Détection de QTL dans le protocole de canard gras

1 Introduction

Les résultats du premier dispositif expérimental mis en place pour la recherche de QTL impliqués dans la variabilité des caractères d'intérêt économique ou biologique chez le canard gras, sont présentés dans ce chapitre. Ce dispositif consiste en 7 familles informatives de type Backcross, obtenues en croisant 2 souches expérimentales, la souche INRA444 (canard Kaiya, résultat d'un croisement original Tsaiya x Pekin) et la souche synthétique de Pékin lourd (INRA37), elle-même issue d'un croisement original de 3 souches lourdes européennes.

Le choix de ce croisement est motivé par les résultats de Rouvier *et al*. (1994) qui ont montré que le poids corporel et le poids de foie des mulards issus des mères Pékin et Tsaiya et ceux issus de deux croisements réciproques sont significativement différents. Ces auteurs concluent que l'utilisation des mères résultant d'un croisement entre Tsaiya et Pékin pour la production des mulards permettent d'obtenir un foie gras de poids raisonnable et de meilleure qualité.

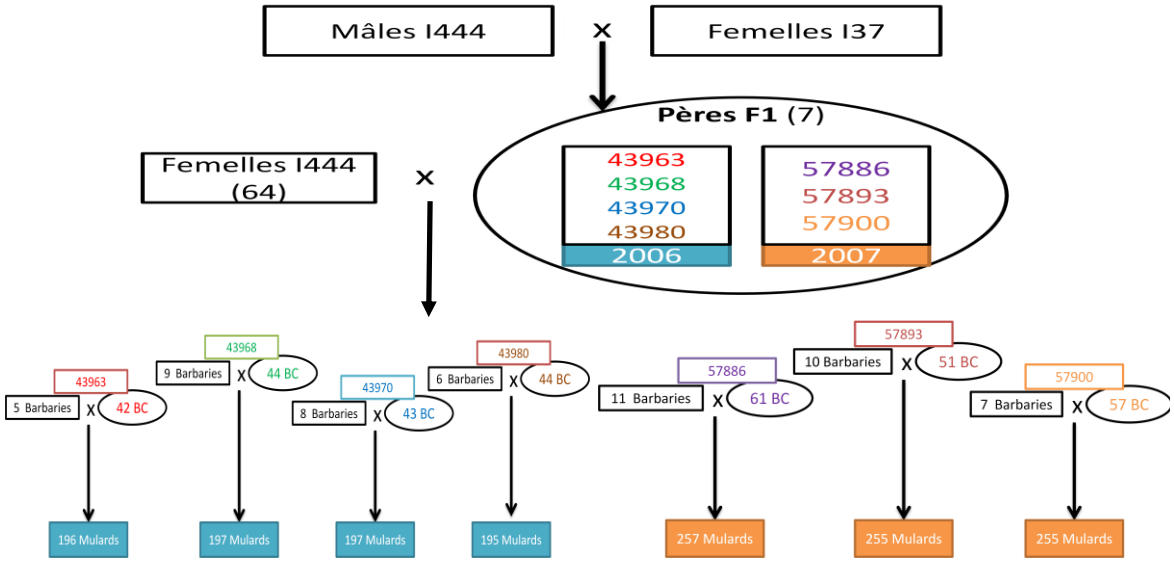
L'utilisation d'un protocole backcross est motivée, en plus d'un certain nombre de contraintes techniques qui ont exclu la mise en place d'un protocole F2, par un effectif nécessaire moins important lorsque les allèles au QTL sont l'un dominant, l'autre récessif. En effet, dans ce cas, le protocole backcross est plus puissant que le protocole F2 pour la détection de QTL s'il est réalisé sur le parent homozygote récessif (Darvasi, 1998), puisque le nombre de descendants nécessaire pour estimer la dominance est alors deux fois moins important que dans un protocole F2. Par contre, lorsque les allèles au QTL sont co-dominants, l'estimation des effets additifs dans un protocole F2 nécessite 30% moins de descendants par rapport à un protocole backcross.

Une large gamme de caractères originaux a été mesurée sur les 1600 canards mulards produits en croisant les femelles Backcross avec des mâles Barbaries. En effet, la plupart de ces caractères (notamment ceux relatifs au gavage) ne sont pas mesurables sur les canes communes Backcross : les mesures sont effectuées sur les mulards plutôt que sur les canes Backcross, ce qui n'est pas classiquement réalisé dans ce type de protocole.

2 Le dispositif détaillé

Le dispositif a été procréé sur 2 années consécutives, chaque année produisant spécifiquement des familles. Ainsi 4 familles de 43 canes BC ont été produites la 1ère année, et 3 familles de 57 canes la seconde année. Annuellement, les mulards issus de ces canes BC étaient produits en 2 lots d'éclosion successifs, chacun d'eux redivisé en 2 lots de gavage.

Figure 4.1 : Dispositif de détection de QTL



Dans notre schéma expérimental (figure 4.1) de production des mulards, différents effets fixés sont identifiés:

- les mulards sont issus de 2 lots d'éclosion avec comme objectif de répartir, pour chaque famille de père F1, la moitié des mulards est issue d'une première éclosion et l'autre moitié d'une deuxième éclosion.
- les mulards sont ensuite élevés (de 0 à 12 semaines) chaque année en 2 fois 8 lots d'élevage d'une cinquantaine de canards.
- Trois gaveurs ont réalisés le gavage des 1600 mulards, 2 gaveurs (G1A et G1B) la première année et 2 gaveurs (G2A et G2C) la deuxième année ; un gaveur (GA) étant commun aux 2 années. Les descendants d'une famille de père sont gavés pour moitié par un gaveur et pour moitié par un autre.

3 Les mesures des caractères

Les caractères enregistrés sur chacun des 1600 mulards peuvent être classés en 4 grands groupes. Il s'agit de caractères de croissance, de caractères liés au métabolisme pendant le gavage, de caractères d'aptitude au gavage et de caractères relatifs aux qualités des produits (foie gras et magret). De plus, quelques aspects comportementaux ont été enregistrés : en élevage, le taux de corticostérone basal et après un stress (pendaison par les pattes, tête en bas) a été dosé et en gavage, le comportement des mulards (facilité de gavage et d'attrapage) a été apprécié.

Pour l'ensemble des caractères, l'analyse de variance qui prend en compte les effets fixes précédemment identifiés (lot d'éclosion, lot d'élevage, gaveur) et la famille de père F1, met en évidence un effet très significatif de la famille du père F1. Les valeurs moyennes des caractères étudiés ainsi que les différences par famille sont présentées ci-après.

Caractères de croissance

Les caractères de croissance (tableau 4.1 ; figure 4.2) ont été mesurés par pesée des animaux à jeun, à différents âges (à 12, 28, 42 et 70 jours d'âge). La balance utilisée est une balance Balea (AGPA - Automate graphique de pesée animale), qui permet l'acquisition d'un poids fiabilisé : en effet, le poids retenu est une moyenne de N poids d'un même animal obtenu en quelques secondes, et dont l'écart-type est inférieur à un seuil S (N et S étant paramétrables). Si un animal bouge ou est dérangé par un autre individu, la prise de mesure ne sera pas faite : la pesée sera poursuivie jusqu'à ce que les conditions de précision soient remplies.

Pour ces différents âges, les poids moyens des animaux sont conformes aux poids de mulards de Kaiya habituellement rencontrés (tableau 4.1) : néanmoins la variabilité de ces poids (coefficient de variation de l'ordre de 20% à 12 jours) est plutôt élevée, surtout à 12 jours d'âge.

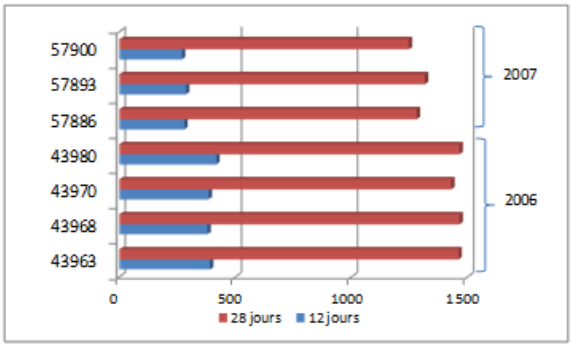
Tableau 4.1 : Descriptions des caractères de croissances.

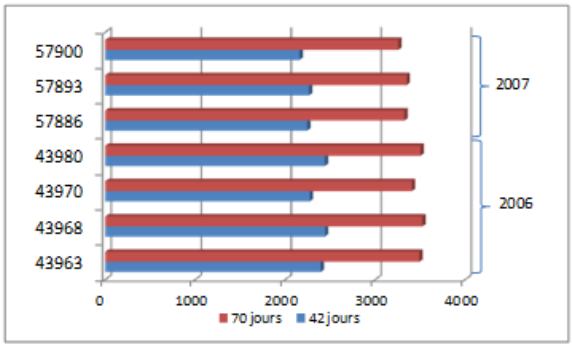
	N	Moyenne	Ecart-type
BW12[1] (g)	1551	337	69
BW28[1] (g)	1549	1373	150
BW42[1] (g)	1545	2306	206
BW70[1] (g)	1508	3396	288

[1] BW12, BW28, BW42, BW70 : poids corporel à 12, 28, 42 et 70 jours.

Au-delà de la variation entre père, un effet « année » (figure 4.2) apparait pour les poids corporels, principalement dans le jeune âge (à 12 et 28 jours d'âge). En effet les mulards nés en 2006 (A2006f1 à A2006f4) ont en moyenne un poids plus élevé que ceux nés en 2007 (A2007f5 à A2007f7). Par contre, les gains de poids moyens sont sensiblement identiques quelles que soient les familles de pères F1.

Figure 4.2 : Poids moyens par famille de père F1
(à 12 et 28 jours à gauche) (à 42 et 70 jours à droite)





Les caractères relatifs au métabolisme

Au début (deuxième repas), au milieu (dixième repas) et à la fin (vingtième repas) de la période du gavage, des échantillons de cinq millilitres de sang ont été collectés pour mesurer le taux de métabolites plasmatiques afin apprécier l'évolution de la stéatose hépatique durant la phase de gavage. Ces collectes, réalisées trois heures après les repas ont permis de déterminer la concentration en glucose, triglycérides et cholestérol dans le plasma sanguin (tableau 4.2 ; figure 4.3), en utilisant des kits spécifiques (tubes EDTA ou acide éthylène diamine tétraacétique).

Deux tubes de sang par canard sont prélevés, l'un des 2 tubes étant centrifugé à 3000 tours/min puis le plasma est aliquoté en 3 petits tubes. L'autre tube est conservé en l'état comme sécurité.

Quel que soit le caractère étudié (triglycérides, cholestérol, glucose), on observe une augmentation des valeurs de dosages sanguins avec l'avancée du gavage (tableau 4.2). De plus, la variabilité des mesures s'accroit aussi de façon importante avec le stade de gavage.

Tableau 4.2 : Descriptions des caractères liés au métabolisme.

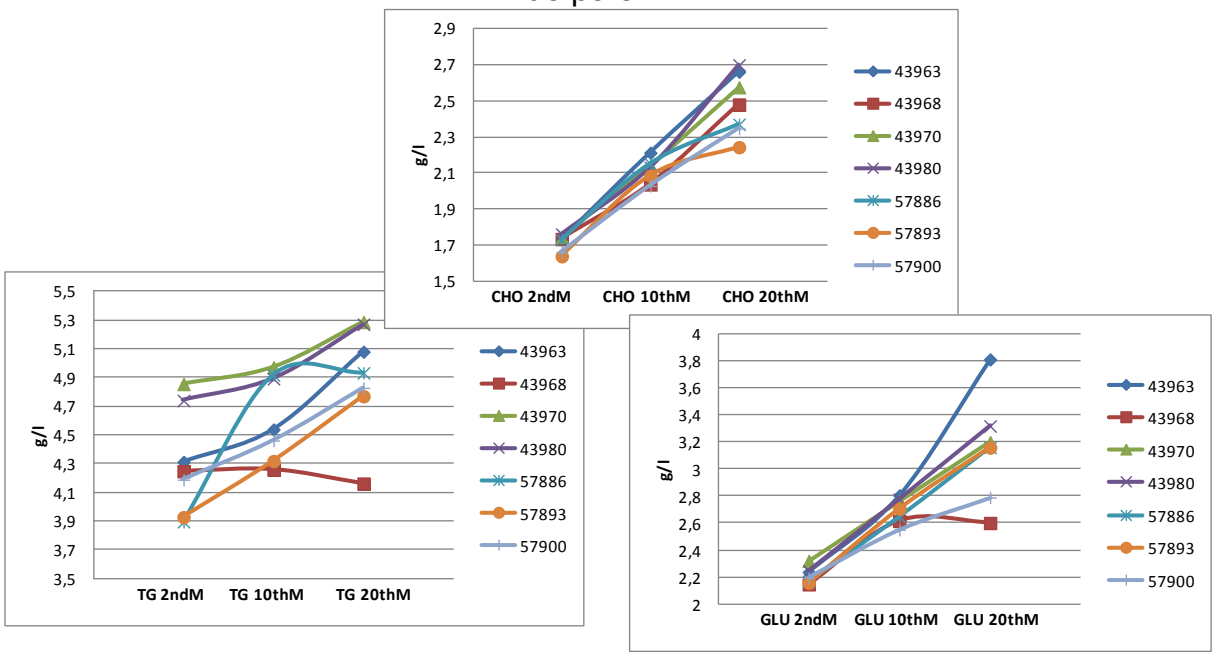
	N	Moyenne	Ecart-type
TG $2^{nd}M^1$ (g/l)	1499	4,27	1,00
TG $10^{th}M^1$ (g/l)	1498	4,61	1,08
TG $20^{th}M^1$ (g/l)	1443	4,89	1,52
CHO $2^{nd}M^1$ (g/l)	1501	1,71	0,25
CHO $10^{th}M^1$ (g/l)	1499	2,11	0,32
CHO $20^{th}M^1$ (g/l)	1433	2,46	0,47
GLU $2^{nd}M^1$ (g/l)	1500	2,20	0,27
GLU $10^{th}M^1$ (g/l)	1498	2,69	0,56
GLU $20^{th}M^1$ (g/l)	1451	3,13	1,08

[1] TG, CHO et GLU : taux de Triglycéride, de Cholestérol et de Glucose dans le sang au 2^{nd}, $10^{ème}$ et $20^{ème}$ repas.

Les taux de cholestérol sont assez comparables pour les différentes familles de père (figure 4.3), ainsi que leur évolution entre les 3 stades de mesures. Pour les taux de triglycérides, on notera toutefois 2 familles atypiques : la famille du père 57886 dont le taux de triglycérides était très élevé en milieu de gavage mais est resté stable ensuite, et la famille du père 43968 qui est la seule dont le taux du triglycéride a légèrement décru avec l'avancée du gavage. Pour les taux de glucose, la variabilité

entre les familles de pères apparait particulièrement en fin de gavage, avec une disparité dans les évolutions de concentration selon les familles : en effet, le taux de glucose entre le 10ème et le 20ème repas augmente très fortement pour le père 43963 alors qu'il reste constant pour le père 43968.

Figure 4.3 : Taux de Triglycéride, de Cholestérol et de Glucose sanguins par famille de père F1



Les caractères de taux de corticostérone avant et après stress

Pour mesurer la réponse au stress des animaux, un test de pendaison est organisé. L'animal est suspendu pendant 10 minutes par les pattes, la tête vers le bas. Des prélèvements sanguins sont effectués avant et après le test pour estimer les niveaux de corticostérone et évaluer la réponse de l'axe hypothalamo-hypophyso-surrénalien (tableau 4.3 ; figure 4.4).

Le niveau de corticostérone s'élève très fortement avec le stress, puisqu'il passe en moyenne de 10 ng/ml à 55 ng/ml (tableau 4.3). Néanmoins, la variabilité des mesures était plus importante avant le stress (coefficient de variation de 107%) qu'après le stress (coefficient de variation de 77%).

Tableau 4.3 : Descriptions des caractères liés au stress

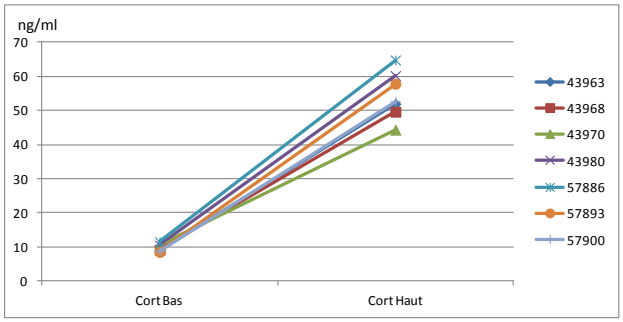
	N	Moyenne	Ecart-type
Cort Bas[1] (ng/ml)	1502	9,66	10,32
Cort Haut[1] (ng/ml)	1502	54,97	42,47
Delta Cort[1] (ng/ml)	1502	45,31	39,37

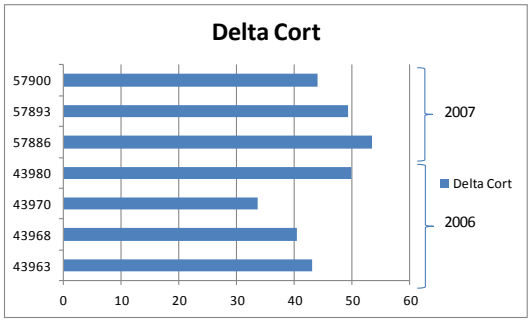
[1] CortL, CortH, DeltaC : Taux de Corticostérone basale, après stress et delta de corticostérone.

