| 1 | On-farm hatching and contact with adult hen post hatch induce sex- | |
|----------|---|---|
| 2 | dependent effects on performance <u>, health and robustness in broiler</u> | a supprimé: and welfare |
| 3 | chickens | |
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| 16 | | |
| 17 | | |
| 18 | Abstract | |
| 19 | To improve the early perinatal conditions of broiler chicks, alternative hatching systems | |
| 20 | have been developed. On-farm hatching (OFH) with an enriched microbial and | |
| 21 | stimulating environment by the presence of an adult hen is a promising solution. Day- | |
| 22 | old chicks were allotted within five hatching and rearing conditions: OFH, conventional | a supprimé: certified JA 757 |
| 23 | hatchery (CH), CH and post-hatching treatment with antibiotics (CH + AB), as well as | a supprimé: different |
| 24 | both hatching systems with an adult hen at hatching (OFH + H, CH + H). <u>To challenge</u> 1 | |

| 29 | the robustness of chickens, they, were exposed on D27 to suboptimal rearing | | a supprimé: On day (D) 27, |
|----|---|-------|--|
| 20 | conditions has explaining for 4 h to prove the house in a new rest of burner to be | | a supprimé: chickens |
| 30 | conditions by combining for 4 n transport in boxes in a new room at a lower temperature | | a supprimé: challenged |
| 31 | and fasting, On their return to the original room, the chicken density was increased, | ***** | a supprimé: for 4 h |
| 32 | and birds were orally vaccinated with the Gumboro vaccine. The impacts of these | | |
| 33 | conditions on hatchability, chick quality score, performance, health and robustness | | |
| 34 | were determined. The OFH chick body weights (BWs) were significantly greater than | | |
| 35 | those of CH chicks at hatching. Whereas there was no effect of hatching conditions, | | |
| 36 | the presence of hens_decreased the hatchability rate, the quality score of OFH chicks | ***** | a supprimé: , categorised according to their behaviour, |
| 37 | and increased mortality at hatching. Treatment of CH chicks with antibiotics (CH + AB) | | |
| 38 | temporarily decreased chicken BW at D19, but the feed conversion ratio (FCR) was | | |
| 39 | not modified. At D19, OFH chicks had the <u>highest BW compared to the other groups</u> , | | a supprimé: best |
| 40 | and the presence of hens at hatching harmed chicken BW regardless of the hatching | | |
| 41 | condition and FCR. An interaction between the effect of experimental rearing | | a supprimé: hatching |
| 42 | conditions and chicken sex was observed later \underline{for}_{r} BW. In males, the OFH chickens | ***** | (a supprimé: in |
| 43 | were the heaviest compared to the other groups at D34 but not at D56. The presence | | |
| 44 | of hens, negatively impacted CH chicken BW at D56. In females, there was no effect | ***** | a supprimé: eventually |
| 45 | of hatching condition on the BWs at D34 and D56, and the presence of hens had a | | a supprimé: eventually |
| 46 | positive impact on OFH chicken BW. There was no effect of hatching conditions on | | |
| 47 | health parameters. In conclusion, the OFH system was a hatching system at least | | |
| 48 | equivalent to the CH system, The presence of the hen, at hatching and during the chick | | a supprimé: , if not better in this study |
| 10 | a facel and a second second second a standard standard the second standard second standard second standards and | | a supprimé: effects |
| 49 | start-up phase on performance interacted with the natching condition and the sex of | | (a supprimé: 's presence |
| 50 | the chickens. | ***** | a supprimé: The health status of hens and brooding behaviour of the hens are essential to ensure the health and welfare of the chicks. |
| 51 | | | and welfare of the chicks. |

| 68 | Introduction, |
|----|--|
| 69 | The integrated management of poultry health includes maintaining health, welfare and |
| 70 | performance throughout the life of animals. This is an even greater challenge in a |
| 71 | global context of reducing the risk of antimicrobial resistance. One axis in the |
| 72 | Ecoantibio2017 plan (Ecoantibio2, 2017) concerns the development of alternatives to |
| 73 | avoid the use of antibiotics. In this context, new poultry rearing systems are being |
| 74 | developed, particularly for the perinatal period. In poultry, the perinatal period is a |
| 75 | stressful period for broiler chicks, which includes the hatching phase and major |
| 76 | physiological changes to adapt to new food resources and environments. In |
| 77 | hatcheries, chicks hatch between 19 and 21 days of incubation. They often stay more |
| 78 | than 12 hours in the hatcher, under optimal temperature, without light and usually |
| 79 | without access to feed and water until placement in farm buildings. The fasting period |
| 80 | of the chicks is further increased by the time needed for hatchery processing, |
| 81 | transportation duration and unloading at the farm, which might last up to the first 72 h |
| 82 | after hatching. Even though chicks can use energy reserves from their yolk sac (van |
| 83 | der Wagt et al., 2020), these conditions induce immediate and long-lasting metabolic |
| 84 | changes (Beauclercq et al., 2019; Foury et al., 2020), behavioural impacts by |
| 85 | increasing fear responses (Jessen et al., 2021) and consequences on chicken |
| 86 | development, performance and welfare (de Jong et al., 2017). |
| 87 | To improve the early perinatal conditions of chicks, alternative hatching systems have |

been developed. On-farm hatching provides the chicks with immediate access to feed
and water according to their needs and avoids the exposure to stressors encountered
in conventional hatcheries (van de Ven et al., 2009). Eggs incubated for 18 days are

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94 transported to the farm and placed either in trays or in the litter where they hatch. The 95 effects of these on-farm hatching systems on broiler health, welfare and performance 96 were recently studied under commercial or more controlled conditions and had shown 97 effects that are not always beneficial. Total mortality and footpad dermatitis in on-farm 98 hatched (OFH) chicks were lower compared to conventionally hatched (CH) fast-99 growing broiler chickens (de Jong et al., 2019; 2020; Giersberg et al., 2021; Jessen et al., 2021). However, day-old chick quality was worse and breast myopathy prevalence 100 101 was higher for OFH than CH chickens (de Jong et al., 2019; Souza da Silva et al., 102 2021).

103 Chicken activity and general behaviour were little affected by the hatching system, with 104 fast-growing OFH chickens being more fearful and less active than CH chickens 105 (Giersberg et al., 2020). Slower-growing broiler chickens hatched in organic farms 106 tended to express less general fearfulness than CH chickens (Jessen et al., 2021a). A 107 positive effect on growth performance was observed during the first week of life until 108 21 days in OFH and CH fed at the hatchery compared to CH chickens (de Jong et al., 109 2020), and longer when parent flocks were young (Souza da Silva et al., 2021). 110 Maintaining optimal health, welfare and performance of chickens is highly dependent

on the gut physiology in interaction with the microbiota and mucosal immune system
(Fortun-Lamothe et al., 2023). Antibiotics have been largely used in poultry production

to improve performance, Growth promotion induced by antibiotics is associated with

- 114 effects on the caecal microbiome at taxonomic, metagenomic, and metabolomic levels,
- 115 which might be targeted via its contribution to host-microbiota crosstalk, particularly by
- 116 acting on the gut barrier function (Broom, 2018; Plata et al, 2022). However, growing
- 117 concerns about the increase of antimicrobial resistance in farm animals led to changes

a supprimé: by acting on the gut barrier function (Broom, 2018)...

es

120 in EU and national legislation governing the use of antibiotics as growth promoters in

121 poultry feed, which resulted in their suppression in 2006 (Council Directive 96/22/EC;

122 Axis 2 and measure 19 of the EcoAntibio2017 plan).

123 Greater attention to the environment during the chick postnatal period, especially the 124 microbial environment, is key to optimising the gut barrier function and more broadly 125 the health and welfare of the chickens and their performance. Naturally, chicks hatch in contact with an adult hen who is a donor of microbiota and a model of learning and 126 maternal care (Edgar et al., 2016). Early implantation of adult microbiota into the chick 127 digestive system accelerates the maturation of the microbiota and immune system 128 129 (Volf et al., 2016; Broom & Kogut, 2018; Meijerink et al., 2020). In addition, chicks 130 reared in the presence of their mothers are less fearful than those raised without their 131 mothers and develop more behavioural synchrony (Perré et al., 2002), even though 132 hen genetics has a strong effect on chick behaviour, with commercial lines being less 133 maternal (Hewlett et al., 2019). The combination of a new hatching system like OFH 134 with an enriched microbiota and stimulating environment from the presence of an adult 135 hen is a possible solution for chick conditions to be improved and could contribute to 136 poultry health and welfare and product quality. 137 In this study, we analysed the benefits/risks of hatching systems (conventional hatcher,

