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Spontaneous steatosis stimulation in geese induces liver fattening but impacts sexual maturation and muscle growth in a sex-dependent manner

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ABSTRACT

Experimental mimicry of premigratory environmental conditions results in a transient corn hyperphagia associated with a spontaneous liver steatosis in male Greylag geese. Our study aimed to i/ determine the feasibility of the induction of a spontaneous steatosis in female geese and ii/ evaluate its reversibility. Six-week-old Greylag Landaise geese (*Anser anser*) were randomly assigned to a control group (40 males and 41 females) or a spontaneous fattening group (**SF**; 118 males and 122 females) separating the sexes. Controls were fed a grower diet and exposed to a 10 h daylight cycle throughout the experiment. SF birds were exposed to a progressive reduction in day length from 10 to 7 h/day and submitted to a quantitative feed restriction with a grower diet between 8 and 19 weeks of age, followed by an *ad libitum* corn feeding period until 31 weeks of age, after which the grower diet was provided again until 33 weeks of age to study the reversibility of steatosis. Ten Control and 30 SF birds per sex were slaughtered at 31 and 33 weeks of age to evaluate liver fattening. Female SF geese had a lower feed intake during the second part of the corn feeding period (25–31 weeks of age, $P < 0.05$), but had equivalent aptitudes to develop a spontaneous steatosis (liver weight and lipid content, $P > 0.05$), than male SF geese. However, breast muscle growth was penalised in females ($P < 0.05$). Compared to controls, sexual maturation was strongly hindered in both SF males and females (gonad weight, $P < 0.05$). Regarding reversibility, in males, SF birds had equivalent liver weights to controls at 33 weeks of age while liver lipid content remained higher ($P < 0.05$). As control females initiated a steatosis, conclusions on reversibility in that sex were difficult to make. Finally, mortality occurred at the end of the corn feeding period in both sexes in SF birds. Our study enabled us to demonstrate that male and female geese had equivalent aptitudes to develop a spontaneous steatosis, associated with a lower feed intake in females. However, coupled with the protein–deficient composition of the corn-based diet, this lower feed intake could lead to exacerbated protein deficiencies in females, evidenced by a penalised muscle growth in these animals. Reversibility of spontaneous steatosis and mechanisms underlying its onset should be further explored in order to preserve the bird's health and welfare.

Implications

The production of waterfowl fatty liver relies on force-feeding, a practice largely questioned for animal welfare reasons. Alternative practices are being investigated. We demonstrated that male and female geese had equivalent aptitudes to develop spontaneous liver fattening. However, female geese had a lower feed intake that could lead to protein deficiencies. Also, sexual maturation was hindered by this practice. Our results provide new insight for potential

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alternative rearing practices to force-feeding in waterfowl fatty liver production. However, steatosis reversibility and associations between spontaneous liver fattening induction and welfare and health should be further characterised before implementing new practices.

Introduction

Waterfowl (geese or ducks) fatty liver, also known as « foie gras », is considered a delicacy and forms a part of the French cultural and gastronomic heritage (Article L.654-27-1 of the Rural Code). However, its production relies on force-feeding of the birds. This practice has been largely questioned by both animal welfare associations and institutional instances, such as the Council of Europe (1999a and 1999b) and the European Parliament (2022), for the last couple of decades. Thus, alternative practices, not resorting to force-feeding, for the production of waterfowl fatty liver have been investigated.

In birds, unlike mammals, the liver represents the main lipid synthesis site and can transitorily store lipids, as it is the case in migratory birds during premigratory and migratory phases, although storage mainly occurs in peripheral adipose tissues (Odum, 1960; Pond, 1978). Increased feed intake is the main contributor to the overall fattening of migratory birds (Bairlein and Gwinner, 1994). Based on these observations, previous studies from our laboratory have demonstrated the possibility to induce a transient hyperphagia, resulting in a spontaneous steatosis, in male Greylag geese (*Anser anser*) by experimentally mimicking premigratory environmental conditions (i.e. performing trials during winter season with a reduction in day length and alternations in food availability with a restricted access to a pellet diet followed by 12 weeks of *ad libitum* corn feeding) (Guy et al., 2013; Fernandez et al., 2016). Although liver weights were extremely variable (45% of variation), an average of 500 g per goose was obtained.

Reversibility of steatosis is a key indicator that hepatic function has not been permanently altered, and become potentially dysfunctional, and that lasting damages have not been induced. In the conventional rearing system, reversibility of steatosis following force-feeding in geese has been scarcely investigated. The sole study conducted on the matter, showed that, 32 d after the end of an 18 d-force-feeding period, liver weights had returned to control values while liver biochemical parameters got back to control levels after 58 d, with some individuals (7%) retaining histological signs of fibrosis (Babilé et al., 1998).

The « conventional » production system based on force-feeding and the alternative one differ considerably in steatosis stimulation duration (17 d of force feeding vs 84 d of *ad libitum* corn feeding), intensity (840 g/day vs 369 g/day on average) and kinetics (Guy et al., 2013; Brachet et al., 2015; Fernandez et al., 2016). Given these strong discrepancies in rearing system and intake kinetics, the biological mechanisms underlying steatosis and its reversibility may differ between the two rearing systems. Also, all previous experiments were solely conducted in male geese. In the conventional system, both sexes of geese are used as performances in terms of liver weight (≈800 g on average) are similar between male and female geese, in spite of a lower live weight in females (Guy et al., 1995; Guy et al., 1998).

Our study therefore aimed to i/ determine the feasibility of the induction of a spontaneous steatosis in female geese and ii/ evaluate the reversibility potential of a spontaneous steatosis.

Material and methods

The *in vivo* experiment was performed between mid-October 2015 and the end of April 2016 at the experimental site Avipôle (Benquet, France; experimental approval number C40-037-1). The experimental protocol was approved by the local ethics committee under the reference number APAFIS#2304-2015101514325358. Technical staff and scientists all had individual authorisations to conduct animal experimentation in accordance with good animal practices issued by the DDCSPP (Direction Départementale de la Cohésion Sociale et de la Protection des Populations) and slaughter was performed according to the European Council regulations (2009).

