 ± 1°C) and humidity (60 ± 5%). All animals were housed individually in separate cages with access to standard rodent food (INCDMM Cantacuzino, Bucharest, Romania) and tap water. All animals were allowed to acclimatize for one week on the normal diet before grouping.

Experimental design: After acclimatization, the rats were randomly divided into four groups: the control group (M) ($n = 8$); HFD group (C) ($n = 8$); experimental group 1 (E1) (n = 8), and experimental group 2 (E2) (n = 8). The control group received standard food (4,178 kcal/g), while the HFD group and E1 group received an HFD (30% fat) (5,66 kcal/g), and E2 group received 50% HFD (30% fat) + 50% IF concurrently, all for 10 weeks. After 10 weeks of Induction stage I (in the absence of hepatic steatosis, confirmed by histopathological analyses) the rats fed with HFD further received HFD with 60% fat, for an additional 5 weeks. All groups were fed with a rate of 35 g/day and with 500 ml of fresh water per week *ad libitum*. Post-induction stage, HFD group continued with HFD and the Experimental 1 group followed to receive IF. E2 group followed the same diet as the previous – HFD 60% (6,41 kcal/g) fat and IF administered concurrently.

Nutritional intervention and monitoring stages: the study line was divided into two distinct stages:

Induction of NAFLD: (i) Induction I with HFD 30% fat – for 10 weeks and (ii) Induction II with HFD 60% fat – for an additional 5 weeks.

Nutritional Intervention with IF: dietary intervention with IF for 17 weeks.

Innovative food: according to the invention, the innovative food we have created, is composed mainly of *Grifola frondosa* and *Cordyceps sinensis* and has 197,51 kcal (828,38 kJ)/100g: 5% fat, 22% carbohydrates, 54% fibers and 16% proteins. The main bioactive components of the innovative food are polysaccharides, polyphenols, cordycepin and prebiotics, with contribution on metabolism, liver protection and gut microbiome. It is currently under patent application at the National Office for Inventions and Trademarks of Romania, with patent application no. A 000412/2024 [16].

Laboratory analyses: To monitor each animal, the following measurements were performed at the end of each interventional stage: body weight; blood glucose; analysis of hepatic biochemical parameters, urinary parameters, and histopathology analysis.

Food and water intake were registered weekly, while body weight, blood glucose, liver parameters from blood, and histopathological analyses were analyzed at the end of each stage.

For body weight, as well as for liver and blood glucose analyses, the animals were anesthetized via inhalation anesthesia using Anesteran (Rompharm), administered by the Biobase's veterinary doctor.

For blood glucose analysis, a glucometer with glucose test strips, Contour plus ELITE was used.

The biochemical liver analyses were performed using the Element RC automatic biochemistry analyzer, manufactured by Scil (Germany), available in the Biobase Department. The analyzer operates based on absorption spectroscopy at different wavelengths. The absorption spectrum is used to detect changes, while the processor calculates the concentration of biochemical substances present in the blood sample. The analyzer was used in combination with Element RC Hepatic test rotors, which measure 11 parameters: albumin (ALB); total protein (TP); globulin (GLOB); albumin/globulin ratio (A/G); total bilirubin (TB); gamma glutamyl transferase (GGT); aspartate aminotransferase (AST); alanine aminotransferase (ALT); alkaline phosphatase (ALP); total biliary acids (TBA); total cholesterol (TC). The reference values included in the Element RC automatic biochemistry analyzer were considered.

After induction and intervention stages, histopathological examinations were performed at the Histopathology Laboratory of George Emil Palade University of Medicine, Pharmacy, Science and Technology of Târgu Mureș.

For statistical analysis, data were analyzed by one-way ANOVA followed by Bonferroni post hoc t-tests and presented as frequencies and percentages. Statistical significance was considered for $p < 0.05$ for all analyses, with two-tailed p-values. Missing data were excluded and isolated analytical outliers were removed from analysis.

Ethical statement

Animal care and experimental protocols were approved by the Scientific Research Ethic Commission of G.E. Palade University of Medicine, Pharmacy, Sciences and Technology (approval number: 2711/27.12.2023) and National Authority for Veterinary Health and Safety Food (approval number: 70/17.01.2024).

Results

Food intake. The average of food intake (kcal/g food) is decreasing in groups fed with HFD compared to the control group with standard food, where de average is increasing. Water intake tends to decrease in all animal groups as the percentage of fat in the diet increases.