- on-farm hatching), with the presence of an adult hen (OFH + H, CH + H) or not (OFH 138
- 139 and CH) on hatchability and chick quality scores. We also explored the effects of these
- 140 hatching conditions and the presence of an adult hen with chicks on performance,
- 141 health and robustness in suboptimal rearing conditions. The combination of CH and
- 142 post-hatching treatment with antibiotics, (CH + AB) was added as an experimental
- 143 control group of antibiotic growth promoter use,

a supprimé:

a supprimé: This made it possible to become aware of the crucial role of the gut barrier and of the quality of the microbiota implanted in the chick's gut at hatching on the physiological and immune development, its robustness in the face of the hazards encountered during the chicks' lives, and consequently on performance.

| a supprimé: (OFH and CH) | |
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| a supprimé: , and | |
| a supprimé: t | |
| a mis en forme : Police :Non Italique | |
| a supprimé: growth promoter | |
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| a supprimé: on hatchability on chick quality score, performance, health and robustness. | |
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| 171 | conventional hatchery (CH) or laid directly in the litter of the pens under infrared heat |
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| 172 | lamps to allow on-farm hatching (OFH). The average temperature of the eggs in the |
| 173 | litter was 37.9°C and under 20 h light per day until OFH chick hatching. The ambient |
| 174 | room temperature was maintained at 25 °C with a fan heater. Day-old CH chicks were |
| 175 | transported for one hour in a transport van before placement in pens to simulate |
| 176 | conventional hatchery processing, which has been described to have long-term |
| 177 | deleterious effects on fear response when combined with delayed nutrition (Hollemans |
| 178 | et al., 2018). The time when CH chicks were placed under heat lamps in pens was |
| 179 | considered D0 as well as for the OFH chicks already in place. Temperature under heat |
| 180 | lamps was decreased from 35–38 °C to 31–32 °C from D0 to D3, then 29–30 °C from |
| 181 | D4 to D6 and 26–27 °C from D7 to D13. The light cycle was 20 h light at the CH chick |
| 182 | placement or until hatching time for OFH chick (D0), 13 h light on D1 (increased dark |
| 183 | time to promote maternal behaviour of hens (Richard-Yris & Leboucher, 1987)), 18 h |
| 184 | on D2 and 16 h on D3 and during the rearing period with minimum 20 lux on 80% of |
| 185 | the lighted surface. |
| 186 | Starting period of chicks in contact with hens |
| 187 | Sixteen Lohmann Brown hens, acting as natural gut microbiota donors and adult |
| 188 | presence, were obtained from a local commercial egg-laying hen farm (La cabane à |
| 189 | Chiron, Benet, France). The hens were aged 31 weeks, vaccinated against Marek |
| 190 | Disease Virus (MDV), Infectious Bursite Disease Virus (IBDV) and Infectious Bronchitis |
| 191 | Virus (IBV) infections, and were sanitary controlled and declared free of Mycoplasma |
| 192 | gallisepticum, Mycoplasma synoviae, Chlamydia psittaci and Salmonella pullorum |

a déplacé (et inséré) [1]

a supprimé: All experimental procedures were approved by the Ethics Committee COMETHEA POITOU-CHARENTES n°84 (APAFIS#24474-2020021816237418 v3) and carried out following current European legislation (EU Directive 2010/63/EU). All steps of hatching, experimentation and rearing were done at the experimental unit (EASM, Poultry alternative breeding facility, INRAE, 17700 Surgères, France, DOI: 10.15454/1.5572418326133655E12).¶

a supprimé: Sixteen Lohmann Brown hens, acting as natural sources of gut microbiota and adult presence, were obtained from a local commercial egg-laying hen farm (La cabane à Chiron, Benet, France). The hens were aged 31 weeks, vaccinated against Marek Disease Virus (MDV), Infectious Bursite Disease Virus (IBDV) and Infectious Bronchitis Virus (IBV) infections, and were sanitary controlled and declared free of Mycoplasma gallisepticum, Mycoplasma synoviae, Chlamydia psittaci and Salmonella pullorum gallinarum. Only Ascaris and Heterakis parasites were detected in hen faeces, and they were at a very low level. \P Each hen was placed separately in a wire-latticed pen $(3\ m^2)$ in the experimental pens described above with a nest box, perch, feed and water ad libitum. Hens were accustomed to their new environment for 12 days, fed with a standard rearing diet for laying hens (30099G25, Arrivé Nutrition Animale, Saint-Fulgent, France) and allowed to deposit faecal and caecal microbiota on litter. The room temperature was 25 °C and the artificial photoperiod was 16 h L:8 h D before egg deposition, 20 h L:4 h D during hatching and the same programme as the chicks afterwards. Two days before chick arrival or egg hatching, a wire-latticed space for chicks was placed in their pen. Eight hens were used for 8 groups of 18 OFH chicks, and eight hens were used for 8 groups of 18 CH chicks. On D0, day-old CH chicks were placed under the pen's wire-latticed space, and OFH chicks were already under this space. Chicks and hens were in visual and auditory contact for a few hours. Then hens were deprived of feed and water from the morning. When lights were switched off, the hens were shut up in their nest boxes, and chicks were placed under each hen as gently as possible for 11 h without any feed and water. Chicks and hens were put physically together in a closed nest for the night to promote maternal behaviour and the acceptance of chicks (Richard-Yris & Leboucher, 1987). The following morning, one hour before the lights were switched on, the nest-box doors were taken away to allow free access to the whole pen. Free in-access feed and water were placed under wire-latticed space for chicks and in raised troughs for hens, not accessible for chicks. Hens were present with chicks for two weeks, the critical period for chick start, and removed on D15. Weight and clinical examinations of the hens were recorded the day before they were installed in the pens and, on D15, when they were removed.

251 gallinarum. Only Ascaris and Heterakis parasites were detected at a very low level in 252 hen faeces. 253 Each hen was placed separately in a wire-latticed pen (3 m²) in the experimental pens 254 described above with a nest box, perch, feed and water ad libitum (Figure 2A). Hens 255 were accustomed to their new environment for 12 days, fed with a standard rearing 256 diet for laying hens (30099G25, Arrivé Nutrition Animale, Saint-Fulgent, France) and 257 allowed to deposit faecal and caecal materials and thus microbiota on litter. An egg 258 was always left in the nest to encourage brooding behaviour. The room temperature 259 was 25 °C and the artificial photoperiod was 16 h L:8 h D before egg deposition, 20 h 260 L:4 h D during hatching and the same programme as the chicks afterwards. Two days 261 before chick arrival or egg hatching, a wire-latticed space (101 x 50 cm) for chicks was 262 placed in their pen (Figure 2B). Eighteen-day embryonated eggs were laid under 263 infrared heat lamps to allow on-farm hatching (OFH) (Figure 2C). Eight hens were used 264 for 8 groups of 18 OFH chicks, and eight hens were used for 8 groups of 18 CH chicks. 265 On D0, day-old CH chicks were placed under the pen's wire-latticed space, and OFH 266 chicks were already under this space. Chicks and hens were in visual and auditory 267 contact for a few hours. Then hens were deprived of feed and water from the morning. 268 When lights were switched off, the hens were shut up in their nest boxes, and chicks 269 were placed under each hen as gently as possible for 11 h without any feed and water. 270 Chicks and hens were put physically together in the closed nest for the night to promote 271 maternal behaviour and the acceptance of chicks (Richard-Yris & Leboucher, 1987). 272 The nest was made of wire mesh covered with a tarpaulin and placed on shavings. 273 The following morning, one hour before the lights were switched on, the nest-box 274 tarpaulins were taken away to allow free access to the whole pen. The nest was 8

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| 275 | present throughout the hen's stay. Free in-access feed and water were placed under |
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| 276 | wire-latticed space for chicks (Figure 2D), not accessible for hens, and in raised |
| 277 | troughs for hens, not accessible for chicks. Chicks could get in and out wire-latticed |
| 278 | space as they pleased. Hens were present with chicks for two weeks, the critical period |
| 279 | for chick start, and removed on D15. Weight and clinical examinations of the hens were |
| 280 | recorded the day before they were installed in the pens and, on D15, when they were |
| 281 | removed. |
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284 Figure 2. Experimental design of chick starting period in contact with hens.

