



69th Annual Meeting of the European Federation of Animal Science

Dubrovnik, Croatia - 27th to 31st August 2018

Evaluation of two laying hen strains kept in different cage systems for liver health

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1.10.2018

INTRODUCTION

- Increasing awareness about welfare of hens bring a great change on housing systems around the world.
- Housing systems can be effective on the types and extent of disorders such as liver disorders.



INTRODUCTION

- Fatty Liver Disorder, characterized by excessive fat deposits in the hen's liver and abdomen, is one of the major causes of mortality in commercial flocks.
- More than 90% of the fatty acids can be synthesised in a birds liver.



INTRODUCTION



Housing conditions and Nutrition are the main factors causing fatty liver in birds, such as lack of exercise and the consumption of high energy diets.



INTRODUCTION

- The study was undertaken to evaluate the differences in blood parameters and fatty liver disorder of two laying hen strains kept in conventional and enriched cages.



MATERIALS AND METHODS

- 532 beak trimmed brown (Lohmann Brown Classic; LB) and 532 beak trimmed white (Lohmann LSL Classic; LW) laying hens (16 weeks old) were kept in conventional and enriched caging systems.
- Both caging systems were installed in the same building.
- Each system consisted of three rows.



MATERIALS AND METHODS

- The conventional cages were designed to house 20 hens on each of them (with dimensions measuring 192 cm width, 62.5 cm depth and 57 cm height).
- Nipple type drinkers (8 nipples/cage) were used.



MATERIALS AND METHODS

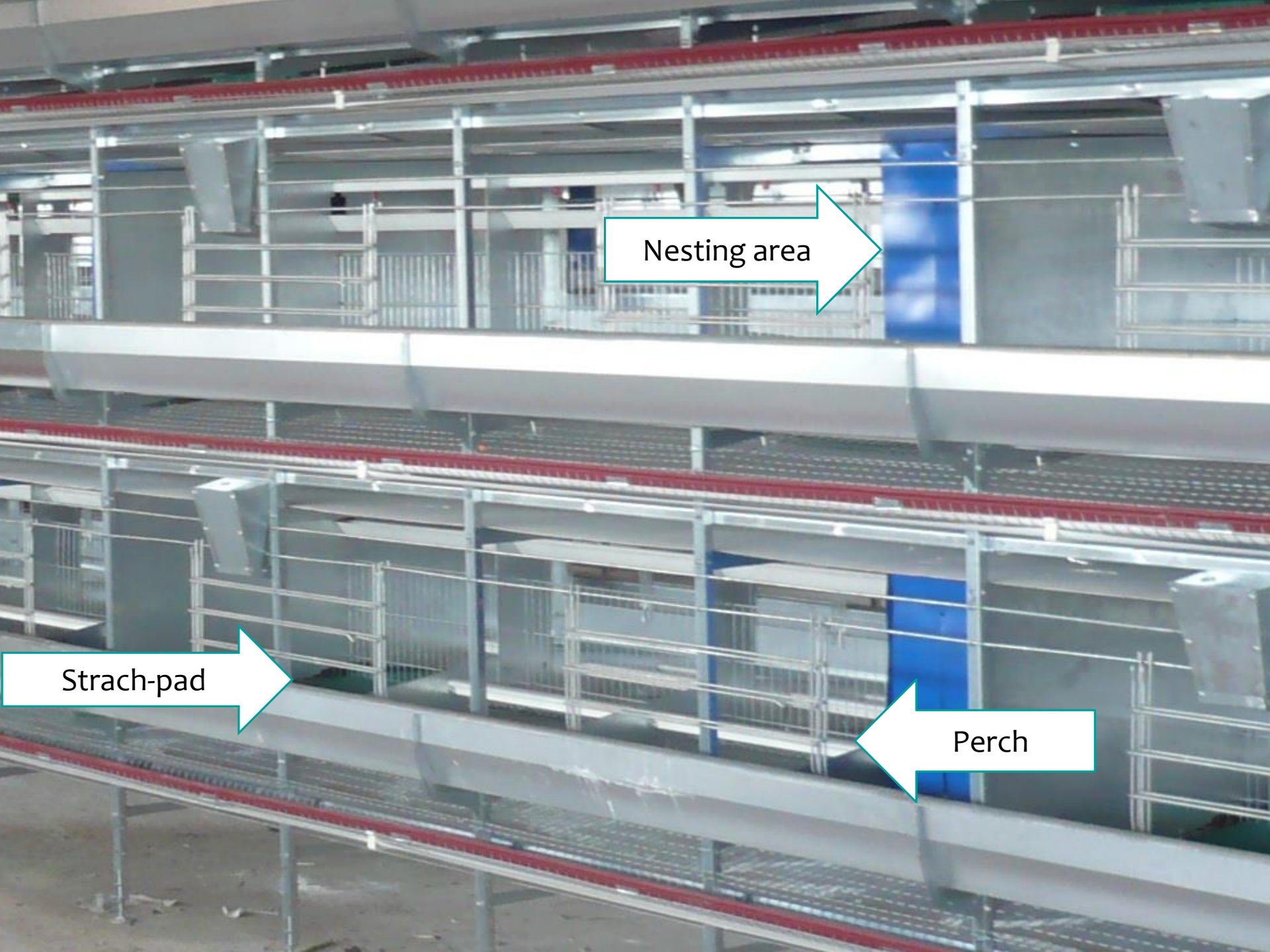
- The enriched cages were designed to house 18 hens in each of them (with dimensions measuring 240 cm width, 62.5 cm depth and 57 cm height).
- The enriched cages included a **nest area** (48 cm width × 62.5 cm depth), **scratch-pad area** (35 cm width × 35 cm length), **perch** and **embossed claw shortener** (12 cm width × 3 cm length).



