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ABSTRACT

Following the various recent avian influenza crises, the shortage of male mule ducklings has led to the use of females, although these are not normally used mainly because of defects in the presentation of the final product. The aim of this study was to examine the evolution of hepatic steatosis induced by overfeeding in female mule ducks with or without a visible network of veins on the surface of lean or fatty livers. The overall evolution of hepatic steatosis (weight gain, gross biochemical composition) was strikingly similar in both types of liver. Histological observations confirm that in both types of livers, there is a steady increase in the accumulation of lipid droplets in hepatocytes throughout the period of overfeeding. At the same time, other parameters (fibrogenesis, measured by the accumulation of hydroxyproline; oxidative status, measured by the activities of the enzymes superoxide dismutase and catalase; contents of reduced and oxidised glutathione and level of hypoxia, measured with Hypoxia 1 and 2 Induced Factors) are also altered similarly in all samples. Nor did the overall activities of genes belonging to different metabolic pathways reveal any major differences when normal and veinous livers were compared. In conclusion, hepatic steatosis induced by overfeeding developed under very similar conditions in the normal and veinous livers of female mule ducks. However, these visible anatomical differences degrade the visual quality of the final product and make veinous livers less attractive to processors and consumers.

Implications

Some (20–40%) female mule ducks present a developed venous network (unknown origins) on the surface of their liver. This is never observed in males. We compared, for the first time, biochemical properties and gene activities in liver female mule ducks, with or without a developed superficial venous network. We report very few differences between the two studied groups of females, both before and after overfeeding. Therefore, the development of hepatic steatosis induced by overfeeding is similar in all females. However, the final presence of a venous network in some of them makes it less desirable for processors or consumers.

Introduction

France is the world's leading producer of "foie gras", accounting for over 70% of the global production. "Foie gras" is an important component of the French gastronomic meal which has been listed by UNESCO (2010) as part of the humanity's intangible cultural heritage. "Foie gras" is the result of hepatic steatosis obtained by overfeeding palmipeds, mainly ducks, for 10–12 days with increasing amounts, twice a day, of corn mixed with water, minerals and vitamins (Bonnefont et al., 2019). As a result, the liver produces a considerable amount of lipids, which are primarily stored *in situ*, resulting in significant liver hypertrophy accompanied by fattening (Herault et al., 2010; Bax et al., 2012; Lo et al., 2020). The result is a liver whose weight is multiplied by a factor of 8–10, accompanied by a profound change in its chemical composition, with the proportion of lipids rising from less than 5% of gross weight to more than 60%. These lipids are mainly triglycerides but although also other lipids such as free fatty acids, phospholipids and cholesterol esters.

In recent years, the global poultry industry has been faced with a series of devastating avian influenza crises, resulting in the deaths of millions of wild and domestic birds (Spackman, 2020). These crises precipitated profound changes in farming methods, mainly through the implementation of new biosecurity regula-

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tions. However, despite these measures, a significant shortage of day-old birds emerged. This shortage was particularly evident in France, where it was most pronounced in duck production, particularly "foie gras" production. The production of this product is traditionally carried out with male mule ducks which are preferably overfed due to sexual dimorphism in BW favourable to males. Females are also known to be more difficult to rear and overfeed (Basso et al., 2014). This is reflected in particular by the fact that females are more active and nervous than males, which makes it more difficult to handle them individually during the overfeeding period. In addition, a significant proportion of females, ranging from 20 to 40% (Marie-Etancelin et al., 2015), have a developed and superficial network of veins on the surface of the liver, making them less attractive to consumers. As a result, these superficial veins have to be removed manually by processors using complex and time-consuming procedures, which ultimately results in a product that is still unattractive but very costly in terms of time and labour. As a result, the practice of overfeeding female mule ducks was gradually abandoned many years ago. However, the shortage of male ducklings has revived interest in female ducks. Recently, Atallah et al. (2024) demonstrated that male and female mule ducks have very similar abilities to develop hepatic steatosis in response to overfeeding. However, to our knowledge, no precise information is available on the specific problem of the presentation of the liver (superficial veinous network) in certain females, nor on the evolution of hepatic steatosis during overfeeding in female mule ducks with or without this anatomical specificity.

The aim of this article is to present a comparative analysis of the development of hepatic steatosis during the overfeeding period in female mule ducks with and without visible veins on the surface of the liver. This is the first time that such a comparison has been attempted.

Material and methods

Animal and liver Sampling

A flock of approximately 500 female mule ducks (*Caïrina moschata* × *Anas platyrhynchos*) was reared for 12 weeks, from hatching, in accordance with standard commercial practices. At the age of 12 weeks, 12 birds (whose live weight was similar to the average live weight of the entire flock) were randomly selected and slaughtered to form the reference group of ducks (0 meal) before the start of overfeeding. After electronarcosis and exsanguination, the ducks were rapidly eviscerated and their livers were extracted for subsequent examination. Four livers were identified as belonging to the "veinous" group, characterised by the presence of clearly visible superficial veins on the outer surface of the liver (Supplementary Fig. S1). The remaining eight livers from female subjects did not have this anatomical feature and were therefore designated as belonging to the "normal" group. The remaining birds were overfed for 21 meals (twice daily for 10.5 days) using a standard overfeeding programme based on moistened corn meal (97.5% corn supplemented with vitamins and minerals). At the start of the overfeeding period, the ducks received 225 g of DM per meal. The quantity of feed was gradually increased until a final value of 480 g was reached for the last meal. Over the entire period of overfeeding, the ducks ingested an average quantity of 8.0 kg of feed (based on the dry matter content of the corn). After the 8th (day 5) and 16th (day 8) overfeeding meals, 12 ducks were randomly slaughtered as described above to constitute the 8- and 16-meal groups, respectively. Three and five livers out of 12 were identified as veinous in the 8- and 16-meal groups, respectively. After 21 overfeeding meals, all the remaining birds were slaughtered, and 24 of them (12 normal livers and 12 veinous livers) were selected to form the 21-meal groups. At this final stage, with data available for the whole flock, the selection of 21-meal livers was made to represent the variability observed in the weight of the livers (517 ± 92 g, CV% = 18) of all birds. All the ducks were slaughtered approximately 12 h after the last meal in a commercial slaughterhouse with all procedures being carried out in accordance with standardised protocols (electronarcosis, bleeding, scalding, plucking). At the end of the slaughter process, 20 min after death, the livers were harvested and weighed. Subsequently, 50 g of tissue was removed from the median lobe and frozen directly in liquid nitrogen before being stored at −80 °C. Another piece of liver was also removed from the same location and preserved in paraformaldehyde buffer (4%) for histological observations. All biochemical measurements were conducted in duplicate after grinding the tissues in liquid nitrogen.