L'évolution de corticostérone avec le stress ne semble pas être affectée par un effet année (figure 4.4). Néanmoins, on peut remarquer que la famille du père 43970 présente une moindre augmentation de son niveau de corticostérone comparé

aux autres familles. Hormis ce père, le classement des familles selon le niveau de corticostérone avant ou après stress reste le même.

Figure 4.4 : Taux de corticostérones par famille de père F1





Les caractères d'aptitude au gavage

L'aptitude au gavage des canards a été mesurée par pesée des animaux en début et fin de la période de gavage, et des différentes pièces de la carcasse (le foie gras, les cuisses, le gras abdominal ou le magret qui correspond aux muscles pectoraux, sa peau et la graisse sous-cutanée qui le couvre). Aussi le poids de la carcasse ressuée (poids plumé, saigné, sans pattes et bout d'ailes) a été enregistré. La consommation journalière de maïs a également été enregistrée pour chaque animal durant le gavage : la dose-repas étant identique pour tous les animaux, sont enregistrés les repas sautés ou les demi-doses données (tableau 4.4 ; figure 4.5).

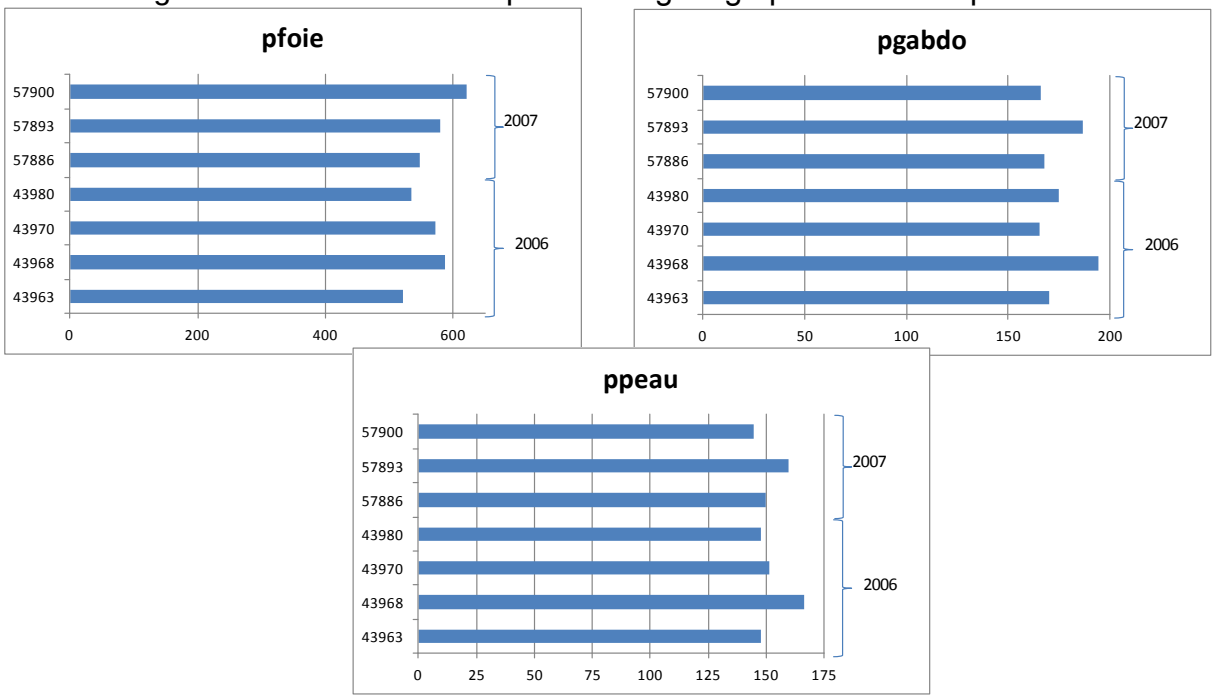
Tableau 4.4 : Description des caractères d'aptitude au gavage.

	N	Moyenne	Std
DFI[1] (g/d)	1498	1325	74
BWbeg[1] (g)	1501	3830	300
BWend[1] (g)	1498	5805	378
OWG[1] (g/d)	1498	1974	247
CW[1] (g)	1474	4902	329
pfoie[1] (g)	1492	567,9	115,3
pgabdo[1] (g)	1476	175,0	28,8
pmMW[1] (g)	1476	256,5	23,6
ppeau[1] (g)	1476	152,4	20,3
TSW[1] (g)	1476	481,9	45,1

[1] DFI, BWbeg, BWend, OWG, CW, FLW, AFW, pmMW, pmSFW, TSW : consommation moyenne journalière, poids du début et fin gavage, gain de poids durant le gavage, poids de la carcasse, du foie, du gras abdominal, du magret, de la peau et de cuisse.

Si aucun effet « année de mesure » n'apparait pour les caractères d'aptitude au gavage, l'effet famille de pères F1 est particulièrement significatif pour 3 caractères : le poids de foie gras, le gras intra-abdominal et le poids de la peau du magret (figure 4.5). Parmi ces caractères en lien direct avec l'engraissement des animaux, les familles de père semblent se comporter de la même façon pour le gras abdominal et pour le poids de peau du magret, mais pas pour le poids de foie gras : ainsi les familles 57893 et 43968 ont un engraissement sous-cutané ou abdominal très développé alors que la famille avec le poids de foie le plus élevé est la 57900.

Figure 4.5 : Caractères d'aptitude au gavage par famille de père F1



En plus des caractères décrits ci-haut, des observations comportementales en gavage ont été effectuées par les gaveurs. La facilité d'attrapage et de gavage sont notées pour chaque canard mulard, la note est attribuée par le gaveur sur une échelle de 1 à 4.

Les caractères de qualité de produits

La qualité du foie gras (tableau 4.5 ; figure 4.6) et du magret (tableau 4.6 ; figure 4.7) a été appréhendée par des mesures de compositions et des mesures de propriétés technologiques.

Ainsi le taux de fonte du foie (pourcentage de perte de graisses après stérilisation de 60g d'un foie pendant 50 min à 105° C) et les pertes à la cuisson du muscle (15 min dans une eau bouillante de 85° C) sont mesurés. Pour le magret le pH à 20 minutes et 24 heures *post-mortem* sont mesurés. Le taux de lipides et de protéines ont été estimés par spectrophotomètre avec la technique *near infra red* (FOSS NIRSystem) sur des échantillons du foie gras. Le taux de lipides du magret est aussi estimé par la même technique. Pour le foie, le taux de collagène a été déterminé selon Woessner (1961). Les indices L*, a* et b* de coloration pour le foie gras et le magret sont mesurés par un chromamètre (CR 300 Minolta). La perte par exsudat sous vide des muscles enveloppés sous un film plastique a été mesurée après 6 jours de stockage à 6° C. Enfin, la tendreté de la viande crue a été mesurée avec le test de Warner-Bratzler : ce test mesure, à l'aide d'un appareil spécifique, la force et l'énergie nécessaire pour couper un morceau de viande.

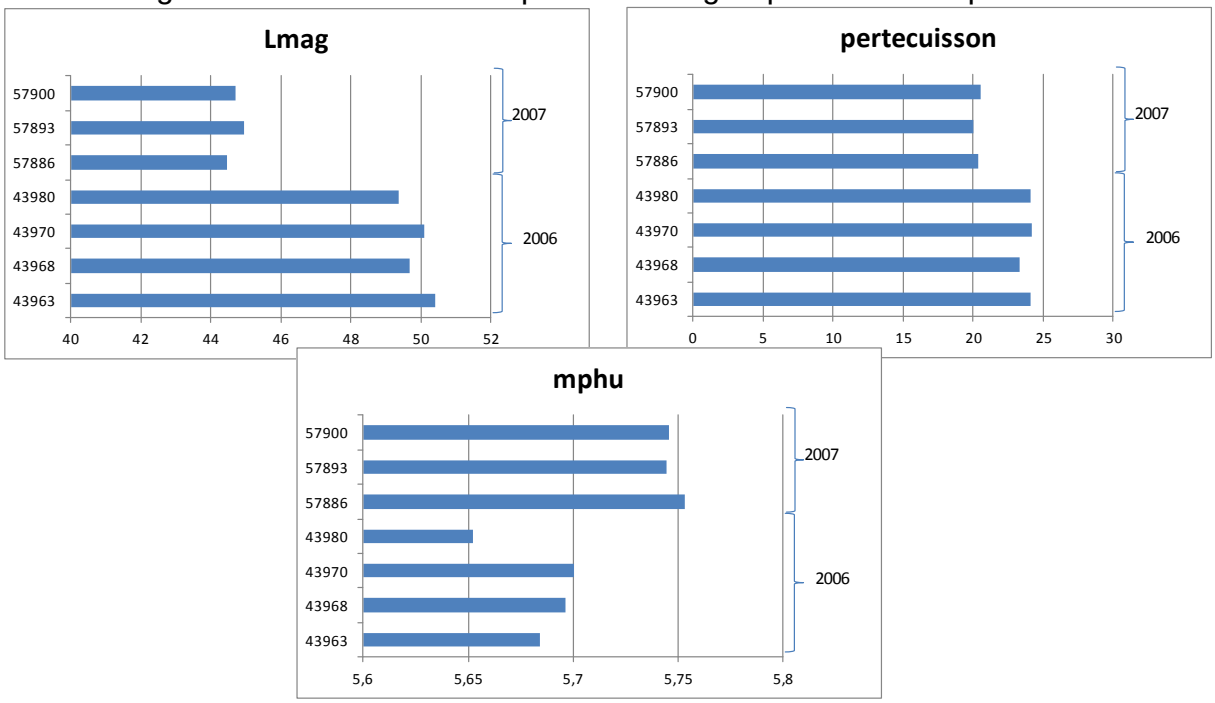
Tableau 4.5 : Description des caractères de qualité du magret.

	N	Moyenne	Std
Lmag[1]	1476	47,3	3,4
amag[1]	1476	20,4	2,5
bmag[1]	1475	7,62	1,46
MpH20[1]	1476	6,01	0,18
MpHu[1]	1476	5,72	0,14
MvacL (%)[1]	1462	1,58	0,84
pertecuisson (%)[1]	1437	22,10	3,84
Fmax[1]	1443	42,49	7,65
Energy (mJ) [1]	1442	149,61	40,16
MlipC (%)[1]	1476	4,93	0,70

[1]ML*, Ma*, Mb*, MpH20, MpHu, MvacL, MCookL, Fmax, Energy, MlipC : luminosité, indice rouge et jaune du magret, le pH du magret 20 post mortem et le pH ultime, Perte d'eau sous vide et à la cuisson du magret, force et énergie nécessaire pour couper un morceau de magret et taux de lipide du magret.

La plupart des caractères de qualité des produits présentent un effet année très marqué : c'est particulièrement le cas pour les 3 indices de couleur des magrets, les caractères de rhéologie et le pH ultime du magret (figure 4.6). Hormis pour le pH 20 minutes post mortem, on observe globalement une grande homogénéité de 3 familles de pères de 2007 et à contrario une plus grande hétérogénéité pour les familles de pères de 2006.

Figure 4.6 : Caractères de qualité du magret par famille de père F1



Un certain nombre de caractères ont aussi permis d'apprécier la qualité du foie gras tel que la texture au doigt sur foie refroidi. Aussi, des caractères comme l'extension des taches dues aux viscères sur le foie, la pigmentation du lobe du foie ou les zones de nécros sont estimés.

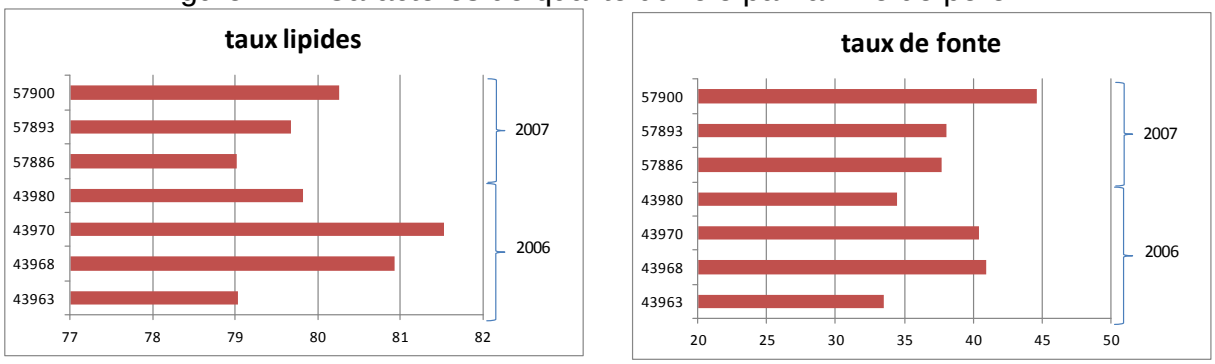
Tableau 4.6 : Description des caractères de qualité du foie.

	N	Moyenne	Std
Taux de fonte[1] (%)	1472	38,7	12,4
Lfoie[1]	1476	72,4	2,36
afoie[1]	1476	9,17	1,77
bfoie[1]	1476	31,2	2,9
Taux de collagène[1] (mg/g)	1436	1,27	0,27
Taux de protéine[1] (%)	1476	7,7	1,1
Taux de lipides[1] (%)	1476	52,4	4,3

[1]MR, LL*, La*, Lb*, LColC, LProtC, LLipC : taux de fonte du foie, luminosité, indice rouge et jaune du foie, taux de collagène, de protéine, de lipide du foie.

Le taux de fonte est le caractère de qualité du foie gras le plus variable avec une coefficient de variation de l'ordre de 32% (tableau 4.6). A l'inverse, la luminance du foie et le taux de lipides font partie des caractères les moins variables (CV inférieurs à 10%).

Figure 4.7 : Caractères de qualité du foie par famille de père F1



On observe des différences entre familles de pères F1 particulièrement marquées pour le taux de fonte et le taux de lipides des foies (figure 4.7). Ainsi, le taux de fonte varie de 33,5% pour la famille du père 43963 à 44,6% pour la famille du père 57900. Néanmoins les différences de taux de fonte d'une famille à l'autre ne semblent pas être directement expliquées par les différences de taux de lipides entre familles.

4 Résultats et discussion

4.1 Article 2

Detection of single and pleiotropic QTL controlling metabolism, meat and liver quality traits of the overfed inter-specific hybrid mule duck

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Soumis le 24 Avril 2012 à *Journal of Animal Science*

QTL in overfed hybrid duck

Detection of single and pleiotropic QTL controlling metabolism, meat and liver quality traits of the overfed inter-specific hybrid mule duck[1]

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[1] This work was supported by the French National Research Agency GENANIMAL, by the Scientific Group of avian genome analysis AGENAVI and the Midi-Pyrénées and Aquitaine regional councils. M. Kileh Wais was supported by a grant from INRA Animal Genetics Division and the University of Djibouti. The technicians of UEPFG Experimental Unit deserve special thanks for the ducks breeding, the phenotypic traits recording and blood sampling. We thank G. Cardinet for her help for the development of the microsatellite markers.

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ABSTRACT
The mule duck, an interspecific hybrid obtained by crossing Common duck (*Anas Platyrhynchos*) females with Muscovy (*Cairina moschata*) drakes, is widely used for fatty liver production. The purpose of the present study is to detect and map single and pleitropic QTL segregating in the Common duck species, having an influence on the expression of traits in their overfed mule ducks offspring. To this end, a Common duck back-cross (BC) design was generated by crossing Kaiya duck and heavy Pekin duck experimental lines, which differ notably in the bodyweight and overfeeding ability of their mule progeny. The BC females were mated to Muscovy drakes and on average, 4 male mule ducks per BC female - 1600 in total - were hatched and measured for growth, metabolism during growth and overfeeding period, overfeeding ability and breast meat and fatty liver qualities. The phenotypic value of the BC females was estimated for each trait by assigning the mean value of its offspring's phenotype, taking the variance, depending on the number of sons measured per BC and the heritability of the trait considered, into account. The genetic map used for QTL detection has 91 microsatellite markers aggregated into 16 linkage groups (LG), covering a total of 778 cM.
Twenty-two QTL were found significant at the 1% chromosome-wise threshold level, using the single trait detection option of the QTLMap software. Most QTL were detected for breast meat and fatty liver qualities: QTL for meat pH 20 minutes *post mortem* were mapped on LG4 (at 1% genome-wide level) and QTL for meat lipid content and cooking losses were found both on LG2a. For the fatty liver weight and composition in protein and lipid, QTL were mainly detected on LG2c and LG9 and multiple traits analyses highlighted pleiotropic effects of QTL in these chromosome regions. Apart for the strong QTL on chromosome Z for plasma triglyceride content at the end of overfeeding period detected in single trait analysis, all metabolic traits QTL were revealed with the multi-traits approach: QTL on LG14 and LG21 affected the plasma cholesterol and triglyceride contents whereas QTL on LG2a seemed to impact glycaemia and the basal plasma corticosterone content. A higher density genetic map will be needed for fine mapping of the QTL.

Key Words: hybrid mule duck, liver quality, meat quality, metabolic trait, pleiotropy, QTL.