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| 285 | A. Hen wire-latticed (3 m ²) with nest box (width 23 cm, length 35 cm, height 40 cm). | a mis en forme : Exposant |
|-----|---|--|
| 286 | perch, and free in access feed and water. B. Wire-latticed space (101 x 50 cm) for | a mis en forme : ANM main text, Justifié |
| 287 | chicks within the hen pen. C. Eighteen-day embryonated eggs laid under infrared heat | |
| 288 | lamps in the chick wire-latticed space and in presence with hen. D. Chicks under the | |
| 289 | wire-latticed space with the possibility to get in and out, and to have free in access feed | |
| 290 | and water. | a mis en forme : Anglais (E.U.) |
| 201 | Receiver conditions | |
| 291 | Rearing conditions, | a supprime: Experimental design |
| 292 | Seven hundred twenty-day-old among which 432 were from a conventional batchery | a mis en forme : litre 3 |
| | Seven hundred twenty day out among which 402 were norm a conventional hadrery | a supprime: , certified JA 757 chicks, |
| 293 | (CH) and 288 were hatched on-farm (OFH), were allocated into five groups: CH, CH + $$ | |
| 294 | antibiotics treatment (CH + AB), CH + hen (CH + H), OFH, OFH + hen (OFH + H) | |
| 295 | (Figure 1). Each group was randomly placed in the room, repeated in eight pens (18 | |
| 296 | chicks/pen, 3 m^2). Antibiotic treatment was only applied in chick drinking water for the | |
| 297 | CH + AB group: ADJUSOL® TMP SULF Liquid (25 mg/kg sulfadiazine and 5 mg/kg | |
| 298 | trimethoprim, VIRBAC, CARROS, France) for 5 days (D2–D6) and SURAMOX 50 (400 | |
| 299 | mg/10 kg, i.e. 20 mg/kg amoxicillin, VIRBAC) for 5 days (D19-D23). Sex was | |
| 300 | determined on D19 and the number of chickens was adjusted to a maximum of 16 per | a supprimé: on tagged chickens on D19, |
| 301 | pen, keeping a balanced ratio between males and females. On D27, chickens were | |
| 302 | exposed for 4h transport in boxes to a new room at a lower temperature (15 °C instead | a supprimé: challenged |
| 303 | of 25 °C) and feed deprivation. On their return to the original room, the pen size was | a supprimé: by combining a supprimé: 4 h of |
| 304 | reduced from 3 m^2 to 1.5 m^2 to increase chicken density, and birds were orally | |
| 305 | vaccinated with the live Gumboro vaccine in drinking water (HIPRAGUMBORO® - G97, | |
| 306 | HIPRA FRANCE, Saint-Herblain, France). These conditions are stress factors that | a mis en forme : Police :Non Italique |
| 307 | chickens may encounter on farms; the objective was to expose chickens to suboptimal | a supprimé: during rearing |

| 315 | rearing conditions, Chickens had ad libitum access to water and to feed without any |
|-----|--|
| 316 | anticoccidial drugs. They were fed with a standard starter diet (raw energy = 4462 |
| 317 | kcal/kg, crude protein = 23.91%) until D19, then a grower diet from D20 to D34 (4527 |
| 318 | kcal/kg, crude protein = 20.51%) and a finisher diet from D35 to D56 (4600 kcal/kg, |
| 319 | crude protein = 19.98%). A wire mesh platform and a perch were used for |
| 320 | environmental enrichment. |
| 321 | •/ |
| 322 | Chick quality scores |
| 323 | Chick quality scores were determined at placement in the pen for CH chicks (D0), |
| 324 | corresponding to 21 days of incubation for OFH chicks, on 24 to 25 chicks from the |
| 325 | three treatments: CH (at the entrance into the pens), OFH and OFH + H (after hatching |
| 326 | within their pen). They were macroscopically defined according to the grid of Tona |
| 327 | (Tona et al., 2003) and modified by adding several other parameters (Guinebretière et |
| 328 | al., 2022). Briefly, the chicks were scored on a total score of 110, including scores of |
| 329 | posture (on 5), down (on 5), legs (on 6), red dot on the beak (on 10), grouped into an |
| 330 | "appearance" score (on 26); activity (on 6), eyes (on 16), leg joint inflammation (on 5) |
| 331 | and leg dehydration (on 5) were grouped into a "tiredness" score (32), and finally, |
| 332 | retracted yolk (on 12), navel (on 12), remaining membrane (on 12), and remaining yolk |
| 333 | (on 16) were grouped in an "abdomen" score (on 52). |

- 334
- 335 **Behavioural observations**

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a supprimé: without inducing pathology or mortality a supprimé: Chickens had ad libitum access to feed without anticoccidial drugs.

a supprimé: Faeces were collected from litter on D14 and D54 for parasite analyses.

a déplacé vers le haut [1]:

Sixteen Lohmann Brown hens, acting as natural sources of gut microbiota and adult presence, were obtained from a local commercial egg-laying hen farm (La cabane à Chiron, Benet, France). The hens were aged 31 weeks, vaccinated against Marek Disease Virus (MDV), Infectious Bursite Disease Virus (IBDV) and Infectious Bronchitis Virus (IBV) infections, and were sanitary controlled and declared free of Mycoplasma gallisepticum, Mycoplasma synoviae, Chlamydia psittaci and Salmonella pullorum gallinarum. Only Ascaris and Heterakis parasites were detected in hen faeces and they were at a very low level.¶ Each hen was placed separately in a wire-latticed pen (3 m²) in the experimental pens described above with a nest box, perch, feed and water ad libitum. Hens were accustomed to their new environment for 12 days, fed with a standard rearing diet for laying hens (30099G25, Arrivé Nutrition Animale, Saint-Fulgent, France) and allowed to deposit faecal and caecal microbiota on litter. The room temperature was 25 °C and the artificial photoperiod was 16 h L:8 h D before egg deposition, 20 h L:4 h D during hatching and the same programme as the chicks afterwards. Two days before chick arrival or egg hatching, a wirelatticed space for chicks was placed in their pen. Eight hens were used for 8 groups of 18 OFH chicks, and eight hens were used for 8 groups of 18 CH chicks. On D0, day-old CH chicks were placed under the pen's wire-latticed space, and OFH chicks were already under this space. Chicks and hens were in visual and auditory contact for a few hours. Then hens were deprived of feed and water from the morning. When lights were switched off, the hens were shut up in their nest boxes, and chicks were placed under each hen as gently as possible for 11 h without any feed and water. Chicks and hens were put physically together in a closed nest for the night to promote maternal behaviour and the acceptance of chicks (Richard-Yris & Leboucher, 1987). The following morning, one hour before the lights were

a supprimé: Hatching and husbandry¶ Hatching conditions ¶

Certified JA 757 18-day embryonated eggs (Galina Vendée, Essarts-en-Bocage, France) were either placed at 37.6°C with 75% relative humidity and no light in a conventional hatchery (CH) or laid directly in the litter of the pens under infrared heat lamps to allow on-farm hatching (OFH). The average temperature of the eggs in the litter was 37.9°C and under 20 h light per day until OFH chick hatching. The ambient room temperature was maintained at 25 °C with a fan heater. Day-old CH chicks wer [1]