Study design, feeding programs and housing management

A total of 158 male and 163 female 6-week-old Greylag Landaise geese (Maxipalm®; *Anser anser*) were randomly assigned either to a control group (Control, n = 40 male and 41 female geese) or a spontaneous fattening group (**SF**; n = 118 male and 122 female geese) (Fig. 1A). The geese were reared in pens of 39–41 animals each, separating the sexes (1 control and 3 spontaneous fattening pens per sex) and BWs were standardised between pens, within each sex (Supplementary Fig. S1). Animals had access to an outside yard from 0715 to 1830 h until 15 weeks of age after which access was limited in time in order to comply with experimental day length monitoring (see below). Water and poultry grit were provided *ad libitum* for the entire duration of the experiment, and temperature was monitored daily.

In the control group, geese were fed a commercial grower diet (Supplementary Table S1; Nutricia, Mont de Marsan, France) and exposed to a 10 h/day light cycle for the entire duration of the experiment. The geese were fed *ad libitum,* except between 15 and 19 weeks of age where feed intake was capped at 320 g/goose per day in order to ensure normal growth while preventing excessive fattening. However, feed intake decreased spontaneously after 17 weeks of age; thus, artificial reduction in feed intake was only applied between 15 and 17 weeks of age in the control group. In the SF group, in order to mimic the autumn shortening in day length, birds were exposed to a 10 h/day light cycle until 17 weeks of age as of which the duration was lowered by 15 min per day in order to reach a 7 h/day light cycle by 19 weeks of age. The geese were fed the same commercial grower diet as the control group, *ad libitum* until 8 weeks of age, then submitted to a gradual quantitative feed restriction in order to mimic feed rarefication before migration and increase ingestion capacity and induce hyperphagia when feed was subsequently provided *ad libitum*. Feed restriction procedure was as follow: 350 g/goose per day from 8 to 9 weeks of age, 300 g/goose per day from 9 to 11 weeks of age, 280 g/goose per day from 11 to 14 weeks of age, and 250 g/goose per day from 14 to 19 weeks of age (Fig. 1B). No refusals were observed between 10 and 19 weeks of age. As of 19 weeks of age, the geese were fed *ad libitum* a whole corn diet (Supplementary Table S1; Sud-Ouest Aliment, Haut-Mauco, France) for a 12-week period, until 31 weeks of age. In order to favour intake, corn was progressively incorporated into the diet during the 3 days prior to exclusive corn feeding. From 31 to 33 weeks of age, birds were returned to an *ad libitum* commercial grower diet feeding in order to study the potential reversibility of steatosis (Fig. 1A).

Mortality occurred in one pen of female geese between 17 and 18 weeks of age (Fig. 1C). Following autopsies of the dead animals by a veterinarian, a bacterial infection (Erysipelas) was detected and all remaining animals, regardless of pen, were vaccinated (Ruvax, Boehringer Ingelheim Animal Health, Lyon, France).

Measurements, slaughter and samplings

Feed intake was recorded daily per pen and expressed in g DM/-day per goose. Mortality (i.e. animals found dead or with deteriorating condition resulting in euthanasia) was evaluated daily and,

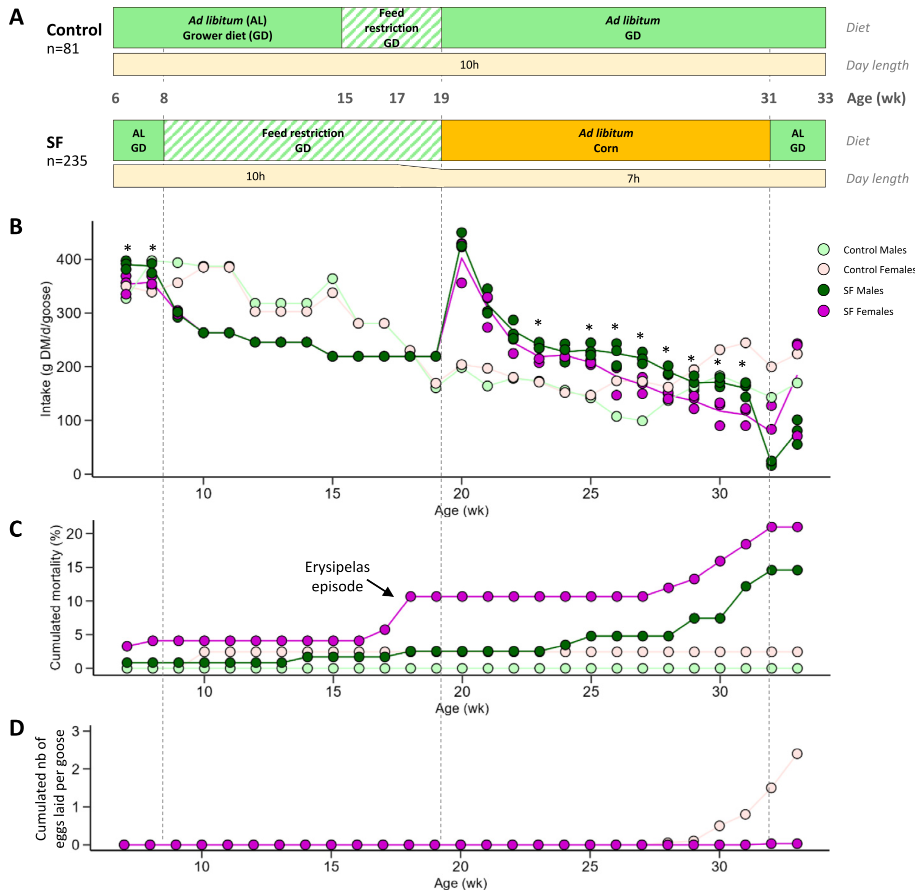

Fig. 1. Experimental design and geese feed intake, mortality and number of eggs laid according to the experimental group and sex. (A) At 6 weeks of age geese were either assigned to a control (C) group (n = 81) or a spontaneous fattening (SF) group (n = 235). Control geese had access to a grower diet (GD) fed *ad libitum* (AL), except between 15 and 19 weeks of age (320 g/goose per day), and were exposed to a 10 h/day light cycle for the entire duration of the experiment. In the SF group, birds were gradually feed restricted with a GD between 8 and 19 weeks of age, followed by an AL corn feeding period until 31 weeks of age, itself followed by an AL feeding period of the GD diet until 33 weeks of age. SF geese were exposed to a 10 h/day light cycle until 17 weeks of age as of which the duration was lowered by 15 min per day in order to reach a 7 h/day light cycle by 19 weeks of age. At 31 and 33 weeks of age 30 SF birds and ten control birds per sex were slaughtered. (B) Feed intake was measured daily by pen (n = 3/sex for the SF group and n = 1/sex for the Control group), averaged for each week and expressed in equivalent of DM. In the SF group, feed intake was compared between sexes using a non-parametric Kruskal-Wallis test. * $P < 0.05$ between SF males and females. (C) Mortality was evaluated daily by pen and expressed as cumulated mortality (%) over time while (D) eggs laid were counted daily and expressed as cumulated number of eggs. Abbreviations: AL = *ad libitum* feeding, GD = grower diet, SF = spontaneous fattening, wk = week.

