ANSWERS TO REVIEWERS COMMENTS

Effects of feeding treatment on growth rate and performance of primiparous Holstein dairy heifers

Yannick Le Cozler, Julien Jurquet, Nicolas Bedere
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Submitted by Nicolas Bedere 2019-09-09 09:22

Round #1

Author's Reply:

Decision

by Luis Tedeschi, 2019-10-29 14:07

Manuscript: https://doi.org/10.1101/760082

Revision Round #1

Dear authors; three reviewers have provided comments and suggestions on various aspects of your study. Before a final recommendation by the PCI ANSC can be made, I invite you to revise your manuscript based on reviewers' feedback. Please, read carefully the information provided for authors at https://animsci.peercommunityin.org/about/help_generic#For%20authors.

Author's Reply: are following the reviewers' comments and are indicated by the 'AU:' prefix and the font is red.

In this special part (answers to reviewer comments), we used references lines of 1st submitted paper and added the references in the new manuscript in red. We wish to thank the reviewer for his advice to improve the present paper and we are now looking forward to receiving the decision. English revisions have been performed by a specialised company (certificate of proofreading attached). We wish to thank in advance reviewers and editor for considering and correcting the present paper. We are now looking forward their decisions regarding the present publication.

Reviews

Round #1

Decision

by Luis Tedeschi, 2019-10-29 14:07

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Dear authors; three reviewers have provided comments and suggestions on various aspects of your study. Before a final recommendation by the PCI ANSC can be made, I invite you to revise your manuscript based on reviewers' feedback. Please, read carefully the information provided for authors at https://animsci.peercommunityin.org/about/help_generic#For%20authors.

Reviews

Reviewed by anonymous reviewer, 2019-10-27 05:42

The study by Cozler et al. investigates the effects of feeding treatment on growth rates and consequences on performance in primiparous Holstein dairy heifers. Overall, the paper is well-written and the concept studied is very straightforward. The experiments are well-designed and the methodology appears to be appropriate. Findings presented in the manuscript contribute to a better understanding of the long-term effects of nutritional management on performance traits in Holstein dairy heifers. Some minor points need to be addressed prior to final publication.

Minor points:

Line 21: "… an experiment was performed…" AU: done line 21

Line 26: "… although they were still…" AU: done line 27

Line 46: ".. or older." AU: done line 49

Line 62: "…impact of accelerated growth" AU: done line 66

Line 66: "…designed and carried out an experiment…" AU: done line 70

Line 84: "…that, due to the fedding…" AU: done line 90

Line 139: "…grass, with the exception of…" AU: done line 166

Line 211: "2 consecutive values were not". Please state the interval between sample collection: AU: this information has been added on line 258-260

Line 211: "positive. Reductions in P4 milk…" AU: done line 260

Line 287: "…difference in the dam's BW…" AU: done line 330
Line 334: "AFS had minimal effects of fertility. Concerning ovarian…" AU: done line 381
Line 352: The present study indicates that …" AU: done line 400
Line 367: "in a study by Johnson et al…" AU: done line 414
Line 370: "…probably explains why no difference…" AU: done line 417
Line 371: "…the amount of milk offered until weaning…" AU: done line 425-426
Line 384: Define IGF1 AU: done line 437
Line 397: In the present study, a negative…" AU: done line 449-450
Line 415: "In the present study, fertility was…" AU: done line 465
Line 415: "…In a previous study on…" AU: done line 465
Line 434: "Collectively, these results indicate that…" AU: done line 483

Reviewed by anonymous reviewer, 2019-10-29 05:31

The objectives of the study were to assess the effects of feeding rearing programs on growth, reproduction and production performance of Holstein cows at nulliparous and primiparous stages. General comments The manuscript covers a relevant topic for dairy production. The material and methods section could be improved if it was more specific and improving the readability.

AU: Thanks to comments and suggestions of the reviewers, and the final language correction, we think that the readability has been improved now.

Materials and methods

L90-92: This sentence states that the expected average age at 1st AI was 15 mo. for SD and ID1 groups and 12 mo. what age for ID2. However, if ID1 groups are on an intensive plain diet wouldn't it be reasonable to expect they would reach the breeding age before the SD group?

AU: we agree, but as the experiment was performed in a seasonal calving herd, it was not possible to start breeding before 15 mo of age (in a non seasonal system, it would start earlier)

L92-93: When does the 1st season of grazing start and finish? When does pasture season 2 start and finish? Please, elaborate.

AU: the information has been added on lines 98-99

L94-95: Are you talking about only milk yield or also milk components? Please, specify.

AU: we specified that it was "yield" and added the information on line 102

L106-108: When did ID1 and 2 calves start to get 15% more since calving or after the pre-experimental phase (i.e., 10 d old)? Please, specify.

AU: this started on day 11 of live (added line 123)

L107: Table 1: In table 1: - Why is PDIE used as an acronym for metabolizable protein and not MP? I don't understand the term "rumen-degradable nitrogen", did you mean rumen-degradable protein? If so, why not us the RDP abbreviation? I don't understand the UFL concept, please explain.

AU: to explain more, we added information lines 109-115. Reference has been added in the list (558-560), and information also given in table 1.

L110: This sentence is confusing. Remove "(i.e. turning out to pasture)" since calves were housed during this period.

AU: this part has been deleted, line 119

L227: Where were you recording all the data before uploading it to the R software?

AU: we added information lines 274-276.

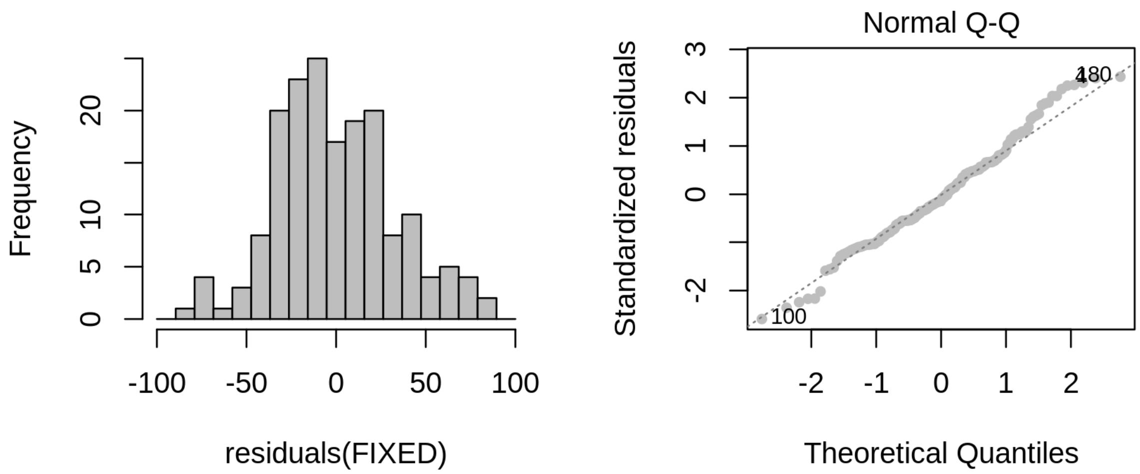
L235: Did you check if assumptions of ANOVA were not violated (e.g., normal distribution of residuals, homoscedasticity, etc.)?