Body weight has an increasing trend. While the female's body weight has an increasing trend in all groups, in males, the body weight growth trend was greater in the control group than in the other two groups with HFD, during the induction stage (Figure 1).

In the control group, the average weight gain in the female group was 60-145 g, and the average weight gain in

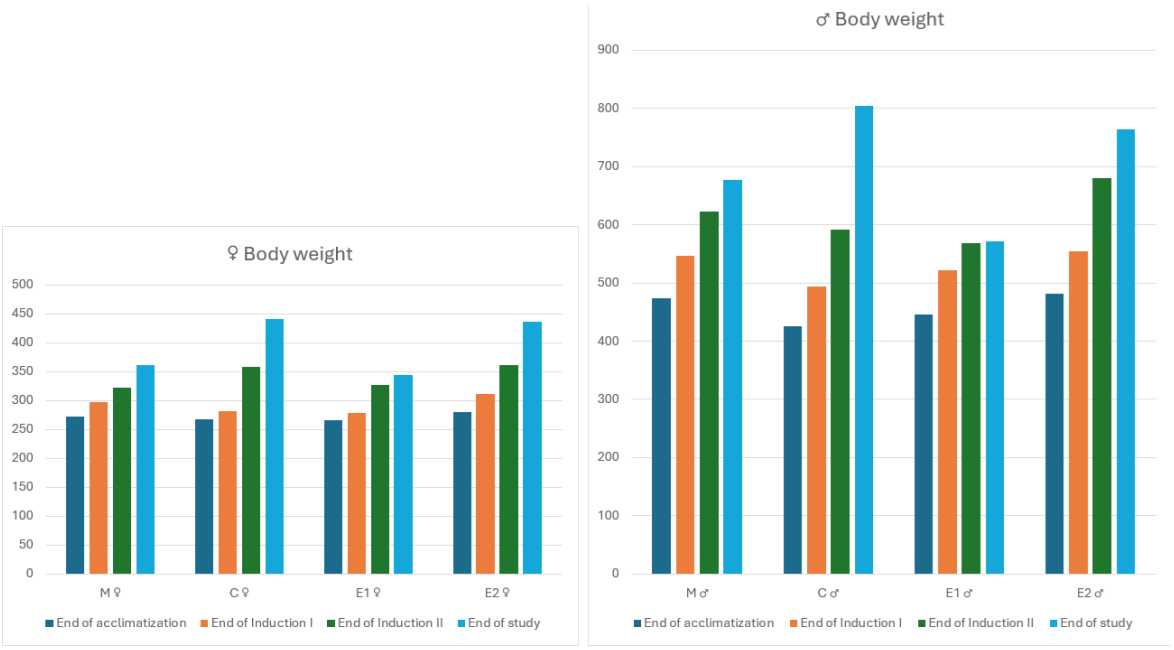

Fig. 1. Females and males body weight modifications during the study: mean value (g) in M group (normal diet), C group (HFD), E1 group (HFD then IF,) and E2 group (HFD + IF concurrently)

the male group was 160-210 g. In HFD group, all animals gained weight over time, especially at the end of the study. HFD female trend shows substantial weight gain over the study, with more weight than control females (max 194 g vs. 145 g), suggesting the HFD increased adiposity or metabolic intake. HFD male trend showed substantially higher maximum weight gain than maximum control males, suggesting growth acceleration is evident in HFD males (Figure 1).

In E1 group we have observed significant increase during induction stage and stabilization after switching to IF intervention ($p < 0.001$). All females gained weight, but less than HFD females. Final weights and gains were intermediate between control and HFD females, suggesting the effectiveness of food intervention after induction, on HFD effects. The male trend shows less weight gain than HFD group males and even the control group.

In the E2 group, all females show substantial weight gain, with greater gain than E1 and control groups. Their gains approach HFD group levels, suggesting E2 intervention was less effective for weight control. In the end of induction stage, E2 and control groups are significantly heavier than E1 ($p < 0.05$). HFD group is intermediate. In the end of study, E2 is significantly heavier than E1 ($p < 0.05$); other pairwise comparisons are not significant after correction.

Sex has a strong and expected effect on body weight across stages, males are larger ($p < 0.005$).

Body weight significant increases across times, with largest overall gain of all groups by study end ($p < 0.001$).