during the corn feeding period, body and livers after autopsy of the dead animals were weighed. Cumulated mortality rate was calculated excluding animals that were slaughtered for experimental purposes and estimated relative to the initial number of animals in each group (Supplementary Table S2, Fig. 1C). When eggs were laid, they were counted and removed daily (Fig. 1D). Animals were weighed at 6 and 14 weeks of age after an 11–13 h fasting and at their date of slaughter, before transportation, without prior fasting. Ten control geese and 30 SF geese per sex (ten per pen) were randomly selected and slaughtered at 31, at the end of the corn feeding period, and 33 weeks of age, after 2 weeks of return to a grower diet feeding (Fig. 1A). Birds were electrically stunned and immediately killed by an exsanguination through a ventral cut of neck blood vessels. After scalding and plucking, carcasses were eviscerated. The liver, abdominal fat and reproductive organs (testicles or ovaries) were carefully removed and weighed. Additional measurements were performed on the liver surfaces. Colour L* (lightness), a* (redness), b* (yellowness) values were recorded with a CR 300 Minolta chromametre (Osaka, Japan). Near Infra-Red Spectra (**NIRS**) measurements were performed using the Labspec 5000 Pro spectrometer (ASD Inc., Boulder, CO) (absorbance range: 350–2 500 nm) to predict biochemical characteristics of the livers as previously described (Marie-Etancelin et al., 2014). A section of the right lobe of the liver was sampled and immediately snap frozen in liquid nitrogen and subsequently stored at −20 °C for further biochemical analyses. After 24 h of chilling, the carcasses were weighed and one breast muscle, the associated breast skin and one whole leg (bone, muscle and skin) were carefully dissected and weighed according to the method of the World Poultry Science Association (Jensen, 1984).

Biochemical analyses of the livers and experimental diets

DM and lipid content of the livers were determined on a representative subsample of the livers (20% of the livers). As lipid contents were high, samples were mixed with fine sand to increase the exchange surface and avoid fat crust formations. DM content was determined by drying grinded fresh liver samples in an oven at 105 °C for 24 h (JOCE, 1971). Total lipid content was determined after cold extraction in a chloroform/methanol mixture (2/1, V/v) and measured gravimetrically according to the method described by Folch et al. (1957), with 1 g of fresh liver for 50 ml of extraction volume. Then, DM and total lipid content were estimated for all liver samples using prediction equations based on NIRS spectra as previously described (Marie-Etancelin et al., 2014). Spectrum data were shortened from 650 to 2 350 nm and transformed with a Standard Normal Variate and Detrend 1, 10, 10, 1 normalisation with the Winisi software (version 4.6.8, FOSS Analytical A/S, Hilleroed, Denmark). Through a modified Partial Least Square analysis, the prediction equations were then generated.

Diet DM was obtained after 24 h at 103 °C and ashes after 5 h at 550 °C. Diet Nitrogen (N) levels were determined with the DUMAS combustion method (AOAC, 2005, method 968.06) with a Leco auto-analyser (model FP-428; Leco Corp., St Joseph, MI, USA) and converted into CP (N × 6.25). Crude fat was determined using Soxtec system H+ (after acid hydrolysis pretreatment) according to the method described by Alstin and Nilsson (1990).

Statistical analyses and graphical outputs

Statistical analyses were performed using R software version 4.2.0. To verify homoscedasticity of the data, a Levene test was performed. Variance being heterogeneous between groups on measurements at slaughter and biochemical analyses, data were log transformed to improve variance homogeneity. For all measurements at slaughter and biochemical analyses, a linear mixed model was applied using the package lme4 (Bates et al., 2015) with sex, time (31 or 33 weeks), experimental group (Control or SF) and their two-way interactions (sex × time, sex × group, time × group) as fixed effects and the pen as random effect. Mean values were compared pairwise within time and group × sex using lsmeans posthoc test from the package lsmeans (Lenth, 2016). In the SF group, feed intake was compared between sexes using a non-parametric Kruskal-Wallis test. No statistical analyses were performed on mortality and the number of eggs laid.

Results

Feed intake

As intended, during the *ad libitum* corn feeding period (19–31 weeks of age), intake of SF birds numerically surpassed that of control geese in both sexes (Fig. 1B; + 1.6 kg DM/goose in females and +6.9 kg DM/goose in males). Discrepancies in feed composition, with a higher energy level (14.0 vs 11.5 MJ/kg) and a lower protein level (68 vs 148 g/kg) in the corn-based diet compared to the grower diet, resulted in a 35 and 86% increase in energy intake and a 50 and 30% decrease in protein intake in female and male SF geese, respectively, when compared to controls. The increased feed intake in SF birds was characterised by a brief and transitory hyperphagia over the two first weeks of corn feeding (Fig. 1B, 419 and 313 g DM/goose per day respectively for weeks 20 and 21 vs ≈ 190 g DM/goose per day in controls) after which feed intake gradually decreased in both sexes.

When investigating the impact of sex on intake, as only one pen per sex was used in controls, no statistical analyses could be performed. In SF birds, feed intake was 22% (−42 g DM/goose per day) lower (P < 0.05) in females compared to males during the end of the corn feeding period (25–31 weeks of age).

