Comparison of the composition and sensory characteristics of goose fatty liver obtained by overfeeding and spontaneous fattening[1]

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ABSTRACT Spontaneous liver steatosis can be experimentally induced in domestic Greylag geese by combining a short photoperiod with a sequence of feed restriction followed by ad libitum corn feeding. This could offer an alternative to the conventional "foie gras" production system based on overfeeding. The present work aimed at comparing the compositional characteristics, sensory profile, and acceptability by a consumer panel of fatty livers obtained by overfeeding and spontaneous fattening. In all, 210 male geese were used: 125 geese were raised over a 31-wk period to produce fatty liver without overfeeding ("alternative livers") and 85 were raised using conventional methods with overfeeding ("conventional livers"). Mean liver weight was over 1 kg (1,102 g) in the conventional group and 445 g in the alternative group. The characteristics of the livers were studied in 2 subpopulations: 44 conventional livers representative of the experimental population (mean liver weight 1,064 g) and 42 alternative livers weighing more than 400 g (mean 702 g). Compared with the alternative livers, livers from the conventional group showed significantly ($P < 0.05$) higher dry matter and lipid contents, lighter color (L^*), and lower yellowness (b^*). The neutral lipids of alternative livers contained significantly less triglycerides and free fatty acids and significantly more cholesterol and cholesterol esters than those from conventional livers. Detailed analysis of the fatty acid composition of triglycerides showed that the proportion of mono- and polyunsaturated fatty acids was significantly higher in the alternative livers. However, covariance analysis suggested that these differences in lipid composition were mainly due to differences in lipid content between both types of livers. The evaluation of cooked livers by a trained expert panel revealed significant differences in the sensory profile between the conventional and alternative livers. The acceptability by a consumer panel was significantly lower in alternative compared to conventional livers. This difference was not related to weight and/or lipid content since livers of similar weight range (800 to 1,000 g) were compared and showed clear-cut differences for hedonic scores.

Key words: goose, spontaneous fattening, force feeding, liver chemical composition, liver quality

INTRODUCTION

Overfeeding geese is a very ancient tradition, used by the Egyptians as early as 2500 BC. The goal of such overfeeding was to fatten the birds in order to provide a high-energy food for human consumption. Overfeeding has been extended to ducks over the last centuries,

with the main objective of producing "foie gras" (fatty liver), a quality delicacy with a high added value. In order to produce "foie gras," grown geese and ducks are overfed with large amounts of corn, delivered twice daily, in order to induce liver steatosis. Depending on the species and the production process, this period, known as "gavage" (cramming), lasts 10 to 17 D. It is generally acknowledged that the efficiency of waterfowl to respond to overfeeding originates from the natural ability of migrating birds to overconsume food in order to store energy before long distance migration (Odum, 1960). Indeed, in birds traveling long distances, the premigratory increase in body weight (**BW**) can reach +100% of the lean mass and the energy needed for migration is for the most part stored as fat (Bairlein, 1987). However, overfeeding is an increasingly questioned practice because the force-feeding procedure, including intubation for corn delivery, is claimed to be stressful and/or painful, and therefore has a negative impact on the birds' well-being (Rochlitz and Broom,

2017). Ethical concerns about "foie gras" production were notably brought to the foreground in France when in 1998, the Scientific Committee on Animal Health and Animal Welfare (SCAHAW) reported to the European Commission that "force-feeding is detrimental to the welfare of the birds." In the late 2000s, our research group initiated a project to explore the possibility of inducing spontaneous liver steatosis without force feeding. We clearly demonstrated that hyperphagia and spontaneous liver steatosis could be experimentally induced in domestic Greylag geese by combining a short photoperiod and a sequence of feed restriction, followed by a period of ad libitum corn feeding during the fall and winter (Guy et al., 2013). These conditions were supposed to mimic premigratory changes. The level of steatosis was lower than that obtained with overfeeding, while individual variability was much higher with coefficients of variation (c_v) of liver weight ranging from 60 to 71%, depending on the age of slaughter. We recently demonstrated that hyperphagia plays a key role in the development of spontaneous liver steatosis. Indeed, the pattern and extent of hyperphagia have been shown to be strongly correlated with the level of hepatic steatosis (Fernandez et al., 2016).

Given the current state of knowledge, the average weight of the livers obtained by spontaneous fattening after 12 wk of corn consumption is roughly half that of livers obtained under the conventional production system based on overfeeding during 13 to 14 D (450 to 500 g vs. 850 to 900 g). It is not known whether the biological mechanisms underlying liver steatosis are the same in both cases, but previous results suggest that in livers obtained by spontaneous fattening (weight range 800 to 1,000 g) the lipid, dry matter, and protein content are very close to those of livers obtained with overfeeding (Guy et al., 2013). However, the composition of the lipid fraction in fatty livers obtained by spontaneous fattening has not been investigated as of yet. Moreover, a direct comparison of the 2 types of livers within the same experimental design has yet to be conducted.

Therefore, in the present work, we compared several characteristics in livers obtained by overfeeding or by spontaneous fattening, in particular the gross chemical composition and detailed composition of the lipid fraction. In addition, because "foie gras" is considered a delicacy, consumer expectations regarding its sensory qualities are high. We therefore also compared the sensory profiles and consumer acceptability of both types of livers.

MATERIAL AND METHODS

Animals were handled according to the recommendations on the protection of animals used for experimental purposes in agreement with the European Communities Council Directive of November 24, 1986 (86/609/EEC). The investigators involved in these experiments were certified by the French governmental authority (authorization no. 31–11 43 501). All experimental facilities were also certified for the breeding, care, and slaughtering of animals (authorization no. A40624).

The experiment was carried out at the INRA experimental unit on waterfowl (Benquet, France), using 210 Greylag geese from the Palmsire strain. The birds originated from 2 hatching batches. The first batch provided 125 one-day-old male goslings that were raised for 31 wk to produce fattened liver without overfeeding. This production system (described in detail below) is hereafter referred to as the "alternative system." The second hatching batch provided 81 one-day-old male goslings that were raised for 14 wk and overfed for 17 D; this production system is hereafter referred to as the "conventional system." Both systems were set up in such a way that birds were slaughtered during the same week.