MATERIALS AND METHODS

- The nesting area was separated from the other areas by a blue curtain composed of plastic strips.
- Two plastic perches 190 and 137 cm in length ran parallel to the feed through in the perch area.
- The scratch-pad areas were green plastic mesh.
- Nipple drinkers were used (8 nipples/cage).





Nesting area

Strach-pad

Perch

MATERIALS AND METHODS

- At 16 weeks of age all pullets were weighted.
- 252 pullets each of LW and LB strains were housed randomly in the enriched cages.
- 280 pullets each of LW and LB strains were housed randomly in the conventional cages.
- Each strain and cage system group consisted of 14 cages.



Table 1. Composition and analysed content of nutrients of experimental diets

	Laying Periods (Weeks Of Age)				
	16-17	18-31	32-45	46-59	60-73
Maize	51.6	52.0	53.4	54.0	54.8
Wheat	7.6	7.80	6.80	6.90	6.90
Soybean meal, 47%	17.4	17.3	17.3	17.1	16.2
Full-fat Soy	8.00	6.52	6.09	4.34	4.10
Sunflower seed meal	5.50	5.70	5.10	6.20	6.40
Limestone	8.00	8.50	9.10	9.40	9.60
Dicalcium phosphate	1.20	1.50	1.50	1.40	1.30
Sodium chloride	0.21	0.21	0.21	0.21	0.21
DL-Methionine	0.17	0.15	0.15	0.15	0.14
Vitamin mineral premixes ¹	0.25	0.25	0.25	0.25	0.25
Sodium bicarbonate	0.10	0.10	0.10	0.10	0.10
Analysed value					
ME, MJ/kg	11.6	11.4	11.4	11.3	11.3
CP, %	17.8	17.3	17.0	16.6	16.2
Ca, %	3.40	3.70	3.95	4.05	4.12
Total P, %	0.58	0.61	0.60	0.58	0.55
Methionine, %	0.44	0.41	0.41	0.41	0.39
Lysine, %	0.87	0.84	0.83	0.80	0.77

¹Supplied per kg of diet: 3.6 mg retinol, 0.06 mg cholecalciferol, 30 mg DL- α -tocopherol acetate, 2.5 mg menadione dimethylpyrimidinolbissulfite, 2.5 mg thiamin, 6 mg riboflavin, 4 mg pyridoxol, 20 μ g cobalamin, 25 mg niacin, 8 mg calcium-D-pantothenate, 1 mg folic acid, 50 mg ascorbic acid, 50 μ g D-biotin, 150 mg choline chloride, 1.5 mg canthaxanthin, 0.5 mg apocarotenoic acid ester, 80 mg Mn, 60 mg Zn, 60 mg Fe, 5 mg Cu, 1 mg I, 0.5 mg Co, 0.15 mg Se.