AU: The 3 assumptions made when running an ANOVA are:

1. independent data
2. Normal distribution of the residuals
3. equal variance

We checked if these assumptions were respected, mainly through visualization techniques.
The independence of the data is inherent to the design.
The Normal distribution of the results can be checked by visualizing their distribution and QQplots (Quantile-Quantile Plots). Here is the example of body weight at first service:

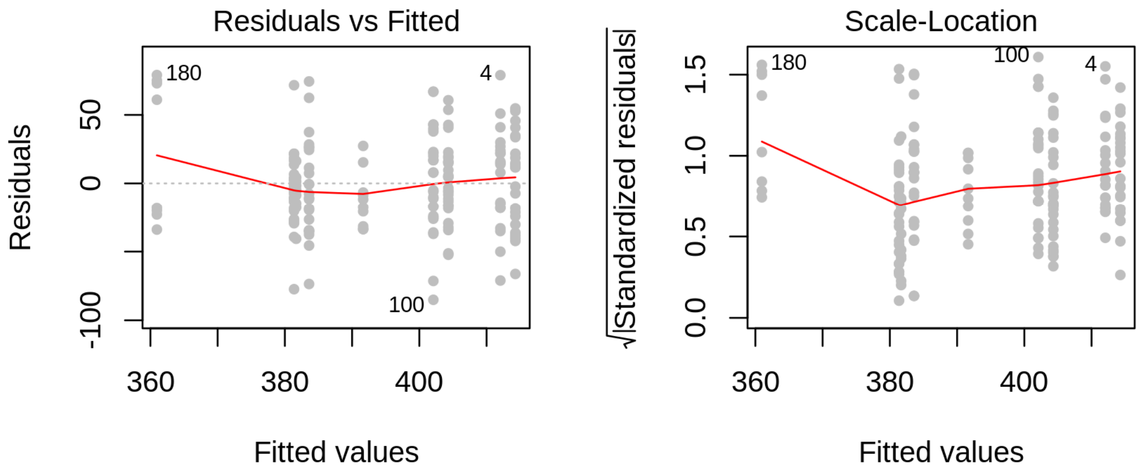
```
> FIXED<-lm(bw_ai1~year+feeding_system,data = data1)
> png("ANOVA_asumptions_normal.png",width=175,height=175/2,units="mm",point
size=12,res=250)
> par(mfrow=c(1,2))
> hist(residuals(FIXED),breaks = seq(-100,100,length.out=20),col = "grey",
main="")
> plot(FIXED,which=2,pch=20,col="grey")
> dev.off()
```



In this example, the residuals distribution (left panel) is very similar to a Normal distribution: e_i~N(0,32.9). The QQplot (right panel) show the quantiles of the standardized residuals in ordinates, against the quantiles of a simulated Normal distribution with the same mean and the same standard deviation. The regression is fairly similar to the dotted line which is y=x. This is another check that shows that the residuals follow a normal distribution.

The homoscedasticity of the variance can be checked by checking the absence of relationship between the residuals and the prediction of the model. With the same example:

```
> png("ANOVA_asumptions_homoscedasticity.png",width=175,height=175/2,units=
"mm",pointsize=12,res=250)
> par(mfrow=c(1,2))
> plot(FIXED,which=c(1,3),pch=20,col="grey")
> dev.off()
```



In this example, neither the residuals (left panel) nor the square root of the standardized residuals (right panel) show any pattern of relationship with the prediction of the model (x-axis). This means that the variance is constant among groups and that the homoscedasticity assumption is respected.
We checked all these for for all our models.
We decided to add few information (lines 278-279)

Results
Please correct the caption of Table 4 for "Association of age at first service with growth and reproductive performance of heifers during the rearing period"
AU: done line 335
Discussion
L389: remove "in"
AU: done line 377

Reviewed by Emilio Mauricio Ungerfeld, 2019-10-28 20:54
The manuscript by Le Cozler et al. tests the hypothesis that late born heifers can compensate growth and reproduce without any disadvantage in production or fertility compared to their earlier born pals. This is an important research that has relevant implications to dairy production.
The manuscript has a clear objective and the experiment and treatments are well designed to test the hypothesis. It is true that the treatments confound the effects of feeding regime and calving date. However, this is perfectly adequate for the objective of the study. Research seems to have been conducted with care and paying attention to details, but some aspects need to be clarified:
i) Did the authors conduct proximal analyses of the total mixed rations or feed components? If so, please add to the Materials and Methods and Results section
AU: For the analysis, we performed analysis on each feed components, at different stages of the experiment and then, recalculated for the mixed ration, using INRAtion Software. We detailed these aspects on lines 180-189
ii) Was pasture intake measured?
AU: Pasture intake was not measured. We added the information on line 190
iii) The statistical model should include year as a random, rather than as a fixed effect
AU: Year was considered in the models to take into account unknown environmental effects (e.g. drought) that might affect the average contemporary group in our analyses. We decided to include it as a fixed effect because there are only 3 levels (year1, year2, year3), which is the best option with this very limited number of levels. Indeed, if we would have include it as a random effect, the variance would have been estimated based on 3 levels only and would not be very accurate. Even with long lasting experiment (e.g. 10 years), the number of levels

in the factor “year” is rather small, this is the reason why considering year as a fixed effect is the statistical approach the most found in the literature (some examples: Dillon et al., 2003. Liv. Prod. Sci 83:21-33; Macdonald et al. 2005. J. Dairy Sci. 88:3363-3375; Horan et al., 2005. J. Dairy Sci. 88:1231-1243; Delaby et al. 2009. Animal 3:6:891-905; Cutullic et al. 2011. Animal 5:5:731-740; Berry at al. 2014. J. Dairy Sci 97:3894-3905, Hazel et al., 2014. J. Dairy Sci 97:2512-2525; Krpalkova et al. 2014. J. Dairy Sci. 97:6573-6582; Manzanilla-Pech. 2016. J. Dairy Sci. 99:443-457...).