Animals, Feeding and Housing Conditions under the Alternative System

A summarized view of the experimental process is provided in Figure 1.

Birds were fed a commercial diet from birth to 19 wk of age (2,800 kcal/kg ME and 180 g/kg CP from birth to 6 wk of age, and 2,800 kcal/kg ME and 160 g/kg CP from 7 to 19 wk of age; Maïsadour, Benquet, France) to meet the National Research Council requirements (NRC, 1994). From 20 wk of age onwards, the birds were fed moist whole grain maize stored in a flexible silo with a capacity of 30 t (storage in oxygen-free conditions) (Laplace, Pau, France). The corn was of standard commercial type and remained at the same dry matter (**DM**) content as at the time of harvesting (DM = 75%). Moist whole grain was preferred to dried corn grain because of its higher palatability for geese (Guy, INRA, Benquet, personal communication) and its higher starch proportion, which favors hepatic lipogenesis (Hermier et al., 1999).

The feeding program was the same as previously described by Guy et al. (2013). Briefly, this feeding strategy was based on a feed restriction period that ensures normal growth while preventing excessive fattening (McLandress and Raveling, 1981), and induces hyperphagia when stopped. The birds were fed ad libitum from birth to 5 wk of age, then 300 g/d during week 6, 350 g/d during weeks 7 to 9, 300 g/d during weeks 10 to 11, 280 g/d during weeks 12 to 14, and 250 g/d during weeks 15 to 19. At 19 wk of age, the geese were weighed before the start of the fattening process. From week 19 onwards, birds had ad libitum access to corn (Figure 1). Whole corn grains were progressively introduced in the pellet diet over the 3 last days before ad libitum feeding to familiarize the geese with the new diet. The birds had free access to water throughout the experiment.

From hatching to 4 wk of age, the birds were raised in a 100 m^2 pen in a building dedicated to the starting of goslings. At the age of 5 wk, the animals were
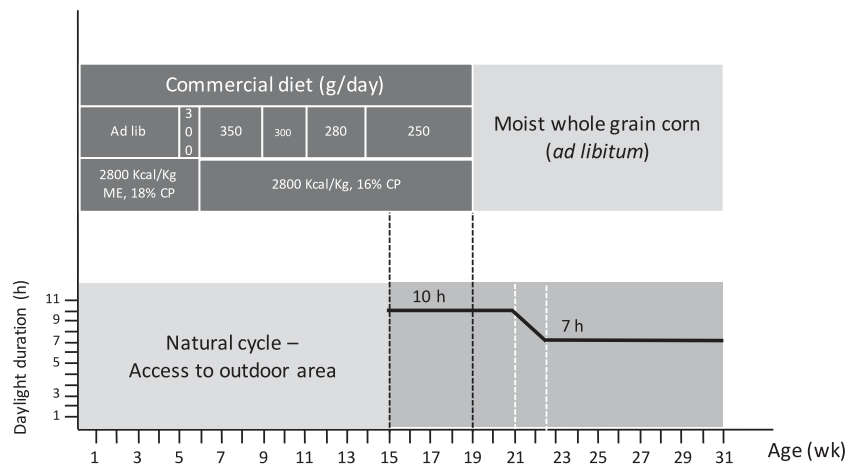
Figure 1. Schematic representation of the experimental design in the alternative production system based on spontaneous liver fattening.

weighed and 6 groups of 19 to 22 birds each were formed so that the mean and variance of live weight were similar between the groups. These 6 groups were transferred to a building with no natural lighting and housed in 6 separate pens. The surface of each pen was 32 m², i.e., 1.5 to 1.7 m²/bird. Each pen provided access to a small outdoor enclosure but at 15 wk of age its access was closed in order to control lighting conditions.

In the first part of the experiment in the starting building, the birds were raised in natural lighting conditions. During this period, the daylight duration fell from about 14 h (beginning of July) to 11 h (15 September). Once transferred inside the second building with no natural lighting at the age of 5 wk, all birds were kept under artificial lighting following a cycle of 10 h light:14 h dark with an average light intensity of 30 lux from 7:00 am to 5:00 pm that remained constant during the experimental period. At the start of week 22, the lighting duration was gradually decreased by 30 min every 2 D until a short-day period of 7 h L:17 h D was reached at almost 24 wk of age. This short photoperiod was maintained up to the end of the experiment (Figure 1).

The feed intake was recorded daily for each pen from 20 to 31 wk of age, i.e., during the corn feeding period, by calculating difference in trough weight between 2 fillings.

Animals, Feeding and Housing Conditions under the Conventional System

From hatching to 14 wk of age, the birds were raised in a 100 m² pen in a breeding building under natural lighting. From the age of 5 wk, they had free access to small outdoor enclosure. The birds were fed the same commercial diet as described above: 2,800 kcal/kg ME and 180 g/kg CP from birth to 6 wk of age (provided ad libitum), and 2,800 kcal/kg ME and 160 g/kg CP from 7 to 14 wk of age. Feed was provided ad libitum until the age of 8 wk. Thereafter, the access to feed was restricted to 4 h/d and then to 2 h/d during the last 4 D before the start of overfeeding. This practice increases crop size and prepares the birds for the phase of overfeeding (Arroyo et al., 2012).

At the age of 14 wk, the birds were weighed before the start of the fattening process. They were then transferred to the overfeeding building where they were housed in collective cages (3 birds/cage). The overfeeding process consisted in the delivery of 31 meals in total (2 meals per day for 16 D, with 1 meal the first day), composed of a soak-corn mixture (38% water, 36% corn flour, and 26% corn grain). Each meal was delivered in 2 boluses of 45 and 55% of the meal volume, respectively. The meal volumes were progressively increased from 235 g dry corn on the first day to 550 g for the last 6 meals. The animals had free access to water.

Slaughter and Measurements

The same slaughtering procedure was used for conventional and alternative birds on 2 different days in the same week. Feed was not withdrawn before slaughter for alternative birds, whereas conventional birds received their last meal the evening before slaughter. The procedure started at 6:00 am. Birds were crated in transport cages (4 birds/cage) and transported to the experimental slaughterhouse (5 min transport). The animals were weighed before slaughter. They were then suspended individually from a shackle with their head downwards and electrically stunned using head-only scissor tongs delivering a 90 V, 50 Hz AC current for 5 s. All birds were slaughtered by a ventral cut of neck blood vessels within 5 s after the end of the stun.