Crude biochemical composition of livers

The DM content was determined by drying the ground liver in an oven at 105 °C for 24 h. The total lipid content was measured according to Folch et al. (1957) after extraction with chloroform: methanol (2:1). Total protein content was determined according to the manufacturer's procedure (Pierce™ BCA protein assay kit, 23227, ThermoFisher, Fisher Scientific, Strasbourg, France)) after extraction with phosphate-buffered saline. The hydroxyproline (**OH-Pro**) content was determined according to the methodology described by Woessner (1961) on the delipidated dry residue obtained after the extraction of total lipids.

Oxidative status

GSH/GSSG analysis: The ratio of reduced glutathione to oxidised glutathione (**GSH/GSSG**) was determined according to the manufacturer's protocol (catalogue #: 239709, Abcam, Cambridge, UK). The results are expressed as μmol/mg protein.

The activities of the enzymes superoxide dismutase (**SOD**, catalogue #: 19160, Sigma, St. Louis, MO, USA) and catalase (**CAT**, catalogue #: KB03012, BioQuoChem, Llanera-Asturias, Spain) were determined according to the procedures outlined by the manufacturers. The results are expressed as U/mg protein.

ELISA tests

The levels of Hypoxia Inducible Factor 1 Alpha (**HIF1α**, catalogue: # MBS065720) and Hypoxia Inducible Factor 2 Alpha (**HIF2α**, catalogue:# MBS9365131) were quantified by ELISA tests using MyBioSource (MBS, San Diego, CA, USA) assay kits in accordance with the manufacturer's instructions. The results are expressed as pg / mg protein.

Histology

Paraffin-embedded and paraformaldehyde-fixed liver tissue sections (3 μm) were stained with hematoxylin (catalogue:# HMM500, CliniSciences, Nanterre, France) and eosin (catalogue:# NB-42-51683, CliniSciences, Nanterre, France) for histopathological analysis. The stained liver sections were analysed blindly according to a scoring system ranging from 0 (no lipid droplets visible) to 4 (almost only large lipid droplets in the hepatocytes). The scoring system was as follows: 1 (only small lipid droplets), 2 (majority of small lipid droplets), 3 (majority of large lipid droplets) and 4 (almost only large lipid droplets in hepatocytes). The mean score values were determined in five independent microscopic fields observed by three independent and trained persons.

Gene expression

Total cellular RNA was extracted from liver samples using TRI Reagent (catalogue:# TR 118, Molecular Research Center Inc., Cincinnati, Ohio, USA). RNA was quantified using a nanophotometer (N60, Implen, Westlake village, CA, USA). Total RNA samples (2 μg) were transcribed using the High Capacity cDNA Reverse Transcription Kit (Catalogue:# 10400745, Applied Biosystems, Foster City, California, USA) for quantitative real-time polymerase chain reaction (qPCR) analyses. Primers were designed in two consecutive exons to avoid amplification of genomic DNA, using the PrimerQuest™ tool (Integrated DNA Technologies, Coralville, Iowa, USA) and the primers for SYBR Green assays are presented in Table S1, Supplementary Information. Amplifications were performed on an Aria Mx real-time PCR system (Agilent, Santa Clara, California, USA). RT-qPCR data were normalised to the GlycerAldehyde-3 Phosphate Dehydrogenase (GAPDH) messenger RNA (mRNA) level and analysed by LinRegPCR (v2021.2). This programme determines the PCR efficiency per sample and takes this into account in a linear regression approach to correct the cycle threshold value for the quantification of the mRNA level. The initial concentration (N0) of each sample is calculated as follows: N0 = threshold / (Effmean × Cq) with Effmean: average PCR efficiency and Cq: quantification cycle.

Statistics

The statistical analyses were performed using the general linear model (Proc GLM) of the SAS software, version 9.4 of the SAS system for Windows. The model included the main effects for group (normal or veinous), the number of overfeeding meals (0, 8, 16, 21), and the group × meal interaction. Where applicable, differences between means were tested using the Tukey-HSD posthoc test, where necessary variables were transformed before analysis to meet the conditions of normality and homoscedasticity ($\log_2$ for RT-PCR and OH-Pro analysis). Values are expressed as means ± SD. The significance level was set at $P < 0.05$.

Results and discussion

From the beginning until the completion of the 16 overfeeding meals, the randomisation of samples made it possible to collect between 25% and 40% female livers with medium to very dark veins out of a total of 12. After the overfeeding period, the proportion of female livers with a veinous pattern remained at approximately 25–30% in the rest of the flock (data not presented). This result is consistent with the observations made by Marie-Etancelin et al. (2015) in female mule ducks following overfeeding. It should be noted that Gerzilov et al. (2013) and Brun et al. (2015) did not report any visible anatomical differences in the livers taken from overfed male and female mule ducks. As observed by Maher (2019) in chickens and El Karmoty and Ayman (2019) in geese, the most apparent veins observed on the surface of the livers in the present study could be identified as left and right hepatic veins and their respective subdivisions in caudodorsal and ventral branches. Our results shown that the presence of visible veins on the surface of the duck's liver was not caused by the overfeeding itself, as this was a characteristic that affected 25–30% of the birds throughout the experiment. We must therefore accept that this trait is common in some female ducks, with a possible link to their genetic background that remains to be identified.

In all birds, overfeeding led to a significant increase in liver weight, from approximately 125 g before to 525 g after (Table 1). This 4.2-fold increase in liver weight in female mule ducks was expected and is similar to what was previously reported by Marie-Etancelin et al. (2015) and Gerzilov and Petrov. (2015). In male mule ducks, the increase in liver weight was comparable (Bonnefont et al. 2019, Atallah et al., 2024). The veinous and non-veinous weights of the liver did not vary significantly on average, indicating that this anatomical feature did not affect the development of liver growth during the overfeeding period. Throughout the overfeeding period, the amount of stored lipids in all livers showed a marked increase from 6% to approximately 60%. It can be concluded that, in females, the quantity of lipids in the liver was similar in the veinous and non-veinous livers before and after overfeeding. As previously reported by Gabarrou et al. (1996) and Bax et al. (2012) in males, the percentages of protein extracted from the livers decreased significantly during the overfeeding period in both groups of female birds. The substantial amount of carbohydrates supplied twice a day by the corn during the overfeeding period induced lipogenesis that accumulated lipids in the liver and consequently, diluted its constituent proteins.