We considered your suggestion, here is an example with body weight at first service (bw_ai1):

```
> FIXED<-lm(bw_ai1~year+feeding_system,data = data1)
> Anova(FIXED,type=3)
Anova Table (Type III tests)

Response: bw_ai1
                 Sum Sq  Df    F value    Pr(>F)
(Intercept)    21880176   1 19741.4624 < 2.2e-16 ***
year              27148   2    12.2472 1.079e-05 ***
feeding_system    11051   2     4.9853  0.007877 **
Residuals        187309 169
---
Signif. Codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> summary(FIXED)
Call:
lm(formula = bw_ai1 ~ year + feeding_system, data = data1)

Residuals:
    Min      1Q  Median      3Q     Max
-85.083 -20.578  -3.997  19.860  79.036

Coefficients:
               Estimate Std. Error t value Pr(>|t|)
(Intercept)     392.443      2.793 140.504  < 2e-16 ***
year1            13.551      3.665   3.698 0.000294 ***
year2             3.582      3.491   1.026 0.306252
feeding_system1   8.288      3.573   2.319 0.021576 *
feeding_system2   6.057      3.601   1.682 0.094401 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 33.29 on 169 degrees of freedom
  (1 observation deleted due to missingness)
Multiple R-squared:  0.1646,    Adjusted R-squared:  0.1448
F-statistic: 8.324 on 4 and 169 DF,  p-value: 3.75e-06

> RANDOM<-lmer(bw_ai1~feeding_system+(1|year),data = data1)
> Anova(RANDOM,type=3)
Analysis of Deviance Table (Type III Wald chisquare tests)

Response:  bw_ai1
                   Chisq Df Pr(>Chisq)
(Intercept)    1866.0475  1  < 2.2e-16 ***
feeding_system    9.8638  2   0.007213 **
```

```
---
Signif. Codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> summary(RANDOM)
Linear mixed model fit by REML ['lmerMod']
Formula: bw_ai1 ~ feeding_system + (1 | year)
   Data: data1

REML criterion at convergence: 1703.3

Scaled residuals:
    Min      1Q  Median      3Q     Max
-2.5455 -0.6410 -0.1231  0.6058  2.4087

Random effects:
 Groups   Name        Variance Std.Dev.
 Year     (Intercept)  224.2   14.97
 Residual             1108.4   33.29
Number of obs: 174, groups:  annee, 3

Fixed effects:
                Estimate Std. Error t value
(Intercept)      392.450      9.085  43.198
feeding_system1    8.298      3.574   2.322
feeding_system2    5.955      3.600   1.654

Correlation of Fixed Effects:
                (Intr) feeding_system1
feeding_system1 -0.085
feeding_system2 -0.081 -0.183

> AIC(FIXED);AIC(RANDOM)
[1] 1720.564
[1] 1713.284
```

Considering year as random gives the same results as fixed in this case. The residual variance is the same, AIC are very similar, the effect sizes for feeding systems are similar between both approaches.
Given these elements, we intend to maintain year as a fixed effect, except if we misunderstood what you had in mind.
Done 272-275
In general, the document can be followed well. However, there are numerous writing and grammar mistakes that need to be fixed. I pointed out some of them under Specific comments. I recommend the authors to have the manuscript proofread by a native English speaker.
AU: Thanks to comments and suggestions of the reviewers, and the final language correction, we think that the readability has been improved now. English revisions have been performed by a specialised company (certificate of proofreading attached).
From their results, I disagree with the authors´ conclusion that using nutrition to help heifers born later in the season catch up in growth did not impair their productive performance. There was a tendency towards a 6 to 7% loss in milk production in the first lactation (lines 297-298), which is not negligible. To me, it is somewhat risky to conclude that there is no loss in milk production. Certainly, this practice will be profitable for some combinations of milk

prices and other costs such as labor and feeding. But while the study provides very useful knowledge, I recommend being more cautious about concluding that heifers born later in the season will not lose milk production if fed more.
AU: we temperate a bit our conclusion, and added “in the present study” (line 462), and added information (line 449 – 451).
Plus, there is no information about subsequent lactations in this study, which should constitute another note of caution.
AU: we added this point at the end of the conclusion (line 486-487)

Results of fat- and protein-corrected milk production need to be calculated and presented.
The Results section needs to be improved. I could not find Table 5. The same with the Feed intake result in the Appendix.
AU: Table 5 missed in this version. It was a mistake. Information regarding feed intake on a kg basis jas been added (figure 3)
In many instances results are presented in the text but the reader is not referred to a table. In other instances P values are not presented in the text.
AU: this has been corrected directly in the text (reference to the table or figure) and P values have been added.
In the Appendix, the curves either do not follow or do not overlap with the observations. Please correct or clarify.
AU: this was an error. No such a problem in the new supplementary figure.
Specific comments
Abstract
Line 30. “there were no differences”
AU: added on line 30
Lines 30, 36, and throughout. Recommend “milk composition” instead of “milk quality”
AU: added on line 30, 36… (please noticed
Line 32. Insert “or” i.e. “or 15.5”
AU: added on line 32
Line 35. “the whole lactation”
AU: added on line 35
Line 37. Suggest “with the growth of heifers born earlier in the season”
AU: added on line 38-39
Introduction
Line 52. “Increasing nutrient uptake”
AU: correction done on line 55
Lines 59-60. Unclear sentence, please re-write.
AU: we re-wrote lines 62-63
Line 62. Perhaps “too rapid growth” instead of “too high a growth”?
AU: added on line 65
Line 66. “conduct” instead of “led”
AU: we changed to “carried out” as proposed by referee 1 (line 70)
Lines 70-71. I do not understand “and results from autumn groups calving strategy could be used in a non-grouping strategy”. Recommend re-writing or eliminating
AU: we re-wrote (lines 74-75)
Lines 71-73. Suggest re-writing as “We examined the possibility for late-born heifers to catch up with the rest of the heifers at 1st artificial insemination (AI) at a minimum BW of 370 to 380 kg, resulting in less than 22 mo at first calving.”
AU: we re-wrote (lines 75-78)
Materials and Methods
Lines 77-81. It is not clear which 3 cohorts are the authors referring to. Is it the three treatments? Perhaps a simple table, including years, number of animals, breed and any other aspect the authors judge important could help the reader understand this aspect of the design better
AU: we re-wrote (lines 84-87)
Line 92. “the end”
AU: done line 99

Line 93. “in season 2” or “in the second season”
AU: we re-wrote line 100
Line 94 “milk production”
AU: we changed to yield, as proposed by referee 1 (in the final version, corrected by two natives, they proposed to write yield instead of production. We did so). Line 102
Line 95. “The experiment”
AU: we added “the” line 1903
Lines 98-100. Can be fused into one sentence
AU: done (line 106-108)
Line 101. Piled straw?
AU: (line 116): no, it was cumulated straw bedding (this sentence is also changed in the definitive text)
Line 104. What is the meaning of “dynamic groups”
AU: we added information (lines 119-120)
Line 112. “concentrate intake”
AU: we re-wrote line 130
Line 119. “the SD and ID1 groups”
AU: we added “the” (line 136)
Line 129. 80 feeders?
AU: No individual feeder. Each animal had its own place and had direct contact with his neighbours.
Lines 130-131. “competition for feed”
AU: we re-wrote line 148
Line 135. “mineral supplements”. Please specify composition, product, brand and origin. Same on lines 139-140 and lines 146 and 150.
AU: we added a section, lines 154-161 on minerals, and lines 180-190 for feed in general
Lines 155-156. Suggest “A classification based on age at first service (AFS) was created a posteriori in order to better understand which factors could lead to…”
AU: done lines 194-195.
Line 157. Suggest “Three groups were created with equal number of animals in each of them”.
AU: done line 196
Line 161. “would occur”
AU: done line 201
Line 172. I suggest “Sampling and measurements”
AU: done line 212
Line 213. “These”
AU: done lines 194-195
Lines 232 and 239. “overall mean”
AU: done lines 293
Results
Table 3 is not cited in the text for the growth results. Please correct
AU: done lines 194-195
Line 248. “animals”
AU: done lines 314, 316, 317
Lines 248-249. “did not get pregnant”
AU: done lines 301
Lines 252-253. Please indicate P value and “(result not shown)”
AU: Table 3 is now available
Line 262. Please indicate “(result not shown)” if not shown in any table
AU: Table 3 is now available
Lines 262-265. Please indicate significance and where are these results show, or otherwise “(result not shown)”
AU: as we wrote “significant” in the text (at least $P < 0.05$) , p value is not necessary. Table 3 is now available too
Lines 267. “services”
AU: Done line 319