MATERIALS AND METHODS

- The lighting program was 16 L:8 D during the laying period.
- The body weight was determined individually at the 73rd week old.
- One hen was (total 14), with the average weight from each group, selected for the blood parameters.
- The blood samples were drawn from the wing veins.



MATERIALS AND METHODS

- The serum was separated by the centrifugation of the samples at 1500 rpm for 15 min.
- The levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol and triglycerides were determined by Vitros 350 autoanalyser (New York, USA; Product code 680–2153) using their accompanying commercial kits (Vitros Chemistry Products, Ortho-Clinical Diagnostics, Johnson-Johnson Company, New York, USA).



MATERIALS AND METHODS

- After the blood was drawn from them, all of the 14 hens were slaughtered.
- The relative liver weight of each hen was calculated (relative liver weight = [liver weight (g)/live weight (g)] × 100).
- Each hen's liver was evaluated macroscopically for **colour** (reddish-brown or yellow), **consistency** (soft or not) and **shape** (sharp or round).



- Then each liver was divided into two halves for the microscopic examination.
- Two slides were prepared for each half of the liver.

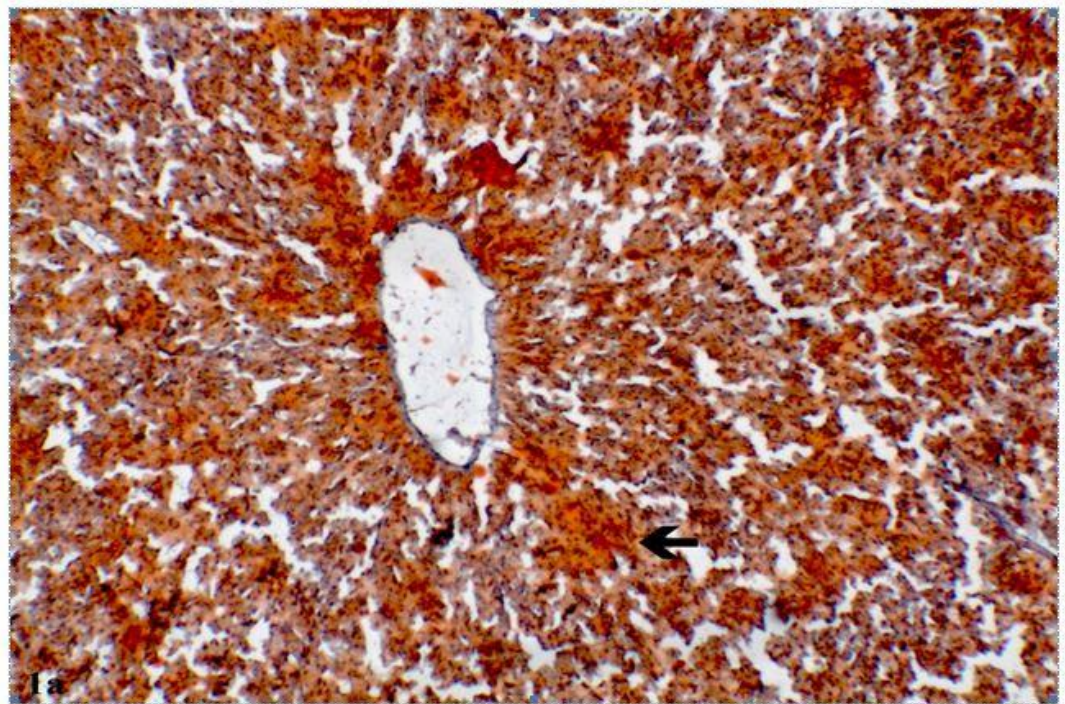


Figure 1a: Many lipid droplets in the hepatocytes in LW group (arrow), moderate, Oil Red O Stain, x100