The results presented in Fig. 1 indicate that the total OH-Pro content in veinous and normal livers showed a similar variation during the overfeeding period. A constant level was observed between 0 and 16 meals, after which a significant increase was observed until the end of the overfeeding period (21 meals). In mice, Montefusco et al. (2022) and Arai et al. (2022) proposed that the increase in OH-Pro content in the liver could be used as an indicator of the development of fibrogenesis, which is generally observed during the transition from simple steatosis to steatohepatitis associated with metabolic dysfunction (MASH). Consequently, the observed variation in the total OH-Pro content of the normal and veinous livers indicated that hepatic fibrogenesis had occurred at the end of the overfeeding period. This finding reflects the fact that all the birds encountered significant difficulties in adapting to the alterations imposed on the liver tissue by the overfeeding programme. However, the comparable OH-Pro levels in the veinous and normal livers after 21 overfeeding meals suggest that this anatomical specificity did not confer protection or favour to the livers in the context of the possible development of hepatic fibrogenesis, which was ultimately induced by overfeeding.

Before the start of the overfeeding period, all hepatocytes observed were categorised with a score of 0 (no visible lipid droplets) in all samples (Fig. 2). This is consistent with the fact that the livers of the two groups initially had similar weights and low lipid contents. Nevertheless, during the overfeeding period, lipids gradually accumulated in the hepatocytes, and mean histological scores increased in the veinous and normal livers. At the end of the overfeeding period (21 meals), these histological scores were not statistically different between the normal and veinous livers (3.08 ± 0.14 and 3.5 ± 0.19, respectively). However, the higher value observed in the veinous group than in the normal group is consistent with the lipid content values previously described in Table 1, although not statistically significant.

As illustrated in Fig. 3, the overfeeding programme resulted in a notable elevation in the levels of Hypoxia Induced Factors (HIF1α and HIF2α), particularly between the initial and final stages of the overfeeding period, in the normal and veinous livers of female ducks. HIF transcription factors are directly involved in the response to initial (HIF1α) or acute (HIF2α) hypoxia (Downes et al., 2018). Therefore, the observed increase in HIF factor content indicates that, independently of the development of the veinous network, the livers of female mule ducks were subjected to progressively hypoxic conditions throughout the overfeeding period. These hypoxic conditions are a consequence of the development of hepatic steatosis, which alters metabolism and disrupts hepatic oxygen homeostasis (Suzuki et al., 2014).

As pointed out by McGarry et al. (2018), a reduction in the efficiency of oxygen supply can also lead to an increase in reactive oxygen species (**ROS**), particularly when associated with a high

Table 1
Evolution of the gross biochemical composition of veinous and normal livers of female mule ducks according to the number of meals during the overfeeding period. Values are mean ± SD.

Liver	Normal				Veinous				P^1		
Meal	0	8	16	21	0	8	16	21	L	M	L × M
LW[2]	$130^{a} \pm 7$	$258^{b} \pm 9$	$395^{c} \pm 19$	$525^{d} \pm 5$	$112^{a} \pm 9$	$253^{b} \pm 11$	$448^{c} \pm 23$	$526^{d} \pm 6$	Ns	***	*
M[3]	$66.72^{a} \pm 0.25$	$48.52^{b} \pm 0.59$	$38.50^{c} \pm 0.76$	$28.83^{d} \pm 0.54$	$67.79^{a} \pm 0.64$	$47.67^{b} \pm 1.76$	$35.50^{c} \pm 0.59$	$29.44^{d} \pm 0.69$	Ns	***	*
L[4]	$5.94^{a} \pm 0.19$	$34.48^{b} \pm 1.29$	$48.86^{c} \pm 3.15$	$60.54^{d} \pm 1.06$	$6.16^{a} \pm 1.04$	$37.78^{b} \pm 4.16$	$53.20^{c} \pm 1.68$	$61.73^{d} \pm 0.77$	Ns	***	Ns
P[5]	$8.15^{a} \pm 0.63$	$7.18^{ab} \pm 0.29$	$5.75^{b} \pm 0.49$	$3.12^{c} \pm 0.13$	$11.81^{a} \pm 1.65$	$7.10^{b} \pm 0.66$	$4.34^{c} \pm 0.21$	$3.27^{c} \pm 0.16$	Ns	***	***

Within a line, means with the same superscripts are not different ($P < 0.05$). Ns = Non-significant ($P > 0.05$), *** = $P < 0.001$, * = $P < 0.05$.
[1] M = influence of the number of meals, L = Influence of the type of liver, MxL = interaction between M and L.
[2] Liver Weight (g).
[3] Moisture (% of raw liver).
[4] Lipids (% of raw liver).
[5] Proteins (% of raw liver).