Mortality

In control birds, mortality was low over the whole experiment (1 female, none in males). In SF geese, cumulated mortality rate reached 21% in females (19 birds) and 15% in males (10 birds) (Fig. 1C, Supplementary Table S2) at the end of the experiment. In SF females, a first numeric increase in mortality occurred between 17 and 18 weeks (8 geese) due to a bacterial infection (Erysipelas) in one pen. Between 18 and 27 weeks of age, mortality was stable in both sexes in SF birds, but numerically increased by 10% (five male geese and six female geese) over the end of the corn feeding period, between 27 and 32 weeks of age. Save one, all birds that died after 27 weeks of age had livers weighing more than 500 g (618 g on average in females, 670 g on average in males, at autopsy without prior exsanguination; Supplementary Table S2).

Body traits of the geese at slaughter

At 31 (end of the corn feeding period) and 33 weeks of age (after 2 weeks of return to a grower diet feeding), 30 SF and 10 Control geese per sex were slaughtered. BW at slaughter was not impacted by experimental treatment nor sex, neither at 31 nor at 33 weeks of age, but decreased overall with time (P < 0.05; Supplementary Table S3). For all body traits, we will first evaluate the impact of sex in controls at each time point (31 and 33 weeks of age), then in SF birds, and finally, we will look into the differences between controls and SF birds.

In control geese, no difference was observed on liver weight between male and female geese. However, control females had a higher liver lipid content (+12 points at 31 weeks, + 17 points at 33 weeks), a higher liver lightness (L*, + 10 points at 31 weeks, + 11 points at 33 weeks), a higher liver level of redness (a*, + 4 points at 31 weeks, + 5 points at 33 weeks) and a higher liver level of yellowness (b*, + 6 points at 31 weeks, + 9 points at 33 weeks) than their male counterparts (P < 0.05) at both 31 and 33 weeks of age (Fig. 2). No impact of time was observed on liver–associated parameters in control geese.

In SF geese, male and female geese had equivalent liver weights, liver lipid content, liver DM content, liver lightness, and liver level of yellowness. Male geese however had a lower liver level of redness than females (−2.6 points at 31 weeks, - 2.4 points at 33 weeks). Liver weight decreased with time (31 vs 33 weeks, P < 0.05) in both sexes in SF birds. This was associated with a decrease in DM content (P < 0.05), lightness (L*, P < 0.05) and level of redness (a*, P < 0.05) in both sexes, and lipid content (P < 0.05) solely in females and level of yellowness (b*, P < 0.05) solely in males. As expected (Guy et al., 2013; Fernandez et al., 2016), it is to be noted that a great variability in liver weight was observed in the SF group in both sexes (CV of 54% in females and 72% in males at 31 weeks of age), correlated with a great variability in lipid content (Fig. 2A /B/C), indicating that some geese seemingly respond to the spontaneous steatosis stimulation, while others do not.

In males, when comparing Control and SF geese, liver weight was 2.2 fold higher in the SF group compared to controls at 31 weeks of age (P < 0.05). This was associated with an increase in lipid content (+20 points, P < 0.05), lightness (L*, P < 0.05) and yellowness (b*, P < 0.05), indicating an increased liver fatness in these animals (Fig. 2A/C and Supplementary Table S3). At 33 weeks of age, although no significant impact on liver weight was observed, SF geese still presented a 16–point higher liver lipid content, and higher liver lightness (L*, P < 0.05) and yellowness (b*, P < 0.05) compared to controls. In females, no significant difference between Control and SF birds was observed on liver–associated parameters.

In control geese, when considering overall fattening and muscle growth, no differences were observed between males and females neither at 31 nor 33 weeks of age. Abdominal fat weight decreased with time in control females (P < 0.05, Supplementary Fig. S2A, Supplementary Table S3), while breast skin and whole leg weight decreased over time in control females and males (P < 0.05) (Supplementary Table S3). Likewise, in SF geese, no differences were observed between males and females neither at 31 nor 33 weeks of age, except on breast muscle weight that was lower in females (P < 0.05) compared to males at 31 weeks of age. In females, abdominal fat weight decreased with time while breast muscle weight increased (Fig. 3A, Supplementary Fig. S2A, Supplementary Table S3). In males, no impact of time was observed. When comparing Control and SF geese, muscle weight was impaired by SF

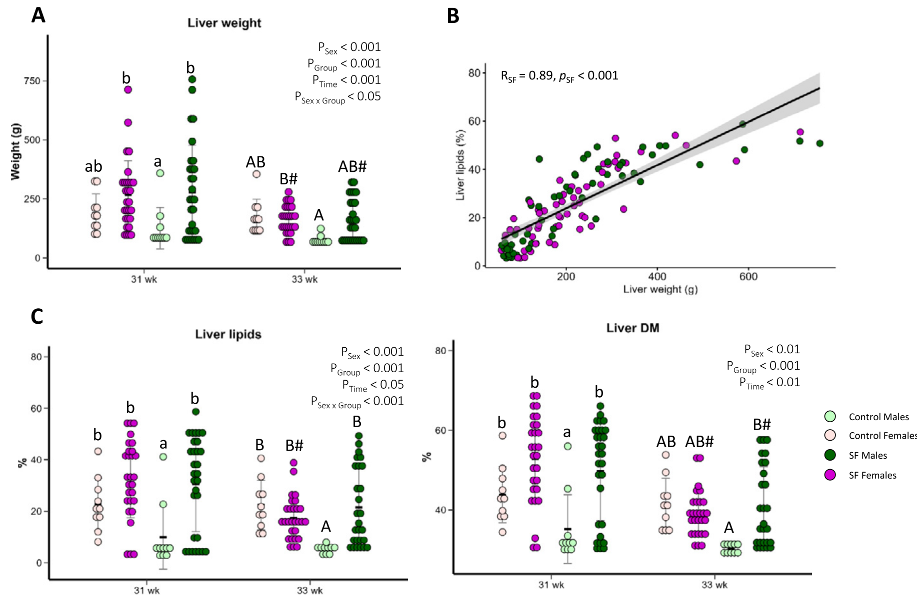

Fig. 2. Geese liver characteristics at slaughter. (A) Liver weight according to the experimental group, sex and time, (B) Spearman correlations between the liver weight in SF birds at 31 and 33 weeks of age and liver lipid content, (C) biochemical composition of the liver at slaughter according to the experimental group, sex and time. A linear mixed model was applied with sex, time (31 or 33 weeks of age), experimental group (Control or SF) and their two-way interactions (sex × time, sex × group, time × group) as fixed effects and the pen as a random effect. Mean values were compared pairwise within Time (31 or 33 weeks of age) and Group × Sex using lsmeans posthoc test. Mean values with a different superscript within a Time differ (P < 0.05); # significantly different (P < 0.05) from 31 weeks of age within an experimental group of the same sex. Data are presented as means (black square) ± SD (error bars). Correlation were considered absent when |R| < 0.3, low when 0.3 < |R| < 0.5, moderate 0.7 > |R| > 0.5, high when 0.9 > |R| > 0.7 and very high when |R| > 0.9. P < 0.05 was considered significant. Abbreviations: SF = spontaneous fattening, wk = week.