Line 297. Where is this result presented?
AU: Sorry, we forgot to include table 5.
Lines 303. Replace “was already shrunk” by “decreased”
AU: changed but rewrote according to referee 1 suggestion.
Line 309. “patterns”
AU: done line 356
Line 327. AFS12.5 and AFS14.0?
AU: done line 373
Line 329. “pic”? (do the authors mean peak?)
AU: done line 377 (referee was right, it was a mistake)
Discussion
Line 352. “The present experiment”. Delete “down”
AU: done 400
Line 354. Delete “down”
AU: done 402
Lines 366-367. Re-write as: “The differences in feed allowance resulted in differences in development and size at 6 and 12 mo of age, but had limited effect on BW at weaning”
AU: done line 413-414
Line 367. “by Johnson”
AU: done line 414
Line 368. Do not understand “in size in pre-weaning performance”
AU: we removed “in size”
Line 370. “total mixed ration”
AU: done line 413
Line 371. Please re-write “low in most practices”
AU: done line 425 “In most commercial…”
Line 374. “the present study” “was around”
AU: done line 427
Line 376. “recommendations”
AU: done line 429
Line 377. “by Ettema” “about the importance”
AU: done line 430
Line 378. “achieved the recommended targets weights, which led to economic losses”
AU: done line 431-432
Line 380. “the milking phase”
AU: done line 434
Line 387. Delete “presented and”
AU: done line 440
Line 388. Delete “achieve the recommended targets and, therefore, this leads to economic losses” and start a new sentence
AU: this was line 431 and changes have been done
Line 394. “effects”
AU: done line 447
Lines 395-396. Please re-write for better understanding
AU: for lines 395 to 405, this has been re-written (lines 446-456)
Line 397. But that was the only lactation evaluated
AU: for lines 395 to 405, this has been re-written (lines 446-456)
Line 398. “was associated” Also, please explain how it was associated i.e. positively associated
AU: for lines 395 to 405, this has been re-written (lines 446-456)
Line 400 seems to contradict what as been said in line 398
AU: for lines 395 to 405, this has been re-written (lines 446-456)
Line 405. Delete “is”
AU: for lines 395 to 405, this has been re-written (lines 446-456)
Lines 406-409. This is important, although I think that the direction and significance of the finding is what really matters, rather than absolute milk production with 24 mo calving
AU: we agree too

Line 409. “the present study” “younger heifers”
AU: done line 462
Lines 411-413. The difference with what? With the meta-analysis discussed above? Please clarify
AU: done line 463 (“in milk production”)
Line 415. “In the present study”
AU: done line 465
Line 418. Recommend repeating “Puberty was reached” instead of the more vague “It occurred”
AU: done line 468
Line 422. Delete “means that it”
AU: in this corrected version, we decided to keep it, and ask the English natives to correct (see final version)
Line 425. “future milk production”
AU: done line 475
Line 426. “by age”
AU: done line 476
Line 429. “years”
AU: done line 479
Line 432. “in the present study”
AU: done line 481
Line 434. Delete “All”
AU: we re-wrote “collectively, these…”(line 483)
Lines 435-437. Re-write for clarity and to eliminate redundancies
AU: done lines 483-487
Tables 3 and 4. Could it be “Conception rate at first service” instead of “Success at first service”?
AU: done
Tables 3 and 4. “Number of services”
AU: done

VERSION WITH REVISION TRACKS AFTER ENGLISH CORRECTION

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Abstract

The objective of this study was to investigate effects of feeding-rearing programs that aim for first calving at 20-27 months (mo) of age on growth, reproduction and production performance of Holstein cows at nulliparous and primiparous stages. We hypothesised that, in a seasonal autumn-calving strategy, heifers born late in the season could catch up to the growth of heifers born earlier and be inseminated during the same period, at a body weight (BW) of at least 370 kg. This approach would result in first calving age at 21-22 mo of age without impairing their later performance. To test this hypothesis, we studied 217 heifers over 3 years. They were split into three treatment groups: control feeding (SD), an intensive-plane diet (ID1) from birth to 6 mo of age or an intensive-plane diet from birth to one year of age. Heifers in groups SD and ID1 were born from September until the end of November, while those in ID2 were born later. The present study showed that late-born heifers (ID2) could catch up with the growth of the others due to the feeding treatment, although they were still 42 kg lighter than the SD and ID1 heifers at first calving. No difference in reproductive performance was observed among groups. Once primiparous, the cows reared with the ID2 treatment tended to produce less milk than SD and ID1 cows (ca. 400 kg less on a 305 d basis throughout lactation), and no differences in milk composition, feed intake, body condition score or BW were observed among groups. Age at first service (AFS) was classified *a posteriori* into three classes: 12.5 ($AFS_{12.5}$), 14.0 ($AFS_{14.0}$) and 15.5 mo ($AFS_{15.5}$) of age. Heifers in $AFS_{12.5}$ grew faster than those in $AFS_{14.0}$ and $AFS_{15.5}$. Once primiparous, the $AFS_{12.5}$ cows tended to produce less milk at peak than $AFS_{14.0}$ and $AFS_{15.5}$ cows (ca. 1.5 kg/d less) although no difference in total milk yield during lactation was observed. No differences in milk composition, feed intake, body condition score or BW were observed among groups. These results support the conclusion that the feeding treatment can enable late-born heifers to catch up to the growth of heifers born earlier in the season. This strategy results in an earlier

first calving that does not impair their reproductive performance but does decrease milk yield slightly during first lactation. Future studies should investigate long-term effects of this strategy.

Key words: dairy cattle, heifer, growth, reproduction, feeding treatment

Implications

Increasing the growth rate of dairy heifers decreased their age at puberty, potentially reducing age at first calving and ultimately shortening the non-productive rearing period. Heifers first calved at 22.5 months (mo) of age or less had similar performances similar to heifers that first calved at 23.8 mo of age or older.