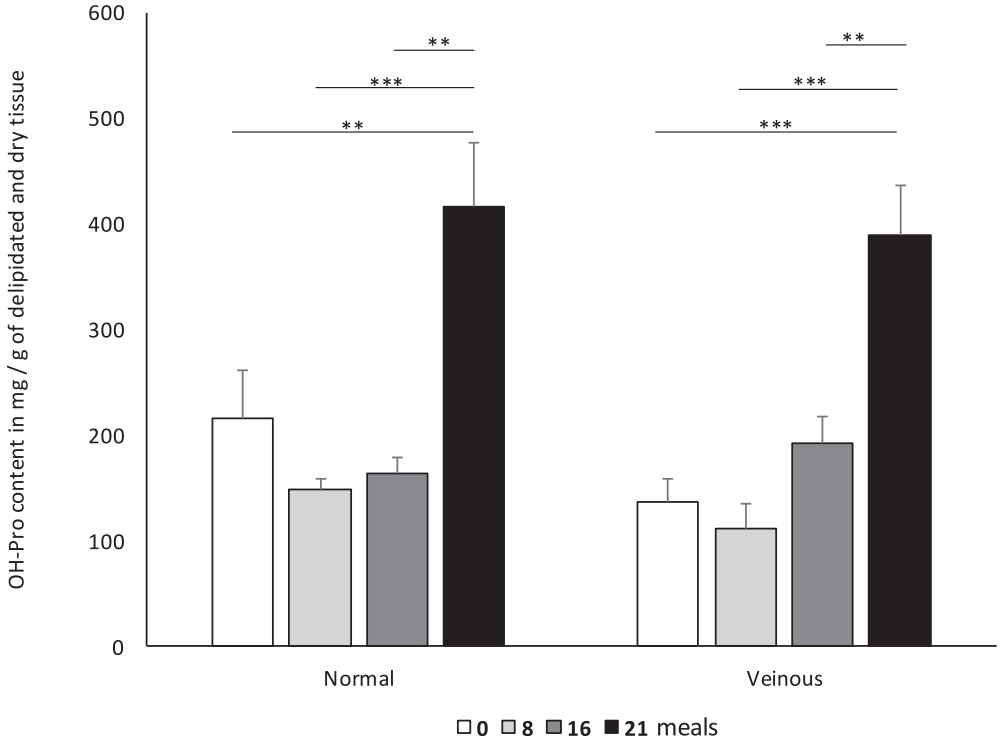

Fig. 1. Evolution of the hydroxyproline contents (OH-Pro) of normal and veinous livers from female mule ducks during the overfeeding period. Values are mean ± SD. ** = $P < 0.01$, *** = $P < 0.001$.

accumulation of lipids in hepatocytes (Chen et al., 2019). These ROS are highly toxic to the cell, requiring a cellular response with multiple antioxidant actions. One of the most common ROS is the superoxide ion O_2–. This can be neutralised by the coupling activities of the enzymes superoxide dismutase and catalase (Perlemuter et al., 2005). The activity of the SOD enzyme was found to be similar in the normal and veinous lean livers of female mule ducks. However, it increased significantly during the overfeeding period (Fig. 4). This change was almost equivalent in the two groups, demonstrating that throughout the overfeeding period, an increasing number of superoxide ions were produced and required neutralisation. The second step in the action of the SOD/CAT couple is supported by the catalase enzyme which converts H_2O_2 into water and dioxygen. In the present study, catalase enzyme activity showed a decrease between the beginning and end of the overfeeding period, with a more pronounced decline observed in normal livers ($P < 0.001$) than in veinous livers ($P < 0.05$) of female mule ducks. Nevertheless, this resulted in a highly significant ($P < 0.001$) decrease in the CAT/SOD ratio in veinous and normal livers of female mule ducks between the beginning and the end of the overfeeding period. The increase in SOD activity is indicative of the liver's response to oxidative stress during the development of hepatic steatosis. Conversely, the decrease in CAT activity is a sign of cellular dysfunction. It can therefore be assumed that these two enzymes must have synchronised activities to support efficient ROS neutralisation. In the present study, SOD and CAT activities underwent opposing changes during the overfeeding period, which may impair the neutralisation of hydrogen peroxide and potentially contribute to lipid oxidation (Gasparin et al., 2018). The glutathione system, present in all cells, is also a powerful system for combating oxidative stress. Supported by specific peroxidases, the transformation of reduced glutathione (GSH) into oxidised glutathione (GSSG) is very effective in maintaining cellular redox homeostasis (Sahoo et al., 2017). Conse-