stimulation at 31 weeks of age, with a lower breast muscle weight in both males (P < 0.05) and females (P < 0.05) (Fig. 3A). Given these results, we wanted to explore if liver growth occurred at the expense of muscle growth in SF birds. However, in these geese, liver weight and breast muscle weight were only weakly negatively correlated (R = –0.26, P < 0.01; Fig. 3D).

In female control birds, ovary weight did not vary over time (Fig. 3B) and had a low positive correlation with liver weight (R = 0.48, P < 0.05; Fig. 3E). Control birds started laying eggs at 28 weeks of age with a linear increase as of 29 weeks of age, reaching a total of 32 eggs at 33 weeks of age (2.4 eggs/goose; Fig. 1D). In female SF geese, ovary weight increased over time (P < 0.05) and had no correlation with the liver weight (Fig. 3E). Solely one egg was laid in that group at 32 weeks of age (Fig. 1D). Sexual maturation was strongly impacted by the experimental treatment. SF females demonstrated an 84% reduction in ovary weight compared to controls (P_{Group} < 0.001, Fig. 3B). This was associated with a marked lower number of eggs laid in the SF group compared to controls (2.4 vs 0.03 eggs/-goose; Fig. 1D).

In both control and SF birds, testicle weight did not vary over time (Fig. 3C). In control males, testicle weight had a moderate positive correlation with liver weight (R = 0.59, P < 0.01), while it had a low negative correlation with liver weight in SF birds (R = –0.36, P < 0.01) (Fig. 3F). SF males displayed a 36% reduction in testicle weight compared to controls (P_{Group} < 0.01).

Discussion

As intended, in the SF group, a transitory hyperphagia was observed in both sexes during the 2 first weeks of corn feeding. Although liver weights were variable, after 12 weeks of corn feeding, this resulted in a spontaneous steatosis as previously observed by Guy et al. (2013) and Fernandez et al. (2016; 2019).

Impact of the sex on the aptitude to spontaneous steatosis

In SF birds, the weight, colour and biochemical characteristics of the livers after 12 weeks of corn feeding were similar between males and females, suggesting a similar aptitude to spontaneous steatosis in both sexes. For equivalent liver characteristics, SF females had a 22% lower intake compared to males during the second part of the *ad libitum* corn feeding period (25–31 weeks of age). These results could indicate a better feed efficiency in females subjected to a spontaneous steatosis stimulation.

In control birds, however, sex had a strong impact on liver characteristics. Higher liver lipid contents, yellowness and lightness were observed in females compared to males at both 31 and 33 weeks of age. Feed intake was numerically higher in control females compared to control males as of 26 weeks of age, potentially explaining these differences. In a small–scale experiment (n = 5), Flock et al. (1937) also observed differences in liver weights between *ad libitum*–fed male and female geese. The increase in liver weight in females occurred concurrently with sexual maturation, evidenced by the presence of eggs in formation, whereas a similar experiment (n = 3) conducted in autumn did not induce steatosis. Although conducted on a low number of animals, these latter experiments could suggest that steatosis occurs concurrently with sexual maturation in females. In agreement, it is well documented that oestrogen induces strong shifts in liver lipid metabolism during egg production in birds (for review, Tramunt et al., 2021). In our experiment, egg laying was observed in control females as of 28 weeks of age and ovary weight was lowly positively correlated with liver weight, thus corroborating this hypothesis.

Spontaneous steatosis stimulation strongly impaired gonad growth in both sexes with little (males) to no (females) correlation with the liver weight, suggesting a direct impact of the spontaneous steatosis stimulation on reproductive organ development. Two main factors diverging between control and spontaneous fat-