483 The scan sampling method was used to follow the behaviour of hens and chicks on 484 days 2, 5, 6, 7, 8, 9, 12, 13 and 14 with the following repertoire: resting (the hen is lying 485 or standing still, eyes closed and without chicks), maintenance (preening, scratching, 486 stretching), feeding behaviour (the hen is eating or drinking), locomotion, exploration 487 (the hen is scratching or pecking at the ground or the environment), observation (the 488 hen is observing the environment with neck movements), maternal behaviour (the hen is making food offering to the chicks, the hen is expressing maternal calls, the hen is 489 490 brooding the chicks by lying down and spreading her wings), fear behaviour (the hen 491 is flying or running from the experimenter, freezing, alert), agonistic behaviour (the hen 492 is chasing the chicks, the hen is pecking the chicks, others (punctual behaviours like 493 vocalisations). To characterise hens' behaviour towards the chicks, each hen was 494 categorised according to the frequencies of agonistic or maternal behaviours. We 495 defined three categories: 1) maternal (M): the hens expressed only maternal 496 behaviours towards the chicks; 2) tolerant (T): the hens expressed both maternal and 497 agonistic behaviours towards the chicks or less than 5% of scans with maternal 498 behaviour; 3) aggressive (A): the hens rejected the chicks and expressed only 499 agonistic behaviour towards them. 500 To evaluate the proximity between chicks and hens, the experimenter also recorded 501 the localisation of four chicks randomly tagged at D0 per pen and the hen within the 502 pen. To that end, the pen was virtually divided into four zones (Figure 3). The 503 observations were conducted between 10 AM and noon and between 3 and 5 PM by 504 the same experimenter. The experimenter walked slowly in front of each pen and 505 recorded the behaviour of the hen and the localisation of the four tagged chicks every

(a déplacé (et inséré) [4]

a supprimé: (Table 2)

a supprimé: 2

a supprimé: Two hens were defined as maternal, six were tolerant, and five were aggressive among the 13 hens analysed (Table 2).¶

a supprimé: randomly tagged

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- 513 eight minutes (approximately), with a total of 10 scans per hen per day and 177 scans
- 514 per hen for the whole period of observation.
- 515



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Figure <u>3</u>, Schematic representation of the pen $(3m^2)$ with the zones used to locate the four tagged chicks and the hen during behavioural observations; A: the nest <u>(23 cm</u> <u>wide x 35 cm long x 40 cm high)</u>, B and D: two halves of the pen and C: the wirelatticed space for the chicks (101 × 50 cm).

- 523 Performance
- 524Body weight (BW) was measured at DQ, D19, D34 and D55. Feed consumption was525measured in each pen for the periods between DQ, D19, D19–D34 and D34–D55, and526then used to calculate the feed conversion ratio (FCR) as the feed consumption-to-BW

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a déplacé vers le bas [2]: Health parameters¶ Droppings deposited on pen litter were collected on D14 and D54 and analysed for parasite detection (*Coccidia*, *Ascaris* and *Heterakis*). Five grams of droppings were homogenised in 70 mL of flotation solution (0.36% of sodium chloride). The mixture was then filtered and pressed through a tea strainer (small mesh) to extract as much of the liquid part as possible. A homogeneous sample was deposited into a McMaster cell counter, and after 5 min of rest, the oocysts and nematode eggs were counted, and their number was expressed per gram of droppings (OPG). Health disorders, mortality and causes of death were registered during the experiment.¶

a supprimé: ¶ Chick quality scores¶

Chick quality scores were determined at placement in the pen for CH chicks, corresponding to 21 days of incubation for OFH chicks, on 24 to 25 chicks from the three treatments: CH, OFH and OFH + H. They were macroscopically defined according to the grid of Tona (Tona et al., 2003) and modified by adding several other parameters issued from the CASDAR QUALICOUV project (Guinebretière et al., 2022). Briefly, the chicks were scored on a total score of 110, including scores of posture (on 5), down (on 5), legs (on 6), red dot on the beak (on 10), grouped into an "appearance" score (on 26); activity (on 6), eyes (on 16), leg joint inflammation (on 5) and leg dehydration (on 5) were grouped into a "tiredness" score (32), and finally, retracted yolk (on 12), navel (on 12), remaining membrane (on 12), and remaining yolk (on 16) were grouped in an "abdomen" score (on 52).¶

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gain ratio per pen during both periods and the entire rearing period. At D56, 16
identified males per group were slaughtered, and *pectoralis major* and *pectoralis minor*(breast) muscles were weighed to calculate their yields relative to BW and ultimate pH.

Ultimate pH was measured as the pectoralis major pH 24 hours after slaughter.

567 Health parameters

a déplacé (et inséré) [2]

- 568 Droppings deposited on pen litter were collected on D14 and D54 and analysed for
- 569 parasite detection (Coccidia, Ascaris and Heterakis). Five grams of droppings were
- 570 <u>homogenised in 70 mL of flotation solution (0.36% of sodium chloride). The mixture</u>
- 571 was then filtered and pressed through a tea strainer (small mesh) to extract as much
- 572 of the liquid part as possible. A homogeneous sample was deposited into a McMaster
- 573 cell counter, and after 5 min of rest, the oocysts and nematode eggs were counted,
- 574 and their number was expressed per gram of droppings (OPG). Health disorders,
- 575 mortality and causes of death were registered during the experiment.
- 576

566

577 Statistical analyses

- 578 Hatching rates between hatchery and on-farm hatchings were compared using chi-
- 579 squared tests. Chick quality parameters were analysed by a non-parametric Kruskal-
- 580 Wallis test, considering the treatment (CH, OFH and OFH + H), followed by Mann-
- 581 Whitney post hoc tests. A 2-way ANOVA was then carried out to test the effects of the
- 582 experimental group, the sex and their interaction on performance. The statistical model
- used was then: $Y_{ij} = \mu + a_i + b_j + ab_{ij} + e_{ij}$ where Y_{ij} is the dependent variable, μ the
- 584 overall mean, ai the Experimental group (CH, CH + AB, CH + H, OFH, OFH + H), bi

| a supprime: , | CH + H | | | |
|--------------------------|---------------|-------------|--------------|-----|
| a supprimé: ⁻ | The normality | of residual | distribution | was |

| | checked with the Shapiro-Wilk test for BW, feed intakes and FCR |
|--------------|---|
| -(| a supprimé: hatching |
| (| a supprimé: condition |
| \mathbb{Z} | a supprimé: and |
| Ľ, | a supprimé: effect |
| Y | a supprimé: , as well as the two-by-two interactions |

| 594 | the Sex effect, abij the two-by-two interaction and eij the residual error term. When there | |
|-----|---|---------------------------|
| 595 | was an interaction between variables, a Fisher (LSD) test was used to determine the | |
| 596 | statistical significance of the difference. Differences were considered significant when | |
| 597 | p-values < 0.05 and a tendency for $0.05 , Analyses were performed using$ | a supprimé: 1 |
| 598 | XLSTAT software (version 2015, Addinsoft, Paris, France). | a supprimé: 05 |
| 599 | Behavioural data did not meet the assumption of normality and homogeneity of | |
| 600 | variances. Non-parametric Mann-Whitney U-tests were used on the mean percentage | |
| 601 | of scans per behavioural category to compare the behaviour of hens in contact with | |
| 602 | CH chicks to the hens in contact with OFH chicks. To compare the proximity of CH and | |
| 603 | OFH chicks towards the hen, Mann-Whitney U tests were conducted on the mean | |
| 604 | number of tagged chicks located in the same area of the pen as the hen over the 177 | |
| 605 | scans recorded per hen. | |
| 606 | | |
| 607 | Results | |
| 1 | | |
| 608 | Hatchability and chick quality | a déplacé (et inséré) [3] |
| 609 | Hatchability | |
| 610 | For conventional hatchers, 97.7% of CH fertile eggs hatched at E21 and 97.2% ± 4.2% | |
| 611 | of OFH fertile eggs hatched at E21 in pens. The presence of hens had a significant | |
| 612 | impact on the OFH condition (p = 0.034). In the presence of hens, $86.8\% \pm 11.9\%$ of | |
| 613 | OFH + H chicks hatched at E21. Unhatched eggs were mainly found in the pens with | a supprimé: (Figure 3) |
| 614 | aggressive hens (9/11) or in the OFH pens next to those with aggressive hens (4/4). | |
| 615 | No mortality of CH chicks or OFH chicks was observed at hatching, whereas 5.6% ± | |
| 1 | Q, | |
| ļ | 15 | |

| 620 | hatching (n = 10) due to three hens' aggressiveness or another reason. Only 3.6% |
|-----|--|
| 621 | (2/56) of chicks had residual yolk sacs at the age of 20 days (one CH and one CH + |
| 622 | AB) and no yolk residue was found at 56 days. |
| 623 | Quality scores of chicks |
| 624 | No difference was shown due to the hatching conditions ($p > 0.05$) on the total quality |
| 625 | scores, with good scores in the three groups considered (OFH: 96.2 \pm 1.5, CH: 97.3 \pm |
| 626 | <u>1.5; CH+H: 95.1 ± 1.7), However, the subtotal score of the appearance was impacted</u> |
| 627 | by, treatment whereas the subtotal scores for tiredness and abdomens of the chicks |
| 628 | were unaffected by treatment ($p > 0.05$, data not shown). Indeed, whereas the subtotal |
| 629 | score for appearance was not different between CH chicks or OFH chicks, it was |
| 630 | deteriorated by the presence of the hen within the hatching pen in OFH + H compared |
| 631 | to OFH chicks (p = 0.01) (Figure 4), |
| 632 | |
| 633 | |