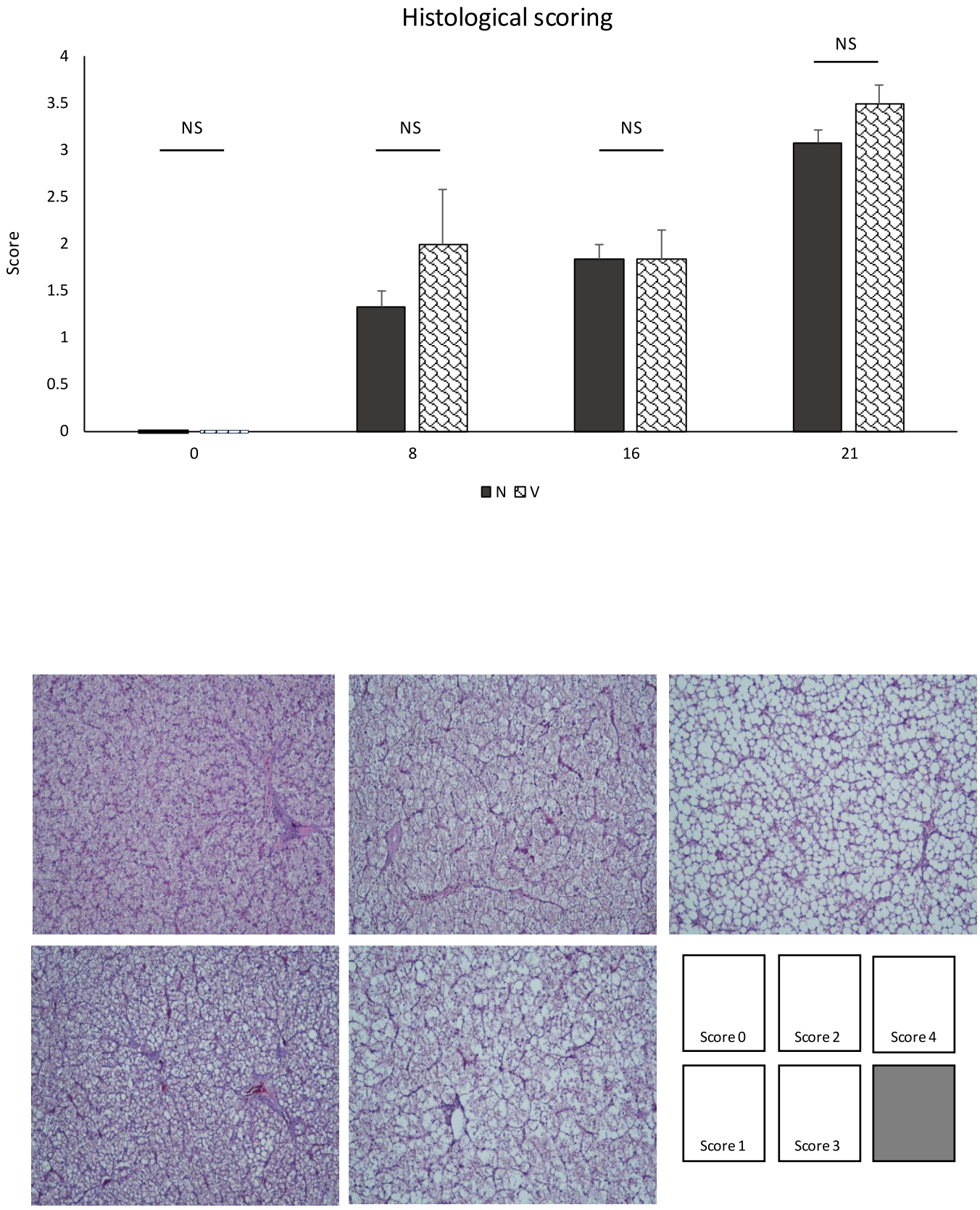

Fig. 2. Histological scoring values for normal (N) and veinous (V) livers from female mule ducks during the overfeeding period (0, 8, 16, 21 meals). Values are mean ± SD. Examples of the scoring grades (Staining Hemalun-Eosin and same magnification (x100) for all pictures).

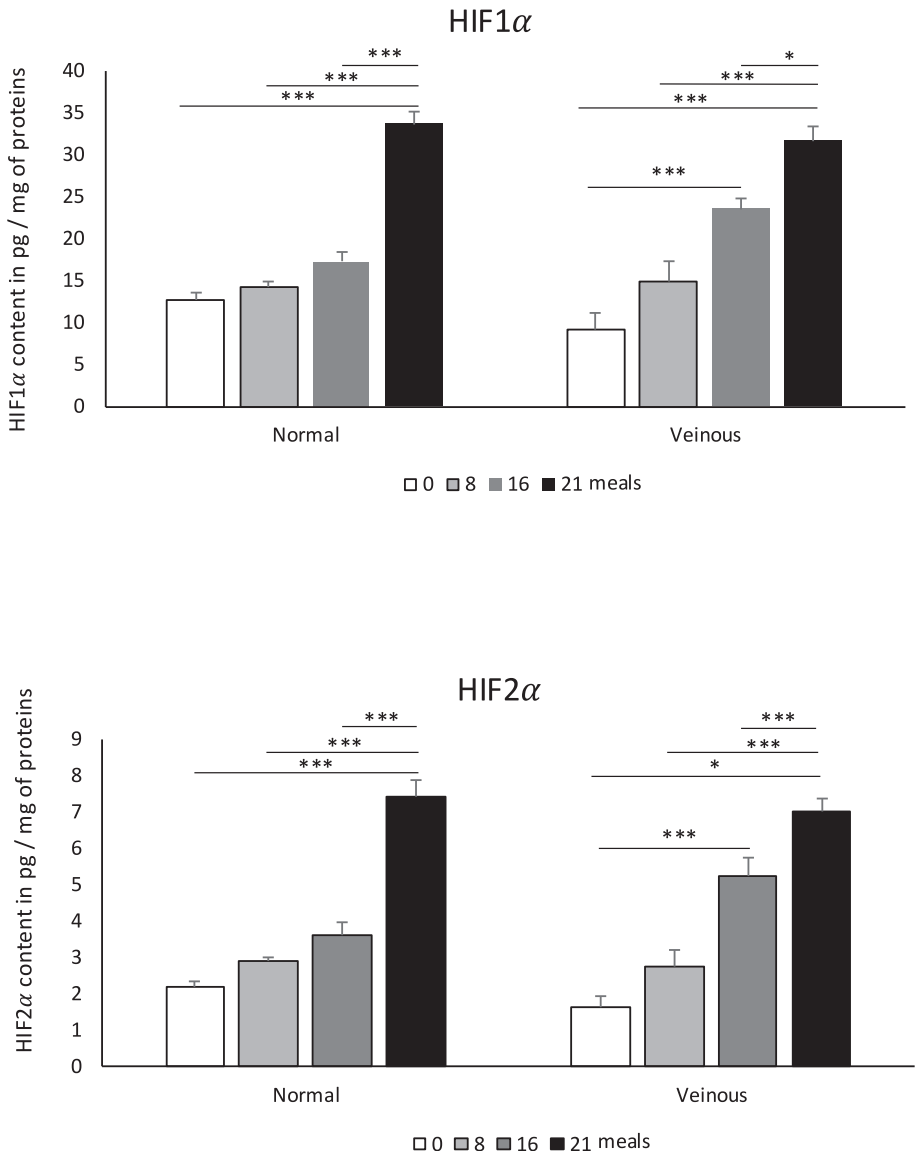

Fig. 3. Evolution of Hypoxia–Induced Factors 1 and 2 (HIF1α and HIF2α) concentrations in normal and veinous livers of female mule ducks during the overfeeding period. Values are mean ± SD. * = $P < 0.05$, *** = $P < 0.001$.

quently, the GSSG/GSH ratio should be considered a main indicator of cellular dysfunction (Alkazemi et al., 2021). A decrease in reduced and oxidised glutathione concentrations was observed between the initial (0 meal) and final (21 meals) stages of the overfeeding period in the normal and veinous livers of female mule ducks (Fig. 5). This indicates that, regardless of the organisation of the veinous network in the livers, the overfeeding programme induced oxidative stress conditions. The observed decreases in GSH and GSSG indicated that hepatocytes utilise glutathione as an antioxidant to neutralise pro-oxidant factors such as H_2O_2. However, this defence mechanism against oxidative stress, in combination with the SOD and CAT enzymes, is insufficient in severe cases of NAFLD or NASH (Sumida et al., 2013) in mice. During the rapid development of hepatic steatosis, an increase in the activity of the ß-oxidation pathway is also observed, which leads to a significant leakage of causing ROS (Geng et al., 2021). These ROS will induce mitochondrial dysfunction, which is part of the described lipotoxicity associated with the development of hepatic steatosis (Guo et al., 2013). This was also likely to be the case in the present study, where antioxidant mechanisms were largely activated during the development of hepatic steatosis induced by overfeeding, whether the liver was veinous or normal.