At about 20 min post mortem, the carcasses were eviscerated and the liver was carefully removed and weighed. Abdominal fat was also collected and weighed.

The color of the liver was measured immediately after slaughter along the ventral face of the big lobe using the trichromatic CIE Lab coordinates system (L^*, a^*, b^*) and a CR-300 Minolta Chroma Meter (Minolta, Osaka, Japan). The average of 3 measurements performed at different sites (top, middle, and bottom of the large lobe) was computed for each liver. An approximately 50 g sample was taken from the upper part of the big lobe. This sample was divided into 2 and both portions were immediately frozen in liquid nitrogen, vacuum packed, and stored at $-80°C$ until subsequent chemical analysis. The livers were then put on ice and chilled at $+4°C$ until processed in the afternoon of the slaughter day.

The carcasses were stored overnight in a $+4°C$ cold room. The chilled carcasses were anatomically dissected, and the following weights were recorded: breast muscle (pectoralis major), breast skin + subcutaneous fat, thigh + shank meat and bone, and the corresponding skin + subcutaneous fat. In addition, the body fattening index which is a rough approximation of total body fat was calculated according to Guy et al. (2013) as follows: abdominal fat + 2^*(breast skin and fat) + 2^*(thigh skin and fat).

Liver Processing

Among the livers collected, only those weighting over 400 g were processed on the afternoon of the slaughter day (core temperature ranged from 6 to 10°C depending on liver size). The main blood vessels were removed by careful trimming, and then each liver was transversally divided into 3 parts, each of which included the 2 lobes. The middle portion was adjusted to a weight of 200 g and placed in a glass jar for further processing. Processing was based on the standard method for whole "foie gras," which consists in the cooking of whole individual fatty livers. Salt (12 g/kg) and pepper (2 g/kg) were added, and the jars were cooked for 1 h in water in an autoclave ("Brouillon Process," Sainte Bazeille, France) at 85°C under a pressure of 0.8 bar to obtain a pasteurization value of 170. The water temperature was monitored, and 2 control jars contained temperature sensors. After 30-min chilling with circulating cold water, the jars were stored at 4°C until analysis.

Chemical and Biochemical Analyses

Liver samples were analyzed for dry matter, protein (total nitrogen), and lipid contents. The dry matter content was determined by oven drying at 105°C for 24 h to constant weight (JOCE, 1971). The nitrogen content was determined according to the Dumas combustion method using the Leco auto-analyzer (model FP-428, Leco Corp., St Joseph, MI) and converted to crude protein using a conversion factor of 6.5. Total lipids were extracted and measured gravimetrically according to Folch et al. (1957).

For the analysis of glycogen, glucose, and lactate, approximately 1 g of frozen liver tissue was homogenized in 10 mL of 0.5 M perchloric acid, and 0.5 mL aliquots of the homogenate were taken after removal of the fat cake, for the enzymatic determination of glycogen and glucose after glycogen hydrolysis with amyloglycosidase (Dalrymple and Hamm, 1973). The rest of the homogenate was centrifuged for 20 min at 2,500 g, and the supernatant was used to determine free glucose and lactic acid (Bergmeyer, 1974). The glycogen content was calculated as the difference between the results of the 2 sets of glucose determination. The results were expressed in $\mu mol/g$ of fat free liver.

Determination of Neutral Lipid Profiles

In the fattened liver of waterfowl, neutral lipids (or nonpolar lipids) are mainly composed of mono-, di-, and triglycerides, free fatty acids, cholesterol, and cholesterol esters (Fournier et al., 1997; Hermier et al., 2003). The separation and quantification of these chemical compounds was performed by gas chromatography (**GC**), according to the method described by Myher and Kuksis (1984), using a Hewlett Packard 5890 Series II chromatograph with an automatic injector (6890, Agilent Technologies, Santa Clara, California, USA). Samples of the chloroform fraction obtained by the method of Folch et al. (1957) were taken and disposed into a vial. The sample volume of the chloroform extract was adjusted according to the known lipid content: 20 μL for samples with a total lipid content greater than 0.08 g/10 mL of chloroform, 30 μL for those with concentrations between 0.07 and 0.08 g/10 mL of chloroform, and 40 μL for concentrations lower than 0.07 g/10 mL of chloroform. Twenty microliters of internal standard was added (Tri- Caprin, Tri C10, 50.5 mg/100 mL heptane) to each sample. After evaporating the chloroform using nitrogen gas (about 3 min), 100 μL of Tri-Sil BSA in pyridine (Thermo Scientific, Waltham, Massachusetts, USA, 49012) was added. The vial was closed with a Teflon cap (to prevent evaporation), vortexed, and placed in a desiccator, since the silylated derivatives are very unstable in moist environments. The separation was performed on a DBS- 15 m $\times$ 0.32 mm $\times$ 0.1 μm column (123–5011 Agilent Technologies). A volume of 1 μL of sample was injected on-column (injection temperature: 103°C). The oven temperature was programmed as follows: 100°C (3 min), then 30°C/min to 170°C, then 20°C/min to 250°C, then 8°C/min to 345°C until the end of the run. The following peaks were identified: free fatty acids (C16:0, C16:1, C18:0, C18:1, C18:2), monoglycerides (monoC16:0, monoC18:1), diglycerides (diC16:0, diC18:1), cholesterol, cholesterol esters (cholC16:0, cholC18:1), triglycerides (triC16:0, 1,3diC16:0–2, C18:1, 1,3diC18:1–2C16:0, triC18:1). All compounds were quantified using

the internal standard, and results were expressed as % of total neutral lipids.