Introduction

In seasonal calving systems, heifers usually first calve at a young age (ca. 24 months (mo)). The first insemination (i.e. service) may be delayed, however, for heifers born at the end of the calving period if an adequate body weight (BW) is not reached (i.e. 360-380 kg for Holstein heifers in French dairy herds; Le Cozler *et al.*, 2008). Increasing nutrient uptake and thus the growth rate of these late-born heifers is one solution to lower this risk. High growth rate during rearing is associated with decreased age at puberty; consequently, first calving may occur as early as 20-21 mo of age. Tozer (2000) concluded that a higher plane of nutrition incurred higher daily feed costs, but these costs were recouped when heifers calved at a younger age through savings on labour, housing and overall feed costs. Regardless of the rearing strategy (group-calving or not), animals need to reach an adequate body size and or body weight before calving to avoid compromising milk production during the first lactation (Bach and Ahedo, 2008). Indeed, an accelerated growth program for dairy heifers cannot focus only on early onset of puberty. Many authors have studied the influence of growth intensity on future performances (Le Cozler *et al.*, 2008). Most studies indicated that a too -rapid growth rate had a negative influence, while some indicated that accelerated growth had little impact. According to Pirlo *et al.* (1997), reducing the age of first calving to 23 to 24 mo was the most profitable procedure, but no less than 22 mo (except in cases of low milk prices and high rearing costs). They concluded that the reluctance to decrease the age of first calving is generally attribute to the belief that early calving is detrimental to milk yield and longevity. We designed and conducted an experiment to determine the influence of feeding treatments on growth parameters, reproduction and the production performance of Holstein primiparous heifers that first calved from 20-27 mo of age in a seasonal calving system. We assumed that genetic improvements in dairy production over the past few decades had yielded animals that could calve earlier than 24 mo of age. We also assumed that results for animals reared in a seasonal calving strategy could be used and generalised for those in a non-grouped strategy. We examined the potential for late-born heifers to catch up to the rest of the heifers by the first artificial insemination (AI) at a minimum BW of 370-380 kg, resulting in a first calving at less than 22 mo of age.

Materials and methods

General design

A total of 217 Holstein heifers, born during the calving season in 2009-10 (n = 65), 2010-11 (n = 73) and 2011-12 (n = 76; September to February), were reared and followed until oestrus synchronisation (12-15 mo of age) at the INRA experimental farm of Méjusseaume (Le Rheu, France). For details of the rearing procedures and strategies used in the present study, see Abeni *et al.* (2019). Calves born from 1 September to 30 November were alternately assigned to 1 of two nutritional treatments (according to birth order) and fed either a standard diet (SD) or an intensive-plane diet (ID1) from 0-6 mo of age. It was expected that heifers fed the SD and ID1 diets would reach 190-200 and 220-230 kg at 6 mo of age, respectively. Heifers born after 1 December (ID2) received the same intensive-plane diet as ID1 heifers from 0-6 mo of age to decrease potential interaction between age and treatment during this period. Thereafter, a supplemental diet was formulated for ID2 heifers to enable them to reach 380 kg at 12 mo of age. The main objective of the ID2 diet was to study the potential for late-born heifers to catch up to the rest of the heifers by the first AI at a minimum BW of 370-380 kg. It was expected that this strategy would correspond to a mean age of 15 mo for SD and ID1 heifers and 12 mo for ID2 heifers. In year one, heifers grazed from mid-May until the end of October. In year two, heifers grazed from the end of March until calving season (starting 1 September). At the end of the first grazing season, all heifers were group-housed until being turned out to pasture in the second season. Three weeks before the expected date of calving, heifers were placed in cow herds and individually fed a similar total mixed ration (TMR). During lactation, milk yield was recorded twice per day and animals were weighed one per day. The experiment ended 15 weeks after calving.

Feeding management

Diets were formulated for each growth stage according to recommendations and procedures developed by Agabriel and Mechy (2007) to reach a targeted average daily gain (ADG) per period, as a function of the initial BW and feeding treatment used. In this approach, energy is expressed per UFL (forage unit for lactation, UFL/kg dry matter (DM)), which is the energy required for lactation (g/kg)/1760. For protein, PDIN (protein digestible in the small intestine, g/kg DM, when degradable nitrogen limits microbiological growth (INRA 2007) and PDIE (protein digestible in the small intestine, g/kg DM, when available energy limits microbial growth) are used. PDIN is the protein supplied by rumen-undegradable protein (PDIA) plus that supplied by microbial protein from rumen-degradable dietary protein. In comparison, PDIE is PDIA plus the microbial protein from rumen-fermented organic matter (INRA, 2007). At the end of the pre-experimental phase (0-10 d), heifers were group-housed indoors on deep straw bedding. They were fed a reconstituted milk replacer (MR) made from 135 g milk powder (23.9% crude protein and 19.0% fat content) and 865 g water per L until weaning (ca. 77 -84 d of age). They were reared in dynamic groups: calves entered the group each week, while others left it at weaning. They were individually fed with automatic milk feeding systems (AMFS), with *ad libitum* access to fresh water, straw and hay. Group size ranged from 8-24 calves per AMFS. From day 11, milk was distributed according to the standard ration routinely used in the experimental herd (SD) or the standard ration increased by 15% (ID1 & ID2). All calves were fed TMR no. 1 (TMR1) *ad libitum* (Table 1).The TMR1 contained 47.5% of maize silage, 47.5%of concentrate 1 and 5% of 18 % CP lucerne pellets.

Table 1. Ingredients and chemical composition of the experimental diets

Item[1] Stage of growth, age	TMR1 (7 d to 4 mo)	TMR2 (4 to 6-8 mo)	TMR3a (9-11 mo)	TMR3b (6-11 mo)	TMR4 (11-15 mo; *winter 1)*	TMR5 (21-26 mo) *(21 d before calving until calving)*	TMR6 (21-26 mo) *Calving + 14 d*	TMR7 (21-35 mo) *(15 d after calving until end of lactation)*
Feeding treatment	All	All	SD, ID1	ID2	All	All	All	All
Ingredient, %								
Maize silage	47.5	72.0	80.0	80.0	79.0	84.5	52.5	65.0
Soyabean meal	-	8.0	20.0	20.0	21.0	9.0	8.0	8.0
18% CP lucerne pellets	5.0						10.0	10.0
Straw						2.5	2.5	2.5
Urea								0.8
Vitamins & minerals	47.5	20.0						
Concentrate 1[2]			1.0	2.0	1.0			1.0
Concentrate 2[3] (kg/head/d)								
Concentrate 3[4] (%)						4.0	25	15.0
Estimated chemical composition								
DM, %	51.4	42.0	42.2	46.0	42.1	38.6	48.8	44.4
PDIE, g / kg DM	93.0	93.1	104.5	103.1	106.2	85.0	93.7	89.6
PDIN, g / kg DM	79.8	84.0	108.7	108.5	111.3	72.8	83.9	91.3
UFL / kg DM	0.96	0.96	0.98	1.00	0.99	0.93	0.93	0.92

[1] abbreviations: TMR: total mixed ration; SD, ID1, ID2: animals fed a standard (SD) or increased-plane (ID1 & ID2) feeding treatment; DM: dry matter; UFL: forage unit for lactation, UFL/kg DM; PDIN: protein digestible in the small intestine when degradable nitrogen limits microbiological growth (g/kg DM); PDIE: protein digestible in the small intestine when available energy limits microbial growth (g/kg DM; INRA, 2007).
[2] Chemical composition: DM 88.7%; PDIE 118 g; PDIN 114 g; UFL 1.05.
[3] Chemical composition: DM 87.9%; PDIE 81 g; PDIN 90 g; UFL 0.96.
[4] Chemical composition: DM 87.7%; PDIE 101 g; PDIN 76 g; UFL 1.05.