Blood glucose measurement. Baseline plasma glucose was comparable across groups (ANOVA, $p > 0.05$). During the induction stages glucose rose in several animals with a significant time effect and a group and time interaction ($p < 0.05$), indicating that glycemic trajectories differed between diet groups (Table 1). During the induction stage, HFD group showed larger glucose increases in multiple animals compared with groups receiving the innovative food. By the end of the study many animals fed with IF showed partial normalization of glucose, consistent with metabolic modulation by the innovative diet.

Liver biochemical analyses. Our results highlight how hepatic parameters reflect responses to HFD exposure and the effects of IF intervention. Hepatic parameters that exceed the reference values are highlighted in Table 2.

Table 1. Blood glucose measurement (mg/dl) in all groups during the study

Rats' code	End of Induction I	End of Induction II	End of study
M [a]	151.5	148.8	142
M [b]	157.5	158.3	160.3
C [a]	111	131.7	130.7
C [b]	143.3	154.3	153
E1 [a]	124.8	140.5	150.7
E1 [b]	155.3	173	148.3
E2 [a]	137.8	149	149.7
E2 [b]	159	154.3	161.7

Data are presented as mean value; [a] - females; [b] - males

Table 2. Liver parameters in all groups during the study

Rats' code	End of Induction I	End of Induction II	End of study
M [a]			
ALB	3.6 L	3.9 L	4 L
TP	6.7	6.3 L	6.5
AST	55 H	52.3	53
ALT	35.3	34.5	29.8
ALP	141.8 H	135.3 H	117.5
TC	99.7	106 H	124.9 H
M [b]			
ALB	3.5 L	3.7 L	3.2 L
TP	6.3 L	6.4	5.8 L
AST	51.5	57.3 H	65.8 H
ALT	29.8	36.3	38 H
ALP	228.3 H	207 H	196.5 H
TC	72.1	82	100
C [a]			
ALB	4.3	3.6 L	3.8 L
TP	7.2	7.6	6.4
AST	57.3 H	59 H	72.7 H
ALT	59.5 H	53 H	55.3 H
ALP	233.3 H	302.7 H	215 H
TC	106.6 H	133.3 H	101.1 H
C [b]			
ALB	3.5 L	3.5 L	3.5 L
TP	6.9	7.9	7
AST	53.5 H	64 H	65.5 H
ALT	49 H	38.5 H	62 H
ALP	366 H	391.3 H	308 H
TC	102.7 H	135.6 H	122.7 H
E1 [a]			
ALB	4.1	4.3	3.5 L
TP	7.1	7	6.3 L
AST	60.3 H	56.8 H	52.7
ALT	54.8 H	65 H	34.3
ALP	311.5 H	220.3 H	110
TC	107.6 H	113.7 H	102.8 H
E1 [b]			
ALB	3.7 L	3.4 L	3 L
TP	6.8	6.5	5.8 L
AST	49.5	63.7 H	51.3
ALT	43.3 H	46 H	39 H
ALP	366.3 H	283 H	169 H
TC	91.5	186.5 H	81.6
E2 [a]			
ALB	4.4	4.2	3.9 L
TP	7.6	6.9	6.2 L
AST	52.3	47.7	53.7 H
ALT	49.3 H	55.7 H	41.7 H
ALP	263.3 H	162.3 H	138.7 H
TC	122 H	124.3 H	118.2 H
E2 [b]			
ALB	3.6 L	3.7 L	3.4 L
TP	6.2 L	6.3 L	6.4
AST	51.8	52.7	54.7 H
ALT	30.3	40 H	46.3 H
ALP	229.8 H	185 H	153.3 H
TC	79.6	91.9	102.6 H

Data are presented as mean value; [a] - females; [b] - males; *L – below the lower reference limit; *H– above the upper reference limit

Serum protein indicators (ALB and TP)

Serum ALB and TP concentrations remained within normal or near to normal physiological limits for Sprague-Dawley rats [27]. No statistically significant differences were observed among dietary groups ($p > 0.05$) across any stage.

In the control group, some low albumin and low TP suggest potential protein malnutrition and standard food formula should be recalculated and adjust. C group (HFD-fed) showed mild fluctuations but no clear hypoalbuminemia, suggesting hepatic synthetic capacity remained intact despite lipid loading. E1 and E2 groups exhibited values comparable to or slightly higher than M, indicating no impairment of protein synthesis and possible protective nutritional effect of IF. HFD did not produce hypoalbuminemia; the IF diet in E1 and E2 maintained normal hepatic protein output, consistent with improved metabolic handling of dietary fat.