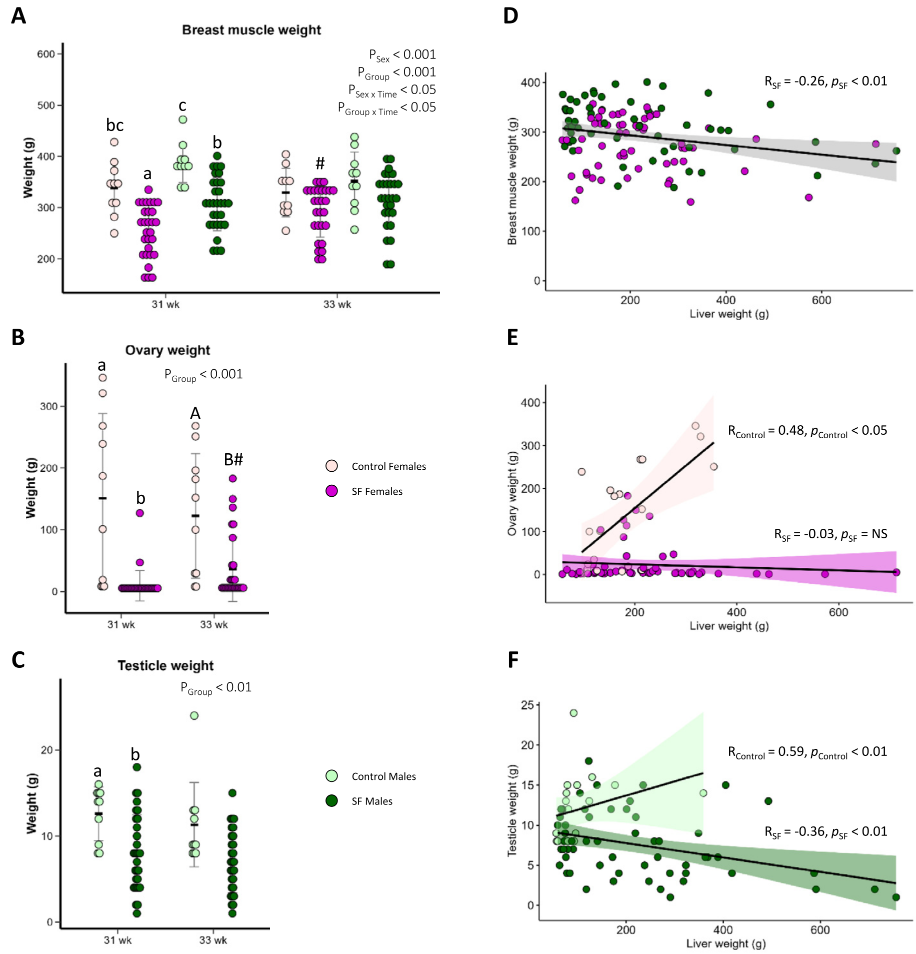

Fig. 3. (A) Geese muscle weight after chilling and (B/C) reproductive organs' weight at slaughter according to the experimental group, sex and time. Spearman correlations between the liver weight in SF males and females at 31 and 33 weeks of age and (D) Geese breast muscle weight, (E) Ovary weight in SF females and (F) Testicle weight in SF males. A linear mixed model was applied with sex, time (31 or 33 weeks of age), experimental group (Control or SF) and their two-way interactions (sex × time, sex × group, time × group) as fixed effects and the pen as a random effect. Mean values were compared pairwise within Time (31 or 33 weeks of age) and Group × Sex using lsmeans posthoc test. Mean values with a different superscript within a Time differ (P < 0.05); # significantly different (P < 0.05) from 31 weeks of age within an experimental group of the same sex. Data are presented as means (black square) ± SD (error bars). Correlation were considered absent when |R| < 0.3, low when 0.3 < |R| < 0.5, moderate 0.7 > |R| > 0.5, high when 0.9 > |R| > 0.7 and very high when |R| > 0.9. P < 0.05 was considered significant. Abbreviations: SF = spontaneous fattening, wk = week.

tening conditions could be incriminated: i/ the light cycle and ii/ the composition of the feed. It is well documented that reproduction in birds is mainly regulated by photoperiod. Increasing day length induces the hypothalamo-pituitary–gonadal axis that is responsible for gonad maturation (Dawson et al., 2001). Thus, gonad weight increases in both male (Leska et al., 2012) and female (Bao et al., 2023) geese during the breeding season, when day length increases. In our experiment, control birds were exposed to an average-day light cycle of 10 h/day while SF birds were exposed to a short-day light cycle of 7 h/day. Thus, the longer daylight cycle in Control birds could have induced the onset of gonad maturation, contrary to what was observed for SF birds. Diet could however also contribute to gonad growth impairment. Indeed, in laying hens, Kim and Kang (2022) evidenced an increase in egg weight when dietary energy levels were risen from 11.30 to 11.70 MJ/kg of ME, while Yu et al. (2009) evidenced a decrease in reproductive performances (egg production and weight) when ME was increased from 11.71 MJ to 14.06 MJ/kg of ME, suggesting a potential deleterious impact of high ME levels. This hypothesis is corroborated in geese, where Chen et al. (2023) showed in late laying stage geese fed 5 different diets ranging from 9.65 to 11.75 MJ/kg that ovarian weight was impacted by dietary ME level, with weight increasing with ME level up until 10.7 MJ/kg and decreasing afterwards, in association with a decreased hatchability of the fertilised eggs and an increased liver weight. In our experiment, the exclusive corn–based diet was rich in energy (14.01 MJ/kg of ME), potentially negatively impacting on reproductive performances. Also, the corn–based diet was poor in proteins, compared to the grower diet, and was below the nutritional recommendations for adult geese (7 vs 13–14%) (Farrell, 2004), thus potentially delaying sexual maturation. Finally, when SF geese were returned to a grower diet (31–33 weeks of age), the light cycle was not altered. However, ovary weight in females at 33 weeks was increased compared to their counterparts at 31 weeks, suggesting a potential impact of the diet on ovary maturation.

Muscle growth, measured through the weight of the breast muscle, was lower in SF geese compared to controls at 31 weeks of age, but this decrease was more marked in females. Muscle growth is particularly impacted by protein intake. The corn diet being deficient in proteins, and intake being lower in SF females compared to males, protein intakes were 30 and 50% lower in, respectively, SF males and females compared to controls, thus explaining the reduced muscle growth. These observations indicate exacerbated protein deficiencies in females when submitted to a spontaneous steatosis stimulation and should be further investigated in order to evaluate the impact of this practice on health and welfare of the birds.

Reversibility of spontaneous steatosis

In control birds, no modulations of liver characteristics were observed between 31 and 33 weeks of age, while liver weight decreased in previously corn fed birds (SF) in both sexes after 2 weeks of return to a grower diet, although lipid content only decreased in females. These decreases could suggest that the induced steatosis is reversible. However, in males, although no significant difference in liver weight was observed at 33 weeks of age between Control and SF geese, differences in lipid content and colour were persistent, evidencing persistent changes in liver metabolism and function. Babilé et al. (1998) showed that 16 d after the end of force-fed induced steatosis, liver weight (187 vs 88 g) and liver lipid content (24.1 vs 5.6%) remained higher in previously force-fed male geese compared to male controls. These values are numerically close to the ones obtained in our study 2 weeks after the end of spontaneous steatosis stimulation. In the study of Babilé et al. (1998), liver weights only returned to control values after 32 d, while liver biochemical parameters got back to control levels after 58 d. Further time might thus be necessary to fully recover after spontaneous steatosis as well. Due to the sanitary context in 2016, with the spread of avian influenza in the South of France, we were unable to perform samplings at ulterior dates, as initially planned. However, these experiments should be performed in order to make conclusions on the reversibility of spontaneous steatosis in geese. Also, it would be pertinent to evaluate the impact of steatosis level at the end of the corn feeding period on the subsequent reversibility. Thus, following liver weight kinetics through non-invasive methods, like ultrasound imaging, or analyses of plasmatic markers such as chemerin, could be of interest to further characterise steatosis and its reversibility in geese (Cobo et al., 2015; Mellouk et al., 2019). Finally, further characterisations of the livers would be pertinent in order to evaluate metabolic and histological changes occurring in the liver with spontaneous steatosis as previously performed in force–fed birds (Atallah et al., 2024).