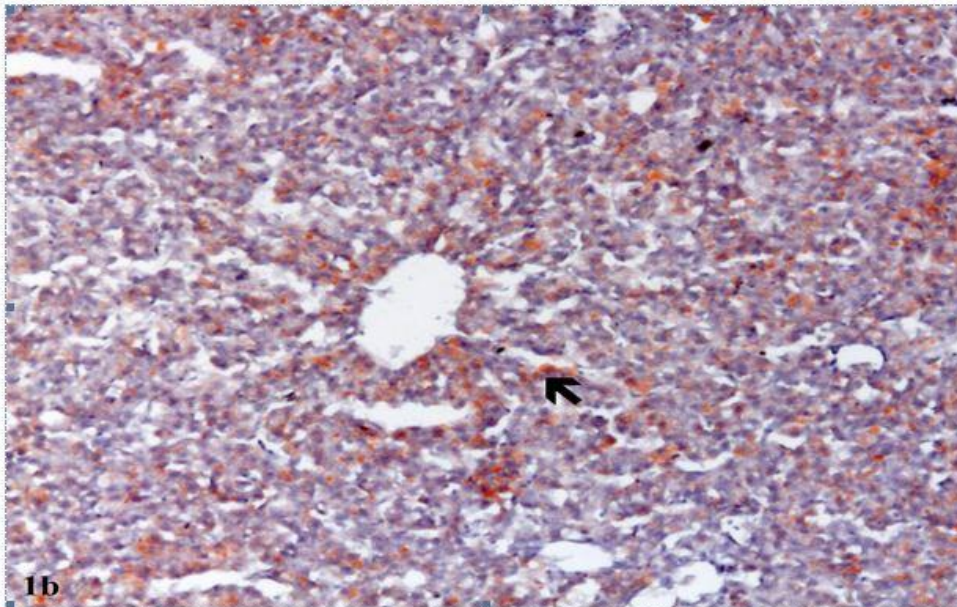


Figure 1b: Some lipid droplets in the hepatocytes in LB group (arrow), slight, Oil Red O Stain, x100

- The diffusion and frequency of lipid droplets for the fatty liver score were evaluated microscopically.

MATERIALS AND METHODS

- Each slide was scored as;
 - negative (droplets less than 10%),
 - slight (droplets between 10–30%),
 - mild (droplets between 30–70%) and
 - severe (droplets more than 70%).



RESULTS AND DISCUSSION

- In this study, serum ALT ($P=0.038$) and AST ($P=0.007$) levels of hens kept in the conventional cages were higher than those of the hens kept in enriched cages.
- Also serum cholesterol level was higher in hens kept in the conventional cages ($P=0.005$).
- In the present study, it showed that the enriched materials affected all these parameters altogether.



Table 2: Some blood parameters and relative liver weight of two different laying hen strains kept in conventional and enriched cages

Cage Type	Strain	ALT ¹ (U/l)	AST ¹ (U/l)	Cholesterol (mg/dl)	Triglycerides (g/l)	Relative liver Weight (%)
Conventional	LB	16.4	96.7	103.8	831	2.45
	LW	17.4	86.5	99.5	813	2.41
Enriched	LB	13.2	76.9	92.2	819	2.31
	LW	17.8	78.3	86.0	810	2.28
Conventional		16.9	91.6	101.6	822	2.43
Enriched		15.5	77.6	89.1	815	2.29
	LB	14.8	86.8	98.0	825	2.38
	LW	17.6	82.4	92.7	812	2.35
Pool SEM		0.324	2.473	2.161	6.182	0.071
P-value						
Cage Type		0.038	0.007	0.005	0.554	0.354
Strain		< 0.001	0.373	0.230	0.285	0.819
Cage type X Strain		0.007	0.249	0.829	0.743	0.940

¹ALT: alanine amino transferase, AST: aspartate amino transferase

RESULTS AND DISCUSSION

- Strain type was significant for serum ALT ($P < 0.001$) levels.
- Serum ALT levels in LB hens were lower than in LW hens.
- It shows that genetic differences are important for serum ALT.
- Serum AST, cholesterol and triglycerides were not differing between strains.
- Cage type and strain interaction was found significant for serum ALT ($P = 0.007$) levels due to the LB hens being more affected by cage type than LW hens.