Hepatic enzyme activity (AST, ALT, ALP)

Serum AST and ALT increased markedly in HFD-fed rats during induction 2 (AST $p = 0.032$; ALT $p = 0.027$), coinciding with the escalation of dietary fat to 60%. Both E1 and E2 groups showed significantly lower enzyme activities than controls (AST: HFD > E1, $p = 0.04$; ALT: HFD > M, $p = 0.03$). At the end of the study, enzyme values in E1 and E2 returned toward baseline, while HFD animals remained mildly elevated. HFD induced transient hepatocellular stress, particularly during the Induction II stage. Switching to or combining with IF mitigated enzyme el-

evations, suggesting anti-inflammatory and hepatoprotective effects of the IF diet.

ALP showed significant group effects (Induction II: p = 0.032; End of study: p = 0.016). The C group demonstrated consistently higher ALP activity than all other groups, especially versus E1 at the study end (p = 0.03). E1 and E2 rats maintained lower ALP levels throughout, reflecting reduced cholestatic or biliary stress. Prolonged HFD 60% elevated ALP, indicating biliary enzyme induction and potential lipid-related hepatic stress. IF supplementation for E1 and E2 groups effectively prevented these changes, supporting improved bile metabolism and hepatobiliary function.

Lipid metabolism marker (TC)

At baseline, TC levels were comparable across groups. During Induction II stage, when HFD group received HFD 60%, TC rose significantly compared with M (p = 0.043) and E2 (p = 0.049). E1 and E2 maintained TC values near normal physiological range (≈100 mg/dL). By the study end, cholesterol normalized across all groups, though C animals remained slightly higher on average. The transient hypercholesterolemia in HFD and E1 groups during the Induction phase, reflects the metabolic burden of high fat feeding. The normalization in E1, after IF intervention and stable values in E2 indicate that IF feeding likely enhanced lipid utilization and prevented diet induced dyslipidemia.

Sex related differences

Males exhibited slightly higher AST, ALT, and ALP, consistent with known sex based hepatic enzyme variability in Sprague-Dawley rats, while females tended toward higher ALB and TC. These variations were not large enough to influence overall group trends. No significant sex differences were detected (p > 0.1).

Histopathological examination. The presence of hepatic steatosis was further confirmed through histopathological examination, which revealed both micro- and macro-vesicular lipid droplets within hepatocytes. Initially, after induction I stage, microscopic images of liver biopsies indicated the absence of lipid deposits (Figure 2).

A complex and organized architecture is noted, with normal appearing hepatocytes arranged in cords

After the Induction II stage, the results of liver biochemical analyses were confirmed by the results of histopathological analyses. Microscopic images of liver biopsies confirmed the presence of lipid deposits in liver tissue taken from rats fed with HFD 60% fat (Figure 3).

On the examined sections, hepatic parenchyma with lipid micro- and macro-vacuoles at the level of hepatocytes is noted

Discussions

Body weight during the study. Males start and end significantly heavier than females, which is expected in Sprague-Dawley rats [16]. Gradual weight increase across all phases in the control group was normal in healthy rats. Their consistent weight gain serves as a baseline. No sudden weight loss is observed, indicating the animals were likely in good health and not under stress from the control conditions. These rats are still growing, particularly the males, so the weight gain aligns with normal developmental patterns [17].

HFD in rodents typically causes increased adiposity, metabolic dysregulation, and sometimes insulin resistance and is evident accelerated weight gain in both sexes compared to controls [18]. HFD exaggerates the natural sex-based growth differences even more than in the control group.

E1 group received a post-induction IF intervention that moderated the weight gain caused by the previous HFD, suggesting the intervention may have limited the weight gain, counteracting some HFD-induced effects. The intervention appears effective in both sexes, with weight trends closer to controls than to the HFD group.