INTRODUCTION

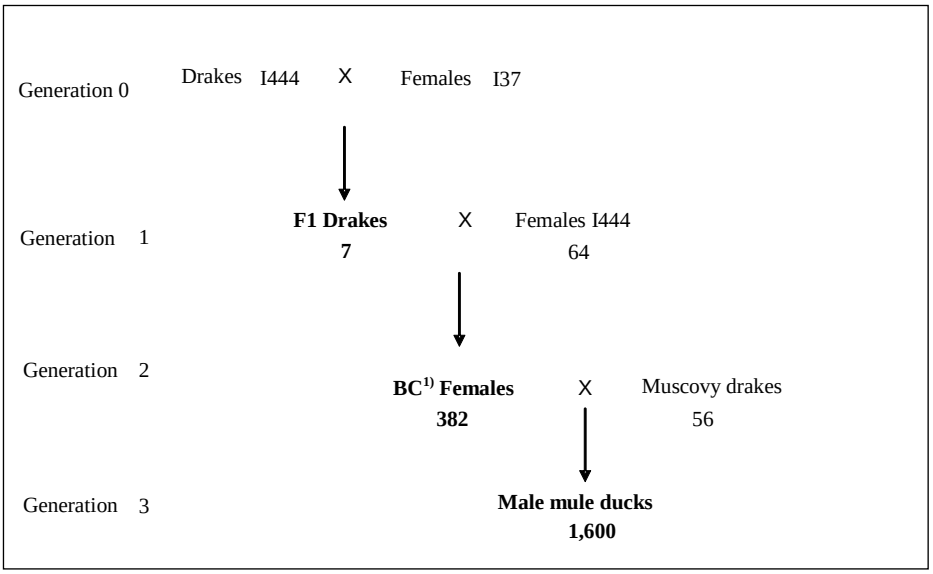
Ninety-five percent of the fatty liver production in France comes from mule ducks, the infertile inter-specific hybrid progeny of Common duck (*Anas Platyrhynchos*) females and Muscovy (*Cairina moschata*) drakes. Genetic improvement of mule duck performances is thus created by selecting both parental species for production traits which are recorded in their progenies. The parental lines are usually specialized in specific traits: Muscovy ducks are mainly selected for body weight and feed efficiency whereas genetic improvement in Common ducks is focused on reproductive traits (Marie-Etancelin *et al*., 2008). Therefore, the evaluation of the breeding values of

purebred reproducer duck candidates has to be done by progeny testing, lengthening the generation interval. Moreover, traits related to the quality of products need expensive measurements, which are destructive for the end products. In this context, it is particularly interesting to identify major genes or QTL in the parental populations having an impact on traits in the mule duck. To investigate QTL segregating in Common duck, an experimental back-cross (BC) using 2 experimental lines was designed. The phenotypic value of these BC females was estimated by averaging the performances recorded on their mule duck offspring. QTL thus measured were detected by genotyping the BC females with microsatellites markers.
The primary objective of the present study was to identify chromosomal regions influencing production traits, mainly fatty liver and breast muscle quality traits of overfed mule ducks. However, to dissect the underlying physiological mechanisms, metabolic traits related to lipid metabolism during overfeeding were also investigated. First QTL detections were done on a trait by trait basis, after which multi-trait analyses were performed, for all co-localized QTL and all correlated traits, in order to identify pleiotropic QTL.

MATERIALS AND METHODS

Animals and husbandry
Experimental procedures were performed in accordance with French National Guidelines for the care and use of animals for research purposes (Certificate of Authorisation to Experiment on Living Animals n° 7740, Ministry of Agriculture and Fish Products).
The experimental design, a grandson design with male mule ducks measured to estimate the value of their mothers, is similar in principle to the granddaughter design proposed for dairy cows (Weller *et al*., 1990), with four generations (Figure 1). Great-grandparents (generation 0) were recruited in two experimental strains of Common ducks - I444, a light Kaiya strain (the crossbreeding product of a Tsaiya duck and an Asian Pekin duck) and I37, a heavy Pekin strain (a synthetic strain created from 3 heavy European Pekin lines). The choice of this cross was motivated by previous results from Rouvier *et al*. (1994) showing that growth and fatty liver performances in mule ducks sons of Tsaiya ducks, of Pekin ducks or of the 2 reciprocal (Tsaiya x Pekin) or (Pekin x Tsaiya) crossbreds were significantly different. Seven “grandson” families were produced, in which the grandsires (generation 1) are thus 7 (I444 x I37) F1 individuals, crossed to a total of 64 Common female I444 ducks, to produce a total of 382 BC females (generation 2). Finally, the BC females were mated to Muscovy drakes, to produce the 1,600 mule ducks (generation 3), which were used to estimate their phenotypic values. However, the number of mule duck progenies per BC female was highly variable, ranging from 1 to 8. In this experiment, all phenotypic measurements were recorded on mule ducks.

Figure 1: Experimental design



1)BC= Back-cross

As described in Marie-Etancelin *et al*. (2011), mule ducklings were bred in batches of 50 animals. They were fed *ad libitum* from 0 to 6 weeks of age with a starting diet (2,820 kcal ME/kg and 17.5% crude proteins) and then were fed restricted from 6 to 10 weeks of age (230 g/day with a growing diet, 2,850 kcal ME/kg and 15.5% crude proteins). With the same growing diet, the “pre overfeeding” period started at 10 weeks of age with 5 days of restriction (200 g/day) and 6 days of gradual increase of feed amount (from 220 to 320 g/j). At 12 weeks of age, ducks were overfed during 12 days in two successive series of 200 animals -with a gap of 2 days between series- starting at 80 and 82 days of age, with 2 different crammers. During this period, animals were bred in collective cages of 4 or 5 individuals and were overfed twice a day with a mix of 35% corn-flour, 25% corn grain and 40% water: the average feed amount ingested by animal and meal varied from 410 g to 825 g. At the end of the overfeeding period, the animals were slaughtered at 92 and 94 days of age, respectively. They were bled after a “head-only” electrical stunning and plucked. The carcasses were refrigerated 24 hours at 4°C. Then, they were eviscerated: fatty liver, breast muscles, legs and abdominal fat were removed. To avoid confusion between a group of full-sibs mule ducks (progeny of a BC dam) and fixed effects, mule ducks were selected to be split up into the 2 annual hatching series, then into different breeding batches and cramming series. For each animal, the hatching, breeding and overfeeding batches were recorded.

Measurements recorded on mule ducks are detailed in Table 1, and classified in 6 types of traits: growth traits before the overfeeding period, corticosterone traits, body weights and metabolic traits during the overfeeding period, overfeeding ability traits measured after slaughter, liver quality traits and muscle quality traits. Body weights were recorded at 12, 28, 42 and 70 days of age. A hanging test, measuring the animal stress response, was organised: at six weeks of age, ducks were hanged by the legs head downwards on a string during 10 minutes, and blood samples were taken before and after the test in order to estimate the corticosterone levels and to assess the response of the HPA (hypothalamic-pituitary-adrenal) axis to this hanging stress. Measurements of plasma metabolites contents (glucose, triglyceride and cholesterol) at the beginning (after the second meal), the middle (after the tenth

meal) and the end (after the twentieth meal) of the 12 days overfeeding period were performed. Body weights at the beginning and the end of the overfeeding period and feed consumption during the whole overfeeding period were recorded. Behavioural information such as the ease of catching and overfeeding was also registered at the beginning, middle and end of the overfeeding period. To appreciate the duck overfeeding ability, the carcass and the component pieces (fatty liver, thigh, breast skin, breast muscle and abdominal fat) were dissected and weighted. Measurements related to liver quality such as melting rate, lipid, protein and collagen contents, colour (L*, a*, b* coordinates in the CIELAB system) were recorded. The external liver traits (texture, pigmentation of lobes, necrosis area, extent of red spots …) were also appraised. Lastly, muscle quality was estimated by measuring the pH 20 minutes *post mortem* and the ultimate pH, the cooking losses and the drip losses under vacuum, the colour descriptive L*, a*, b* values and the lipid content recording. The raw meat tenderness was measured with the maximal shear force and the energy at the maximum of the Warner-Bratzler test. All meat quality measurements were carried out on the breast meat (*Pectoralis major* muscle). Genetic parameters of all these traits are detailed in Marie-Etancelin *et al*. (2011).

Table 1: Traits descriptions: abbreviations, means and standard deviation (n ranging from 340 to 342)

Trait		Means	s.d.
Growth measurements			
BW12, kg	Body weights (at 12, 28, 42, 70 days of age)	0.34	0.06
BW28, kg		1.37	0.13
BW42, kg		2.31	0.16
BW70, kg		3.40	0.21
BWG12-28, g/d	Body weight gain	60.92	4.58
BWG12-42, g/d		63.49	4.17
BWG12-70, g/d		51.84	3.07
BWG28-42, g/d		62.11	4.82
BWG28-70, g/d		47.04	3.48
BWG42-70, g/d		37.64	4.36
BW_A, kg	Asymptotic body weight	4.07	0.25
T_i, d	Age at inflexion point	23.51	1.60
Corticosterone traits			
CortL, ng/ml	Corticosterone level before stress	9.77	5.80
CortH, ng/ml	Corticosterone level after stress	55.95	28.16
DeltaC, ng/ml	Corticosterone difference between before and after stress	46.18	26.27
Body weights and metabolic traits during overfeeding period			
DFI, kg/d	Daily feed intake	1.32	0.04
BWbeg, kg	Body weight at beginning of overfeeding period	3.83	0.22
BWend, kg	Body weight at end of overfeeding period	5.80	0.25
OWG, kg	Weight gain during the overfeeding period	1.97	0.15
TG 2^{nd}M, g/l	Plasma triglyceride content (at 2^{nd}, 10^{th} and 20^{th} meal)	4.30	0.64
TG 10^{th}M, g/l		4.60	0.65
TG 20^{th}M, g/l		4.90	0.88
CHO 2^{nd}M, g/l	Plasma cholesterol content (at 2^{nd}, 10^{th} and 20^{th} meal)	1.71	0.14
CHO 10^{th}M, g/l		2.11	0.19
CHO 20^{th}M, g/l		2.46	0.30
GLU 2^{nd}M, g/l	Plasma glucose content	2.21	0.15

GLU 10thM, g/l	(at 2nd, 10th and 20th meal)	2.69	0.29
GLU 20th M, g/l		3.12	0.67
FC 2ndM[1)]	Catching ease (at 2nd, 10th and 20th meal)	2.55	0.60
FC 10thM[1)]		2.57	0.64
FC 20thM[1)]		2.53	0.65
FO 2ndM[1)]	Overfeeding ease (at 2nd, 10th and 20th meal)	1.97	0.69
FO 10thM[1)]		1.31	0.36
FO 20thM[1)]		1.24	0.36
Overfeeding ability traits			
CW, kg	Bled-plucked carcass weight	4.90	0.22
FLW, kg	Fatty liver weight	0.57	0.07
pmMW, kg	*Pectoralis major* muscle weight	0.26	0.02
pmSFW, kg	Breast skin+subcutaneous fat weight	0.15	0.01
TSW, kg	Thigh+shank weight	0.48	0.03
AFW, kg	Abdominal fat weight	0.17	0.02
Liver quality traits			
MR, %	Liver melting rate	38.67	8.56
LL*	Liver lightness	72.40	1.48
La*	Liver redness	9.19	1.16
Lb*	Liver yellowness	31.20	2.19
LLipC, %	Liver lipid content	52.37	2.72
LProtC, %	Liver protein content	7.69	0.48
LColC, mg/g	Liver collagen content	1.27	0.16
TFL[1)]	Texture of fatty liver	1.29	1.09
ERS[1)]	Extent of red spots	1.11	0.43
EG[1)]	Type of grain	0.95	0.54
ESV[1)]	Extent of spots due to the viscera	1.45	0.52
PL[1)]	Pigmentation's lobes	0.41	0.29
Muscle quality traits			
Energy, mJ	Energy needed to cut the muscle	149.58	26.37
Fmax	Maximal shear force	42.37	5.13
ML*	Muscle lightness	47.34	3.91
Ma*	Muscle redness	20.38	2.39
Mb*	Muscle yellowness	7.64	1.00
MpH20	Muscle pH20 minutes *post mortem*	6.01	0.11
MpHu	Muscle ultimate pH	5.72	0.07
MCookL, %	Muscle cooking losses	22.20	2.50
MvacL, %	Muscle drip losses	1.57	0.42
MWC, %	Muscle water content	71.69	0.54
MLipC, %	Muscle lipid content	4.92	1.27

[1)] *Non-continuous traits.*

Genotyping and map construction

Microsatellite markers were selected from 2 data sets: (i) from 158 duck markers developed by the INRA-LGC laboratory (Marie-Etancelin *et al*., 2006), which were submitted to the European Nucleotide Archive in December 2010 (see Appendix 1)

and (ii) from the international nucleotide sequences data-bases (GenBank/EMBL/DDBJ). In order to optimize the markers choice along the duck genome, we took advantage of the great similarity between the duck and chicken karyotypes: all duck nucleotide sequences markers were located by sequence similarity in the chicken genome to predict their positions in the duck genome. The blast e-value cutoff for first-pass mapping of duck microsatellites onto a chicken chromosome was $1e^{-4}$; for locating more precisely markers included in a duck genetic LG already assigned to a chicken chromosome the value was equal to $1e^{-15}$.
One hundred and sixteen microsatellites markers were selected amongst the most informative markers (at least 3 of the 7 F1 sires heterozygous at the marker position) and well distributed throughout the chicken genome. They were used to genotype the BC female ducks, their parents (sires F1 and dams I444) and their paternal grand-parents. Fluorescent microsatellite analyses were performed on ABI3100 and ABI3730 DNA sequencers (Applied Biosystems). The analyses were performed using GeneMapper V4.0 software, and genotypes were integrated in a local INRA database (GEMMA, LIMS) in which correct Mendelian inheritance could be checked. Linkage groups (LG) were built using the Crimap software 2.4 (Green *et al.,* 1990).

Statistical methods
Growth traits were modeled following the Weibull model according to Maruyama *et al.,* (1999, 2001). Parameters -the asymptotic weight (BW_A) and the age at the inflexion point (t_i) of the growth curve- were estimated with the following model:

$$BW_t = BW_A - (BW_A - B)exp^{\left[-\left(\frac{C-1}{C}\right)\left(\frac{t}{t_i}\right)^C\right]}$$

where BW_t is the body weight at age t, BW_A is the asymptotic weight and t_i is the age at the inflexion point. The parameters B and C have no biological interpretation. These parameters were estimated by non-linear regression with the NLIN procedure of SAS (2002) taking into account all available weights from birth to slaughter, i.e. at 12 days, 28 days, 42 days and 70 days of age.
Before the linkage analysis aiming at locating QTL, all mule duck traits (recorded or computed traits) were previously corrected for environmental fixed effects with a GLM procedure of SAS (2002). For all traits, the “hatching batch” effect were taken into account, adding the “breeding batch” effect for traits related to growth, or the “overfeeding batch” effect for traits related to overfeeding, or the “batch of measurement set” effect for traits linked to corticosterone. The residual effects of the previous linear model were kept and the performance for each BC female was computed as the average of its mule duck sons’ residual. A few traits (catching ease, overfeeding ease, liver texture, liver pigmentation's lobes or extent of red spots …) were not distributed on a continuous scale (Table 1), but were categorical data. Nevertheless, when mule duck residuals were averaged to be assigned to a given BC female, these traits could be considered as continuous. Thus, threshold traits were treated as normal traits.
QTL detection was carried out with the QTLMap software (Elsen *et al*., 1999; Gilbert *et al*., 2008; Filangi *et al*., 2010) in order to implement a linkage analysis according to the interval mapping method (Lander and Botstein, 1989). For each chromosome, probabilities of each possible phase of the F1 male founders were first estimated from their progenies’ marker information. The most likely sire phases were assumed to be the correct ones: for a set of tested positions (practically every 1 cM), the probabilities of corresponding chromosomal segments transmission to the offspring

were estimated. Then, QTL detection was carried out by within-sire linear regression (Knott *et al*., 1996). The model was the following:

$$Y_{ij} = s_i + (2p_{ij} - 1)a_i + e_{ij}$$

where the dependent variable Y_{ij} is the average performances (previously corrected for fixed effects) of the n_{ij} mule ducks sons of BC *j* of sire *i*. For each location on the genome, s_i is the male founder *i* effect, a_i half the substitution effect of the putative QTL carried by the sire *i*, and p_{ij} the probability, for daughter (BC) *j,* of inheriting one arbitrarily defined QTL allele from her sire *i*, given the marker information. The residual variance e_{ij} was defined within sire families to improve robustness to unlinked QTL segregation between families (Goffinet *et al*., 1999).

In our "grandson design" with recorded phenotypes belonging only to the third generation, phenotypes assigned to the BC generation had a variance expressed as:

$$var(z_j) = \sigma^2 \frac{1 + \frac{1}{4}(n-1)h^2}{\frac{1}{4}n}$$

with $\sigma^2 = \sigma_g^2 + \sigma_e^2$, *n* the number of sons of BC and σ_g^2, σ_e^2, σ^2, the genetic, residual and total variances, and $h^2 = \sigma_g^2/\sigma^2$ the heritability of the trait (Kileh-Wais and Elsen, 2012). Those variances were computed with the Varcomp procedure of SAS software (2002). For the multi-trait approach, we took into account the covariance between traits S and T as

$$cov(z_j) = \frac{n_{ST} + \frac{1}{4}(n_S n_T - n_{ST})r_{ST}}{n_S n_T}$$

with n_S the number of mule ducks, sons of the BC j for trait S, n_T this number for trait T, n_{ST} the number of sons of BC j for both traits S ant T and r_{ST} the ratio between genetic and phenotypic covariances between traits S and T.