RESULTS AND DISCUSSION

- Relative liver weights were not affected by cage type and strain.
- At macroscopic examination all livers were in general swollen, with blunt edges and mottled at varying sizes with yellow colouration.
- Especially in livers of LW hens an increase of intensity and softening of consistency were noticed.



Table 3. Fatty liver score of two different laying hen strains kept in conventional and enriched cages

Cage Type		Strain	Fatty liver score* (%)				
			0	1	2	3	1+2+3
Conventional			6.25	37.5	31.3	25.0	93.8
Enriched			18.8	31.3	25.0	25.0	81.3
	<i>P</i> -value		0.600	0.710	1.000	1.000	0.600
		LB	25.0	50.0	12.5	12.5	75.0
		LW	0	18.8	43.8	37.5	100.0
	<i>P</i> -value		0.101	0.063	0.113	0.220	0.101

* 0: droplets less than 10%, 1: droplets between 10–30%, 2: droplets between 30–70% and 3: droplets more than 70%

RESULTS AND DISCUSSION

- At microscopic examination lipid vacuoles were seen in sections of the cytoplasm of hepatocytes stained with Oil Red O. These vacuoles were round/oval in form and red stained.

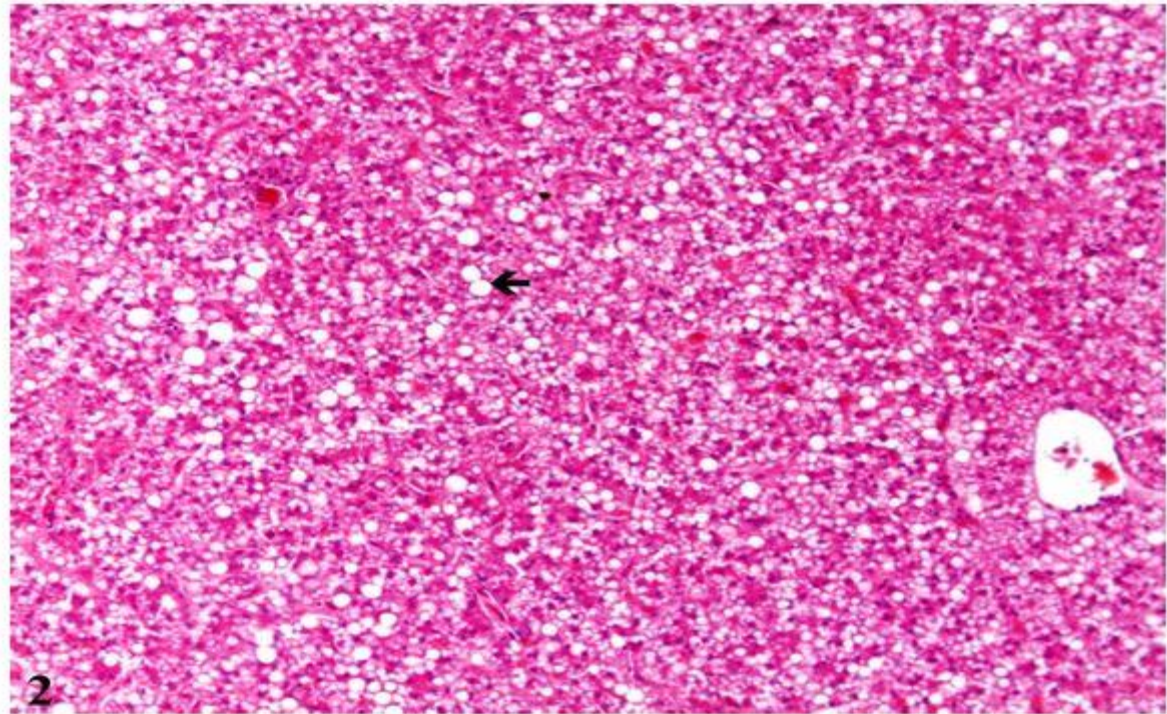


Figure 2: Varying sizes, round/oval form and clearly demarcated lipid vacuoles in cytoplasm of the hepatocytes (arrow), Hematoxylin-Eosin Stain, x40