E2 group showed the largest final body weight, significantly higher than E1 at induction stage and study end. This suggests that the concurrent HFD + IF mix did not

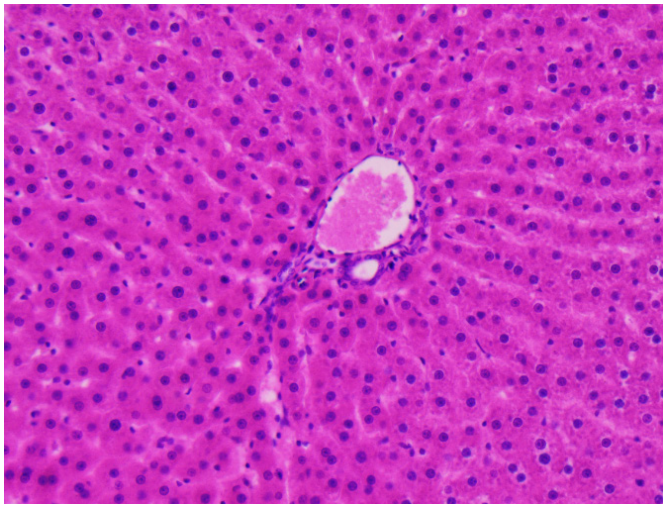

Fig. 2. Microscopic description of liver biopsy from C3 female rat post-induction I stage; HE 20x.

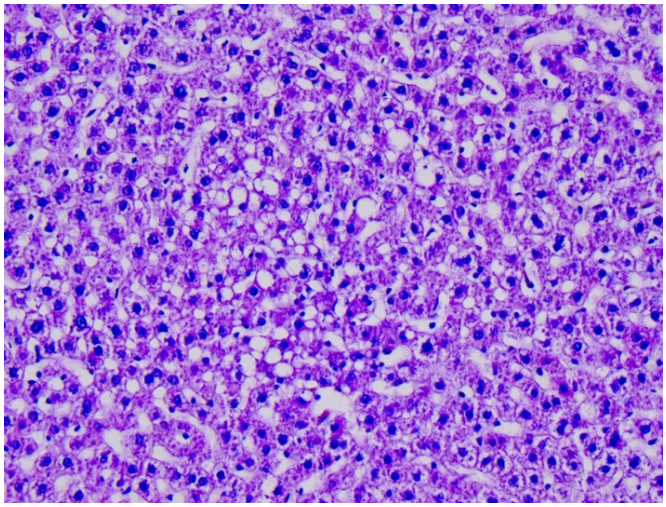

Fig. 3. Microscopic description of liver biopsy from E1.1 female rat post-induction II stage; HE 20x.

prevent weight gain; in fact, animals on mixed diet gained most, possible due to higher total caloric intake, palatability or metabolic effects of combined diets.

Selection of a high-fat dietary model of NAFLD. To investigate the efficacy of potential therapeutic food and validate the preclinical study, reliable, simple and reproducible tools are needed. For that purpose, animal models for diet-induced NAFLD models were developed to mimic the human disease [19]. **S**prague-Dawley rats on HFD develop NAFLD with elevated ALT and cholesterol after just two weeks, escalating to steatosis, inflammation, and fibrosis over 10–20 weeks [20].

However, our dietary pattern during Induction I stage (30% fat), was insufficient to induce liver steatosis in 10 weeks. This is the reason why we extended the induction stage (Induction II) for another 5 weeks, and we increased the proportion of fat to 60%.

A high fat diet, which includes 30%–75% of total calories derived from saturated fat, induced metabolic changes leading to fat storage, insulin resistance, and NAFLD development. However, the HFD dietary regime may give variable results, which depend on rodent species and strain. Sprague-Dawley rats fed an HFD develop NAFLD and NASH symptoms, which are associated with their diet-dependent susceptibility to obesity [21].

During the Induction stages, in both groups fed with HFD, the liver parameters were higher as the fat percentage increased in induction II vs induction I stages. After the hepatic steatosis induction, liver function tests confirmed morphological and functional hepatic impairment, as evidenced by markers of hepatocellular injury, hepatic insufficiency, and steatosis [14, 15].

Rats in the HFD group show clear signs of liver stress and potential damage (high AST, ALT, ALP), metabolic imbalance (high TC), and potential protein metabolism disturbances (low ALB and TP). These effects are typical and expected from prolonged HFD exposure in Sprague-Dawley rats [21].

Our results of liver biochemical analyses were confirmed by histopathological analyses, meaning no lipid deposits after the Induction I stage. After the Induction II stage, microscopic images of liver biopsies confirmed the presence of lipid deposits in the liver tissue sampled from rats fed an HFD.