Determination of Triglyceride Profiles

Triglycerides were separated using high-performance liquid chromatography (HPLC), according to Héron et al. (2007). The HPLC chain (Agilent Technologies) was used with an Evaporative Light-Scattering Detector (ELS-D 2100, Polymer Laboratories). Samples of the chloroform fraction obtained by the methods of Folch et al. (1957) were diluted according to their total lipid content and manually injected using a 20-μL injection valve. The flow rate was set to 1 mL/min, and the temperature of column was maintained at 15°C. A Phenomenex (Phenomenex, Le Pecq, France) 2.6 μm C18 100 Å, 150 × 4.6 mm (Kinetex) column was used with a binary acetonitrile-dichloromethane gradient to ensure adequate separation of the different lipid compounds. The detector nebulization and evaporation temperatures were respectively 60°C and 80°C; the gas flow and gain were set at 1.0 SLM (Standard Liter per Minute) and 2.1, respectively. Signal integration was carried out using specialized software (GC ChemStation, Agilent Technologies). The integrated signals were quantified using the calibration curves of different standard compounds. Based on previous data obtained for duck fatty liver in our laboratory (unpublished results), the following triglycerides were expected to be found and quantified: linoleyl 1, di-oleyl 2,3 glycerol (LOO), palmitoleyl 1, di-oleyl 2, 3 glycerol (PoOO), palmityl 1, oleyl 2, linoleyl 3, glycerol (POL), di-palmityl 1,2, linoleyl 3, glycerol (PPL), triolein (OOO), palmityl 1, di-oleyl 2,3, glycerol (POO), palmityl 1,3, oleyl 2, glycerol (POP), tripalmitine (PPP), stearyl 1, di-oleyl 2, 3, glycerol (SOO), palmityl 1, oleyl 2, stearyl 3, glycerol (POS), di-palmityl 1,2, stearyl 3, glycerol (PPS), palmityl 1, di-stearyl 2,3, glycerol (PSS)/palmityl 2, di-stearyl 1,3, glycerol (SPS). Data were expressed as % of the total triglyceride content. Five peaks could not be identified. They accounted for 5 to 10% of total triglycerides, depending on the sample considered, and were not included in data calculations.

Sensory Analysis and Acceptability Testing

The sensory and acceptability tests were conducted by the Agrotec Food Technology Resource Centre (Agen, France). The 3 following groups of livers, representative of both production systems and different weight classes, were assessed:

- Livers from the conventional system in the weight range 800 to 1,000 g (n = 13; 914 ± 45 g, mean ± SD), reflecting the standard weight objective under current production conditions in France.
- Livers from the alternative system in the weight range 800 to 1,000 g (only 9 livers fell in this range;

908 ± 55 g), to allow comparison with conventional livers of the same weight.
- Livers from the alternative system in the weight range 600 to 800 g (n = 13; 697 ± 49 g); this weight range is representative of the subpopulation of alternative livers that were retained for chemical analysis (mean liver weight 702 g in this subpopulation).

For the evaluation of the sensory profile by a trained expert panel, fatty livers were trimmed of all visible fat lost during cooking. They were cut into slices of the same thickness, covered with cling film, and stored at 4°C until the evaluation that occurred within 2 h after preparation. The samples were presented individually and in a different order between experts. Each expert scored the samples on a 10-point scale (0 to 10) for 19 sensory indicators related to the aspect (color intensity, color homogeneity, quantity of visible fat, yellow color of fat), the smell (global smell, alcohol smell; though that was not the case in the present study, alcohols may be used in fatty liver preparation), the texture (soft, melting, creamy, texture homogeneity), the taste (salty, bitter), and the flavor (global flavor, lean liver aroma, goose aroma, pepper aroma, alcohol aroma, foreign aroma, aroma persistence). For a given sample, the experts were not allowed to change a characteristic they had already evaluated.

A consumer panel of 69 members (43 women and 26 men) was asked to evaluate liver acceptability. Panel members were selected to be representative of the distribution of age classes and employment ranges in the French population. They were all regular consumers of fatty liver. The samples were prepared and served as described for the determination of sensory profile. The panel members were asked to first score the global acceptability and then the aspect, the smell, the texture and the taste, using a 10-point scale from 0 (very bad) to 10 (excellent).

Statistical Analyses

Because of the sampling method used to form the 2 experimental subsamples compared for carcass and liver characteristics, and the groups selected for sensory evaluation, the conditions required for the general linear model were not met. We therefore evaluated the effect of the production system using a non-parametric test. The NPAR1WAY procedure of SAS (SAS Institute Inc., 1987, Cary, North Carolina, USA) was used to calculate Wilcoxon scores (rank sums). Based on the score calculations, Kruskal–Wallis's test gave the level of probability of differences between production systems (with 2 modalities: conventional vs. alternative). In the case of sensory profiling and consumer acceptability data, 3 groups of livers were compared (conventional 800 to 1,000 g vs. alternative 600 to 800 g vs. alternative 800 to 1,000 g). The NPAR1WAY procedure was also used

Table 1. Geese liver weight and body characteristics in initial and subsampled populations in the conventional (force feeding) and the alternative (spontaneous fattening) production systems.

	Conventional system				Alternative system			
	Mean	SD	Min	Max	Mean[1]	SD	Min	Max
Liver weight (g)								
Initial population	1,102	208	694	1,533	445***	266	69	1,130
Subsampled population	1,064	222	694	1,510	702	201	410	1,130
Slaughter weight (g)								
Initial population	9,286	620	8,027	11,111	8242*	1,097	5,610	11,359
Subsampled population	9,254	600	8,140	10,433	8,666	1,079	6,290	11,359
Weight gain (g) [2]								
Initial population	2,604	291	1,735	3,241	1,886*	925	−795	5,081
Subsampled population	2,599	259	1,888	3,160	2,211	890	88	5,081
Abdominal fat (g)								
Initial population	530	82	352	777	577*	163	153	1,010
Subsampled population	524	81	352	693	639	155	316	1,010
Body fattening index (g)[3]								
Initial population	1,630	207	1,207	2,325	1,573**	378	551	2,664
Subsampled population	1,625	202	1,207	2,083	1,731	355	904	2,664

[1]For a given phenotype, differences between initial and subsampled populations were tested using Student's t-test and the results reported as: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

[2]Weight gain during force feeding (conventional system) or during stimulation for spontaneous liver fattening (alternative system).