An adapted version of the QTLMap software (Elsen *et al*., 1999) was developed to take into account the phenotypic variance heterogeneity in single and in multi-trait analyses (Filangi *et al*., 2010).

For each trait and each linkage group, 1,000 within-family permutations were performed to estimate empirical chromosome-wide significance level of the test statistics (Churchill and Doerge, 1994). The conservative genome-wide thresholds were derived from chromosome-wide significance levels, using an approximate Bonferroni correction:

$$P_{genome\ -wide} = 1 - (1 - P_{chromosome\ -wide})^{1/r}$$

where r is the ratio between the length of a specific linkage group and the length of the genome considered for QTL detection (778 cM). The 95% confidence intervals of the QTL locations were estimated by lod drop-off. In practice, the bounds of the interval are the two locations where likelihood was equal to the maximum likelihood minus 3.84 ($= \chi^2_{(1,0.05)}$). The QTL effect was expressed in phenotypic deviation units

(SD), and estimated as: $\alpha = \frac{1}{SD} \times \frac{1}{n} \sum_{i=1}^{n} |\alpha_i|$, where *SD* is the phenotypic standard deviation and *n* is number of sires (Roldan *et al*., 2010).

QTL detection was first carried out on a single trait basis. As it has been shown that in the case of pleiotropic QTL (named plQTL), the fact of using simultaneously phenotypic information from different correlated traits increases the QTL location precision and possibly increases the power of evidencing effects too small to be detected in single trait analysis (Gilbert and Le Roy, 2003), multiple traits QTL detection were also performed. Traits to be included in the multiple trait analyses were grouped according to two main criteria: (1) the "co-location criteria" (Co-located QTL: CQ) for traits for which two or more significant QTL (at 5% chromosome-wide threshold) were located in the same chromosome area by single trait approaches and (2) the "correlation criteria" (Phenotypic Correlation: PC) for traits presenting phenotypic correlations higher than 0.55 (see Appendix 2). The CQ criteria aims at refining the QTL location if the single traits analyses indeed correspond to a single plQTL, whereas the PC criteria is used to detect new plQTL that could not be detected by the single traits approach. Finally, as an additional phenotypic approach, metabolism traits during overfeeding were grouped by meal (2^{nd}, 10^{th} or 20^{th} meal) and component (CHO, TG and GLU) in multiple traits QTL detection. The multi-traits detections were carried out by recursive backward approaches: among the n co-located or correlated traits for which a significant QTL was found, all sub-groups of traits (2 by 2, 3 by 3, etc ...) were tested to identify the underlying pleiotropic QTL. The most significant combinations traits which *P*-value were higher than the highest single trait *P*-value, were retained and presented in the multi-traits QTL Tables.

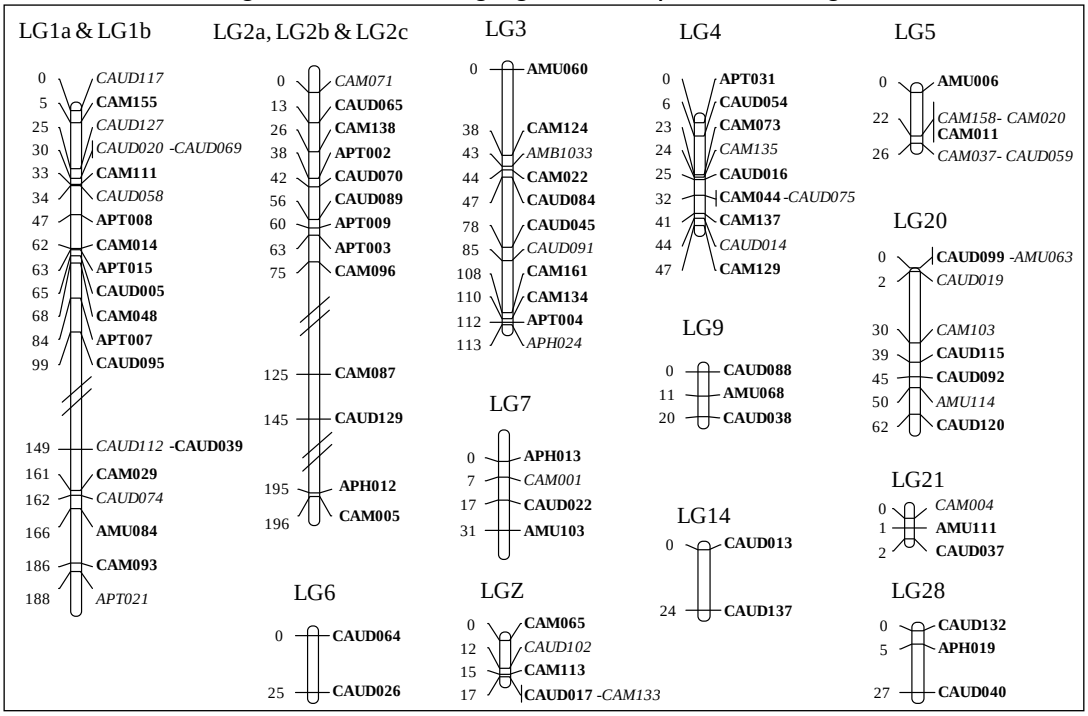
RESULTS

Genetic maps

A genetic map consisting of 91 markers aggregated into 16 linkage groups (LG) and covering a total of 778 cM was obtained (Figure 2). The sequence alignment by BLAST analysis of the duck microsatellite markers to the chicken genome sequence allows the assignment of the 16 duck LG to 13 different chicken chromosomes. Previous reports showed the strong conservation of synteny between duck and chicken (Fillon *et al*., 2007; Skinner *et al*., 2009), allowing to propose a nomenclature of duck chromosomes based on that of chicken (Skinner *et al*., 2009).

In these studies, the major rearrangement between the duck and chicken karyotypes found is that the ancestral chromosomes 4 and 10, fused in the chicken lineage to give GGA4, remain separate in duck. Apart for this only interchromosomal rearrangement, conservation of synteny between the two species was demonstrated, including all microchromosomes analyzed. We thus consider here that duck chromosomes APL1 to APL9 correspond to chicken chromosomes GGA1 to GGA9, then APL10 corresponds to GGA4p and finally, the rest of the karyotype is offset by one, with GGA10 corresponding to APL11 and so on. Therefore, the number of the LG in our genetic map was the number of corresponding duck chromosome (APL). As some of our independent LG were located by BLAST search on the same chicken chromosome, we used letters in the nomenclature to distinguish them (e.g. LG1a and LG1b for two independent LG assigned to GGA1).

Figure 2: Sex-average genetic map in centiMorgan

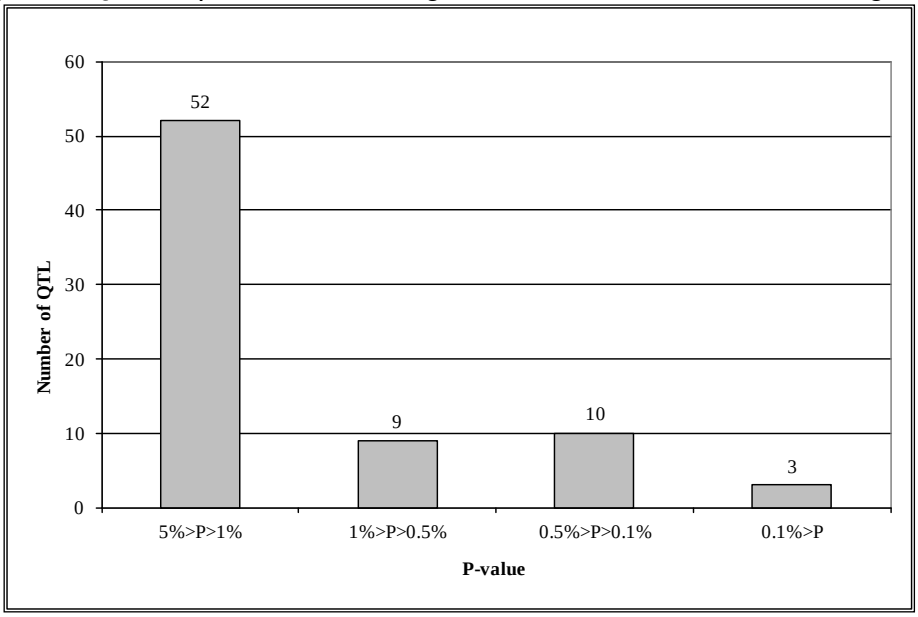


LG: Linkage Group
Markers in boldface belong to the framework map and markers in italics belong to the putative map.

QTL detection

Single trait QTL analysis on all the measured traits allowed the detection of 74 QTL (Figure 3). Out of these, 22 were significant at 1% threshold at the chromosome-wide level. The presentation of the results will focus on these most significant QTL, most of which mainly concern overfeeding ability and product quality traits. QTL will be first presented as three groups of traits involved in functions which are agronomically related and for which physiological links can be suspected: “growth”, “body weight and metabolism during overfeeding” and “overfeeding ability and quality of products”. These will be presented using first single trait and subsequently multi-traits approaches, the latter allowing the detection of plQTL. Finally, multi-trait detections will be presented for plQTL concerning more than one of our three groups of traits. QTL upper the 1% chromosome-wide threshold (in single trait and multi-traits detections) are detailed in Tables 2 to 8 and QTL under the 1% chromosome-wide threshold (in single trait detections) presented in Appendix 3.

Figure 3: QTL frequencies according to the chromosome-wide level significance



Traits related to growth

QTL for growth traits are presented in Table 2 for the single trait approach and Table 3 for the multi-traits plQTL approach.

Two QTL significant at 0.5% threshold at chromosome-wide (and 5% at genome-wide) - one for body weight at 28 days of age and one for body weight gain between 12 and 28 days - were detected by the single trait analyses on linkage group 3. Six others QTL significant at the 5% threshold chromosome-wide, for BW42, BW70, BWG12-28, BWG12-42, BWG12-70, BWG28-42, and BWG28-70, were co-localized on LG2b (suppl. 3). However, none of these reached the 1% chromosome-wide threshold. Thanks to the Weibull curve approach, 3 new QTL were detected: 2 QTL for Ti respectively on LG7 (at 1% threshold) and LG1b (at 5% threshold – suppl. 3) and 1 QTL for BW_A on LG20 (at 5% threshold – suppl. 3). The Ti QTL on LG7 presented a relatively strong QTL effect of 0.30 in phenotypic deviation units. The lack of highly significant QTL for BW_A is consistent with the absence of QTL for the bodyweight at 70 days, since both traits are highly correlated (0.78).

Table 2: Growth traits – in a single trait analysis

LG	Traits[1)]	Flanking markers[2)]	Location[3)] (cM)	LRT_x[4)]	*P*-value[5)]	Effect of QTL substitution[6)]	Confidence interval (cM)
3	BW28	CAM022-CAUD084	46	29.10	** / †	0.14	33 - 64
3	BWG12-28	CAUD084-CAUD045	51	27.75	** / †	0.19	35 - 65
7	Ti	APH013	0	21.93	*	0.30	0 – 6

[1)] See Table 1 for the definitions of the traits.
[2)] Flanking markers of the most probable QTL position.
[3)] Most probable QTL position.
[4)] Maximum likelihood ratio for x locus.
[5)] Level of significance of P-value - at chromosome-wide: 0.01>P>0.005; ** 0.005>P>0.001; *** 0.001>P*
- at genome-wide: †0.05>P>0.01; †† 0.01>P
[6)] QTL effect in phenotypic deviation units (SD), and estimated as $\alpha = \frac{1}{SD} \times \frac{1}{n} \sum_{i=1}^{n} |\alpha_i|$.

In multi-traits analyses, neither the co-localized QTL approach nor the correlation between traits approach allowed to confirm QTL or to identify a new one. The multi-traits detection for BW28 and BWG12-28, which are strongly correlated (r = 0.92) and presented 2 co-localized QTL on LG3, did not reveal any plQTL reaching 1% threshold on LG3. However, by combining the two Weibull parameters (Ti and BW_A) in a multi-traits analysis, a new plQTL significant at 1%, appeared in LG21 (Table 3). This is consistent with the observation that in single trait analyses the LRTs on LG21 for Ti and BW_A respectively are nearly significant.

Table 3: Growth traits – in a multiple traits analysis

LG	Traits group & type[1)]	Traits[2)]	Flanking markers[3)]	Location[4)] (cM)	LRT_x[5)]	*P*-value[6)]	Effect of QTL substitution[7)]	Confidence interval (cM)
21		BW_A	CAM004	0	25.96	*	0.12	0 - 2
	PC	Ti					0.11	

[1)] Criteria to select the traits for multiple trait analysis: CQ for QTL Co-located – PC for Phenotypic Correlation.
[2)] See Table 1 for the definitions of the traits.
[3)] Flanking markers of the most probable QTL position.
[4)] Most probable QTL position.
[5)] Maximum likelihood ratio for x locus.
[6)] Level of significance of P-value - at chromosome-wide: 0.01>P>0.005; ** 0.005>P>0.001; *** 0.001>P*
- at genome-wide: †0.05>P>0.01; †† 0.01>P
[7)] QTL effect in phenotypic deviation units (SD), and estimated as $\alpha = \frac{1}{SD} \times \frac{1}{n} \sum_{i=1}^{n} |\alpha_i|$.

Traits related to bodyweight and metabolism during the overfeeding period

QTL related to metabolism traits during overfeeding (P<0.01) are presented in Tables 4 and 5 for single and multiple traits analysis, respectively.

Table 4: Metabolic and body weight traits during the overfeeding phase – in a single trait analysis

LG	Traits[1)]	Flanking markers[2)]	Location[3)] (cM)	LRT_x[4)]	*P*-value[5)]	Effect of QTL substitution[6)]	Confidence interval (cM)
2a	FO 20th	CAM138-APT002	28	24.67	*	0.34	15 - 37
4	FC 2nd	CAM137	41	34.71	*** / ††	0.23	38 - 44
Z	TG 20th	CAM065-CAUD102	1	26.54	**	0.48	0 - 8

[1)] See Table 1 for the definitions of the traits.
[2)] Flanking markers of the most probable QTL position.
[3)] Most probable QTL position.
[4)] Maximum likelihood ratio for x locus.
[5)] Level of significance of P-value - at chromosome-wide: 0.01>P>0.005; ** 0.005>P>0.001; *** 0.001>P*
- at genome-wide: †0.05>P>0.01; †† 0.01>P
[6)] QTL effect in phenotypic deviation units (SD), and estimated as $\alpha = \frac{1}{SD} \times \frac{1}{n} \sum_{i=1}^{n} |\alpha_i|$.

Only one strong QTL was found for plasma triglyceride content after the 20th overfeeding meal on LGZ (corresponding to the Z gonosome) with a *P*-value lower than 0.5% at the chromosome-wide level and a QTL substitution effect of 0.48 phenotypic standard deviations. None of the other traits, related to blood plasma contents (glucose, triglyceride or cholesterol) or to the stage of the overfeeding process (2nd, 10th or 20th meal), reached the 1% threshold at chromosome-wide level.

For the 2 traits related to the ease of handling the animals during overfeeding (overfeeding per se and catching), 2 QTL reached the 1% threshold: 1 on LG2a for overfeeding ease at the 20th meal and one highly significant QTL (P<0.001) on LG4 for catching ease at the 2nd meal. Finally, for this group of metabolic and behavior traits during overfeeding, 15 QTL, significant at 5% threshold at the chromosome-wide level (suppl. 3), were scattered on 9 different LG: 4 linkage groups with co-localized QTL (LG2a, 2c, 14, and 21) and 5 with isolated QTL (LG1b, 3, 6, 7 and 28). To sum up, the plasma cholesterol contents were affected by QTL on LG2c, 3, 14 and 21, while the plasma triglyceride contents were influenced by QTL on 2 of these 4 LG (LG14 and LG21). The main QTL involved in the genetic determinism of glucose contents were located on 3 others LG (LG2a, 6 and 28). Finally, LG7 and LG28 carried QTL affecting the body weights at the beginning of the overfeeding period, whereas 2 QTL on LG9 and LG21 affected respectively the weight gain during the overfeeding period and the weight at the end of overfeeding period.
In multiple trait analyses, the co-location criteria on LG2a, LG14 and LG21, and the correlation criteria between cholesterol and triglyceride contents after the 10th meal revealed 4 potential plQTL areas, 2 of which are located on LG14. A strong plQTL (P<0.001) was revealed on LG2a by combining corticosterone level before stress and glucose content at the 2nd meal. Another strong plQTL reaching the same *P*-value threshold (P<0.001) was confirmed on LG21 for cholesterol and triglyceride contents at the 10th overfeeding meal. This plQTL was revealed both by the analysis of two co-located uni-trait QTL on LG21 and by the analysis of correlated traits. The correlated-traits approach for triglyceride and cholesterol contents after the 10th meal also revealed a weaker but new plQTL on LG14, this result being consistent with the plQTL (at 0.5% threshold) obtained on LG14 after the analysis based QTL co-localisation of cholesterol content after the 10th meal and triglyceride content after the 2nd meal. For this last plQTL, the correlated-traits approach has also reduced the confidence interval to 0-17 cM, whereas it was respectively 0-22 cM and 0-24 cM for TG2nd and CHO10th in single trait detections. Thus, two plQTL were found for triglyceride content after the 2nd or the 10th meal, combined with cholesterol contents after the 10th meal, on LG 14: indeed, both single trait QTL for TG2nd and CHO10th have *P*-values around 0.03 whereas for TG10th the *P*-value reached only 0.06. The close location of the 2 plQTL suggests that they may be confounded into one plQTL concerning the three traits.