5.9% (from 0 to 16.7% according to the pen) OFH + H chicks died or were removed at

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a supprimé: Figure 3. Number of live hatched chicks according to hatching conditions; conventional hatchery (CH) condition performed in one hatchery (one value); on-farm hatching (OFH) and on-farm hatching with hen (OFH + Hen) conditions were repeated in eight pens each, each pen contained 18 embryonated eggs or chicks; values are expressed as means ± standard error

a supprimé: Whereas n

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(a supprimé: ;

a supprimé: when considering the subtotal scores linked to the appearance, the tiredness or the abdomens of the chicks, it appeared that

a supprimé: score

a supprimé: changed depending on

a supprimé: the

a supprimé: (Figure 4), with the two other subtotals not being significantly changed

a supprimé:

a supprimé: a supprimé: The deterioration of chick quality with hens was probably relateddue to the hen aggressiveness.



access to their own space) and the later behavioural analysis. However, the chicks

were kept in the analysis as they were in contact with their hen during hatching and

with the microbiota the hen deposited in the pen. There was no significant difference

in the behaviour of the hens, regardless of the hatching condition of chicks, except for

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| the frequency of the | behaviour "observe"; | OFH hens | tended to | o observe | their | | |
|------------------------------|---|--|--|--|--|---|---|
| environment less than C | CH hens (<u>Additional file</u> | e: Table <mark>S1</mark>). | | | | a supprimé | : 1 |
| | | | | | | | |
| <u>Table S1. Behaviour c</u> | of hens according to | the chick ha | tching co | nditions | | a mis en fo | rme : Anglais (E.U.) |
| | | | | _ | | a mis en fo | rme : Anglais (E.U.) |
| | Hatching co | onditions | | | | a mis en fo | rme : Anglais (E.U.) |
| | the frequency of the environment less than (<u>Table S1. Behaviour o</u> | the frequency of the behaviour "observe"; environment less than CH hens (Additional file Table S1. Behaviour of hens according to Hatching co | the frequency of the behaviour " <i>observe</i> "; OFH hens environment less than CH hens (<u>Additional file:</u> Table <u>S1</u>). <u>Table S1. Behaviour of hens according to the chick ha</u> Hatching conditions | the frequency of the behaviour " <i>observe</i> "; OFH hens tended to environment less than CH hens (<u>Additional file:</u> Table <u>S1</u>). <u>Table S1. Behaviour of hens according to the chick hatching con</u> Hatching conditions | the frequency of the behaviour " <i>observe</i> "; OFH hens tended to observe environment less than CH hens (<u>Additional file:</u> Table <u>S1</u>). <u>Table S1. Behaviour of hens according to the chick hatching conditions</u> | the frequency of the behaviour "observe"; OFH hens tended to observe their environment less than CH hens (Additional file: Table <u>S1</u>). <u>Table S1. Behaviour of hens according to the chick hatching conditions</u> | the frequency of the behaviour "observe"; OFH hens tended to observe their environment less than CH hens (Additional file: Table S1). Table S1. Behaviour of hens according to the chick hatching conditions Hatching conditions |

<u>OFH</u>

<u>1.37 ± 0.72</u>

<u>31.16 ± 22.74</u>

<u>4.92 ± 1.95</u>

Hen behaviour

Agonistic

Rest/Comfort

Fear

<u>CH</u>

<u>2.54 ± 3.74</u>

<u>17.72 ± 7.16</u>

7.07 ± 3.39

| | Feeding | <u>18.10 ± 4.52</u> | <u>19.45 ± 11.56</u> | <u>0.731</u> | | | | |
|------------|------------------------------------|-------------------------|-------------------------|-----------------------------|-------------------------------------|--|---|--------|
| | Locomotion | <u>6.78 ± 4.12</u> | <u>3.39 ± 2.95</u> | <u>0.146</u> | | | | |
| | Observation | <u>17.53 ± 7.45</u> | <u>9.52 ± 4.76</u> | <u>0.045</u> | | | | |
| | Exploration | <u>22.62 ± 7.62</u> | <u>19.77 ± 10.78</u> | 0.656 | | | | |
| | Maternal | 1.32 ± 1.94 | 3.39 ± 7.98 | 0.732 | | | | |
| | <u>Others</u> | 6.32 ± 2.31 | 7.02 ± 7.02 | 0.470 | | | | |
| | CH = conventional hate | <u> </u> | ching on-farm (n = 7) | | | a mi | is en forme : Anglais (E.U.) | |
| | Behaviour observations | s (mean ± SD of scan pe | | a mi | is en forme : Anglais (E.U.) | | | |
| | <u>p-value < 0.05 = signifi</u> | cant difference between | hatching conditions (Ma | ann-Whitney l | <u>J-test)</u> | (a mi | is en forme : Anglais (E.U.) | |
| | k | | _ | _ | | a mi | is en forme : Anglais (E.U.) | |
| 678 | Υ | | | | | a su | ıpprimé: ¶ | |
| 679 | V | | | | | | | |
| 1 | | | | | $\langle \cdot \rangle$ | 1 | | |
| 680 | Hens' behaviour towa | irds the chicks was ca | tegorised according to | o the freque | ncies of | $\setminus \left \frac{1}{4} \right $ | | |
| 681 | agonistic or materna | l behaviours. Two he | ens were defined as | maternal, s | ix were | | | |
| C02 | televent en d.C. | | - 40 h | T = - - 4 \ | | a su chicl | pprime: Table 1. Behaviour of hens according k hatching conditions | to the |
| 682 | tolerant, and five were | e aggressive among th | e is nens analysed (| | | a su | ıpprimé: 2 | |
| 1 | | | | | | | | |

<u>P-value</u>

0.550

<u>0.181</u>

0.384

- -

_ _ _

_ _ _

| Hatching | Hen bel | naviours | Catagon | |
|--|----------------------------|---------------------------------------|---------------------------------------|--|
| conditions | Agonistic | Maternal | Calegory | |
| CH1 | 7.91 ± 0.27 | 0 | А | |
| CH2 | 0 | 0.57 ± 0.07 | т | |
| СНЗ | 0.56 ± 0.07 | 0.56 ± 0.07 | т | |
| CH4 | 0 | 5.08 ± 0.22 | М | |
| CH5 | 0 | 1.69 ± 0.13 | т | |
| CH6 | 6.78 ± 0.25 | 0 | А | |
| OFH1 | 1.13 ± 0.11 | 0 | А | |
| OFH2 | 0 | 21.47 ± 0.41 | М | |
| OFH3 | 1.69 ± 0.13 | 0.56 ± 0.07 | т | |
| OFH4 | 1.69 ± 0.13 | 0.56 ± 0.07 | т | |
| OFH5 | 1.13 ± 0.11 | 1.13 ± 0.11 | т | |
| OFH6 | 1.69 ± 0.13 | 0 | А | |
| OHF7 | 2.26 ± 0.15 | 0 | А | _ |
| Behaviour ol A = Agressiv T = Tolerant | bservations (n ve | nean ± SD of s | scan percenta | ages over 9 days) |
| M = Materna | al | | | |
| | | | | |
| The mean significantly 0.05), | number of c ≀between CH | hicks observ I <u>(0.42 ± 0.14</u> | ved in the s l <u>, n = 6)</u> and | ame area as the hen did not dif OFH (0.39 ± 0.21 ; n = 7) chicks (|
| , | | | | |
| | | | | |