Exposure to the IF intervention. HFD induced significant weight gain in both sexes, and males had more pronounced increases than females. Post-induction IF intervention demonstrated a clear reduction in weight gain compared to HFD (Figure 1). Final weights were closer to control group levels, suggesting potential efficacy in mitigating diet-induced obesity.

A substantial number of publications regarding nutritional intervention demonstrated effective reductions in liver injury markers in HFD fed rats [22-26].

Liver parameters interpretation. In all groups, we observed that some animals had mild hypoalbuminemia (<4.1), especially in male rats during and after induction/intervention stages. HFD and both experimental groups showed reduced ALB alongside low TP over time, likely indicating liver stress or compromised protein synthesis. Albumin is a liver-synthesized protein; low levels indicate impaired liver synthetic function. In long-term high fat feeding, serum albumin typically decreases due to hepatic dysfunction [27].

AST is an enzyme released with hepatocyte injury. AST elevation was most prominent in the HFD group, suggesting hepatocellular injury. HFD group had the highest AST at each post-induction time, and the control group had the lowest ($p=0.004$).

HFD group had markedly higher ALT than the control at each induction point, indicating substantial liver injury. These trends align with published data, high fat diets significantly elevate ALT [19]. Elevated AST and ALT enzymes in our HFD group mirror the literature publications showing acute liver stress under HFD [28].

Alkaline phosphatase (ALP) was the most elevated in the HFD group. E1 and E2 groups showed attenuation of this trend, with E2 having the best normalization ($p=0.037$). ALP spikes align with cholestatic or biliary injury observed in HFD models. Indeed, studies report an HFD-induced ALP increase (~24%) [20].

HFD is well-known to induce dyslipidemia with significantly higher total cholesterol (TC), and LDL in HFD rats [26]. In our HFD group, we had marked hypercholesterolemia, particularly after Induction II stage and during Intervention. E1 group shows the best cholesterol control after the intervention stage, especially in males. E2 female group had elevated TC but generally lower than HFD and some normalization toward study end; occasional elevations, but many values stayed near or below the upper limit. E2 male group exhibited the greatest improvement in cholesterol regulation, indicating a potential protective effect of IF intervention on lipid metabolism. Total cholesterol increases match HFD-driven dyslipidemia trends. E1 and E2 group interventions reduced liver enzymes and cholesterol, and reflect similar improvements seen with resveratrol [9], curcumin [22] and β-sitosterol [23].

Standard laboratory rat food is generally formulated to meet basic nutritional needs and maintain health. However, even standard food can influence liver parameters, especially in young adult rats, due to some critical factors [27]. Standard rat food, while considered "neutral," may cause mild hepatic enzyme elevations due to its energy content, composition variability, and the metabolic sensitivity of young Sprague-Dawley rats. These effects can be especially pronounced in studies involving metabolic stress or long-term feeding protocols [29].

Lifestyle and diet, probiotics and prebiotics, GLP-1 RA, and SGLT2i may become effective and safe treatments to alleviate NAFLD [30]. We have demonstrated that in combination with a low-fat diet, IF treatment decreased serum

TC and AST/ALT and ALP levels by restoring impaired lipid metabolism in rats previously fed HFD. Our innovative food also effectively enhanced weight loss, which may have occurred through a decrease in visceral fat, as previously reported in the literature [31], but the mechanisms remain to be identified.

The expanding knowledge regarding the beneficial effects of medicinal mushrooms on metabolic diseases (especially NAFLD and NASH), has driven researchers to pursue potential efficacious and safe therapies using nutrition as medicine. Cordycepin, polysaccharides and polyphenols have been widely used as a food for the treatment of diseases associated with inflammation and oxidative injury, such as hepatotoxicity and liver injury [32, 33].

Our current study thus provides an alternative approach to ameliorate NAFLD by supplementation of caloric-restricted diets with IF that is proposed for the invention approval.

Conclusion

In summary, prolonged high fat feeding (30–60%) in Sprague–Dawley rats led to transient hepatocellular stress, as indicated by elevated transaminases, alkaline phosphatase, and serum cholesterol. The species divergence in weight gain, plus a longer interval to achieve significant hepatic lipid droplet accumulation in the mice may be attributed to species, composition of diets (30% versus 60% fat), and the duration of high fat feeding.