Table 5: Metabolic and body weight traits during the overfeeding phase – in a multiple traits analysis

LG	Traits group & type[1)]	Traits[2)]	Flanking markers[3)]	Location[4)] (cM)	LRT_x[5)]	*P*-value[6)]	Effect of QTL substitution[7)]	Confidence interval (cM)
2a	CQ (2a)	GLU2ndM	CAM071-CAUD065	12	40.55	***/†	0.11	0 - 18
		CortL					0.15	
14	CQ(14)	CHO10thM	CAUD013-CAUD137	6	35.31	**	0.11	0 - 17
		TG 2ndM					0.15	
14		CHO10thM	CAUD013-CAUD137	20	27.33	*	0.11	6 - 24
	PC	TG 10thM					0.08	
21	CQ(21)	CHO10thM	CAUD037	2	37.48	***	0.10	0 - 2
	PC	TG 10thM					0.10	

[1)] Criteria to select the traits for multiple trait analysis: CQ for QTL Co-located – PC for Phenotypic Correlation.
[2)] See Table 1 for the definitions of the traits.
[3)] Flanking markers of the most probable QTL position.

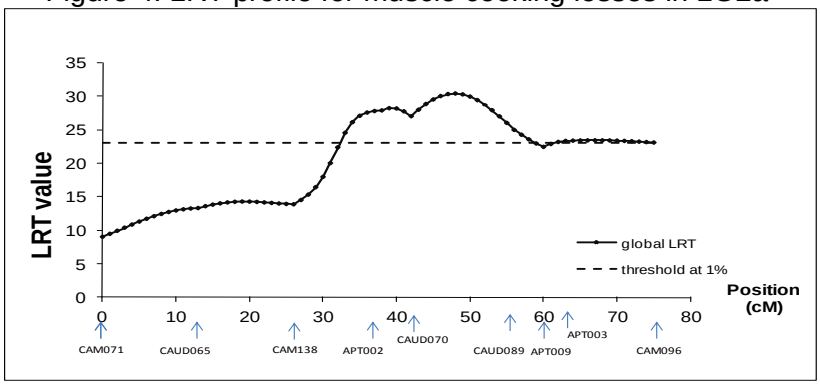
[4] Most probable QTL position.
[5] Maximum likelihood ratio for x locus.
[6] Level of significance of P-value - at chromosome-wide: 0.01>P>0.005; ** 0.005>P>0.001; *** 0.001>P*
- at genome-wide: †0.05>P>0.01; †† 0.01>P
[7] QTL effect in phenotypic deviation units (SD), and estimated as $\alpha = \frac{1}{SD} \times \frac{1}{n} \sum_{i=1}^{n} |\alpha_i|$.

Traits related to overfeeding ability and quality of products

QTL related to the overfeeding ability and product quality traits are presented in Tables 6 and 7 for the single and multiple trait analyses, respectively.

In single trait detection, 16 QTL were identified at the 1% chromosome-wide threshold in 7 different LG, with LG2a gathering QTL related to muscle quality traits, whereas LG2c and LG9 are particularly involved in QTL for fatty liver quality traits. The 2 strongest QTL (at 1% at genome-wide level of significance) were for muscle pH 20 minutes *post mortem* on LG4 and for muscle cooking losses on LG2a (Figure 4).

Figure 4: LRT profile for muscle cooking losses in LG2a



Still on LG2a, but not at the same position, 2 QTL for lipid contents in muscle and, with a smaller *P*-value, for muscle maximal shear force, were revealed. Finally for breast meat quality, a QTL on LG6, significant at 0.5% and explaining 0.23 phenotypic standard units of muscle cooking losses variation was found. The variability in fatty liver weight was partly controlled by 3 QTL on LG21, LG9 and LG2c. Moreover, the latter 2 linkage groups (LG9 and LG2c) have also QTL related to fatty liver quality. On LG2c, 2 QTL for lipid and protein contents were co-localized with fatty liver weight QTL and interestingly, these 3 traits are highly correlated. The QTL substitution effect for liver protein content on LG2c reached 0.31 phenotypic standard deviations. On LG9, 2 QTL for protein content and liver redness were detected at 1% threshold but only the QTL for LprotC was co-localized with the QTL chromosome-wide level, for carcass weight, fatty liver texture and fatty liver weight.

Table 6: The overfeeding ability and product quality traits – in a single trait analysis

LG	Traits[1)]	Flanking markers[2)]	Location[3)] (cM)	LRT_x[4)]	*P*-value[5)]	Effect of QTL substitution[6)]	Confidence interval (cM)
2a	MLipC	CAUD065-CAM138	18	27.40	**/†	0.10	3 - 26
2a	MFmax	CAM138-APT002	28	25.37	*	0.23	22 - 33
2a	MCookL	CAUD070-CAUD089	48	30.43	***/††	0.18	34 - 55
2c	FLW	APH012	0	19.10	*	0.21	0 - 1
2c	LLipC	APH012	0	20.10	*	0.22	0 - 1
2c	LProtC	APH012	0	21.26	**	0.31	0 - 1
3	ESV	AMU060-CAM124	9	25.13	*	0.20	0 - 26
4	MpH20	APT031	0	30.27	***/††	0.22	0 - 5
6	ESV	CAUD064-CAUD026	19	17.28	*	0.20	0 - 25
6	MCookL	CAUD026	25	21.39	**	0.23	7 - 25
9	La*	CAUD088	0	25.75	**	0.22	0 - 10
9	LProtC	CAUD038	20	23.28	*	0.25	0 - 20
9	FLW	CAUD038	20	21.64	*	0.20	5 - 20
21	CW	AMU111	1	22.47	**	0.25	0 - 2
21	TFL	CAUD037	2	23.49	**	0.06	0 - 2
21	FLW	CAUD037	2	23.25	**	0.21	0 - 2

1) See Table 1 for the definitions of the traits.
2) Flanking markers of the most probable QTL position.
3) Most probable QTL position.
4) Maximum likelihood ratio for x locus.
5) Level of significance of P-value - at chromosome-wide: 0.01>P>0.005; ** 0.005>P>0.001; *** 0.001>P*
- at genome-wide: †0.05>P>0.01; †† 0.01>P
6) QTL effect in phenotypic deviation units (SD), and estimated as $\alpha = \frac{1}{SD} \times \frac{1}{n} \sum_{i=1}^{n} |\alpha_i|$.

The multiple traits analysis revealed 9 pleiotropic QTL, 8 of which related to products quality and mainly to liver quality. Four plQTL on LG2c, LG3 and LG9 with very significant thresholds (reaching 0.5% or 0.1% threshold at the chromosome-wide) were suggested by the co-localization of the single-trait QTL, whereas 5 others plQTL were new ones, identified on LG1b, LG3, LG5 and LG7 by the joint analysis of 3 couples of traits. However, most of these 5 plQTL only reached the 1% threshold. These multi-traits analyses confirmed the importance of LG2c for the variability of the liver melting rate, when this trait is associated with the liver structural composition (such as protein contents or collagen contents) and, to a lesser extent, that of LG9 for liver composition variability. LG3 seemed to contain three plQTL: one for liver yellowness and spots on liver due to viscera at the beginning of the linkage group, for which the confidence interval (between 0 to 16 cM) was reduced as compared to single trait detections (confidence intervals between 0 to 19 cM for Lb* and between 0 to 26 for ESV) ; and 2 others in the middle (at 85 cM when combining melting rate and liver protein contents) and at the end (at 108 cM when combining liver protein and lipid contents). One strong plQTL for liver composition traits appeared (at 0.1% threshold) on LG7, which is a totally new information since none of the traits forming this group has a QTL (even at 5%) with the single trait approach. Lastly, the combination of 2 muscle rheological traits (energy needed to cut the breast muscle and maximal shear force) allowed the discovery of a plQTL on LG5.

Table 7: The overfeeding ability and product quality traits – in a multiple traits analysis

LG	Traits group & type[1)]	Traits[2)]	Flanking markers[3)]	Location[4)] (cM)	LRT_x[5)]	*P*-value[6)]	Effect of QTL substitution[7)]	Confidence interval (cM)
1b	PC	MR LProtC	APT021	39	32.21	*	0.10 0.07	32 - 39
2c	CQ(2c) PC	MR LProtC	APH012	0	35.34	***	0.11 0.14	0 - 1
2c	CQ(2c)	MR LcolC	APH012	0	27.33	**	0.12 0.11	0 - 1
3	PC	LProtC LLipC	CAM134-APT004	108	35.26	*	0.12 0.10	98 - 110
3	CQ(3)	Lb* ESV	AMU060-CAM124	6	47.41	***/††	0.11 0.14	0 - 16
3	PC	MR LProtC	CAUD091	85	34.34	*	0.10 0.13	78 - 94
5	PC	Fmax Energy	CAM037	26	27.51	*	0.11 0.07	10 - 26
7	PC	LProtC LLipC	APH013-CAM001	6	33.67	***/†	0.07 0.11	0 - 12
9	CQ(9) PC	LProtC LLipC	CAUD038	20	34.94	**	0.12 0.11	0 - 20

[1)] Criteria to select the traits for multiple trait analysis: CQ for QTL Co-located – PC for Phenotypic Correlation.
[2)] See Table 1 for the definitions of the traits.
[3)] Flanking markers of the most probable QTL position.
[4)] Most probable QTL position.
[5)] Maximum likelihood ratio for x locus.
[6)] Level of significance of P-value - at chromosome-wide: 0.01>P>0.005; ** 0.005>P>0.001; *** 0.001>P*
- at genome-wide: †0.05>P>0.01; †† 0.01>P
[7)] QTL effect in phenotypic deviation units (SD), and estimated as $\alpha = \frac{1}{SD} \times \frac{1}{n} \sum_{i=1}^{n} |\alpha_i|$.

plQTL influencing phenotypes across groups of traits

The most significant multi-traits plQTL detections, acting simultaneously on two or more traits belonging to different groups of traits “growth”, “bodyweight and metabolism during overfeeding” and “overfeeding ability and quality of products” quality), are presented in Table 8. As these traits can concern very different physiological functions, correlations between traits were usually low (all under 0.55) so the multi-traits QTL detection across group was only performed for traits having co-localised QTL.

Four plQTL were obtained on LG7, LG9 and LG21, among which 3 reached the 0.1% threshold. Association of weight gain during the overfeeding period and fatty liver weight traits on LG9 allowed to detect a strong plQTL (at 0.1% threshold) with a smaller QTL confidence interval, reduced from positions 5 - 20 cM and 8 - 20 CM for FLW and OWG respectively, down to positions 11 - 20 cM for the plQTL. On LG21, a plQTL was detected by combining carcass weight and body weight at the end of overfeeding period. Although the Bonferroni correction for multiple tests is very conservative, the maximum likelihood ratio for this plQTL reached the 5% genome-wide threshold. This plQTL is probably mostly determined by CW for which a QTL was also detected on LG21 at 1% threshold in single trait analysis. Still on LG21, another plQTL, significant at 0.1% threshold, was observed by associating overfeeding ease measured at the 10th meal and liver collagen contents. This plQTL

is characterized by a sharp increase in the detection power since the single trait detection threshold was only 5% for both traits. However, the biological explanation for this is unclear. Finally, thanks to the combination of body weight at the beginning of overfeeding period and body weight gain between 42 and 70 days, a plQTL on LG7 appeared while this linkage group was not previously identified as impacting late growth.

Table 8: mixed group – in a multiple traits analysis

LG	Traits group & type[1)]	Traits[2)]	Flanking markers[3)]	Location[4)] (cM)	LRT_x[5)]	*P*-value[6)]	Effect of QTL substitution[7)]	Confidence interval (cM)
7		BWbeg	APH013	0	25.00	*	0.18	0 – 14
	CQ(7)	BWG42-70					0.17	
9		OWG	CAUD038	20	39.02	***/†	0.10	11 – 20
	CQ (9)	FLW					0.11	
21		BWend	AMU111	1	53.57	***	0.18	0 - 2
	CQ(21)	CW					0.17	
21		FO10th	CAM004	0	30.19	***	0.12	0 - 2
	CQ(21)	LColC					0.10	

[1)] Criteria to select the traits for multiple trait analysis: CQ for QTL Co-located – PC for Phenotypic Correlation.
[2)] See Table 1 for the definitions of the traits.
[3)] Flanking markers of the most probable QTL position.
[4)] Most probable QTL position.
[5)] Maximum likelihood ratio for x locus.
[6)] Level of significance of P-value - at chromosome-wide: 0.01>P>0.005; ** 0.005>P>0.001; *** 0.001>P*
- at genome-wide: †0.05>P>0.01; †† 0.01>P
[7)] QTL effect in phenotypic deviation units (SD), and estimated as $\alpha = \frac{1}{SD} \times \frac{1}{n} \sum_{i=1}^{n} |\alpha_i|$.

DISCUSSION

This paper reports the first QTL detection work carried out on overfed inter-specific mule ducks. Single trait analyses, as a first step of our exploration, allowed to detect 22 QTL significant at 1% threshold at the chromosome-wide level. The ratios between the number of QTL detected and the number of traits recorded for a physiological function are very different: very few QTL were detected on traits related to metabolism (blood lipid metabolism traits during overfeeding and corticosterone level during growth) or growth traits, while, in proportion, many QTL were detected for liver and muscle qualities traits. Subsequently, with the multi-traits detection, 18 pleiotropic QTL (plQTL) were identified, with at least 7 new chromosomal regions not previously identified by the univariate analyses. Indeed, the multi-traits QTL analyses were done either i) on groups consisting of correlated traits, or ii) on traits QTL located close to one another after the single QTL detection analyses. With this second type of grouping, the estimated positions of the pleiotropic QTL were similar to one of the positions found in the single trait QTL analyses, and moreover, the confidence interval was generally reduced. This is consistent with Gilbert and Le Roy (2003) who demonstrated that using information from different correlated traits sharing a pleiotropic QTL increases the precision of the estimated location of this QTL. This approach highlighted the most interesting chromosomal regions especially for fatty liver and breast muscle quality traits. The other grouping method (correlation

criteria) allowed to identify new QTL. These QTL could not be detected in single trait analyses because of their small effects: the information gain obtained thanks to the combination of these correlated traits was particularly interesting for duck metabolism traits.
The statistical analyses were performed with some approximations. The data were pre-corrected for significant nuisance effects evidenced by SAS-GLM. This two steps procedure greatly simplified the calculations, in particular for multi-trait analyses, and is generally considered as safe in terms of QTL location estimation (Mc Rae *et al*., 2005; Lillehammer *et al*., 2009). As demonstrated by Crooks *et al*. (2009) for polygenic effect, when data are pre-corrected, possible confusion between certain levels of a nuisance effect and transmitted QTL alleles could result in underestimation of QTL effect and loss of power. It must be emphasized that this approach is conservative and should not result in false QTL detection. The traits analyzed being means of BC offspring (mule ducks) performances: the variability of their variances had to be considered in the model. Both heritabilities and genetic to phenotypic covariance ratios were given as parameters and not estimated with QTL effects. This is clearly an approximation which supposed that the QTL effects are not too strong. This two steps procedure is in the line of the GRAMMAR model for association analysis developed by Aulchenko *et al*. (2007). The backward approach used for multi-trait detection generated many statistical tests, and the significance of the results must be often considered with caution. However, the improvement of QTL localization generated by this approach is probably free of this drawback.

Twelve among the 22 QTL concerned original traits such as liver quality (7 QTL) and muscle quality (5 QTL) for overfed ducks. Some of these traits, especially the latter correspond to traits studied in chicken. The karyotypes of the two species are very similar, with only one inter-chromosomal rearrangement detected to date (Fillon *et al*., 2007; Skinner *et al*., 2009), suggesting that QTL located on orthologous chromosomes could be equivalent. However, further investigations to refine duck QTL location and chicken-duck intra-chromosomal rearrangements will have to be done. The strongest QTL related to breast muscle quality traits (concerning cooking losses, lipid content and maximal shear force to cut the muscle) were mainly gathered on linkage group 2a: QTL for MCookL and for MLipC reached respectively 1% and 5% genome-wide thresholds. In Beijing-You chickens, Ye *et al*. (2010) assessed the association of single nucleotide polymorphisms (SNP) in the fatty acid binding proteins (*A-FABP*) gene, located on GGA2, with the content of intramuscular fat (IMF). Another QTL for muscle cooking losses was detected on LG6: Huang *et al*. (2007a) have already found a QTL for MCookL trait on CAU6, although the breast muscle was not fattening by overfeeding. Regarding pH 20 minutes *post mortem*, 1 strong QTL reaching the 1% genome-wide threshold, was revealed on LG4. On chicken, Wright *et al*. (2006) and Nadaf *et al*. (2007) already mapped QTL for breast meat ultimate pH with QTL Express software on chromosome 4 at 233 cM and 201 cM, respectively. Although this is not exactly the same trait involved in the 2 poultry species (ultimate pH in chicken versus pH 20 minute *post mortem* in duck), this same QTL location on LG4 between species is surprising especially as the chicken is a white meat type species and the duck is a red meat type. Finally, the multi-traits analysis brought up a pleiotropic QTL for meat texture (energy needed to cut the muscle and maximal shear force traits) on LG5, but to our knowledge, there is no publication on this type of trait on chicken.