[3]The body fattening index is an approximation of total body fat calculated as: abdominal fat + 2*(breast skin and fat) + 2*(thigh skin and fat).

to evaluate the effect of the group (with 3 modalities) and, when appropriate, Duncan's multiple range test was used to compare the means.

RESULTS AND DISCUSSION

Main Body Characteristics in the Initial Populations

Conventional System The liver weight in the initial population from the conventional system was higher (mean weight > 1 kg; Table 1) than that usually reported in literature (between 600 and 960 g; Salichon et al., 1994; Davail et al., 2000; Mourot et al., 2006; Su et al., 2009; Arroyo et al., 2017). However, a direct comparison of different studies is difficult because fatty liver weight varies greatly depending on the pattern of corn delivery and environmental conditions during overfeeding. Interestingly, the amount of abdominal fat was greater in the present study compared with that observed in the above-cited studies (530 g vs. 400 to 493 g, respectively), suggesting enhanced overall fattening. Nevertheless, this observation did not hold true when comparing the weight gain during overfeeding (2,604 g) which fell in the same range as for the previously mentioned studies (2,400 to 2,700 g).

Alternative System The average liver weight in the initial population from the alternative system was slightly lower than that reported in our previous studies in which geese were reared in the same conditions and following the same procedure (514 and 497 g as reported by Guy et al., 2013 and Fernandez et al., 2016, respectively, vs. 445 g in the present study). Both the abdominal fat weight and body fattening index were however very close to those recorded by Guy et al. (2013): 577 g in the present study vs. 596 g in Guy et al. (2013) for

abdominal fat, and 1,573 g vs. 1,567 g for the body fattening index. The mean weight gain during the 12 wk of corn consumption was 1,886 g. This result was similar to our previous findings (1,845 g; Fernandez et al., 2016), even though the live weight at slaughter was higher in the present work (8,666 g vs. 7,862 g in Fernandez et al., 2016). This observation suggests that the weight gain during the fattening period in the alternative production system is not dependent upon the live weight at the start of the period. This was confirmed by the calculation of the correlation coefficient between the live weights at the beginning and at the end of the fattening period which was close to zero in the present work (r = 0.07). Overall, it can be stated that there were no marked differences in the performances under the alternative system as compared with previous experiments carried out under the same conditions.

Comparisons of Initial and Subsampled Experimental Populations

The determination of liver characteristics was conducted on 2 groups of experimental animals that were subsampled from the initial populations raised using the conventional (44 geese selected among the 85 slaughtered) and the alternative production systems (42 geese selected among the 125 slaughtered). The criteria used for subsampling was the liver weight. Figure 2 shows liver weight distribution for the initial and subsampled populations for each production system. For birds from the conventional production system, the liver weight distribution of the selected subsample was representative of the initial population (Figure 2A). None of the livers in this group weighted less than 600 g. Moreover, for the conventional production system, there were no significant differences for carcass traits between the
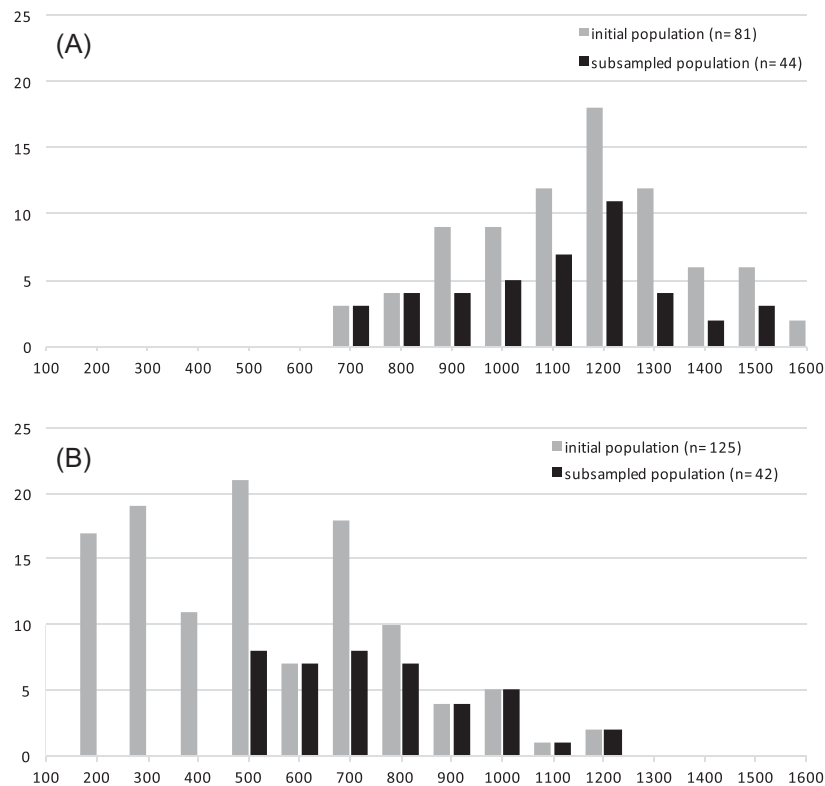
Figure 2. Liver weight distribution in the initial and subsampled populations from the conventional (A) or the alternative (B) system (graduations on the x axis show the upper value of the 100-g weight class; y axis shows the number of livers in each weight class).

initial and subsampled population (Table 1). For the alternative production system, we purposely chose to select only livers weighting over 400 g (Figure 2B). The main reason for this choice is that in France, geese livers weighting less than 400 g are not considered as "foie gras" and cannot be commercialized as such. In addition, we tried to include in the subsampled population the same number of livers from each weight class. The only exception being the "heaviest classes," each containing only a few livers that were all selected. Therefore, the subsampled population from the alternative system significantly differed from the initial population in regard to main body traits, as shown in Table 1. Average liver weight and slaughter weight were significantly higher in the subpopulation (702 g and 8,666 g, respectively) compared with the initial population (445 g and 8,242 g, respectively). This result is consistent with the higher weight gain observed during the fattening procedure (Table 1). Body fattening indicators (abdominal fat and body fattening index) were also significantly higher in the subsample than in the initial population from the alternative system.