Table 1. Classification of hen according to the frequenciesof maternal or agonistic behaviours expressed towards chicks



¹⁹

| 726 | Performance | |
|-----|--|---|
| 727 | Hatching conditions significantly influenced chick DW from batching to eleventer ago | |
| 121 | Hatching conditions significantly influenced chick BW from hatching to slaughter age. | |
| 728 | The OFH chick BW was significantly greater than that of all CH chicks at hatching, | |
| 729 | whether hens were present or not (p \leq 0.002, Figure 5). A sex effect was observed | |
| 730 | from D19 onwards; male chicken BWs were greater than those of females (males: 503 | |
| 731 | \pm 46g, females: 469 \pm 37g, p = 0.0001). Treatment of CH chicks with antibiotics | |
| 732 | temporarily decreased chicken BW at D19 ($p = 0.035$) (Figure 5) due to a decrease in | |
| 733 | weight gain in females (Table 2) compared to CH chickens, while feed intake (data not | ~ |
| 734 | shown) and FCR were not different (Table 2). At D19, OFH chickens had the best BW | |
| 735 | compared to all other groups of chicks (p \leq 0.0003) (Figure 5) and the best weight | |
| 736 | gained per chicken (Table 2). At this time, the presence of hens at hatching with CH | |
| 737 | and OFH chicks had a remnant negative impact on chicken BW regardless of the | |
| 738 | hatching condition (p < 0.0001), as well as on weight gain and FCR for the period D1- | |
| 739 | D19 (Table 2). Both the feed intake per chicken (CH: 624 ± 12g ^a , CH + AB: 600 ± | |
| 740 | 27g ^{ab} , CH + H: 603 ± 25g ^{bc} , OFH: 652 ± 33 ^a , OFH + H: 615 ± 34 ^c , p = 0.001) and the | |
| 741 | weight gained per chicken (Table 2) decreased compared to the other groups, and the | |
| 742 | FCR increased (Table 2). An interaction between the effect of the experimental group | |
| 743 | and chicken sex on BW was observed later at D34 (p = 0.012) and D56 (p = 0.022) on | |
| 744 | BW, even though the FCR was not affected (Table 2). At D34, a week after the | |
| 745 | challenge, the OFH male chickens were the heaviest compared to the other groups (p | |
| 746 | \leq 0.033) and the best weight gain (Table 2). The presence of hens at hatching harmed | |
| 747 | chicken BW ($p \le 0.0004$), regardless of the hatching condition (Figure 6A) <u>and the FCR</u> | |
| 748 | was not affected (Table 2). In females, there was no effect of hatching condition or | |
| 1 | 20 | |

a déplacé vers le haut [3]: Hatchability and chick

a déplacé vers le haut [3]: Hatchability and chick quality ¶ Hatchability ¶ For conventional hatchers, 97.7% of CH fertile eggs hatched at E21 and 97.2% \pm 4.2% of OFH fertile eggs hatched at E21 in pens. The presence of hens had a significant impact on the OFH condition (p = 0.034). In the presence of hens, 86.8% \pm 11.9% of OFH + H chicks hatched at E21 (Figure 3). Unhatched eggs were mainly found in the pens with aggressive hens (9/11) or in the OFH pens next to those with aggressive hens (4/4). No mortality of CH chicks or OFH chicks was observed at hatching, whereas 5.6% \pm 5.9% OFH + H chicks died or were removed at hatching (n = 10); due to three hens' aggressiveness or another reason. Only 3.6% (2/56) of chicks had residual yolk sacs at the age of 20 days (one CH and one CH + AB) and no yolk residue was found at 56 days.¶

| a supprimé: | Independently of the treatment, a |
|------------------------------------|--|
| a supprimé: <mark>0.0001</mark> | CH: 497 ± 38g, CH + AB: 486 ± 37g, p = |
| a supprimé: | 3 |

| a sunnrimé · 3 | _ | | | | | |
|---|---|----|-----|----|----|---|
| , , , , , , , , , , , , , , , , , , | а | su | nnı | im | é: | 3 |

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|---|
| a supprimé: (CH: 455 ± 37g ^b , CH + AB: 445 ± 37g ^c , CH + H: 421 ± 40g ^d , OFH: 471 ± 42g ^a , OFH + H: 425 ± 47g ^d , p = 0.0001) |
| a supprimé: 3 |
| a supprimé: hatching condition |

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| (| a supprimé: 3 |
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a supprimé: 3

| 828 | presence of hens on the BW at D34 (Figure 6A). At slaughter age (D56), there was no | | |
|-----|---|-----|--|
| 829 | effect of hatching condition on the male chicken BW, but the presence of hens at | | |
| 830 | hatching harmed CH chicken BW (p = 0.0008) (Figure 6B) and weight gain for the | | |
| 831 | period D34 – D56 (Table 2). There was a pen effect in CH + H (p = 0.016) and OFH + | a | supprimé: 3 |
| 832 | H chickens (p = 0.001), the pen with the lightest CH + H males was in the presence of | | |
| 833 | an aggressive hen, and the heaviest OFH + H males were in a pen in the presence of | | |
| 834 | a tolerant hen, but all combinations were observed (Additional file: Figure S1). In | a | supprimé: Figure 7 |
| 835 | females, there was no effect of the hatching condition on the BW. The presence of | | |
| 836 | hens at hatching had a positive impact on OFH female chickens compared to CH | | |
| 837 | female chicken BW (p = 0. 0096), with the OFH + H chickens being the heaviest | | |
| 838 | compared to the other CH female conditions (Figure 6B), and having the best weight | | |
| 839 | gain for the period D34 - D56 (Table 2). There was no significant pen effect between | a | supprimé: 3 |
| 840 | CH + H and OFH + H female chickens (p = 0.447). | | |
| 841 | | | |
| I | × | a s | supprimé: Table 3. Performance according to the ching conditions of chicks |

| Day ranges | | Female | | | | D | | | Male | | | D |
|---|-------------------|---|---------------------|-------------------|--------------------|----------|------------|-------------|------------|------------|------------|---------|
| | СН | CH + AB | CH + H | OFH | OFH + H | P-value | СН | CH + AB | CH + H | OFH | OFH+ H | P-value |
| D0 - D19 | 437 ± 26b | 425 ± 30c | 407 ± 33d | 451 ± 29a | 414 ± 42cd | < 0.0001 | 474 ± 36b | 468 ± 29b | 436 ± 40c | 488 ± 44a | 437 ± 50c | < 0.000 |
| D19 - D34 | 683 ±58b | 680 ± 62b | 694 ± 72ab | 702 ± 57ab | 712 ± 77a | 0.046 | 801 ± 89bc | 822 ± 83ab | 778 ± 90c | 837 ± 69a | 816 ± 67ab | 0.002 |
| D34 - D55 | 1104 ± 13b | 1127 ± 15b | 1134 ± 15b | 1122 ± 95b | 1217 ± 16a | < 0.0001 | 1485 ± 17a | 1437 ± 17ab | 1409 ± 18b | 1481 ± 16a | 1501 ± 16a | 0.030 |
| | | | | | | | | | | | | |
| Day ranges | | Fee | d conversion ratio | o (g/g) | | P-value | | | | | | |
| , · | СН | CH + AB | CH + H | OFH | OFH + H | | | | | | | |
| D0 - D19 | 1.370 ± 0.024c | 1.350 ± 0.066c | 1.416 ± 0.049ab | 1.388 ± 0.022bc | 1.447 ± 0.035a | 0.001 | | | | | | |
| D20 - D34 | 1.807 ± 0.030 | 1.773 ± 0.042 | 1.769 ± 0.039 | 1.795 ± 0.035 | 1.787 ± 0.057 | 0.355 | | | | | | |
| D35 - D55 | 2.194 ± 0.091 | 2.213 ± 0.055 | 2.188 ± 0.054 | 2.201 ± 0.049 | 2.141 ± 0.038 | 0.173 | | | | | | |
| D0 - D55 | 1.904 ± 0.036 | 1.902 ± 0.025 | 1.913 ± 0.040 | 1.910 ± 0.022 | 1.912 ± 0.015 | 0.924 | | | | | | |
| Experimental | group: convention | onal hatchery (Cl | H), CH + antibiotic | s treatment (CH + | + AB), CH + hen (C | CH + H), | | | | | | |
| on-farm hatching (OFH), OFH + hen (OFH + H) | | | | | | | | | | | | |
| Values are expressed as mean ± standard error | | | | | | | | | | | | |
| a.b.c. d Differ | ent letters corre | it letters correspond to significant differences between treatment groups | | | | | | | | | | |