Table 2 illustrates the relative expression of several genes involved in various metabolic pathways. In particular, two-way ANOVA revealed no significant effect related to liver type (veinous or normal). On the other hand, the effect of the number of overfed meals, observed in 12 out of 20 cases, induced significantly different transcription levels of the observed genes. The transcriptional activity of different genes has already been described as varying during the development of hepatic steatosis in overfed male mule ducks (Andrieux et al., 2023; Massimino et al., 2021; Pioche et al., 2020; Tavernier et al., 2018). These results are consistent with those of the present study. These variations confirm that during the overfeeding period, normal and veinous livers profoundly modify their metabolisms, mainly towards a much more active synthesis of different types of lipids (genes Scd1, Soat, Cpt1a, Acox1, Plin2, Fabp4) with carbohydrates serving as the main substrate (genes Hk1, Glut2, and Eno1). Of the 6 regulator genes analysed, only 2

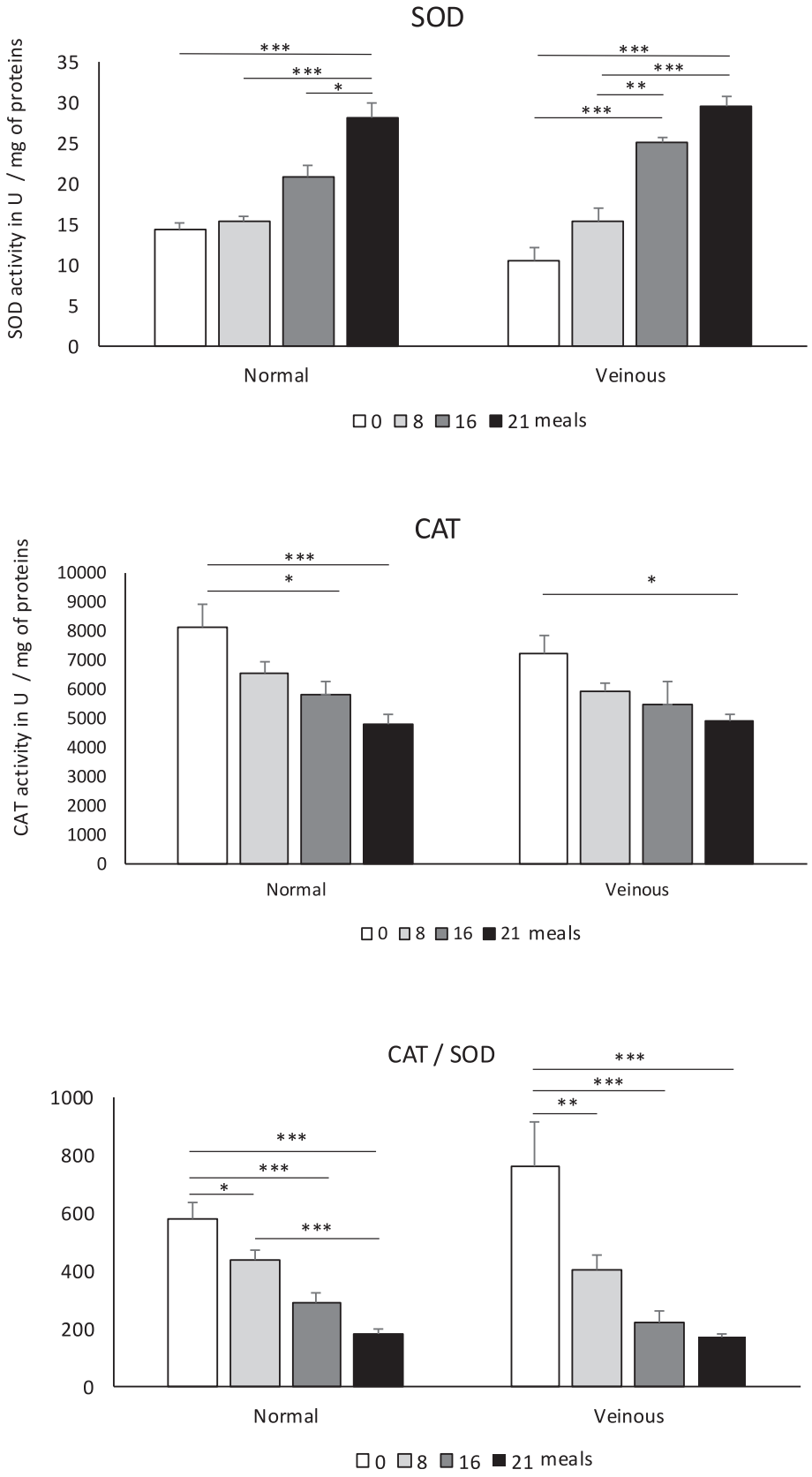

Fig. 4. Evolution of SOD (Super Oxide Dismutase) and CAT (CATalase) enzymes activities in normal and veinous livers of female mule ducks during the overfeeding period. Values are mean ± SD. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