Comparison of Body Traits and Liver Characteristics

BW at slaughter was higher in the conventional system (9,254 g vs. 8,666 g for the alternative system;

Table 2). This was mainly due to a higher weight gain during overfeeding compared with spontaneous fattening (2,599 g vs. 2,211 g) since the BWs before fattening were very similar in both groups (6,655 g and 6,455 g for the conventional and alternative production systems, respectively; data not shown). The accumulation of abdominal fat was significantly higher following spontaneous fattening than with overfeeding (Table 2), suggesting greater peripheral fat deposits in geese from the alternative system. However, this was not confirmed by skin and fat weight measurements at the 2 anatomical locations; indeed, the skin and fat weight tended to be higher for birds from the alternative system at the breast level ($P = 0.08$), but in contrast, the level of thigh subcutaneous fat was significantly higher for birds from the conventional system. Overall, there was no difference in body fattening index between the 2 production systems, suggesting that the overall body fattening did not differ. The differences in weight gain between the 2 groups are mainly due to differences in liver weight (1,063 g vs. 702 g in the conventional and alternative systems, respectively). It is worth noting that this difference remained significant despite the selection of livers above 400 g in the subpopulation from the alternative system. Overfeeding therefore remains far more efficient than spontaneous fattening for the production of fatty liver.

There were significant differences in liver color between the 2 production systems (Table 2). The

Table 2. Geese production traits and liver characteristics in the conventional (force feeding) and the alternative (spontaneous fattening) production systems.

	Conventional system (n = 44)	Alternative system (n = 42)	SEM	P^1
BW at slaughter (g)	9,254	8,666	100	**
BW gain during fattening (g)[2]	2,599	2,211	74	***
Carcass weight (g)	5,852	5,808	60	ns
Abdominal fat (g)	524.0	639.2	14,9	***
Breast muscle	274.9	288.9	4.0	$P = 0.08$
Breast skin and fat	226.4	242.4	4.6	$P = 0.09$
Thigh muscle and bones	420.0	403.6	4.4	ns
Thigh skin and fat	330.1	303.7	6.3	*
Body fattening index[3]	1,625	1,731	32	ns
Liver weight (g)	1,063	702	31	***
Liver color				
L*	69.3	58.6	0.7	***
a*	7.8	7.4	0.2	ns
b*	28.5	31.7	0.4	***
Liver composition:				
Dry matter (%)	67.2	63.9	0.4	***
Lipids (%)	56.6	53.2	0.5	***
Proteins (%)	6.1	6.5	0.1	ns
Glucose (μmol/g fat free liver)	35.2	44.8	1.1	***
Lactate (μmol/g fat free liver)	36.1	34.2	0.7	ns
Glycogen (μmol/g fat free liver)	69.7	102.5	6.4	**

[1]Level of probability of the effects of the production system: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, $P > 0.10$.
[2]Weight gain during force feeding (conventional system) or stimulation for spontaneous liver fattening (alternative system).
[3]The body fattening index is an approximation of total body fat calculated as: abdominal fat + 2*(breast skin and fat) + 2*(thigh skin and fat).

luminance was significantly higher in livers from the conventional system, probably due to a higher level of fattening than in the alternative system (56.6 vs. 53.2% lipids, respectively; $P < 0.001$). Conversely, the yellow index (b*) was significantly higher in livers from the alternative system. The differences in the overall amount of corn consumed during the fattening process (14 vs. 28 kg, for the conventional and alternative systems, respectively) probably induced differences in the levels of pigments such as carotenoids that accumulate in liver lipids. In a previous report, we clearly showed that the increase in liver weight during the spontaneous fattening process was associated with a progressive rise in liver yellowness, as measured by the b* coordinate (Guy et al., 2013).

The concentrations of glycogen and glucose in livers obtained with the alternative system were higher than those obtained with the conventional system, whereas lactate contents did not differ significantly. In the alternative system, feed was available until the animals were collected for slaughter, whereas in the conventional system the last meal was delivered the evening before slaughter. Leprettre et al. (1997) showed that liver glycogen concentrations in overfed geese decrease very quickly as the time elapsing from the last meal increases. This could explain the difference in liver glycogen content observed between the 2 production systems.

Lipid Composition of the Livers

During overfeeding, the quantity of hepatic lipids is known to increase drastically (i.e., from 5% to more than 50% of the liver's weight). Several reports have previously shown that triglycerides represent more than 95% of all lipids accumulated during the process of liver steatosis in geese (Blum et al. 1992; Salichon et al., 1994; Fournier et al., 1997) and ducks (Hermier et al., 2003). Cholesterol esters usually range from 1 to 2% and free cholesterol from 0.5 to 1%. The figures we report here for livers obtained using overfeeding (conventional system) are consistent with the previous data in the literature (Table 3). They also confirm that monoglycerides are present only at very low levels and that free fatty acids represent less than 1% of total lipids, as previously shown in Greylag goose liver by Salichon et al. (1994) and Fournier et al. (1997). The neutral lipid profile of livers obtained with the alternative system differed significantly from that of the conventional livers: they contained significantly less triglycerides and free fatty acids, although the differences were of low magnitude (–0.4 and –0.12 points, respectively), and significantly more cholesterol and cholesterol esters (+0.1 and +0.5 points, respectively). The differences in triglycerides and cholesterol contents between the production systems are probably due to the degree of liver steatosis, because the production system effect was not significant when covariance analysis was performed including liver weight as a covariate (data not shown). The effects observed for free fatty acids, diglycerides, and cholesterol esters content are not due to differences in liver weight. However, the magnitude of these differences is very small and probably not of biological significance.

As shown in Figure 3, the 3 main triglycerides observed in livers obtained by overfeeding contain at least 1 monounsaturated fatty acid (oleic acid, C18:1). In addition, we found that triolein (OOO) and palmityl

Table 3. Composition of the neutral fraction of liver lipids (data are given as % of total neutral lipids) in the conventional (force feeding) and the alternative (spontaneous fattening) production systems.