Incorporation of innovative food, either as a dietary switch (E1) or concurrent mix (E2), normalized hepatic enzyme activity and stabilized lipid metabolism, demonstrating a significant hepatoprotective and metabolic-modulating effect.

The innovative food has proven to be highly effective in reducing body weight, evidenced by the significant decrease in body mass in the E1 group compared to the HFD group, in both female and male rats.

The biochemical data support the hypothesis that IF contributes to hepatic resilience under dietary fat overload without impairing protein synthesis or metabolic balance.

Authors' contribution

TR: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Writing – original draft

IMM: Conceptualization; Formal analysis; Investigation; Methodology; Supervision; Writing – review & editing

Conflict of interest

None to declare.

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References

1. Eslam M, Newsome PN, Sarin SK, et al. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. J Hepatol. 2020;73(1):202–209
2. Younossi ZM. Non-alcoholic fatty liver disease – A global public health perspective. Jour Hepatol. 2019; 70(3):531-544
3. Riazi K, Azhari H, Charette JH, et al. The prevalence and incidence of NAFLD worldwide: A systematic review and meta-analysis. Lancet Gastroenterol Hepatol. 2022;7(9):851–861
4. Lim S, Meigs JB. Epidemiology of NAFLD and type 2 diabetes: Health burden and clinical implications. Nat Rev Endocrinol. 2021;17(1):63–74
5. Pipitone RM, Ciccioli C, Infantino G, La Mantia C, Parisi S, Tulone A, Pennisi G, Grimaudo S, Petta S. MAFLD: a multisystem disease. Ther Adv Endocrinol Metab. 2023; 14: 1–23
6. Hydes T, Alam U, Cuthbertson DJ. The Impact of Macronutrient Intake on Non-alcoholic Fatty Liver Disease (NAFLD): Too Much Fat, Too Much Carbohydrate, or Just Too Many Calories? Front Nutr. 2021;16(8):640557
7. Zhao L, Clay-Gilmour A, Zhang J, Zhang X, Steck SE. Higher ultra-processed food intake is associated with adverse liver outcomes: a prospective cohort study of UK Biobank participants. Am J Clin Nutr. 2024;119(1):49-57
8. Song M, Bai Y, Song F. High-fat diet and neuroinflammation: The role of mitochondria. Pharmacol Res. 2025;212:107615
9. Su X, Li Q, Yang M, Zhang W, Liu X, Ba Y, Deng Q, Zhang Y, Han L, Huang H. Resveratrol protects against a high-fat diet-induced neuroinflammation by suppressing mitochondrial fission via targeting SIRT1/PGC-1α. Exp Neurol. 2024;380:114899
10. Ji J, Wu L, Wei J, Wu J, Guo C. The Gut Microbiome and Ferroptosis in MAFLD. Journal of Clinical and Translational Hepatology. 2023;11(1):174–187
11. Semmler G, Wöran K, Scheiner B, et al. HFD-fed rodent models for metabolic fatty liver disease—An update and critical review. Nutrients. 2021;13(9):3154
12. Petrasek J, Iracheta-Vellve A, Szabo G. The evolving role of rodent models in NAFLD: Bridging nutritional interventions and mechanistic insights. Hepatology Communications. 2023;7(1):e01123
13. Svegliati-Baroni G, Marzioni M. Preclinical models of MAFLD: From nutritional overload to fibrosis and NASH. Nat Rev Gastroenterol Hepatol. 2023;20(5):318–331
14. Rohbeck P, Lindner D, Schierwagen R, et al. Impact of dietary fat type and content on MAFLD progression in rats. Nutrients. 2022;14(18):3779
15. Delli Bovi AP, Parola M, Cannito S. Lipids, inflammation, and fibrosis in MAFLD: Experimental models and translational perspectives. Cells. 2023;12(3):490
16. Matran IM, Banescu CV, Talmaci R. Aliment adjuvant in prevenirea/tratamentul steatozei hepatice nealcoolice. BOPI. 2024;11:15
17. Stanciu GD and Coman C. Tratat de medicină experimentală: şoareci şi şobolani de laborator. Ed. „Gr. T. Popa", Iaşi 2022, 59-96
18. Jensen VS, Hvid H, Damgaard J, Nygaard H, Ingvorsen C, Wulf EM, Lykkesfeldt J, Fledelius C. Dietary fat stimulates development of NAFLD more potently than dietary fructose in Sprague–Dawley rats. Diabetol Metab Syndr. 2018;10(4):1-13
19. Tessari P, Coracina A, Cosma A, Tiengo A. Hepatic lipid metabolism and non-alcoholic fatty liver disease. Nutrition, Metabolism & cardiovascular Diseases. 2009; 19:291-302
20. Maciejewska D, Lukomska A, Dec K, Skonieczna-Zydecka K, Gutowska I, Skorka-Majewicz M, et al. Diet-Induced Rat Model of Gradual Development of Non-Alcoholic Fatty Liver Disease (NAFLD) with Lipopolysaccharides (LPS) Secretion. Diagnostics (Basel). 2019; 9(4):205-215
21. Xu ZJ, Fan JG, Ding XD, Qiao L, Wang GL. Characterization of High-Fat, Diet-Induced, Non-alcoholic Steatohepatitis with Fibrosis in Rats. Dig Dis Sci. 2009;55(4):931–940
22. Li S, You J, Wang Z, Liu Y, Wang B, Du M, Zou T. Curcumin alleviates high-fat diet-induced hepatic steatosis and obesity in association with modulation of gut microbiota in mice. Food Research International, 2021; 143: 110270
23. Abo-Zaid OA, Moawed FS, Ismail ES, Farrag MA. β-sitosterol attenuates high- fat diet-induced hepatic steatosis in rats by modulating lipid metabolism, inflammation and ER stress pathway. BMC Pharmacol Toxicol, 2023; 24(1):31
24. Song L, Qu D, Zhang Q, Jiang J, Zhou H, Jiang R, Li Y, Zhang Y, Yan H. Phytosterol esters attenuate hepatic steatosis in rats with non-alcoholic fatty liver disease rats fed a high-fat diet. Nature Scientific Reports. 2017;7: 41604