In females, no difference was observed on liver characteristics between SF and control geese 2 weeks after the end of the spontaneous steatosis stimulation. However, it is important to note, as stated prior, that control levels were high in females, likely in association with the sexual maturation. This maturation did not occur in SF females. Thus, liver kinetics appear different between control and SF birds, with no impact on liver weight in Controls but a decrease in liver weight in SF birds, making conclusions on reversibility in that sex difficult to make. Further studies are needed to conclude on the steatosis kinetics in female geese and its links with sexual maturation onset.

Also, a 10% increase in mortality (five male geese and six female geese) was observed in the SF group at the end of the corn feeding period (27–32 weeks of age) in both sexes, while no mortality occurred in the control group during that period. Previous studies (Guy et al., 2013; Fernandez et al., 2016) did not evidence any mortality during that period, but mortality did occur as of 33 weeks of age when the corn feeding period was extended (Fernandez et al., 2016), suggesting a detrimental impact of prolonged exclusive corn feeding. Although no evident cause of death was determined, all geese that died in the SF group, save one, had high liver weights (>500 g), suggesting liver steatosis in these animals. Thus, the association between spontaneous steatosis induction and health should be further characterised.

Conclusion

Our study enabled us to demonstrate that male and female geese had equivalent aptitudes to develop a spontaneous steatosis, associated with a lower feed intake in females. However, coupled with the protein–deficient composition of the corn-based diet, this lower feed intake could lead to exacerbated protein deficiencies in females, evidenced by a penalised muscle growth in these animals. This should be further investigated in order to evaluate the impact of spontaneous steatosis stimulation on health and welfare. Also, our study evidenced the onset of sexual maturation in control geese, associated with a steatosis onset in females, while sexual maturation was hindered in geese submitted to a spontaneous steatosis stimulation. The association between sexual maturation and steatosis stimulation in waterfowl should thus be further explored.

Regarding reversibility, in males, we hypothesise that, as in the conventional system based on force-feeding, additional time is required for full reversibility. In females, given the interactions between the liver fattening kinetics and sexual maturation, further studies are needed in order to determine the reversibility in these animals. Finally, mortality occurred at the end of the corn feeding period in both sexes. Association between spontaneous steatosis induction and health should thus be further characterised, and potential modifications made to the rearing system to preserve the bird's health and welfare.

Supplementary material

Supplementary Material for this article (https://doi.org/10.1016/j.animal.2025.101533) can be found at the foot of the online page, in the Appendix section.

Ethics approval

The experimental protocol was approved by the local ethics committee under the reference number APAFIS#2304-2015101514325358. Technical staff and scientists all had individual authorisations to conduct animal experimentation in accordance with good animal practices issued by the DDCSPP (Direction Départementale de la Cohésion Sociale et de la Protection des Populations), and slaughter was performed according to the European Council regulations (2009).

Data and model availability statement

The datasets and R codes were not deposited in an official repository but are available from the authors upon request.

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

J.A. is currently employed by EURALIS GASTRONOMIE (Maubourguet, France), and M.D.B. is currently employed by DUSSAU DISTRIBUTION (Pecorade, France). All other authors declare that they have no competing interests.

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References

Alstin, F., Nilsson, M., 1990. The Soxtec® hydrolysis system improves the official methods for determining total fat content. Industries Alimentaires et Agricoles 107, 1271–1274.

Association of Official Analytical Chemists (AOAC), 2005. AOAC Official Method 968.06. Protein (Crude) in Animal Feed. Dumas Method - Chapter 4. In AOAC Official Methods of Analysis (ed. Horwitz, W.). AOAC, Gaitherburg, MD, USA, p. 25-26.

Atallah, E., Trehiou, S., Alquier-Bacquie, V., Lasserre, F., Arroyo, J., Molette, C., Remignon, H., 2024. Development of hepatic steatosis in male and female mule ducks after respective force-feeding programs. Frontiers in Physiology 15, 1392968. https://doi.org/10.3389/fphys.2024.1392968.

Babilé, R., Auvergne, A., Dubois, J.P., Bénard, G., Manse, H., 1998. Réversibilité de la stéatose hépatique chez l'oie. Actes des 3èmes Journées de la Recherche sur les Palmipèdes à Foie Gras, 27 octobre 1998, Bordeaux, France, p. 45-48.

Bairlein, F., Gwinner, E., 1994. Nutritional mechanisms and temporal control of migratory energy accumulation in birds. Annual Review of Nutrition 14, 187–215. https://doi.org/10.1146/annurev.nu.14.070194.001155.

Bao, Q., Gu, W., Song, L., Weng, K., Cao, Z., Zhang, Y., Zhang, Y., Ji, T., Xu, Q., Chen, G., 2023. The photoperiod-driven cyclical secretion of pineal melatonin regulates seasonal reproduction in geese (Anser cygnoides). International Journal of Molecular Sciences 24, 11998.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67, 1–48. https://doi.org/10.18637/jss.v067.i01.

Brachet, M., Guy, G., Fernandez, X., Arroyo, J., Fortun-Lamothe, L., 2015. Environmental impacts of the goose foie gras production: comparison of systems with or without force-feeding. Actes des 11èmes Journées de la Recherche Avicole et Palmipedes à Foie Gras, 25 et 26 mars 2015, Tours, France, p. 950–954.

Chen, X., Yu, T., Dou, Y., Ji, Q., Guo, L., Geng, Z., 2023. High dietary energy decreased reproductive performance through increasing lipid deposition in Yangzhou geese at late laying stage. Poultry Science 102, 102915. https://doi.org/10.1016/j.psj.2023.102915.

Cobo, E., Molette, C., Touze, J.L., Venturi, E., Bernadet, M.D., Staub, C., 2015. Mise en place d'une méthodologie en imagerie pour prédire des caractères de production chez les palmipèdes à foie gras. Actes des 11èmes Journées de la Recherche Avicole et Palmipedes à Foie Gras, 25 et 26 mars 2015, Tours, France, p. 1234-1238.