- In sections stained with H&E, clearly demarcated vacuoles with round/oval form were encountered in the cytoplasm of the hepatocytes.

RESULTS AND DISCUSSION

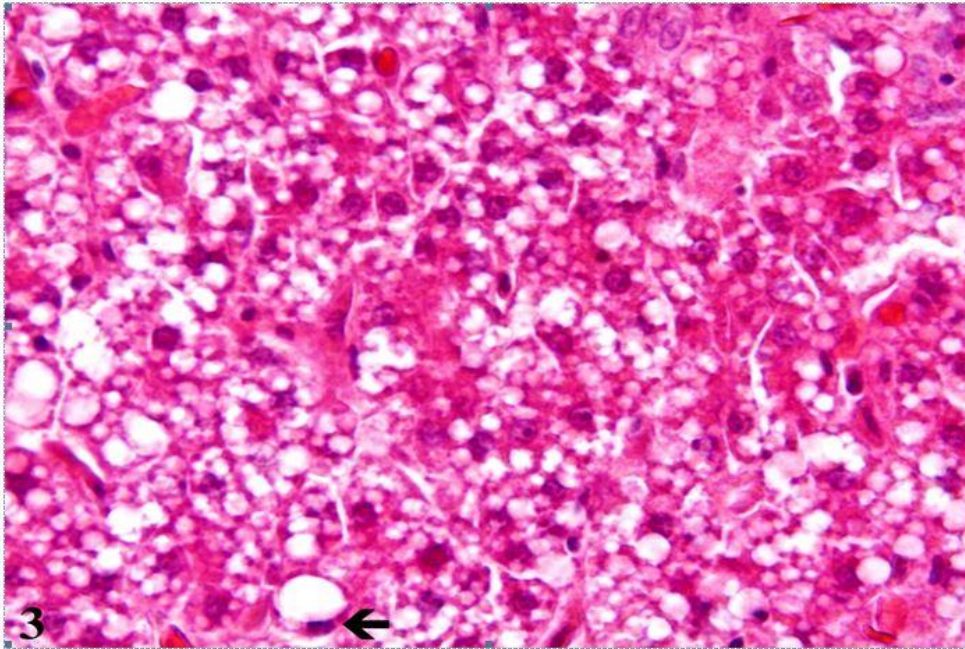


Figure 3: Flattened hyperchromatic nuclei and degenerated /necrotic hepatocytes (arrow), Hematoxylin-Eosin Stain, x400

- Many hepatocytes were degenerated and necrotised.
- In some cases, hyperchromatic nuclei of hepatocytes were pushed into a corner and flattened.
- Accumulations of lipid droplets were graded.



RESULTS AND DISCUSSION

- Fatty liver syndrome was observed in 94% of hens kept in conventional cages and 81% of hens kept in enriched cages.
- Also fatty liver syndrome was seen 75% in LB and 100% in LW hens.
- Cage type and strain did not affect the fatty liver score.



RESULTS AND DISCUSSION

- Fatty liver syndrome is important in laying hens and many factors affect it, the lack of exercise being important.
- But the present study showed that enrichment as given here was not enough for reducing fatty liver score.



CONCLUSION

- The results of the present study suggest that the enrichment of the cages was ineffective in reducing liver problems, except in altering enzyme activities related to improved liver function.
- The strain type was important for some blood parameters.
- The cage type and strain interactions were not found for the examined parameters, except for serum ALT levels.



Thank you for your attention!

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