25. Lin HY, Chen CC, Chen YJ, Lin YY, Mersmann HJ, Ding ST. Enhanced amelioration of high-fat diet-induced fatty liver by docosahexaenoic acid and lysine supplementations. Biomed Res Int. 2014;2014:1-11
26. Lasker S, Rahman MM, Parvez F, Zamila M, Miah P, Nahar K, Kabir F, Sharmin SB, Subhan N, Ahsan GU, Alam MA. High-fat diet-induced metabolic syndrome and oxidative stress in obese rats are ameliorated by yogurt supplementation. Nature Scientific Reports. 2019; 9:20026
27. Pettrino C, Argentino-Storino A. Clinical chemistry and haematology historical data in control Sprague-Dawley rats from pre-clinical toxicity studies. Exp Toxicol Pathol. 2006, 57(3):213-219
28. Jia YJ, Liu J, Guo YL, Xu RX, Sun J, Li JJ. Dyslipidemia in rat fed with high-fat diet is not associated with PCSK9-LDL-receptor pathway but ageing. J Geriatr Cardiol. 2013;10(4):361–368
29. Kucera O, Garnol T, Lotkova H, Stankova P, Mazurova Y, Hroch M, Bolehovska R, Rousar T, Cervinkova Z. The effect of rat strain, diet composition and feeding period on the development of a nutritional model of non-alcoholic fatty liver disease in rats. Physiol Res. 2011;60(2):317-328
30. Mantovani A, Dalbeni A. Treatments for NAFLD: State of Art. Int J Mol Sci. 2021;22:2350
31. Rodríguez-Correa E, González-Pérez I, Clavel-Pérez PI, Contreras-Vargas Y, Carvajal K. Biochemical and nutritional overview of diet-induced metabolic syndrome models in rats: what is the best choice? Nutrition and Diabetes. 2020;10:24-39
32. Lan T, Yu Y, Zhang J, Li H, Weng Q, Jiang S, Tian S, Xu T, Hu S, Yang G, Zhang Y, Wang W, Wang L, Zhu Q, Rong X, Guo J. Cordycepin Ameliorates Nonalcoholic Steatohepatitis by Activation of the AMP-Activated Protein Kinase Signaling Pathway. Hepatology. 2021;74(2):686-703
33. Hu R. Grifola frondosa may play an anti-obesity role by affecting intestinal microbiota to increase the production of short-chain fatty acids. Front Endocrinol (Lausanne). 2023;13:1-11