Table 2. Performance according to the experimental group of chicks

a supprimé: Table 3. Performance according to the hatching conditions of chicks

| | Table 3. Per | rformance acc | ording to the e | exper | | | | |
|-------------|--|--------------------|--------------------|-----------|--|--|--|--|
| | | | | | | | | |
| | Day ranges | | | | | | | |
| | | СН | CH + AB | (| | | | |
| | D0 - D19 | 437 ± 26b | 425 ± 30c | 40 | | | | |
| | D19 - D34 | 683 ±58b | 680 ± 62b | 69 | | | | |
| | D34 - D55 | 1104 ± 13b | 1127 ± 15b | 11 | | | | |
| | | | | | | | | |
| | Day ranges | | Fee | d con\ | | | | |
| | Duy rungeo | СН | CH + AB | (| | | | |
| | D0 - D19 | 1.370 ± 0.024c | 1.350 ± 0.066c | 1.416 | | | | |
| | D20 - D34 | 1.807 ± 0.030 | 1.773 ± 0.042 | 1.76 | | | | |
| | D35 - D55 | 2.194 ± 0.091 | 2.213 ± 0.055 | 2.18 | | | | |
| | D0 - D55 | 1.904 ± 0.036 | 1.902 ± 0.025 | 1.91 | | | | |
| | Experimental group: conventional hatchery (CH), CH | | | | | | | |
| | on-farm hatcl | ning (OFH), OFH | I + hen (OFH + H | H) | | | | |
| | Values are ex | pressed as mea | n ± standard err | or | | | | |
| a supprimé: | a,b,c, d Differ | ent letters corres | spond to significa | ant diffe | | | | |





Figure 5. Body weight at D₀ and D19 and according to the hatching conditions: conventional hatchery (CH), CH + antibiotics treatment (CH + AB), CH + hen (CH + H), on-farm hatching (OFH), OFH + hen (OFH + H); values are expressed as means ± standard error; different letters correspond to significant differences between treatment groups



Figure 6. Weight at D34 (A) and D56 (B) of male and female chickens according to the hatching conditions: conventional hatchery (CH), CH + antibiotics treatment (CH + AB), CH + hen (CH + H), on-farm hatching (OFH), OFH + hen (OFH + H); values are expressed as mean ± standard error: different letters correspond to significant differences between treatment groups

OFH + H



Figure <u>S1</u>, Body weight at D56 of male chickens according to the behaviour of the hen present at the starting period, M: maternal, T: tolerant, A: aggressive, AR: aggressive

CH + H

and removed from the pen; CH + H: chicks hatched in the hatchery and in the presence of hens; OFH + H: chicks hatched on-farm in the presence of hens; median \pm SD (n \leq 9).

885

Breast weight was not affected by the hatching conditions (6.99 \pm 0.06, p = 0.357) and ultimate pH was not modified either (5.7 \pm 0.1, p = 0.951).

888 Health and robustness

Coccidia was detected in variable amounts in the droppings of all the pens at D54 (200–85500 OPG) without any significant effect of the hatching conditions in the presence of hen or not (p = 0.606). No clinical signs were observed during the experiment. In all hatching conditions combined, the viability rate of the chickens was 95.3%. The mortality rate during the whole experiment was 3.19% (23/720). Seventeen

a supprimé: 7

chicks died during the first week of life, 11 OFH + H and 5 CH + H in the presence of
hens and one OFH chick for an unknown reason. Six CH chickens died during the rest
of the experiment, five of which were due to heart problems (2 CH, 1 CH + AB, 2 CH
+ H) and one to unknown causes (CH + H). Eleven chicks were additionally eliminated
after hatching in pens in the presence of hens (4 at D1, 4 at D2, and 1 at D4) and two
later (D33 and D55) for morphological reasons.

901

902 Discussion

New hatching systems are being developed in Europe, and the enrichment of the rearing environment is also in full development, notably by optimising the microbial environment of the chicks to limit the use of antibiotics. In this study, we analysed the benefits/risks of hatching systems (OFH and CH, treated with antibiotics or not) and of the presence of an adult hen or not on hatchability, chick quality score, performance, health and robustness.

909 Hatching conditions

| The hatching conditions compared within the present study concerned a combination |
|--|
| of environmental parameters diverging for both hatching conditions (hatcher or on- |
| farm), from the light regimen to the hatching temperature and the relative humidity, and |
| the egg position. Additionally, there was a partial contact with the litter through the |
| floor-hatching device compared to the hatcher crate. The BW of OFH-certified JA757 |
| chicks was significantly greater than that of CH chicks at hatching, even though the |
| hatchability rate and the quality score of chicks were comparable between the two |
| conditions, and no mortality was reported. These results agree with other studies |
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| 918 | performed on larger number of fast-growing broilers in terms of BW, but not in terms |
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| 919 | of chick quality, which was lower in OFH chicks than in CH chicks (de Jong et al., 2020; |
| 920 | Souza da Silva et al., 2021). In OFH-slow-growing organic broilers, BW was also |
| 921 | reported greater, as well as the hatchability, and of lesser chick quality than that of CH |
| 922 | chicks at hatching (Jessen et al., 2021a; Jessen et., 2021b). However, in our study, |
| 923 | there was no effect of hatching conditions, but the presence of hens decreased the |
| 924 | hatchability rate, the appearance quality score of OFH chicks and increased mortality |
| 925 | at hatching. The negative effect on these indicators could be linked to the very few |
| 926 | hens expressing a clear maternal behaviour towards the chicks (n = 2/16) some of |
| 927 | them even showed agonistic behaviour. However, this genetic line was chosen |
| 928 | because the studied practice could favour the possibility to use culled hens in breeding, |
| 929 | and because of their rather tolerant behaviour, it may be possible to optimize their |
| 930 | brooding behaviour, Improvements could be obtained by carrying it out in a season |
| 931 | with days with greater light amplitudes (spring) to facilitate brooding behaviour, which |
| 932 | was not the case in this study (winter), and by selecting hens with brooding behaviour |
| 933 | to facilitate maternal behaviour (Shimmura et al., 2010). Light color and intensity are |
| 934 | also known to influence social interaction between hens, and tuning both the color and |
| 935 | the intensity could be a management strategy to decrease aggressive behaviour such |
| 936 | as pecking but whose effects vary according to age, genetics and activities (Du et al, |
| 937 | 2022). In addition, in our experimental design, the chicks had to feed under the wire- |
| 938 | lattice space, which was not accessible to the hen. As they obtained both food and |
| 939 | warmth under this space, the hens probably did not have enough tactile stimulation |
| 940 | from the chicks to fully express their maternal behaviour with no agonistic behaviour. |
| 941 | Indeed, in addition to the physiological state, tactile stimulations from chicks play an |

a supprimé: on OFH performed in slow (Jessen et al., 2021) and fast-growing broilers

a supprimé: for the BW but not for other parameters that were reported higher for the hatchability, lower for the quality score of chicks and lower for the mortality

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| | a supprimé: This may be explained by the genetic line of hens used (Lohmann Brown), which is highly selected for laying. and counter-selected for brooding |
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960 important role in the expression and maintenance of maternal behaviour in hens

961 (Richard-Yris & Leboucher, 1987),

962 Starting period

Hatching conditions and the presence of hens for 15 days after placement significantly 963 964 influenced chick performance during the starting period. At D19, OFH chicks had the 965 highest BW compared to the other groups. No significant differences were observed in 966 the behaviour of hens present with OFH and CH chicks, except for OFH hens, which 967 were found to observe their environment less than CH hens. With our small sample 968 size, this result could be explained by the behaviour of one OFH hen, which spent much of the time resting. The CH and OFH chicks did not differ in their proximity 969 970 towards the hen. The mean number of chicks observed in the same area as the hen 971 was very low (less than 1 chick), indicating that they were rarely in contact with the 972 hen. However, chick performance was affected by the presence of the hens, including 973 lower feed intake and consequently lower weight gain and higher FCR. This could be 974 explained by the agonistic behaviour of some hens towards chicks, the attempt of the 975 hens to eat the chick feed and the stress that this may have caused the chicks.