More specifically, QTL related to fatty liver quality cannot be compared with any previously published results, since the present work is the first design for QTL detection in overfed waterfowl. On chromosomal regions 2c, 7 and 9, remarkable clusters of QTL impacting simultaneously at least 2 of these 4 traits (liver melting rate, lipid, protein or collagen contents) are noticeable. These trait associations are very consistent as collagen and proteins are considered important for structuring the fatty liver tissue: a variation of this liver "extracellular matrix" would logically impact on lipids storage capacity and on the liver melting rate. Indeed, recent work has highlighted the role of the protein fraction in the determinism of the technological quality of fatty livers (Théron *et al*., 2011). On LG2c, the strongest improvement of the QTL detection power was obtained by associating melting rate and liver protein content, whereas the pleiotropic QTL on LG7 and LG9 were more related to liver composition (lipid and protein contents).
Regarding the overfeeding ability traits, two of the 3 QTL influencing the fatty liver weight were on LG2c and on LG9, co-localized with liver quality trait QTL. However, higher *P*-values of pleiotropic QTL were obtained by associating two co-localized liver quality traits rather than by associating liver weight and a liver quality trait. The co-localization of QTL for traits measured independently consolidates the reliability of our results, but the chromosomal regions that we identified didn't match with previously published results in other birds. Indeed, on duck or on chicken, most of the liver weight QTL detected did not intersect our QTL localizations: 3 significant areas on LG4, LG6 and CAU7 (corresponding to our LG20) in lean Pekin ducks (Huang *et al*., 2007a) and 3 others on GGA1, GGA8 and GGA12 (corresponding to our LG13) for chicken (Wright *et al*., 2006). Only Gao *et al*. (2009) revealed a liver weight QTL on the GGA2 which a confidence interval covering half of the chromosome in a F2 cross between Silkies fowl and White Plymouth Rock chicken.

For the growth traits, only 3 QTL were detected: 2 for growth between 12 and 28 days of age on LG3 (at 5% genome-wide threshold) and 1 on LG7 (at 1% chromosome-wide threshold). These results are consistent with those found in chicken, as GGA3 is an interesting chromosome for growth traits, with more than 50 QTL identified (www.animalgenome.org, Hu *et al.,* 2010) and numerous publications highlighting QTL related to young ages (Siwek at al., 2004; Ambo *et al*., 2009; Wahlberg *et al*., 2009; Atzmon *et al*., 2008). Maruyama *et al*. (2001) and Vitezica *et al*. (2010) demonstrated that the Weibull function is the best model for fitting duck body weight data, compared to others models such as Gompertz, logistic, von Bertalanffy, Morgan-Mercer-Flodin or Spline. Performing QTL detections from Weibull parameters has highlighted a new area of interest for growth traits in LG7, which has a pleiotropic QTL for growth between 42 and 70 days of age and bodyweight at the beginning of the overfeeding period. Moreover, GGA7 is also known to contain 28 growth traits QTL in chicken (Zhou *et al*., 2007; Atzmon *et al*., 2008; Ambo *et al*., 2009). Nevertheless, the Pekin duck experimental design analyzed by Huang *et al*. (2007b) revealed 4 chromosomal areas (at 5% genome-wide threshold - 2 QTL on LG1 and 2 others QTL on LG2) impacting growth traits which were different from ours QTL localizations.
The multi-traits approach within growth traits has only brought one new QTL when gathering both Weibull parameters (Ti and BW_A) on LG21. By comparing this result with the QTL detected for the bodyweight at the end of overfeeding and the carcass weight on LG21, we could hypothesize that this QTL area modified both the growth curve and the final product as carcass weight. In a specific intercross between two

chicken lines divergently selected for juvenile body-weight, Wahlberg *et al*. (2009) identified 7 regions harboring QTL influencing growth (GGA1, GGA3, GGA4, GGA7 and GGA20) including a network of interacting loci on GGA7. Knowing that GGA20 corresponds to APL21 and the short arm of GGA4 to APL10 (Skinner *et al*., 2009) for which we have no marker yet, we could highlight the consistency between our linkage groups containing QTL for growth traits in young age, and the results by Wahlberg *et al*. (2009) in chicken.

Regarding metabolism traits, we detected only one QTL for triglyceride contents in blood at the end of the overfeeding period. This QTL, located on LGZ (at 1% on the chromosome-wide level) was not identified in chicken QTL literature: only a QTL for glucose content was identified by Zhou *et al*. (2007) on this sexual chromosome. Thanks to the multi-traits analyses, 3 new areas of interest for metabolic traits during the overfeeding phase appeared on LG2a, LG21 and, with a lower likelihood on LG14. On LG14 and LG21, chromosome areas appeared to be involved in the control of lipid metabolism downstream of the liver. On LG2a, the plQTL impacting plasma corticosterone level before stress and glucose contents, is consistent with the fact that corticosterone is involved in the glucose metabolism.
The QTL results for metabolic traits published in chicken are very diverse. To our knowledge, no publications referenced a QTL for corticosterone response to a constraint on GGA2, although Buitenhuis *et al*. (2003) detected their strongest significant QTL for severe feather peaking on GGA2 in a chicken F2 population established from a cross between two commercial lines of laying hens differing in their propensity to feather pecking. Usually, the corticosterone response to a given stress is considered to be associated with feather pecking behavior (Buitenhuis *et al*., 2003). For blood glucose content, the strongest QTL published are spread out on GGA2 and GGA7 (Zhou *et al*., 2007- in a broiler-Fayoumi cross) and on GGA20 and GGA27 (Park *et al*., 2006- in a cross between low and high weights White Plymouth Rock). These last authors have already detected a QTL for cholesterol levels on GGA20 (corresponding to APL21) and a QTL for plasma triglyceride contents on GGA2.
Lastly, Nadaf *et al*. (2009) found a QTL for plasma glucose with a genome-wide level of significance on GGA13 (orthologous chromosome of APL14). However, no triglyceride or cholesterol assay had been made in this study, making any comparison difficult. All these results suggested that metabolic traits are regulated by a large number of QTL with small effects and it was therefore difficult to highlight areas of interests shared by ducks and chickens.

Finally, although multi-trait analyses done with correlated traits revealed new QTL regions, multi-trait analyses done with traits whose QTL were co-localized allowed us to better dissect the results previously obtained in single trait analyses. Whatever the traits, this approach evidenced the 2 main traits contributing to the pleiotropic effect. It is interesting to note that most significant likelihoods were obtained with a combination of two traits but never more than 2.
In single or in multiple trait analyses, the scarcity of QTL obtained for growth traits in relation to the number of recorded traits, yet very heritable (Marie-Etancelin *et al*., 2011), may be explained by a highly polygenic genetic determinism of growth and the absence of QTL with strong effects for this trait. In contrast, we hypothesized that the more complex traits such as glucose, cholesterol and triglyceride contents – particularly weakly heritable at the beginning of the overfeeding period (Marie-

Etancelin *et al*., 2011) - were depending on numerous small QTL. Even if we have obtained here a large number of QTL for liver and meat quality traits, we need to keep in mind that the high number of traits tested for QTL detection (a total of 1008 analyses computed in single trait approach and 366 computations in multi-traits analysis) generates an increased risk of false positive QTL.

CONCLUSION

This duck experimental design, the first one for overfed waterfowl, and the first with trait measurements in a sterile inter-specific hybrid, was based on a crossing of two Common ducks lines with progeny testing. It allowed the detection of 74 QTL significant at 5% threshold at chromosome-wide level, 22 of them reaching the 1% threshold. Most of them affected original traits such as the product quality traits of overfed ducks or their overfeeding ability. The multi-traits QTL approach was effective since it allowed to reveal new chromosomal areas not previously identified by the univariate approach. Moreover it suggested QTL with pleiotropic effects and sometimes reduced the QTL confidence interval.
Objectives of future work will be to increase the genetic map density with SNP markers in order to cover at least duck chromosomes 8, 10, 11 and 12 missing in the present analysis and to complete macro-chromosomes 5, 6 and 9. We will also plan to refine the map for QTL affecting fatty liver quality traits which should be studied as a priority, because of their economic importance and the complexity of their measures.

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Appendix 1: microsatellite markers sequences and accession number

Table 1a: *Anas Plathyrynchos*

Accession	Locus	Sequence Primer 1	Sequence Primer 2	Microsatellite
FR750405	CAM001	AAGGTCTTTCCAGCCTAAATG	CATCAGCATCAAAGCAGAAC	$(CCTT)^{15}$
FR750406	CAM003	ACAGCCTAAGGTGTTTTTCC	TTTTCCTTTGGTCAGTGTGG	$(AC)^{11}$
FR750407	CAM004	AGCAGCTGAGGACTGTATGC	CAAAGGCACTTTTTGTTTGC	$(AC)^{7}$
FR750408	CAM005	TGAATAGACATTGTACTGCTGAGG	TGAAAGTGAGGACAGAAATCC	$(GT)^{4}$
FR750409	CAM006	GGTAATTCTGGCTTTTGTCC	TGCGAAGAATTAGACTTGAGC	$(GT)^{6}$
FR750410	CAM007	TGTCAGGTGTTGTGAGAAAGG	TGGGAGTTTCAGAAGTTTATGG	$(TG)^{3}TA(TG)^{4}$
FR750411	CAM010	TTGAATTTATCAACTGCCTTAGAA	TCATTGGACTGAGGAAGGTC	$(AC)^{13}$
FR750412	CAM011	AGTATCATTTGCTACTGCACGC	CCAGGGCTTCAAATGGAGAAT	$(AC)^{11}$
FR750413	CAM012	TTGTTTTGGCCTGTCTGTATGT	TGCAAGAGATATGGTGCACAAA	$(CA)^{9}$
FR750414	CAM013	GGAATGATGTTTACAGTTCATAGG	TGCTTTGAAGCACTACATGG	$(GTTT)^{4}$
FR750415	CAM014	GCAGAGAAAATGAAATCCTACC	AGCTTAATTCTGCAAGCCTA	$(TG)^{11}$
FR750416	CAM021	GGGAAGACACAAACAGCCTT	TTTGTCCGCTGCATCCTTG	$(CAGA)^{3}$
FR750417	CAM022	TGGTCACTGCCTTACCATTT	GTTGACTGAGAACATGGCATT	$(AAAC)^{3}$
FR750418	CAM023	GCAAAGACTGGTACTTGGAACA	TCCCTTGCCACTTCTCCTATT	$(TAAA)^{5}$
FR750419	CAM026	GAGGTAGGTCTGCAAAGAAACA	GGGGCGATTGAATGAGTGTAT	$(GGA)^{8}$
FR750420	CAM027	GGGGGAAAGAGAATACGCATAT	CACCAGACCACAAGGACATCT	$(TGAA)^{3}$
FR750421	CAM028	ACAGATGTCAGTACTGCCGG	TTAGGGGCAAGTTAGGAGCC	$(GT)^{3}GA(GT)^{6}$
FR750422	CAM029	ATAGGGCCATGGATTGTTTTGC	CCGGTGGATTTCATGCTATTGT	$(GTT)^{4}$
FR750423	CAM030	GTTGACAGATGCAAGCTGTAAC	GCACGTGAGATGATTCTTTGG	$(GACA)^{4}$
FR750424	CAM031	TTGCTGTTGTAGCTGTGAGATT	GCTTTGCTGCTGTTTATTCAGT	$(AAAT)^{3}$
FR750425	CAM034	AGCGTGTTTCTTTAAGCTCTGT	CAGTGTAATTCCAGCCTGCTG	$(TG)^{6}(GT)^{3}(T)^{4}(GT)^{5}$
FR750426	CAM037	TGGGCTCAGGATTTGAAGAAT	ACCAGTTTGCATGATAGGTGT	$(CA)^{10}$
FR750427	CAM040	CTTCTTGCTGCAGAATGAAT	TCACCAAATTTGAGCTTTGG	$(GT)^{5}AT(GT)^{10}$
FR750428	CAM044	GCATGGGAAGCCAATGTTTT	CGCATGATTTCTGCTTCTCTAT	$(GT)^{13}$
FR750429	CAM047	AGTGACTTGAAGGTGAAGGT	CTCCCCAAACCCCATATAAG	$(TC)^{5}$
FR750430	CAM052	GACTGGCAATCCAAAGCA	CACAGCTCCAGAAACAGTCC	$(GT)^{10}$
FR750431	CAM053	GCCATGGCTCCACAGCAG	ATCTCCCACATTGGGTCACG	$(GT)^{15}$
FR750432	CAM062	TTACTTTGTGTGGTCGTCTT	CTTCCACCTATATGTGAGCTG	$(AC)^{5}AT(AC)^{4}(AT)^{5}$
FR750433	CAM065	CCCATACATGTATATGCTGTGC	CTATGCAATGGGCTTCGTAGT	$(AAGG)^{22}AAAG(AAGG)^{3}$
FR750434	CAM071	GCAATATCCCTGGCAGTACC	ACCAGAAATGAGAAGCAGACC	$(GT)^{16}$
FR750435	CAM073	AGGATGCAGTCTACATTTGCA	CCAGTTTCACCAGTTGAGAAG	$(CA)^{14}$
FR750436	CAM076	CTTCCACAAGTGGCAGTGAC	GGAAGAGGGGATTAGACAAAA	$(GT)^{4}GA(GT)^{12}$
FR750437	CAM080	TCCAAACTTAAGCAATTTTTC	CGGTGACCGACCTACCTA	$(GGAA)^{6}(GA)^{11}$
FR750438	CAM081	CACTTAACACTCCAGACCGATA	TTGGGAATTTGTCTCCTTGT	$(AC)^{10}$
FR750439	CAM082	TTTTCATGCTGTGTTTGAGAA	GGTTCTGGGAAGAAAATCAA	$(TC)^{4}$
FR750440	CAM085	TCCTGAGAAGATGGAAATCTG	TCCTTGTCAGCAATTGAGAA	$(CA)^{8}$
FR750441	CAM087	TCCACTGTGCATTTGAGTAAA	CACTTAAAGCTCAGGCAAGG	$(CA)^{21}$
FR750442	CAM093	TGTATCGACTCATTTTGCTCA	GCCTGACTCTTTTTATTTTCCA	$(CTTC)^{6}(CTTT)^{8}$
FR750443	CAM101	GTCAAGTCAGTGCCTCACAA	GTATCTCTTCCAGGCCAACC	$(TTTTG)^{3}(TTTG)^{2}$
FR750444	CAM103	CGGGAAGCTGTAGATAAGCA	TGGCTGGAGAGAGCACCT	$(T)^{6}A(T)^{5}$
FR750445	CAM106	AACACCACCACCAAAGAAAT	TAAACCGGTGGAATCTTTCT	$(TAT)^{4}$
FR750446	CAM111	GTGGCTAGTGCTGATGAATG	TGAACCACTTTCTCACAGGA	$(GT)^{13}$
FR750447	CAM113	TGCTCGGAGGACTGTTAGAT	GCTGCATGCATCTTCTCTTA	$(GT)^{12}$
FR750448	CAM119	CAATGACCAGAAAAGCAACA	AAGAACTTCCCCCTCTTCTG	$(GT)^{10}$
FR750449	CAM121	ACATCTGGAAAAAGCCAGAG	CATTTCAGGTCCTGTTTCCT	$(AG)^{9}$
FR750450	CAM127	TGTAGACTGAAGTGCAACCAA	AGGAATCATGCTGGAAAAAT	$(AT)^{7}$
FR750451	CAM128	CAGCCCTAGAGCTCAAAAAG	GCACTTAAGAGCATGCACAC	$(CA)^{15}$
FR750452	CAM130	TTGCTCCCTTTTGTAGTTCC	GTGGTGGATTGCACTGTAAG	$(ATTT)^{5}$
FR750453	CAM131	CAACGTTCCTATGTCTCACG	AGCTACAGGGTAACTGCACA	$(GT)^{10}$
FR750454	CAM133	CTGTACTTACCCCTGCCATC	CTTTCCTAACCTGCGTTTTC	$(CA)^{10}TA(CA)^{5}$
FR750455	CAM134	ACCCAAGATTTCAGTCTCCA	CAGTATCCAGAGGTGGAAGC	$(CT)^{12}$
FR750456	CAM135	CCATTCAAAGACTCAATGGTT	TGTACACTCACATAGAAGAA	$(TTCC)^{3}CTCC(TTCC)^{5}$ & $TTCT(TTCC)^{7}$
FR750457	CAM138	AGGTTCAAAAGGCAGTTGTC	GCTGAAGCTTGGAGAATACC	$(AAAC)^{2}GAAC(AAAC)^{3}$
FR750458	CAM150	TGCACGTGGTATTTTACACA	GATGCTCTGCTCATACCTGA	$(AG)^{5}$
FR750459	CAM151	GTATGCTTTGTGGTCCATGA	TGCTTGAGATGAAACACTCC	$(GA)^{3}A(GA)^{3}$
FR750460	CAM153	GAGATTTCCAGCTTGCATTT	GGAAGCAGAAGTCTGAAAAGA	$(TG)^{6}T(TG)^{3}$
FR750461	CAM155	GGCAGCAAATTAAGGTTGAC	GACAAGGCTATCCTGGCTTA	$(TG)^{14}$
FR750462	CAM157	ATAACGGGAATCAGCTCTTG	GGGGATGGTTCACATTTTTA	$(CT)^{6}TTTCC(CT)^{3}$
FR750463	CAM158	TGCACTCAGGTTTTCTTTGA	ATCCTTTGCCTGCTTGATAA	$(TC)^{12}$
FR750464	CAM161	TAAAGCAGAGCCAGGCAGT	CACTGAGACACTTTTGTGCAG	$(GGAA)^{16}$