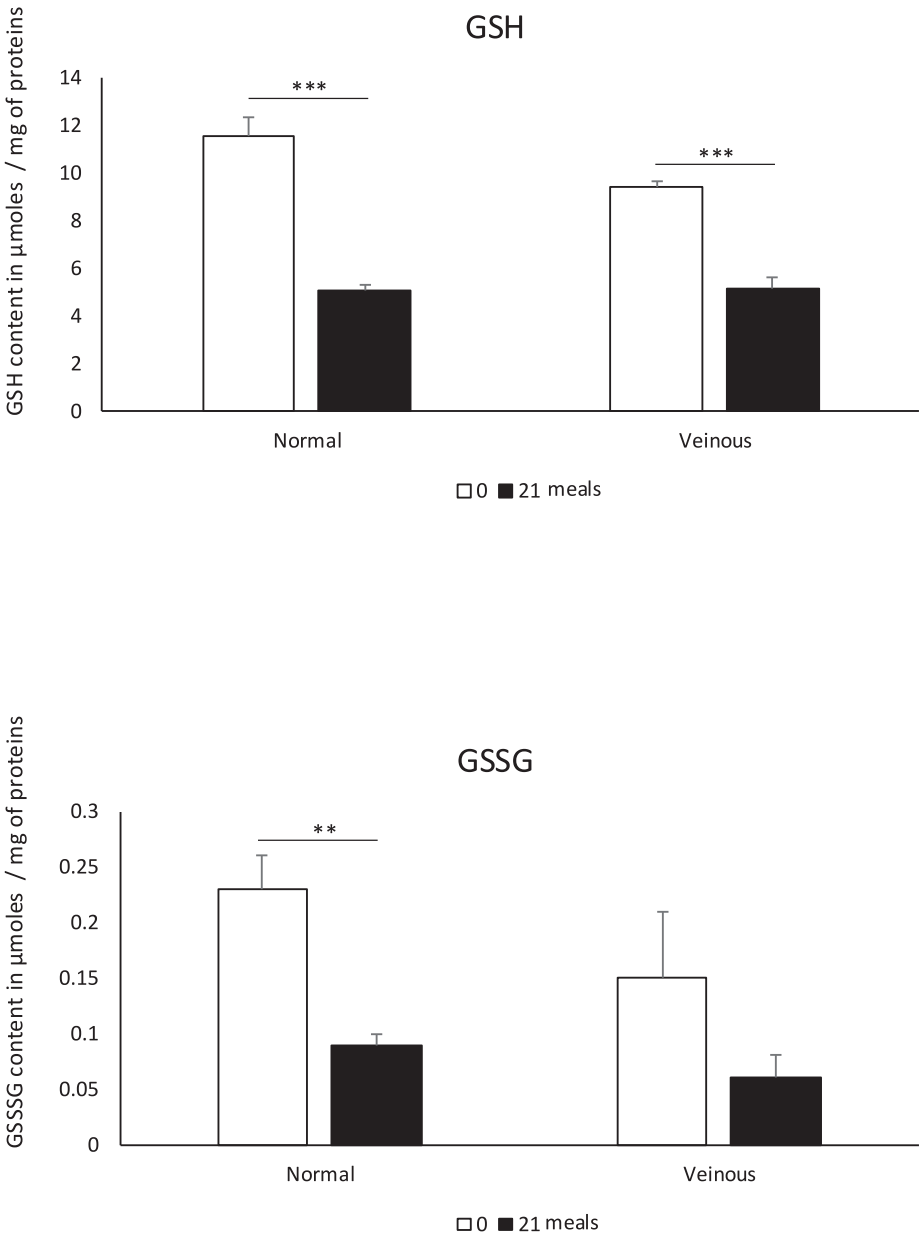

Fig. 5. Evolution of reduced (GSH) and oxidised (GSSG) glutathione concentrations in normal and veinous livers of female mule ducks during the overfeeding period. Values are mean ± SD. ** = $P < 0.01$, *** = $P < 0.001$.

(Bcl6 and Chrebp) were significantly affected by the number of overfeeding meals. It should also be noted that at the end of the overfeeding period, signs of inflammation were noticeable by the significant increase in transcription levels of the IL18 genes in the veinous and normal livers and Tnfα in the normal livers. A total of two genes (Scd1 and Hk1) out of the 20 examined showed a statistically significant interaction between the number of meals and the type of liver (M × L, as shown in Table 2). This suggests that the Hk1 and Scd1 genes have a distinct level of expression depending on the duration of the overfeeding period (or the number of meals) and the type of liver (normal or veinous). Indeed, for the Hk1 gene, its level of transcription decreased during the overfeeding period in normal livers whereas it tended to increase in veinous livers. Hexokinase 1 (encoded by the Hk1 gene) is a carbohydrate kinase that catalyses the phosphorylation of glucose into glucose-6 phosphate. Given that the overfeeding regime provided hepatocytes with a considerable amount of glucose, one might expect the transcription level of the Hk1 gene to increase in response to overfeeding. In this study, we observed that the level of transcription decreased or increased and then decreased in normal and veinous livers, respectively. These unexpected variations in Hk1 gene transcription levels can be attributed to variations in the duration of feed withdrawal which tend to modify the amounts of glucose immediately delivered to hepatocytes during the corn digestion process. The transcription levels of the Scd1 gene increased during the overfeeding period in both types of livers, but only significantly after 16 overfeeding meals in normal livers whereas they were already significant after eight overfeeding meals in veinous livers. The enzyme stearoyl-CoA desaturase (encoded by the Scd1 gene) is involved in the biosynthesis of fatty acids, mainly the synthesis of oleic acid. According to Carrillo et al. (2016), oleic acid is the most abundant fatty acid in the livers of overfed ducks, accounting

Table 2
Relative expression of selected genes[#] involved in metabolisms of normal and veinous livers of female mule ducks according to the number of meals during the overfeeding period. Values are means ± SD.