	Conventional system (n = 44)	Alternative system (n = 42)	SEM	P[1]
Free fatty acids	0.42	0.30	0.02	***
Monoglycerides	0.004	0	0.002	ns
Diglycerides	1.54	1.37	0.04	***
Triglycerides	96.0	95.6	0.07	**
Cholesterol	0.45	0.54	0.01	***
Cholesterol esters	1.73	2.20	0.07	***

[1]Level of probability of the Wilcoxon test for the effects of the production system: ***, $P < 0.001$; **, $P < 0.01$; ns, $P > 0.10$.
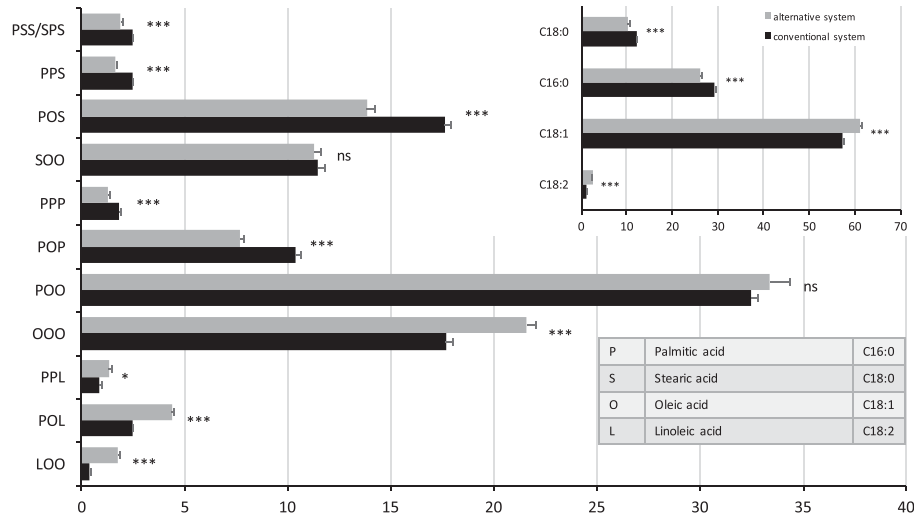

Figure 3. Triglyceride profiles (main figure) and fatty acid composition (inlaid figure) of hepatic lipids in the conventional (force feeding) and the alternative (spontaneous fattening) production systems. Data are expressed as % of total triglycerides or % of triglyceride fatty acid chains (horizontal bars show the standard error of the mean; the effect of production system is reported as ***, $P < 0.001$; *, $P < 0.05$; ns, $P > 0.10$).

1, di-oleyl 2, 3, glycerol (POO) represent 50% of total triglycerides. The calculated proportion of the C18:1 oleic acid was close to 60% of total fatty acids, whereas the proportion of polyunsaturated fatty acid (C18:2 linoleic acid) remained below 3%, thus confirming previous observations on goose fatty liver after overfeeding (Salichon et al., 1994; Fournier et al., 1997).

The proportions of triglycerides containing linoleic acid (PPL, POL, and LOO) were significantly higher in livers from the alternative system (Figure 3). The livers obtained by spontaneous fattening also contained a significantly higher proportion of triolein (OOO), while the proportion of POO and SOO did not differ significantly between the 2 groups. Consequently, the proportion of mono- and polyunsaturated fatty acids was significantly higher in livers from the alternative group, which in turn resulted in a significantly lower proportion of saturated fatty acids. These differences did not seem to depend on differences in liver weight since they remained significant when a model of covariance analysis was used with liver weight as a covariate (data not shown).

Hepatic steatosis results from a large increase in de novo lipogenesis from the carbohydrates ingested by the animal. In ducks, 75% of the glucose ingested is converted into fatty acids and stored in hepatocytes (Evans, 1972). Palmitic acid (C16:0), the main lipid produced by de novo lipogenesis, is the starting point for the synthesis of the various fatty acids, including mono- and polyunsaturated fatty acids, that are incorporated in triglycerides, together with the fatty acids ingested directly in the food. Various types of elongating and desaturating enzymes are involved in the synthesis of liver fatty acids. In the present study, geese received the same type of food (corn) during the fattening period in both production systems. The differences observed in the saturation level of the fatty acids that form the component chains of triglycerides are most likely due to different regulation patterns of the enzymes involved in de novo fatty acids synthesis. It would be of interest to compare the metabolism of fatty acids in liver steatosis obtained by overfeeding and spontaneous fattening.

Sensory Characteristics and Consumer Acceptability of Cooked Livers

The sensory profiles of each of the 3 groups of livers assessed are shown in Figure 4. Among the 19 indicators evaluated, 9 differed significantly, 8 did not differ
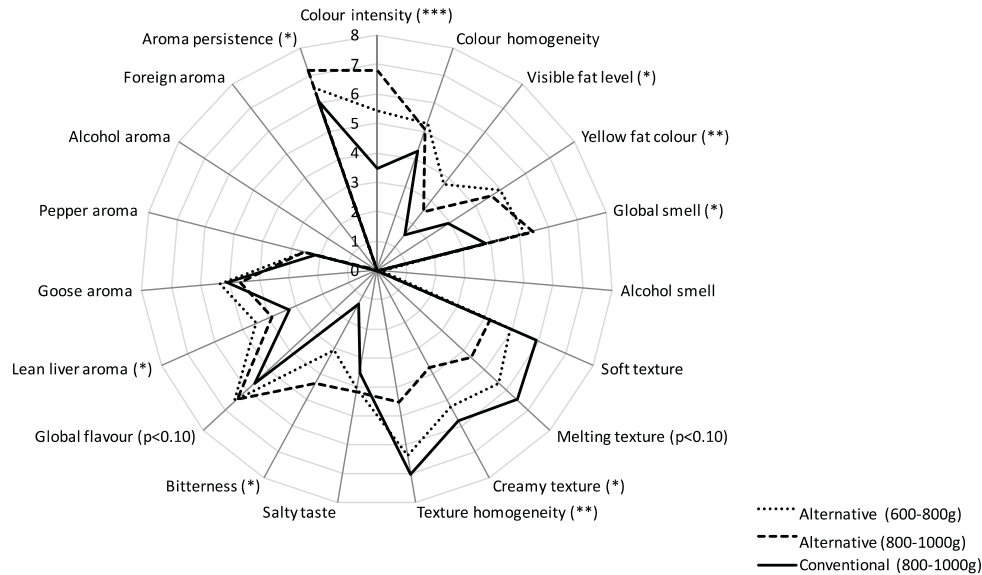
Figure 4. Sensory profile of livers according to weight range in the conventional (force feeding) and the alternative (spontaneous fattening) production systems. Each of the sensory descriptors is scored on a 10-point scale (the result of the Wilcoxon test for the group effect is reported between brackets for each sensory descriptor as: ***, $P < 0.001$; **, $P < 0.01$; *, $P > 0.05$; ns, $P > 0.10$).