From weaning to 6-8 mo of age, calves were housed on deep straw bedding with *ad libitum* access to fresh water and straw. Until 4 mo of age, the SD group received TMR1 *ad libitum* until the maximum daily allowance of concentrate intake reached 2 kg DM/head/d. No restriction was applied for ID1 or ID2 heifers. From 4 to 6-8 mo of age, TMR2 was distributed *ad libitum* until concentrate intake reached

2.0, 2.5 and 2.5 kg DM/head/d for SD, ID1 and ID2 heifers, respectively i.e. total daily allowance of 10.0, 12.5 and 12.5 kg DM/head/d, respectively. These amounts did not change until being turned out to pasture. The TMR2 contained 72% of maize silage, 8% of soya bean meal and 20% of concentrate 1.

SD, ID1 and ID2 heifers were turned out to pasture from mid-May, mid-May and mid-June, respectively, and rotationally grazed on a perennial ryegrass sward. After a 5-d transition phase and throughout the grazing season, the SD and ID1 groups received a supplement of 1 kg DM/heifer/d of concentrate 2. The ID2 group received 1 kg DM/heifer/d of maize silage and 2 kg DM/heifer/d of concentrate 2. Grass availability and/or quality were insufficient to maintain the desired growth rates during summer. SD and ID1 heifers then received up to 2.5 kg DM/heifer/d of additional TMR3a, plus 1 kg DM/heifer/d of concentrate 2. ID2 heifers received up to 3 kg DM/heifer/d of TMR3b, plus 2 kg DM/heifer/d of concentrate 2. To reach 380 kg at the end of the grazing season (when oestrus synchronisation started), the expected ADG for SD and ID1 heifers was ca. 600 g/d during this period, with a feeding regime based on grass plus 1 kg DM/heifer/d of concentrate 2, and 800 g/d when receiving grass plus TMR3a. For ID2 heifers, it was estimated that grass alone was not sufficient to reach 900 g/d during the same period, so TMR3b was used (Table 1). In the pasture area, a permanent headlock barrier (80 places on a concrete floor) was used daily to feed concentrate to SD and ID1 heifers. Heifers were locked in for 1 hour while eating to decrease competition between heifers for feed. Since the ID2 group had *ad libitum* access to the ration, its heifers were not locked in. At the end of the first grazing season (the first week of November), heifers were group-housed (8 heifers/pen) on deep straw bedding and received 3.8 kg DM/head/d of a diet containing 79% maize silage and 21% soya bean meal. They had *ad libitum* access to fresh water, straw and mineral supplements.

Vitamins and minerals, when not included in the concentrate during rearing, were included in mineral blocks that contained 2.5% Ca, 2.0% Mg and 32.5% Na per kg of DM, as well as (in mg/kg) Zn (10 000), Mn (8250), Cu (1500), I (200), Se (20) and Co (13). The concentrates during growth contained 4% P, 27% Ca, 5% Mg, plus vitamins (in UI/kg; 1 000 000 vitamin A, 350 000 vitamin D3 and 8 000 vitamin E). They also contained (in mg/kg) Cu (1500), Zn (10 000), I (200), Co (100) and Se (10). During lactation, the mineral supplement contained 7% P, 22% Ca and 4% Mg, plus vitamins (in UI/kg; 500 000 vitamin A, 100 000 vitamin D3 and 1 500 vitamin E). It also contained (in mg/kg) Cu (1000), Mn (3500), Zn (4530), I (80), Co (35) and Se (22).

After a 2-week adaptation period, heifers' oestrous cycles were synchronised (see below), and the same rearing procedure was applied to all heifers. Heifers were turned out to pasture (generally in March) based on the date of successful insemination. They were reared in a single group and received no additional feed except for grass, along with the supplemental vitamins and minerals.

All heifers were housed indoors three weeks before the expected date of calving, along with multiparous cows, in a cubicle barn with fresh straw bedding that was distributed daily. Heifers were

fed individually and received TMR5 daily, composed of maize silage (84.5 %), soya bean meal (9%), concentrate (4%) and straw. From calving to 14 d post-calving, cows individually received TMR6, which contained maize silage (52.5%), soya bean meal (8%), concentrate (25%), dehydrated lucerne (1%), vitamin/mineral supplements, urea and straw (Table 1).
From day 14 after calving, cows individually received TMR7, which contained maize silage (65 %), soya bean meal (8%), concentrate (15%), dehydrated lucerne (1%), urea and vitamin/mineral supplements (7% P, 22% Ca and 4% Mg). All cows were fed *ad libitum* during lactation assuming at least 10% refusal per day. Feed was distributed twice per day (08:00 and 17:00), and refusals were collected each morning, (7:00) before fresh TMR was distributed.
The chemical composition of TMR ingredients produced on-farm (maize silage, straw) was determined at harvest, and an average sample of each, came from daily sample, was analysed. Another analyse was also done when the storage silo of maize silage changed. However, DM was determined at least once a week for all TMR ingredients. A similar procedure was applied to concentrate feed. The manufacturer analysed the feed (e.g. concentrate, soya bean) before delivering it, and we compared it to the average sample when changing feed. The estimated chemical composition of TMR was then determined using INRAtion® software (INRA, 2010) based on these analyses and the percentage of each ingredient in the TMR. Due to potential changes in composition (e.g. DM or grain content of maize silage), TMR composition was checked regularly, and the amount of each ingredient was adapted accordingly. Grass intake was not measured. All heifers and cows housed indoors had *ad libitum* access to fresh water during the entire experiment.

Age at first service

Age at first service (AFS) was then classified to understand better which factors could influence AFS and how future performance may be related to AFS. Three classes were created, with nearly an equal number of animals in each (Table 2).