Table 1a: *Caïrina Moschata*

Accession	Locus	Sequence 1	Sequence 2	Microsatellite
FR750465	CAM002	TTTCATTCCCTCCCCATC	CGGGTTTATAATATCATTTTGAGG	$(AC)^{8}$
FR750466	CAM009	ACCTGGCTACAAAGCATCAC	AATAGATGGACAAAAGATGCAA	$(GGAA)^{13}$
FR750467	CAM015	CCACATGTCACTTTGTGAACAC	TGGAACGTGTCAGGTGGAA	$(CA)^{14}$
FR750468	CAM018	TCTCCTACTTTCTGCTTACATGG	AATTTTGCCAGGCACCTATG	$(GT)^{12}$
FR750469	CAM019	TGGTCACCCTTTTCCATCTT	CATTCTGTCTCGTTTACTTTCTCC	$(CA)^{10}$
FR750470	CAM020	AGCAACTGAGCTACCAGAAAAA	GTCTCCACCAGCCTATTTCTG	$(CA)^{9}$
FR750471	CAM025	TGAGGGTTGACTTTTAAGGCTT	TGTGGCACTAGACTTTGCAAA	$(AATT)^{3}$
FR750472	CAM032	GTGTTGACTAGCTCTCCGTTTA	GACGCTCACGAACACTTAGG	$(ATTT)^{2}$
FR750473	CAM033	TCCAACCCAAAGGTCTCTTCA	GCTTCATAGAGGCCCAGGTT	$(AAAAAAG)^{3}$
FR750474	CAM035	GCCCCACTAGGGTTGGTAAG	CTTCTGGTCGTCTCCTGGTA	$(GT)^{10}$
FR750475	CAM036	GGTCAGAGTCAAATACGGAGTC	GCACTCCACCCACTTGTTT	$(CA)^{5}AA(CA)^{7}$
FR750476	CAM039	CTGAGAGGCCCCTGCTGT	CACAGCTCCTCCCCAGGT	$(CA)^{3}TG(CA)^{5}CGCA$ &$(CCCA)^{3}CA(CCCA)^{3}(CA)^{8}$
FR750477	CAM041	AACCAAGAAGAATTTCAGCAAA	ATTGCAGCAGGAACTTCATT	$(AAGG)^{7}$
FR750478	CAM042	CTGTGTCTGTGTGAAGCCTA	CCCCATGGAAAGAACTGACAAA	$(TG)^{10}$
FR750479	CAM043	TTACCAGCTCTTGGAAAAT	AAGCATTTGCTTCTTGTGAC	$(ATT)^{3}$
FR750480	CAM045	CTTACCCCTGGAAACTCCTT	TCACGAGAAAAGAAATGCAG	$(GGAA)^{14}$
FR750481	CAM046	GCTGCTTTCTGTTCATTTAGCT	TCTGACCACATGCTTTCTCAT	$(AACATTTGAAG)^{2}$
FR750482	CAM048	GAGGCAGTTGGTTAGTGGTT	CAAATGCCCACAATATAGCA	$(GCCT)^{4}(TCCT)^{6}$ & $(TACT)^{2}(TCCT)^{4}$
FR750483	CAM049	TCCTTGCTCTCCTGCTTCTG	ACCTCACAGTAGCAGAAAAC	$(GATTTGAG)^{2}$
FR750484	CAM054	CTCAGTAAACCTGGTACCTT	CTGTGCTAAGTAACAGCTCAC	$(TG)^{10}$
FR750485	CAM055	TCATTAAAACAAAAATAAAATGCAG	CAAGCAAATGTTCGTGTGTT	$(TG)^{6}TA(TG)^{11}$
FR750486	CAM057	TTCTCAGTTTGCTTGCTCTG	CGTGGACGTATAAACTGCTG	$(TCTCT)^{2}$
FR750487	CAM058	TTCTGTATGGAAAGCAAGATTAAC	ATCGAGCATGTGGAGATTTT	$(TTCC)^{10}$
FR750488	CAM061	CAACTCACAGGTTGAGATAAGG	ACGTGGTCAATCATCTCTCATT	$(AAT)^{5}$
FR750489	CAM063	CGGTTTGCTGTATTTTCACT	GGGAGAGTTGTAAAATGAAGC	$(CT)^{11}$
FR750490	CAM064	CAGGTTGCAAGTCAACAGAC	GTCCTGGAAGTGAAGAGAAGT	$(CA)^{10}$
FR750491	CAM067	GCAAACCGATAACAATGGGA	TAGAGCCTCATGGTACAGGC	$(AAGG)^{3}$
FR750492	CAM068	AGAGCAGAGATTGGGGCAAT	TTTGTCAGCCCTTGGGACA	$(TG)^{9}$
FR750493	CAM070	TGGGTAGTCAATTTGCCTTAGT	CTACAGCTGGTGGGACTATAG	$(TG)^{9}$
FR750494	CAM072	TTTACACAGCTAGAAGGCTTGA	TGGCTCCACATTAACAGATG	$(ATTT)^{5}$
FR750495	CAM088	TGCGTCAACAGCTACATACA	ATAGCAGCCATTCCCATAGA	$(TTG)^{5}$
FR750496	CAM092	GTGCAGACTTGCTGAAAGAA	TGCAGTCTGTAGCTTGTGGT	$(GTT)^{5}$
FR750497	CAM096	CAGCATGAGATTGTTTAGAGGA	AAGCTTTGTGCAATTGATGA	$(AG)^{15}(AAAG)^{20}$
FR750498	CAM098	TAACAGCAGCAAGTCGAAAG	CTTCGGTGCTCCTAGAGGT	$(CA)^{7}CC(CA)^{3}CC(CA)^{6}$
FR750499	CAM099	TCATTTGGGAAGGTCTCAAC	CTCGGAACTCAATGTTAGCC	$(GA)^{14}$
FR750500	CAM100	AAATGTTATTCTCCCGTTGG	GAACAGACCTCTGCCAAAAG	$(TTTTA)^{4}$
FR750501	CAM104	GGAACCTACCGCAATTTATG	TCTCCCTGTTGACAGGTAATC	$(AAC)^{3}$
FR750502	CAM107	GCCCAAATTTAAAGGTTCTG	GAAAGAGGGAGAGTGTGAGG	$(CTTT)^{3}(CT)^{13}$
FR750503	CAM108	CCAAAAGGTAACCAAGGAAC	TGTTGACCAGGAAAATATGG	$(CCTT)^{13}$
FR750504	CAM110	TGTGTGTGACAAACCTCTGA	TGTTTTCCTTTTCAAGCACA	$(AC)^{13}$
FR750505	CAM117	ATGATGCATGAAGCAGAGTG	CCTAAGGTTTGCACAGGTTT	$(GT)^{10}$
FR750506	CAM118	GTGCAGACTTGCTGAAAGAA	TGCAGTCTGTAGCTTGTGGT	$(TG)^{6}$
FR750507	CAM120	TCTCCCAAAGAAAAATCTGG	AGAGGGAGAAAGAGTGAGAGC	$(CCTT)^{19}$
FR750508	CAM124	TCAACTTTGCAAACATAGGG	TTAGTCCCTTTCCCAACAGA	$(AG)^{20}(AAAG)^{5}$
FR750509	CAM126	AATGCTCCTTCTCTGAGCTG	GGGTATTCCATGCTGCTATT	$(AG)^{5}$
FR750510	CAM129	GAAGCTGTTTGTGTTTGCAC	TGTGTAGCTGCATGTTTCCT	$(CCTT)^{22}$
FR750511	CAM132	CAATCTGCTGCTTCTTCTGA	AGCCAGCTGCAAAGAGAATA	$(TTCC)^{21}$
FR750512	CAM136	GAAACAAAGGGCGACATAAT	ATCTGATGGGGTTTTGACAC	$(GA)^{12}$
FR750513	CAM137	CCTTGCTGAGGTCTAAATCC	TGTAAAAGCCATTGTGGTCA	$(TG)^{11}$
FR750514	CAM139	ATGGCAATGGTTGTCCTACT	TTCTGAACTTGCATTGTTTGA	$(TTCC)^{20}$
FR750515	CAM141	TAGCCTAAGAAGAGCCCAGA	TTAAATGCTTGGCTGAACTG	$(AG)^{8}$
FR750516	CAM142	TTCATCTTGAGGTACCTTGG	CATGTTCCTGAGAAGCACAG	$(GGAGA)^{2}$
FR750517	CAM143	CTTTAACACCAGTGGGTTCC	CCCTTGACAATTAGCCTTTG	$(ATT)^{3}G(ATT)^{2}$
FR750518	CAM144	AATGCCTTGTGGAAACTGAT	TTTTATTGGGCAACCTAACC	$(GTTTT)^{6}$
FR750519	CAM146	TGGAAGCAAGATATGTGAGC	TGCAGGATTAATGTCAGCAT	$(TTCC)^{27}$
FR750520	CAM147	TTGTAGCTCCCTGCACAGTA	GGTTCTCTGAGTTTCCTTGC	$(CTG)^{4}$
FR750521	CAM149	AAGCCATAGATTCCTCAGATG	ATATGCTGTGTGCTGGAAAA	$(AAGG)^{29}$
FR750522	CAM152	GAATTAAAATCCCTTTGCTTCT	AAGACATGCAGGCTATTTCA	$(CTCCT)^{8}$
FR750523	CAM154	CGATGAATGCAGTCTCTGAC	AGGTACATCTCACAAGCAACC	$(AT)^{6}$

Appendix 2: Tables representing the correlation coefficients between the different traits studied classified by their physiological functions.

Table 1a: growth traits

	BWG 12-28	BWG 12-42	BWG 28-42	BWG 12-70	BWG 28-70	BWG 42-70	BW12	BW28	BW42	BW70
BWG12-28	1	**0.87**	0.50	0.58	0.19	-0.07	**0.62**	**0.92**	**0.92**	**0.70**
BWG12-42		1	**0.86**	**0.72**	0.42	0.01	0.38	**0.72**	**0.93**	**0.75**
BWG28-42			1	**0.66**	0.54	0.08	0.01	0.31	**0.68**	0.58
BWG12-70				1	**0.90**	**0.69**	0.26	0.49	**0.67**	**0.96**
BWG28-70					1	**0.87**	-0.01	0.11	0.33	**0.79**
BWG42-70						1	-0.03	-0.05	0.00	**0.60**
BW12							1	**0.87**	**0.68**	0.53
BW28								1	**0.91**	**0.70**
BW42									1	**0.80**
BW70										1

Table 1b: traits related to stress

	cortL	cortH	deltaC
cortL	1	0.42	0.23
cortH		1	**0.98**
deltaC			1

Table 1c: metabolic traits

	CHO 2^{nd}M	CHO 10^{th}M	CHO 20^{th}M	TG 2^{nd}M	TG 10^{th}M	TG 20^{th}M	GLU 2^{nd}M	GLU 10^{th}M	GLU 20^{th}M
CHO 2^{nd}M	1	0.36	0.37	0.22	0.19	0.00	0.11	0.02	0.09
CHO 10^{th}M		1	0.39	0.09	0.27	0.16	-0.09	0.15	0.25
CHO 20^{th}M			1	0.28	0.22	0.31	0.02	0.13	0.34
TG 2^{nd}M				1	0.37	0.25	0.30	0.19	0.18
TG 10^{th}M					1	0.38	0.10	0.19	0.32
TG 20^{th}M						1	0.16	0.12	0.37
GLU 2^{nd}M							1	0.12	0.06
GLU 10^{th}M								1	0.31
GLU 20^{th}M									1

Table 1d: carcass traits

	FLW	AFW	TSW	pmMW	pmSFW	BWbeg	BWend	CW
FLW	1	0.13	-0.12	-0.12	0.07	-0.05	0.34	0.35
AFW		1	0.44	0.20	0.58	0.49	0.52	0.56
TSW			1	0.48	0.52	**0.70**	**0.68**	**0.67**
pmMW				1	0.28	**0.69**	0.57	0.54
pmSFW					1	0.51	0.57	0.59
BWbeg						1	0.81	**0.80**
BWend							1	**0.97**
CW								1

Table 1e: product quality traits

	Fmax	Energy	MWC	MLipC	Mb*	ML*	Ma*	MpH20	MpHu	MvacL	MCookL	MR	Lb*	LL*	La*	LProtC	LLipC	LColC
Fmax	1	**0.82**	-0.01	-0.20	0.25	-0.47	0.49	-0.35	0.20	0.21	-0.18	0.22	-0.21	0.04	0.13	0.38	0.07	-0.15
Energy		1	-0.07	-0.10	0.29	-0.38	0.47	-0.22	0.12	0.13	-0.16	0.19	-0.21	0.04	0.11	-0.35	0.06	-0.11
MWC			1	**-0.79**	-0.47	0.00	-0.30	0.23	0.02	0.39	0.12	-0.12	-0.11	-0.09	0.08	0.13	-0.03	0.15
MLipC				1	0.36	0.41	-0.04	-0.25	-0.14	-0.38	0.18	0.09	0.25	0.20	-0.22	0.02	0.11	-0.14
Mb*					1	-0.29	**0.68**	-0.12	0.26	-0.10	-0.38	0.08	-0.24	-0.02	0.15	-0.37	-0.08	-0.06
ML*						1	**-0.84**	-0.07	-0.45	-0.14	0.65	-0.11	0.40	0.23	-0.38	0.47	0.15	0.00
Ma*							1	-0.08	0.43	0.01	**-0.66**	0.16	-0.43	-0.12	0.33	-0.55	-0.12	-0.05
MpH20								1	-0.08	-0.02	-0.05	-0.10	-0.17	-0.17	0.10	0.06	-0.08	0.10
MpHu									1	0.01	-0.49	0.03	-0.30	-0.10	0.15	-0.32	-0.04	0.03
MvacL										1	-0.03	0.07	-0.18	0.04	0.06	-0.17	0.08	-0.06
MCook											1	-0.07	0.39	0.17	-0.27	0.40	0.12	0.01
MR												1	-0.15	0.38	-0.35	**-0.75**	**0.79**	-0.38
Lb*													1	-0.05	-0.13	0.43	0.05	-0.04
LL*														1	**-0.79**	-0.37	0.46	-0.41
La*															1	0.18	-0.46	0.32
LProtC																1	**-0.63**	0.42
LLipC																	1	-0.42
LColC																		1

Appendix 3: QTL detection in single trait analysis with *P*-value ranging from 1% to 5% chromosome-wide level

Growth traits

LG	Traits[1)]	Flanking markers[2)]	Location[3)] (cM)	LRT_x[4)]	*P*-value[5)]	Effect of QTL substitution[6)]	Confidence interval (cM)
1a	Ti	APT015	36	20.55	0.050	0.49	20 - 43
2a	BW70	APT003-CAM096	64	20.38	0.031	0.58	35 - 75
2a	BWG12-70	APT003	63	19.86	0.036	0.57	33 - 75
2a	BWG12-28	APT009-APT003	61	17.74	0.050	0.49	37 - 75
2a	BWG12-42	APT003-CAM096	66	17.18	0.050	0.48	35 - 75
2a	BWG28-42	APT003-CAM096	66	16.26	0.050	0.5	31 - 75
2a	BWG28-70	APT003	63	16.95	0.050	0.56	32 - 75
2a	BW42	CAUD089-APT009	59	17.69	0.050	0.46	37 - 75
3	BWG12-42	CAUD084-CAUD045	62	23.25	0.018	0.31	41 - 74
7	BWG42-70	APH013	0	18.32	0.024	0.75	0 - 13
14	BW12	CAUD137	24	17.56	0.033	0.32	0 - 24
20	BW_A	CAUD099	0	15.57	0.016	0.55	0 - 16
28	BW70	APH019	5	13.72	0.050	0.61	0 - 27

1) See Table 1 for the definitions of the traits.
2) Flanking markers of the most probable QTL position.
3) Most probable QTL position.
4) Maximum likelihood ratio for x locus.
5) Level of significance of P-value at chromosome-wide.
6) QTL effect in phenotypic deviation units (SD), and estimated as $\alpha = \frac{1}{SD} \times \frac{1}{n} \sum_{i=1}^{n} |\alpha_i|$.

Metabolic and body weight traits during the overfeeding phase

LG	Traits[1)]	Flanking markers[2)]	Location[3)] (cM)	LRT_x[4)]	*P*-value[5)]	Effect of QTL substitution[6)]	Confidence interval (cM)
1b	DFI	CAUD039-CAM029	8	19.70	0.032	0.21	0 - 16
1b	FC 10^{th}M	CAM093	37	23.60	0.013	0.25	26 - 39
2a	CortL	CAM071-CAUD065	10	19.91	0.035	0.24	0 - 31
2a	GLU 2^{nd}M	CAM071	0	20.84	0.028	0.23	0 - 18
2c	CHO 2^{nd}M	CAM005	1	15.41	0.030	0.13	0 - 1
2c	FC 2^{nd}M	CAM005	1	13.57	0.050	0.17	0 - 1
3	CHO 10^{th}M	CAUD084-CAUD045	71	22.35	0.024	0.48	47 - 82
6	GLU 2^{nd}M	CAUD064-CAUD026	1	13.45	0.044	0.24	0 - 20
7	BWbeg	APH013	0	19.29	0.018	0.29	0 - 25
7	FO 2^{nd}M	APH013	0	18.11	0.025	0.21	0 - 26
9	OWG	CAUD038	20	17.12	0.044	0.16	9 - 20
14	CHO 10^{th}M	CAUD013-CAUD137	7	17.77	0.031	0.64	0 - 24
14	CortL	CAUD137	24	15.92	0.050	0.11	0 - 24
14	TG 2^{nd}M	CAUD013-CAUD137	7	17.08	0.038	0.14	0 - 22
21	CHO 10^{th}M	CAUD037	2	18.59	0.014	0.64	0 - 2
21	FO 10^{th}M	CAM004	0	18.60	0.014	0.25	0 - 2
21	BWend	CAM004	0	19.60	0.010	0.14	0 - 2
21	TG 10^{th}M	CAUD037	2	15.19	0.045	0.30	0 - 2
28	GLU 2OthM	APH019-CAUD040	26	15.73	0.030	0.29	0 - 27
28	BWbeg	APH019-CAUD040	14	13.60	0.050	0.26	0 - 27

1) See Table 1 for the definitions of the traits.
2) Flanking markers of the most probable QTL position.
3) Most probable QTL position.
4) Maximum likelihood ratio for x locus.
5) Level of significance of P-value at chromosome-wide.
6) QTL effect in phenotypic deviation units (SD), and estimated as $\alpha = \frac{1}{SD} \times \frac{1}{n} \sum_{i=1}^{n} |\alpha_i|$.