significantly, and 2 tended ($P < 0.10$) to differ between the groups. The intensity of color, the level of visible fat, and the yellow color of the fat were significantly lower in livers from the conventional system than in the 2 other groups. There was no difference for these traits between the 2 liver weight groups from the alternative production system (results from multiple mean comparisons, data not shown). The level of visible fat was similar between the 2 groups of the weight range 800 to 1,000 g from both production systems. Color homogeneity did not differ between the 3 groups. It is interesting to see that the score for visible fat was the highest in the alternative 600 to 800 g group, for which the lowest levels of liver fat were measured. External visible fat was trimmed of the livers so this score probably reflects the presence of fat inclusions within the slice. As previously observed with the effect on the b* trichromatic coordinate, the intensity of the yellow color is higher in livers from the alternative group, probably due to the accumulation of carotenoid pigments in the liver lipids. The higher intensity of the yellow colored fat also probably explains why overall color intensity is higher in livers from the alternative system. The global smell intensity was significantly higher in livers from the alternative system and, at the present time, we have no explanation for this observation. Concerning the texture, significantly higher scores were recorded in livers from the conventional system for the melting and creamy texture, as well as for texture homogeneity. The alternative 600 to 800 g group was intermediate between the two 800 to 1,000 g groups in regard to creamy texture (it did not differ significantly from the 2 other groups) and texture homogeneity (it differed significantly from each of the 2 other groups). Differences in fat levels between the conventional and alternative livers could perhaps explain the effects observed on texture. How-

ever, the position of the alternative 600 to 800 g group is not consistent with that because this group scored better for texture indicators than the alternative 800 to 1,000 g group. The perception of bitterness was significantly higher in livers from the alternative groups. Global flavor and lean liver aroma were significantly lower in livers from the conventional group. The highest score was observed for the alternative 600 to 800 g group, the alternative 800 to 1,000 g being intermediate (it did not differ significantly from the 2 other groups). Aroma persistence was significantly higher in livers from the alternative 800 to 1,000 g group compared with those from the 2 other groups that show similar scores for this indicator.

In summary, the livers obtained with spontaneous fattening differed from those obtained with overfeeding by higher scores for the indicators related to the aspect, lower scores for the indicators related to texture, and higher scores for bitterness and flavor intensity. This sensory profile helped to characterize each group of livers with a relative large number of indicators but it did not give any indications on the hedonic perception of the products. This was evaluated using a consumer acceptability test, and the results are shown in Figure 5. Livers from the conventional system were clearly preferred than those from the alternative system, as shown by the global score of acceptability (6.83 for the conventional system vs. 5.77 and 5.59 for the alternative livers of the 600 to 800 g and 800 to 1,000 g groups, respectively). This result reflects the better scores obtained by the livers from the conventional group for the 4 descriptors (texture, aspect, taste, and smell). There was no statistical difference between the 2 alternative groups for any of the descriptors. These results clearly show that the quality of livers obtained by spontaneous fattening is lower than that of livers obtained by
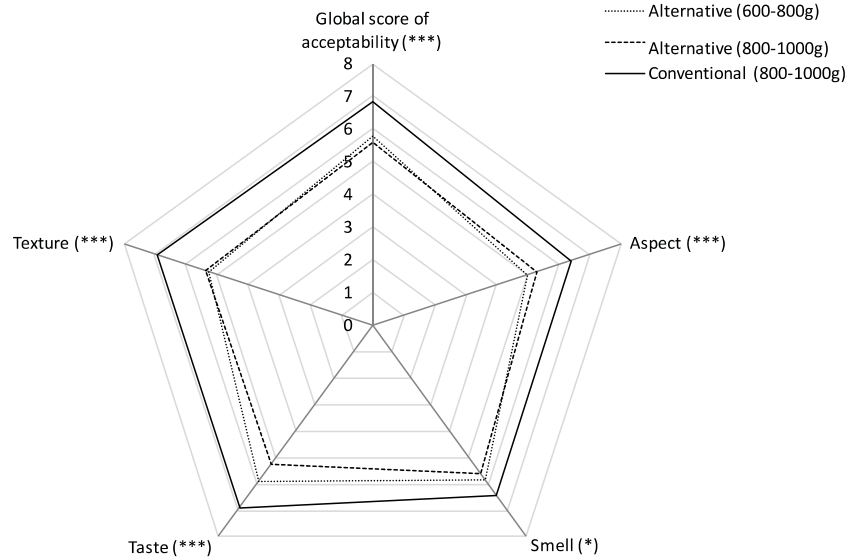
Figure 5. Consumer evaluation of livers according to weight range in the conventional (force feeding) and the alternative (spontaneous fattening) production systems. Each evaluated trait is scored on a 10-point scale (the result of the Wilcoxon test for the group effect is reported between brackets for each trait as: [***], $P < 0.001$; [*], $P > 0.05$).

overfeeding, as perceived by the consumers. This difference is not related to liver weight and/or lipid content since the two 800 to 1,000 g groups show clear-cut differences for the hedonic scores.

CONCLUSIONS

This work is the first to compare the characteristics of livers obtained with the conventional production system, based on overfeeding, and an alternative production system based on spontaneous fattening, the latter being still under development. Overfeeding is far more efficient than spontaneous fattening to induce extended liver steatosis. Although significant differences in the levels of some chemical compounds were recorded (i.e., triglycerides and fatty acid composition in particular), there were no drastic differences in the chemical characteristics of the 2 types of liver. However, the sensory profiles were clearly different and the consumers considerably less appreciated the livers obtained with the alternative system compared with those obtained by overfeeding. The alternative production system is still under development, and future innovations in animal management aimed at optimizing production performances will have to take into account the impact on liver sensorial quality.

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