Table 2. Description of the classes of age (in mo) at first service (AFS)

Characteristic	AFS$_{12.5}$	AFS$_{14.0}$	AFS$_{15.5}$
AFS[1]	12.6 (0.73)	14.2 (0.36)	15.4 (0.65)
Total heifers	58	57	60
Heifers in SD	16	29	29
Heifers in ID1	15	27	30
Heifers in ID2	27	1	1

[1]*Mean (and standard deviation) of age at first service (AFS)*

Oestrus synchronisation

All heifers were inseminated after oestrus synchronisation during the second winter of rearing so that calving would occur at ca. 24 mo of age. At the end of November, oestrus was synchronised for nearly half of the heifers using a progestin ear implant (Norgestomet®, Intervet, Angers, France) along with an intramuscular injection of oestrogen (Crestar®, Intervet, Angers, France), without considering ovarian activity. A second synchronisation was performed three weeks later for the remaining heifers. The ear implant was removed after 9 d of treatment. Heifers generally showed signs of oestrus within 24-96 h and were inseminated when oestrus was detected. Heifers that failed to conceive but exhibited further signs of oestrus were inseminated at the end of the reproductive season (April). Ultrasonography was conducted an average of 42 d after insemination to determine pregnancy. Non-gestating heifers were excluded from the rest of the experiment.

Sampling and measurements

Heifers were weighed every 14 d from birth to weaning, every 21 d from weaning until being turned out to pasture and every 28 d until the end of the experiment. BW was interpolated to compare the BW of heifers at similar stages of growth. ADGs were then calculated. Heifer health and care information was recorded throughout the experiment. The body condition score (BCS) was recorded three weeks before the expected date of calving and then once a month. The method and scale (ranging from 0-5) developed by Bazin *et al.* (1984) was used. BCS was scored by three trained technicians, whose scores were averaged.

Five measurements were recorded to monitor morphological traits during rearing and first lactation: heart girth (HG), chest depth, wither height (WH), hip width and backside width. A tape measure was used to measure HG, while a height gauge was used for the other measurements. The measurements were recorded only for the two first cohorts (2009-10 and 2010-11: Supplementary Fig. 1). Results were interpreted by class of age at first service calving (AFC), which was created later (not shown or discussed in the present article).

Daily feed intake was calculated individually as the daily feed allowance minus refusals. The allowance and refusals were assumed to have the same composition. DM of silage was determined five times per week, while DM of the pellets was determined once per week. Feed composition was estimated from average samples for maize silage, straw, soya bean and concentrate. Composition was not available for fresh grass (Table 1).

Milk content analysis

Milk yield was automatically recorded at each milking (i.e. twice per day). During six successive milkings (Tuesday-Thursday), milk samples were collected and analysed for each cow to determine the fat and protein contents (Milkoscan, Foss Electric, Hillerod, Denmark). Fat- and- protein- corrected milk (FPCM, kg) was calculated using the following equation (INRA, 2018):

$$FPCM = MY \times \frac{[0.42 + 0.0053 \times (FC - 40) + 0.0032 \times (PC - 31)]}{0.42}$$

where FC is milk fat content (g/kg), PC is milk protein content (g/kg) and 0.42 is the UFL value for 1 kg of milk containing 40 g/kg of fat and 31 g/kg of protein.

Milk progesterone analysis

Morning milk samples were collected Monday, Wednesday and Friday from calving to two weeks after the service that induced pregnancy, or five weeks after the end of the breeding season (i.e. July), and were then stored at -20°C to determine progesterone using commercial ELISA kits (Milk Progesterone ELISA, Ridgeway Science Ltd., England). Coefficients of variation among assays for ELISA on 5 ng/ml control samples ranged from 8-14% among experimental years.

Determining Luteal Activity

Two progesterone (P4) milk concentration thresholds were defined, following Petersson *et al.* (2006) and adapted by Cutullic *et al.* (2011), to distinguish (i) the baseline P4 level in milk from the luteal phase level (threshold 1) and (ii) a low luteal phase level from a high luteal phase level (threshold 2). P4 values were classified as negative (< threshold 1), positive (> threshold 2) or intermediate. An increase in P4 milk concentrations was considered to be induced by corpus luteum activity when at least two consecutive values were not negative and at least one was positive. Due to the sampling schedule (Monday, Wednesday and Friday), the interval between samples was 2 d or 3 d. A decrease in P4 milk concentration was considered to result from luteolysis of the corpus luteum when at least one value became negative. These definitions helped to identify and distinguish luteal phases from inter-luteal phases.

Qualifying Progesterone Profiles

Physiological intervals were calculated for each luteal phase: commencement of luteal activity (CLA), cycle length (IOI), luteal phase length (LUT) and inter-luteal interval (ILI; for details, see Cutullic *et al.*, 2011). Ovulation was considered to induce a prolonged luteal phase (PLP) if the luteal phase exceeded 25 d. Ovulation was considered to be delayed if the inter-luteal interval exceeded 12 d. Based on these definitions, P4 profiles were classified as (i) normal, (ii) PLP profile (when at least one PLP was observed), (iii) delayed (D; if CLA > 60 d), (iv) interrupted (I; when at least one ovulation > 2 was delayed) and (v) disordered (Z; when luteal activity appeared irregular but could not be assigned to another abnormality class).

Calculations and statistical analysis

All data on dairy cows (e.g. reproduction, milk yield, feed intake) was automatically stored in a dedicated recording system. Analyses of heifer growth and performance, as well as data on progesterone, were recorded in Microsoft Excel files. All data manipulation and statistical analyses were performed in R software using the *lm* procedure for ANOVA or *glm* for logistic regressions (R Core Team, 2019). Normal distribution of the residuals, equality of the variance and non-dependent data were checked for all models. Quantitative traits (i.e. growth, age, BW, milk yield, BCS, CLA, cycle lengths) were studied using the following ANOVA model:

$$y_{ij} = \mu + year_i + \left|\begin{matrix} AFS_j \\ T_j \end{matrix}\right| + e_{ij}$$

where y_{ij} is the variable of interest, μ is the overall mean of the variable of interest, $year_i$ is the fixed effect of the experimental year (i=1, 2 or 3), AFS_j is the fixed effect of AFS (j= 12.5, 14.0 or 15.5 mo) or T_j is the fixed effect of feeding treatment (j= SD, ID1 or ID2) included in the model, and e_{ij} is the random residual effect. Year was included as a fixed effect because there were only three levels (year1, year2, year3), and this approach seemed the most appropriate option given the small number of levels. Had year been included as a random effect, variance would have been estimated from only three levels, rendering it inaccurate.

Dichotomous traits (i.e. reproductive success and type of cyclicity pattern) were studied using the following logistic regression model:

$$log\left[\frac{P(y_{ij}=1)}{1-P(y_{ij}=1)}\right] = \mu + year_i + \left|\begin{matrix} AFS_j \\ T_j \end{matrix}\right| + \beta \times PRI_{ij}$$

where y_{ij} is the variable of interest, μ is the overall mean and the fixed effects ($year_i$, AFS_j or T_j) are the same as previously described.

For the reproductive performance of heifers, the covariate PRI_{ij} was added; it describes the effect of the interval from the removal of the last progesterone-releasing implant until insemination. This covariate was not required for the performance of cows because only heifers were synchronised. Effects were considered highly significant at P<0.001, significant at P<0.05 and a trend at P<0.10.