The overfeeding ability and product quality traits

LG	Traits[1)]	Flanking markers[2)]	Location[3)] (cM)	LRT_x[4)]	*P*-value[5)]	Effect of QTL substitution[6)]	Confidence interval (cM)
1a	Lb*	APT008-CAUD058	55	21.77	0.037	0.15	50 - 89
1a	MLipC	CAUD095	0	20.35	0.050	0.08	0 - 5
2a	La*	CAM071-CAUD065	2	19.64	0.038	0.28	0 - 27
2a	Mb*	CAM071-CAUD065	6	19.56	0.039	0.24	0 - 27
2a	pmMW	APT002-CAUD070	40	21.27	0.025	0.16	37 - 49
2c	Lb*	CAM005	1	14.53	0.029	0.15	0 - 1
2c	MR	APH012	0	16.60	0.018	0.15	0 - 1
2c	LColC	APH012	0	14.28	0.044	0.15	0 - 1
2c	MpHu	CAM005	1	14.92	0.037	0.10	0 - 1
3	Lb*	AMU060-CAM124	2	21.26	0.033	0.11	0 - 17
6	MpH20	CAUD064-CAUD026	15	13.31	0.046	0.24	0 - 25
7	Ma*	AMU103	31	15.34	0.050	0.02	20 - 31
7	MpH20	APH013	0	18.56	0.022	0.22	0 - 13
9	LLipC	CAUD088	0	21.43	0.011	0.21	0 - 20
9	MR	CAUD088-AMU068	5	17.30	0.042	0.18	0 - 20
14	LL*	CAUD013	0	18.18	0.026	0.25	0 - 10
21	LColC	AMU111	1	16.48	0.030	0.16	0 - 2
21	Fmax	CAUD037	2	15.25	0.045	0.01	0 - 2
Z	MpHu	CAM113	15	20.44	0.019	0.01	9 - 15

[1)] See Table 1 for the definitions of the traits.
[2)] Flanking markers of the most probable QTL position.
[3)] Most probable QTL position.
[4)] Maximum likelihood ratio for x locus.
[5)] Level of significance of P-value at chromosome-wide.
[6)] QTL effect in phenotypic deviation units (SD), and estimated as $\alpha = \frac{1}{SD} \times \frac{1}{n} \sum_{i=1}^{n} |\alpha_i|$.

4.2 QTLs significatifs à 5% au niveau du chromosome

Dans l'article 2, seuls les QTLs significatifs au seuil de 1% au niveau du chromosome sont discutés. Néanmoins, 52 autres QTLs significatifs entre 5% et 1% au niveau du chromosome, obtenus via l'approche uni-caractère et listés dans l'appendix 3 de l'article 2 seront discutés ici.

Pour garder une cohérence dans les notations, les noms des caractères sont toujours en anglais. Aussi, la nomenclature des groupes de liaison et des marqueurs est homogénéisée avec celle de l'article 2.

4.2.1 Les caractères de croissance

Treize QTLs de croissance, significatifs à 5% au niveau du chromosome ont été détectés (appendix 3 – tableau 1). Ces QTLs sont répartis sur 6 groupes de liaisons, mais le GL2a regroupe à lui seul 7 de ces QTLs. Chez la poule, le chromosome homologue Gallus Gallus 2 est connu pour être impliqué dans la variabilité des caractères de croissance. Par exemple Zhou *et al*. (2006) ont mis en évidence plusieurs QTLs pour des caractères de croissance (poids à 42 jours d'âge, ou gain de poids entre 14 et 28 jours d'âge ou entre 28 et 42 jours d'âge) sur le chromosome GGA2. Sur ce même chromosome Ou *et al*. (2009) ont identifié un QTL impliqué dans la variabilité du poids à 70 jours d'âge.

Contrairement aux analyses uni-caractères, aucun QTL n'est mis en évidence sur le groupe de liaison 2a avec les analyses multi-caractères. Sachant que seuls les QTLs significatifs au seuil de 1% ont été retenus dans le cadre de ces dernières analyses, on peut conclure que le regroupement des caractères (quelque soit le critère de regroupement) n'a aucun apport en termes de puissance par rapport aux analyses uni-caractères.

4.2.2 Les caractères liés au métabolisme durant le gavage et les caractères liés au stress (taux de corticostérone avant et après stress)

Onze QTLs significatifs à 5% au niveau du chromosome ont été détectés pour des caractères liés au métabolisme durant le gavage (appendix 3 – tableau 2). Il s'agit des taux de cholestérol, de glucose et de triglycéride dans le sang et le taux basal de corticostérone. Des QTLs ont été identifiés pour tous ces caractères chez la poule. En effet, Zhou *et al*. (2007) et Nadaf *et al*. (2009) ont respectivement mis en évidence des zones chromosomiques impliquées dans la variation du taux de glucose dans le sang sur les chromosomes GGA2 et GGA6. Les QTLs obtenus pour le taux de triglycérides ne semblent pas être connus chez la poule et seul un QTL pour le taux de cholestérol sur le groupe de liaison 3 est déjà identifié chez la poule par Park *et al*. (2006). Aucun QTL pour le taux basal de la corticostérone n'est identifié pour la poule sur les chromosomes GGA2 et GGA13, chromosomes qui correspondent aux groupes de liaison de nos deux QTLs pour ce caractère.

4.2.3 Les caractères d'aptitude au gavage

Dix QTLs significatifs à 5% au niveau du chromosome sont détectés pour des caractères d'aptitude au gavage des animaux (Appendix 3 – tableau 2).

Hormis le poids de foie mesuré sur des canards maigres ou sur du poulet, les caractères d'aptitude au gavage ne peuvent pas être comparés avec les résultats des poules, celles-ci n'étant pas gavées. Dans notre analyse aucun QTL de poids de foie gras significatif entre 1 et 5% au niveau du chromosome n'a été détecté.

4.2.4 Les caractères de qualité de produits

Dix-huit QTLs significatifs à 5% au niveau du chromosome, contrôlant des caractères de qualité du foie gras et du magret sont présentés dans le tableau 3 de l'appendix 3. Dix de ces QTLs sont relatifs à la qualité du foie gras et 8 à celle du magret.

Les groupes de liaison 2c et 9 semblent être des GL d'intérêt pour la qualité du foie gras car en plus des QTLs significatifs détaillés dans l'article 2, on y retrouve 5 QTLs significatifs à 5% au niveau de chromosome pour ce type de caractère : 3 QTLs relatifs au taux de fonte, au taux de collagène et à la coloration jaune apparaissent sur LG2c et 2 QTLs impactant le taux de fonte de le taux de lipide du foie sur LG9.

Au-delà de LG2c, 2 autres QTLs significatifs à 5% au niveau du chromosome sont cartographiés pour la coloration jaune du foie (GL1a et GL3). Ce caractère associé à l'extension des taches due aux viscères permet d'améliorer en puissance et en précision le QTL sur le GL3 dans l'analyse multi-caractère (Lb* et ESV). À ce jour, le seul QTL identifié chez la poule pour le caractère de coloration du foie l'est sur le chromosome GGA4 (Wright *et al*., 2006).

Sur le groupe de liaison 2a, en plus des 3 QTLs significatifs à 1% au niveau du chromosome, 2 QTLs pour des caractères de qualité de magret (pour la coloration jaune du magret et le poids du magret) significatifs à 5% sont détectés. On détecte aussi sur le GL7, deux QTLs significatifs à 5% au niveau du chromosome pour la coloration rouge du magret et le pH de celui-ci 20min post mortem. A noter qu'aucun QTL significatif à 1% n'est cartographié sur le GL7 pour les caractères de qualité du magret.

Les chromosomes GGA2 et GGA7 semblent être des chromosomes d'intérêt pour les caractères de qualité de la viande de volaille. En effet, trois QTLs impliqués dans la variabilité du pH (pH mesuré 20 minutes *post mortem* et le pH ultime) dans le muscle et dans la coloration du magret sont identifiés chez la poule (Wright *et al*., 2006). Nadaf *et al*. (2007) ont confirmé le QTL obtenu sur GGA2 pour le pH de la viande. Pour la coloration de la viande, le QTL identifié sur le groupe de liaison 2a semble être connu chez la poule : Van Kaam *et al*, 1999 ont mis en évidence une zone chromosomique impliquée dans la variabilité de la coloration de la viande de la poule sur GGA2.

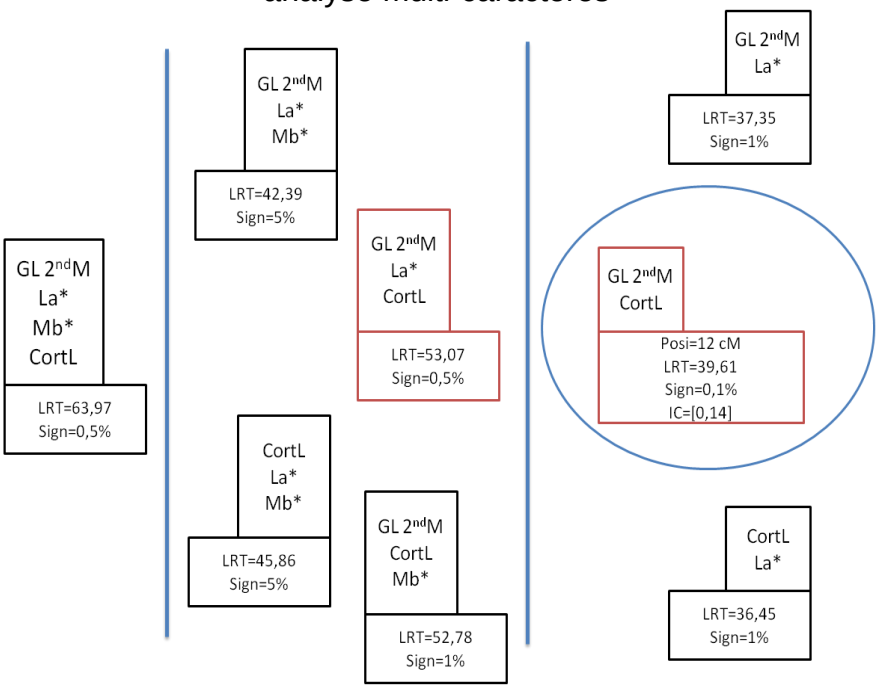
4.3 Les analyses multi-caractères

Comme décrit dans l'article, une analyse multi-caractères a été menée en complément de l'analyse uni-caractère. Les caractères ont été regroupés suivant deux critères : i) caractères très corrélés phénotypiquement, ii) caractères présentant des QTL à 1% co-localisés. L'objectif de cette approche multi-caractère était d'identifier les QTLs pléiotropes. Les résultats de ces analyses multi-caractères ont été présentés dans l'article 2, mais la démarche suivie pour aboutir à ces résultats mérite d'être détaillée.

Initialement, des groupes de 2, 3, 4 ou 5 caractères sont constitués selon les critères précédemment décrits. Lorsqu'il y a N caractères impliqués (avec N supérieur à 2), il est nécessaire de savoir combien de caractères parmi ces N caractères sont impliqués dans la pléiotropie. Des détections de QTL sont effectuées par étape afin d'identifier la combinaison des caractères pour lequel le QTL est le plus significatif. On retiendra au final ce sous-groupe de caractères.

Ci-dessous l'exemple du GL2a est détaillé (figure 4.8). Initialement, 4 QTLs à 5% étaient co-localisés en début de groupe de liaison grâce aux analyses uni-caractères (tableaux 2 et 3 de l'appendix 3) et concernaient les caractères de taux de glucose sanguin au 2ème repas de gavage, le taux de corticosterone sanguin basal à 6 semaines d'age, l'indice de rouge des foies gras et l'indice de jaune des magrets.

Figure 4.8 : Exemple de la démarche suivie pour arriver au résultat final d'une analyse multi-caractères



Description : La première colonne correspond à la première étape de l'analyse où tous les caractères sont analysés ensemble, la deuxième colonne correspond à l'étape de l'analyse où l'on considère les caractères 3 par 3 et la dernière colonne correspond à l'étape de l'analyse où l'on décortique, en effectuant des analyses bi-caractères, le groupe de caractère qui possède le plus grand LRT dans l'étape

précédente. Chaque analyse est représentée ici par deux rectangles. Dans le rectangle du haut sont recensés les caractères analysés ensemble et dans celui du bas on trouve les résultats de l'analyse (maximum LRT et la significativité atteinte).

Dans la première colonne l'analyse simultanée de tous les caractères ayant des QTLs co-localisés en uni-caractère (au seuil de 5 % au niveau du chromosome), soit 4 caractères différents, est effectuée. Puis, les 4 combinaisons de ces caractères pris 3 par 3 font l'objet de nouvelles détections multi-caractères (résultats dans la deuxième colonne) : sera retenu le sous-groupe qui présente la plus grande valeur LRT à condition que la significativité de ce QTL soit supérieure à celle du QTL obtenu pour le groupe complet (à significativité égale, on retient le groupe qui possède le moins de caractères). Ce sous-groupe de 3 caractères fera à son tour l'objet du même traitement : les 3 combinaisons de ces caractères pris 2 à 2 seront testées. Au final, l'association de GL 2^{nd}M et CortL permet d'obtenir un QTL significatif à 0,1% au niveau du chromosome (et 5% au niveau du génome).
On conclut ici, qu'il existe un QTL pléiotrope qui impacte préférentiellement le taux de glucose dans le sang au deuxième repas de gavage (GL 2^{nd}M) et le taux de corticostérone basale (CortL) en élevage avant stress (cf l'article 2).

Remarque : s'agissant d'un groupe constitué avec le critère de co-localisation, les autres combinaisons à deux caractères ont aussi fait l'objet d'analyses QTL pour voir s'il n'y avait pas un deuxième QTL pléiotrope. Ces analyses n'ont donné aucun QTL.

Cette démarche permet au final de retenir le groupe de caractères qui permet de maximiser la significativité du QTL et/ou de réduire l'intervalle de confiance. Elle nous a aussi permis d'épurer des groupes, les caractères qui n'apportent rien en termes de significativité et/ou de précision à l'éventuel QTL pléiotrope. Pour les groupes de caractères formés sur le critère « niveau de corrélation, cette méthode a permis de mettre en évidence des nouveaux QTLs (par rapport à l'analyse uni-caractère). En effet, la combinaison des caractères a dans ce cas permis de mettre en évidence un QTL pléiotrope alors qu'aucun des caractères composant ce groupe, pris isolément ne franchissait le seuil de 5% au niveau du chromosome. Le principal inconvénient est la multiplicité des détections de QTL engendrée par cette approche (au moins 8 analyses pour un groupe de 4 caractères), qui risque de générer la création de faux-positifs.

Toujours sur le même groupe de liaison GL2a mais dans la partie distale, les analyses uni-caractères ont permis de cartographier 7 QTLs relatifs à des caractères de croissance (tableau 1 Appendix 3) et significatifs à 5% au niveau du chromosome à des positions très proches (tous dans l'intervalle [59cM-66cM]). Cependant aucune analyse multi-caractère n'a permis d'améliorer en terme de significativité ces résultats, ce qui nous laisse penser qu'il ne s'agit pas d'un QTL pléiotrope mais de plusieurs QTLs positionnés dans cette courte portion du génome. Les corrélations très fortes (pouvant atteindre 0,96) entre les caractères de croissance peuvent aussi expliquer le peu d'apport des analyses multi-caractères : l'apport d'information d'un deuxième caractère très fortement corrélés au premier est faible.

L'approche multi-caractère est aussi un outil permettant de renforcer nos résultats uni-caractères. Il en a été ainsi des groupes de liaison 2c et 9 qui semblent être très impliqués dans la variabilité de la qualité du foie. En effet on détecte dans le

cadre des analyses uni-caractères 6 QTLs relatifs à la qualité du foie sur le GL2c (qui sont le poids de foie, les taux de lipides, proteines et collagène dans le foie, le taux de fonte et l'indice de jaune) et 5 QTLs sur le GL9 (qui sont le poids de foie, l'indide ce rouge, les taux de protéines et lipides ainsi que le taux de fonte). Ces QTLs sont confirmés par des analyses multi-caractères, les regroupements de ces caractères ont même permis de renforcer les significativités des QTLs (tableau 6 de l'article 2). Néanmoins, ces deux groupes de liaison sont très faiblement couverts par des marqueurs dans notre étude : deux marqueurs distants de 1cM pour le GL2c et trois marqueurs répartis sur 20 cM pour le GL9. Ces deux groupes de liaison devront donc être densifiés en marqueurs pour confirmer (ou infirmer) nos résultats.