976 Treatment of CH chicks with antibiotics, assessed as growth promoters, temporarily 977 decreased chicken BW at D19, but FCR was not modified. This effect was not 978 observed later, but growth promotion was not observed in CH chicks treated with 979 antibiotics. This result is not in agreement with the use of antibiotics as growth 980 promoters in farm animals, but the relative lack of published data on chicken 981 performance limits knowledge of the actual effects of antibiotics on animal performance 982 (Kumar et al., 2018; Broom, 2018; Plata et al. 2022). Their effects also result from their 983 interaction with the microbiota and the variables chosen in the experimental studies.

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989 The effects observed in farms are dependent on the sanitary conditions present, which 990 are different from the much more controlled sanitary conditions in the experimental 991 studies and may contribute to different effects of treatment with antibiotics.

992 Growth period

993 An interaction between the effect of hatching conditions and chicken sex was observed 994 on BW after the challenge on D27. In males, the OFH chicken group was the heaviest 995 compared to the other groups at D34 but not at D56. These results are consistent with 996 a previous study that observed the beneficial effects of OFH on BW only until D21 (de 997 Jong et al., 2020), and not until slaughter time, as reported in various studies when 998 post-hatching feed deprivation time was at least 36 h (de Jong et al., 2017). This may 999 reflect late compensatory growth in CH chickens that have feed deprivation after 1000 hatching. Indeed, weight gain between CH and OFH chickens was no longer different 1001 from D19 for females, and from D34 for males. Alternatively, this may also be a result 1002 of the response to the challenge experienced by the chickens at D27, including 1003 transport, exposure to low temperature, transient feed deprivation, vaccination and a 1004 change to a higher rearing density, but in fact there is no ultimate positive impact of 1005 OFH on BW at slaughter time. Moreover, in our conditions, the presence of hens 1006 eventually negatively impacted male chicken BW, but only for CH chickens at D56. In 1007 females, there was no effect of hatching conditions on the BW at D34 and D56, and 1008 the presence of hens eventually had a positive impact on OFH female chicken BW. 1009 These results were unexpected, but it is known that early stress induces sex-specific, 1010 immediate and life-long effects on the stress response, behaviour, sex hormones, and 1011 hypothalamic and blood gene expression in chickens (Madison et al., 2008; Elfwing et 1012 al., 2015; Foury et al., 2020), with the males being more reactive than the females. The

a supprimé: but the a supprimé: is that 1015 results observed in this study raise questions about the consequences of hatching 1016 conditions in the presence of a hen according to the sex of the chicks. It can be 1017 assumed that male, chicks developed more fear and stress responses than females 1018 when placed in the presence of a hen, and this had negative effects on their growth 1019 until slaughter age for CH chicks. For male OFH chicks, in which the effect of hen 1020 presence on their growth was only observed during the growth phase, the 1021 communication between hens and embryonated eggs before hatching (Edgard et al, 1022 2016) and with chicks at hatching that may have a more limited effect on their growth, 1023 This could even have had negative consequences on hatchability and mortality rates, 1024 but the sex of the chicks was not recorded at that time. The presence of hens with the 1025 female OFH chicks did not affect their performance and even had a beneficial effect 1026 on their growth at slaughter age. These differences observed between treatments and 1027 chick sexes for performance are not likely explained by a difference in proximity 1028 between hens and chicks, which was low in this experiment.

1029 Health and Robustness

1030 There were no effects of hatching conditions on health parameters (parasitic load, 1031 clinical signs, rate of mortality), even after exposure of chickens during their growth 1032 phase to an environmental and vaccine challenge. One limitation of the experiment is 1033 that it does not reflect the farm environment which may include an accumulation of 1034 stressors in a more complex health environment, An infectious challenge could test the 1035 potential benefits of these rearing conditions. However, the challenge used in this study 1036 could have accentuated the differences in the effects of hatching conditions on 1037 performance parameters between males and females, but we did not perform the 1038 unchallenged rearing conditions to assert this. The implantation of adult microbiota into

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| a supprimé: | OFH | |
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a supprimé: increased their fear and stress responses and therefore harmed their growth.

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| 1048 | the chick digestive system by the presence of hens should be nevertheless beneficial | |
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| 1049 | for the maturation of the chick microbiota and gut immune system and still needs to be | |
| 1050 | assessed. | |
| 1051 | Altogether, on-farm hatching of certified broilers was a hatching system at least | |
| 1052 | equivalent to the hatchery hatching system, in this study. The possibility of adding the | |
| 1053 | presence of a hen at chick start-up remains tricky. The health status of the hens was | |
| 1054 | controlled to ensure that no pathogens were transmitted to the chicks. However, the | |
| 1055 | presence of hens, categorised according to their behaviour, revealed deleterious | |
| 1056 | effects on hatching rate, the appearance quality score and hatching mortality. So, the | |
| 1057 | health status and behaviour of the hens are essential to ensure the health status and | |
| 1058 | welfare of the chicks. Moreover, the effects of the hens' presence at hatching and | |
| 1059 | during the chick start-up phase on performance interacted with the hatching condition | |
| 1060 | and the sex of the chickens. To better study hen-egg/chick interaction, the sex effect | |
| 1061 | could be better characterized by in ovo sexing. Further studies should be done to | |
| 1062 | assess the effects of these hatching and chick-starting conditions, in the presence or | |
| 1063 | absence of hens, on the implantation and maturation of the chicks' gut microbiota and | |
| 1064 | mucosal immunity. New devices enabling interactions between hens and chicks should | |
| 1065 | also be tested. | |
| 1066 | | $\langle \rangle$ |

1067 Ethics approval

- 1068 All experimental procedures were approved by the Ethics Committee COMETHEA
- 1069 POITOU-CHARENTES n°84 (APAFIS#24474-2020021816237418 v3) and carried out
- 1070 following current European legislation (EU Directive 2010/63/EU).
- 1071 Author contributions

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a supprimé: In this case, the health status and brooding behaviour of the hens are essential to ensure the health and welfare of the chicks.

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LAG, AB, CS, KG and AC designed the study with the help of CB. LAG, CB, <u>AC and</u> <u>CS</u> performed the experiment with the technical help of SC for the organisation of the experiment and AH for parasitic analyses. CB and LR collected the performance and health parameters. LAG analysed data with the help of AB and CB for the behaviour data. LAG, AB and CB wrote the paper with the help of KG and AC. All the authors reviewed and approved the manuscript.

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- 1095

1096 Acknowledgements

1097 We are grateful to all the members of the RIMEL network whose shared thinking made 1098 the design of this study possible. We thank the staff of the poultry alternative breeding 1099 INRAE, 17700 experimental unit (EASM, Surgères, France, DOI: 10.15454/1.5572418326133655E12) for the development of the experimental set-up 1100 1101 and the conduct of the experimentation. We are very grateful to the staff of the MOQA 1102 team (INRAE, 37380 Nouzilly, France) for their help during the experimentation. The 1103 manuscript has been professionally proofread.

| 1105 | Funding | | |
|--------------|---|---|-----------|
| 1106 | This research was supported by a grant from INRAE, Department of Animal Physiology | | |
| 1107 | and Livestock Systems for the RIMEL network. | | |
| 1108 | Data and model availability statement | | |
| 1109 | The datasets used during the current study are available on line: | | |
| 1110 | https://doi.org/10.57745/6INVYL | | |
| 1111 1112 | Conflict of interest disclosure | a mis en forme : Police :(Par défaut) Aria | , Anglais |
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