Liver	Normal				Veinous				P^1		
Meals	0	8	16	21	0	8	16	21	M[1]	L[1]	MxL[1]
Glycolysis											
Glut2	0.91^{ab} ± 0.06	0.64^{b} ± 0.03	1.01^{a} ± 0.07	1.19^{a} ± 0.12	1.18 ± 0.26	0.73 ± 0.11	1.48 ± 0.22	0.98 ± 0.12	**	Ns	Ns
Eno1	0.97^{b} ± 0.08	1.24^{ab} ± 0.14	1.39^{ab} ± 0.09	1.42^{a} ± 0.12	1.06 ± 0.16	1.20 ± 0.20	1.40 ± 0.26	1.33 ± 0.17	Ns	Ns	Ns
Hk1	1.42^{a} ± 0.44	1.00^{b} ± 0.01	0.47^{c} ± 0.13	0.33^{c} ± 0.08	0.15^{b} ± 0.06	0.67^{ab} ± 0.17	1.27^{a} ± 0.49	0.28^{b} ± 0.09	**	Ns	***
Lipids											
Cpt1a	0.70^{c} ± 0.08	2.03^{ab} ± 0.62	1.45^{b} ± 0.15	2.73^{a} ± 0.25	1.60 ± 0.52	1.38 ± 0.12	1.20 ± 0.12	3.21 ± 0.60	**	Ns	Ns
Acad11	1.03^{a} ± 0.08	0.56^{b} ± 0.08	0.63^{b} ± 0.06	0.58^{b} ± 0.04	0.93 ± 0.17	0.59 ± 0.13	0.57 ± 0.06	0.54 ± 0.06	***	Ns	Ns
Acox1	0.93^{b} ± 0.1	0.73^{b} ± 0.09	0.93^{b} ± 0.07	1.45^{a} ± 0.12	1.14^{ab} ± 0.16	0.65^{b} ± 0.15	1.04^{ab} ± 0.11	1.46^{a} ± 0.16	***	Ns	Ns
Soat	0.88^{b} ± 0.08	0.77^{b} ± 0.06	1.00^{b} ± 0.07	1.53^{a} ± 0.14	1.23^{ab} ± 0.25	0.74^{b} ± 0.18	1.02^{ab} ± 0.14	1.48^{a} ± 0.15	***	Ns	Ns
Dgat2	1.17 ± 0.44	0.59 ± 0.27	0.39 ± 015	0.70 ± 0.20	0.66 ± 0.20	0.42 ± 0.23	1.18 ± 0.48	0.42 ± 0.14	Ns	Ns	Ns
FasN	1.16 ± 0.14	0.94 ± 0.11	1.21 ± 0.22	1.08 ± 0.11	0.67 ± 0.22	0.83 ± 0.12	1.28 ± 0.16	0.92 ± 0.10	Ns	Ns	Ns
Scd1	1.18^{b} ± 0.14	1.72^{ab} ± 0.12	2.35^{a} ± 0.15	2.27^{a} ± 0.25	0.64^{b} ± 0.27	2.19^{a} ± 0.39	2.54^{a} ± 0.26	2.08^{a} ± 0.23	***	Ns	*
Plin2	1.11^{b} ± 0.27	0.68^{b} ± 0.12	1.88^{ab} ± 0.54	2.18^{a} ± 0.33	0.77^{c} ± 0.25	0.79^{bc} ± 0.30	2.01^{ab} ± 0.16	2.21^{a} ± 0.23	***	Ns	Ns
Fabp4	0.86^{d} ± 0.08	6.07^{c} ± 2.75	14.45^{b} ± 3.33	87.29^{a} ± 8.95	1.28^{d} ± 0.28	4.45^{c} ± 0.99	25.25^{b} ± 3.81	64.76^{a} ± 8.77	***	Ns	Ns
ApoB	1.01 ± 0.26	0.46 ± 0.17	1.08 ± 0.62	0.73 ± 0.28	0.99 ± 0.36	0.68 ± 0.50	0.91 ± 0.43	0.83 ± 0.17	Ns	Ns	Ns
Inflammation											
Il18	0.94^{c} ± 0.13	1.10^{c} ± 0.13	1.72^{b} ± 0.21	2.68^{a} ± 0.17	1.25^{b} ± 0.32	1.23^{ab} ± 0.21	1.67^{ab} ± 0.19	2.19^{a} ± 0.19	***	Ns	Ns
Tnfα	1.04^{ab} ± 0.06	0.95^{b} ± 0.22	1.10^{ab} ± 0.14	1.46^{a} ± 0.13	0.93 ± 0.18	1.01 ± 0.17	1.23 ± 0.12	1.12 ± 0.10	Ns	Ns	Ns
Regulators											
Bcl6	0.96^{b} ± 0.23	0.69^{b} ± 0.09	1.29^{ab} ± 0.24	1.76^{a} ± 0.23	1.08 ± 0.21	0.79 ± 0.24	1.11 ±0.22	1.45 ± 0.13	***	Ns	Ns
Pparα	0.98 ± 0.05	0.92 ± 0.17	1.04 ± 0.07	1.15 ± 0.08	1.05 ± 0.18	0.77 ± 0.03	0.98 ± 0.15	1.08 ± 0.11	Ns	Ns	Ns
Srebp	1.07^{a} ± 0.10	0.54^{b} ± 0.09	0.84^{a} ± 0.07	0.75^{a} ± 0.10	0.85 ± 0.23	1.20 ± 0.42	0.96 ± 0.15	0.98 ± 0.14	Ns	Ns	Ns
Chrebp	1.05^{a} ± 1.00	0.74^{a} ± 0.09	0.81^{a} ± 0.05	0.38^{b} ± 0.04	0.89^{a} ± 0.05	0.82^{a} ± 0.16	0.81^{a} ± 0.14	0.37^{b} ± 0.05	***	Ns	Ns
Pparγ	0.97 ± 0.06	0.91 ± 0.14	0.91 ± 0.05	1.02 ± 0.06	1.06 ± 0.14	0.74 ± 0.11	0.99 ± 0.06	0.99 ± 0.13	Ns	Ns	Ns

Within a line, means with the same superscripts are not different ($P < 0.05$). *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, Ns = $P > 0.05$.
[1] M = Influence of the number of meals, L = Influence of the type of liver, MxL = interaction between M and L.
[#] Acronyms of genes are detailed in Supplementary Table S1.

for 45% of total fatty acids. Given that the increase in the level of transcription of the Scd1 gene was more quickly significant in veinous livers than in the normal livers of overfed female ducks, it can be hypothesised that hepatocytes of the veinous livers responded more rapidly to the need for lipogenesis than those of the normal livers. This hypothesis is also illustrated by the slightly higher lipid levels in veinous than in the normal livers after eight overfeeding meals, even if the interaction M × L for lipids was not significant (Table 1).

Conclusion

In the present study, we found few differences related to the development of hepatic steatosis induced by overfeeding in the veinous or normal livers of female mule ducks, with the exception of certain gene activities. The results also show that, regardless of the type of organisation of the veinous network in the liver, female mule ducks are just as likely to develop "foie gras" as males, which never have this anatomical characteristic. Consequently, from a physiological point of view, it is not difficult to envisage the overfeeding of female Mule ducks for the production of "foie gras". However, from a practical point of view, the presence of a well-developed and highly visible veinous network on the surface of the liver of a significant proportion (20–40%) of female mule ducks represents a challenge for processors. As this anatomical difference observed in females was present in the same proportions before and after the overfeeding period, the impact of this feeding method must be ruled out and a genetic origin must be considered as a priority. If the need to overfeed females becomes a recurring problem, further research will be required to examine hepatic angiogenesis in male and female mule ducks. From a physiological point of view, it would also be essential to assess the advantages and disadvantages associated with this anatomical feature.

Supplementary material

Supplementary Material for this article (https://doi.org/10.1016/j.animal.2025.101502) can be found at the foot of the online page, in the Appendix section.

Ethics approval

In France, the overfeeding of palmipeds for the production of "foie gras" is totally legal and controlled by the French government in accordance with national (98/58 CE) and EU regulations (243/2008 CE). We do not modify the way of production and we collected samples at the end of a regular slaughter line in a processing plant which is legally registered for doing this with an agreement delivered by the French authorities. Therefore, there is no need for a specific ethical agreement because we do not change anything in the way of producing and slaughtering the birds. We indicate in the M&M section that all samples were commercial ones collected in a regular processing plant.

Data and model availability statement

None of the data were deposited in an official repository. Data are available on requests to the corresponding author.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

Authors Julien ARROYO and Caroline MOLETTE were employed by Euralis Gastronomie. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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