Conseil de l'Europe, 1999a. Recommandation concernant les canards de Barbarie (*Cairina moschata*) et les hybrides de canards de Barbarie et de canards domestiques (*Anas platyrhynchos*) - Article 24. Comité Permanent de la Convention Européenne pour la protection des animaux dans les élevages, Strasbourg, France.

Conseil de l'Europe, 1999b. Recommandation concernant les oies domestiques (*Anser anser f. domesticus, Anser cygnoides f. domesticus*) et leurs croisements - Article 25. Comité Permanent de la Convention Européenne pour la protection des animaux dans les élevages, Strasbourg, France.

Dawson, A., King, V.M., Bentley, G.E., Ball, G.F., 2001. Photoperiodic control of seasonality in birds. Journal of Biological Rhythms 16, 365–380. https://doi.org/10.1177/074873001129002079.

Farrell, D.J., 2004. Management, nutrition and products of domestic geese: a review. Proceedings of the Australian Poultry Science Symposium, 9 -11 February 2004, Sydney, New South Wales, Australia, p. 139–144.

Fernandez, X., Guy, G., Laverze, J.B., Bonnefont, C., Knudsen, C., Fortun-Lamothe, L., 2016. A kinetic study of the natural induction of liver steatosis in Greylag Landaise geese: the role of hyperphagia. Animal 10, 1288–1295. https://doi.org/10.1017/s1751731116000161.

Fernandez, X., Lazzarotto, V., Bernadet, M.D., Manse, H., 2019. Comparison of the composition and sensory characteristics of goose fatty liver obtained by overfeeding and spontaneous fattening. Poultry Science 98, 6149–6160. https://doi.org/10.3382/ps/pez289.

Flock, E., Bollman, J.L., Hester, H.R., Mann, F.C., 1937. Fatty livers in the goose produced by overfeeding. Journal of Biological Chemistry 121, 117–129. https://doi.org/10.1016/S0021-9258(18)74331-7.

Folch, J., Lees, M., Sloane Stanley, G.H., 1957. A simple method for the isolation and purification of total lipides from animal tissues. Journal of Biological Chemistry 226, 497–509.

Guy, G., Rousselot-Pailley, D., Gourichon, D., 1995. Comparaison des performances de l'oie, du canard mulard et du canard de Barbarie soumis au gavage. Annales De Zootechnie 44, 297–305. https://doi.org/10.1051/animres:19950308.

Guy, G., Baéza, E., Salichon, M.R., Juin, H., Rousselot-Pailley, D., 1998. Influence of goose breeding conditions on the meat and fatty liver production. Annales De Zootechnie 47, 215–224.

Guy, G., Fortun-Lamothe, L., Benard, G., Fernandez, X., 2013. Natural induction of spontaneous liver steatosis in Greylag Landaise geese (Anser anser). Journal of Animal Science 91, 455–464. https://doi.org/10.2527/jas.2012-5492.

Jensen, J.F., 1984. Method of dissection of broiler carcasses and description of parts. In: Working Group, V (Ed.), World's Poultry Science Association European Federation. Papworth's Pendragon Press, Cambridge, UK, pp. 32–61.

Journal officiel de l'Union européenne (JOCE), 1971. Dosage de l'humidite. Deuxième directive de la commission du 18 novembre 1971 portant fixation de méthodes d'analyse communautaires pour le contrôle officiel des aliments des animaux. JOCE L279, 8-11.

Journal officiel de l'Union européenne (JOCE), 2009. Council regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing. JOCE, Brussels, Belgium.

Journal officiel de l'Union européenne (JOCE), 2022. European Parliament resolution of 10 June 2021 on the European Citizens' Initiative 'End the cage age' (2021/2633(RSP)). JOCE C67, 131-136.

Kim, C.H., Kang, H.K., 2022. Effects of energy and protein levels on laying performance, egg quality, blood parameters, blood biochemistry, and apparent total tract digestibility on laying hens in an aviary system. Animals (basel) 12, 3513. https://doi.org/10.3390/ani12243513.

Lenth, R.V., 2016. Least-squares means: the R package lsmeans. Journal of Statistical Software 69, 1–33. https://doi.org/10.18637/jss.v069.i01.

Leska, A., Kiezun, J., Kaminska, B., Dusza, L., 2012. Seasonal changes in the expression of the androgen receptor in the testes of the domestic goose (Anser anser f. domestica). General and Comparative Endocrinology 179, 63–70. https://doi.org/10.1016/j.ygcen.2012.07.026.

Marie-Etancelin, C., Vitezica, Z.G., Bonnal, L., Fernandez, X., Bastianelli, D., 2014. Selecting the quality of mule duck fatty liver based on near-infrared spectroscopy. Genetics Selection Evolution 46, 38. https://doi.org/10.1186/1297-9686-46-38.

Mellouk, N., Ramé, C., Barbe, A., Venturi, E., Touzé, J.L., Staub, C., Bernadet, M.D., Chartrin, P., Bordeau, T., Froment, P., Baeza, E., Dupont, J., 2019. La chemerinemie et l'echnographie: des indicateurs de la steatose hépatique chez le canard? Actes des 13èmes Journées de la Recherche Avicole et Palmipedes a Foie Gras, 20-21 March 2019, Tours, France, p. 426–431.

Odum, E.P., 1960. Premigratory hyperphagia in birds. The American Journal of Clinical Nutrition 8, 621–629.

Pond, C.M., 1978. Morphological aspects and the ecological and mechanical consequences of fat deposition in wild vertebrates. Annual Review of Ecology

and Systematics 9, 519–570. https://doi.org/10.1146/annurev.es.09.110178.002511.
Tramunt, B., Montagner, A., Tan, N.S., Gourdy, P., Rémignon, H., Wahli, W., 2021. Roles of estrogens in the healthy and diseased oviparous vertebrate liver. Metabolites 11, 502. https://doi.org/10.3390/metabo11080502.
Yu, D.-J., Na, J.-C., Choi, H.-C., Bang, H.-T., Kim, S.-H., Kang, G.-H., Kang, H.-K., Suh, O.-S., 2009. Effects of varying levels of dietary metabolizable energy and crude protein on performance and egg quality of organic laying hens. Korean Journal of Poultry Science 35, 367–373. https://doi.org/10.5536/KJPS.2009.35.4.367.