Results

Of the 217 heifers in the experiment, 175 successfully calved. The 42 that did not either died during rearing (7), were culled due to injuries (6) or were not pregnant within the breeding period considered for the present study (29).

Growth and reproductive performance of heifers

Mean BW at birth was 41.3 kg (± 5.2) and did not differ significantly among all groups (i.e. not associated with the feeding treatment, P = 0.85, or AFS, P = 0.15; Table 3; Table 4).

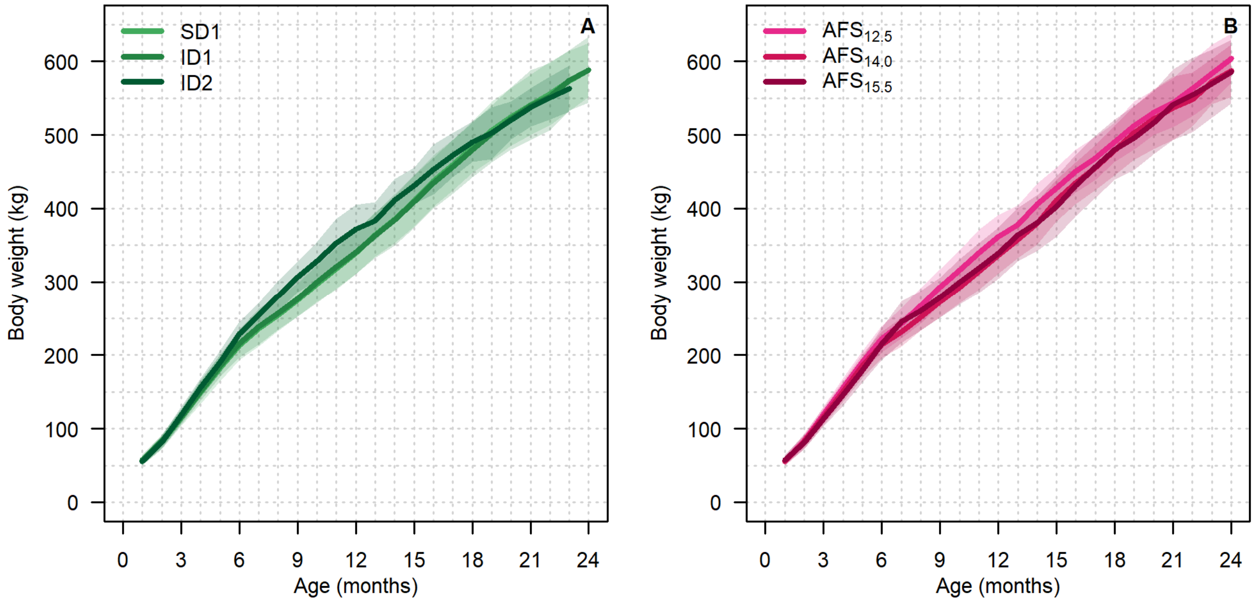

Figure 1. Mean body weight of heifers during the rearing period by (A) feeding treatment (SD, ID1, ID2: animals fed a standard (SD) or increased-plane (ID1 & ID2) feeding treatment) and (B) class of age at first service (AFS). Shaded areas are the dispersions of the data around the means ($\pm$ one standard deviation).

The feeding treatment had little effect on growth during the milking phase, and heifers reached 117 kg (± 11.8) at 3 mo of age (immediately after weaning). From weaning to 6 mo, heifers in the ID2 treatment were heavier than those in the SD and ID1 treatments (229 kg *vs* 213 kg and 217 kg at 6 mo, respectively; $P < 0.001$; Fig. 1A). The highest ADG was observed for ID2 heifers from 0-6 mo (1042 *vs* 958 and 976 g/d for ID2, SD and ID1, respectively; $P < 0.001$, Table 3). This difference remained significant from 6-12 mo of age (789, 703 and 699 g/d for ID2, SD and ID1 heifers, respectively; P 0.01, Table 3). However, from 12-18 mo, ADG was significantly lower for ID2 heifers than for SD and ID1 heifers (660 *vs* 800 and 774 g/d, respectively; $P < 0.001$, Table 3).

The feeding treatment had no effect on reproductive performance (Table 3), although ID2 heifers tended to have fewer services than SD or ID1 heifers (1.5 vs 1.9 or 1.8, respectively). Cows in the three feeding treatments had a similar interval from the start of the breeding season to the first service (13.5 d), similar success at the first service (ca. 62% of heifers pregnant) and a similar pregnancy rate by the end of the breeding season (94%).

No difference in calf BW (37.9 kg) was observed, despite a difference in their dam's BW at the first service and first calving (ID2 heifers were lighter than SD and ID1 heifers Table 3 and 5). ID2 heifers calved at a younger age than SD or ID1 heifers (ca. 2 mo earlier, $P < 0.001$; Table 3).

Heifers inseminated at the youngest age (a mean of 12.5 mo; $AFS_{12.5}$) tended to have a higher growth rate from 0-6 mo of age than those inseminated at 14.0 ($AFS_{14.0}$) or 15.5 ($AFS_{15.5}$) mo of age (1001 *vs* 960 or 978 g/d, respectively; $P < 0.10$; Table 4). This difference increased from 6-12 mo of age (759 *vs* 688 and 698 for $AFS_{12.5}$, $AFS_{14.0}$ and $AFS_{15.5}$, respectively; $P < 0.01$; Table 4; Fig. 1B).

Table 3. Effects of feeding treatment on the growth and reproductive performance of heifers during the rearing period

	Feeding Treatment			Model[1]		Significance levels[2]
	SD1	ID1	ID2	R^2_{adj}	RSE	
Number of heifers	74	72	29			
Growth						
BW[3] at birth (kg)	41.2	41.7	41.1	0.00	5.19	0.85
BW at first AI (kg)	400.7[a]	398.5[a]	378.1[b]	0.14	33.29	★★
ADG[4] 0-6 months (g/d)	958[a]	976[a]	1042[b]	0.09	97.7	★★★
ADG 6-12 months (g/d)	703[a]	699[a]	789[b]	0.31	116.8	★★
ADG 12-18 months (g/d)	800[a]	774[a]	660[b]	0.11	133.2	★★★
Reproduction						
Start of breeding season to first service interval (d)	13.9	12.8	14.0	0.00	5.76	0.46
Pregnancy rate at first service (%)	64	58	66	NA	NA	0.64
Number of services	1.9	1.8	1.5	0.21	0.78	●
Pregnant (%)	95	96	90	NA	NA	0.67
Age at first calving (months)	24.0[a]	23.9[a]	21.9[b]	0.32	1.26	★★★
Calf BW (kg)	38.4	37.6	37.2	0.32	4.02	0.37

[1]adjusted coefficient of determination: R^2_{adj}; residual standard error: RSE
[2]★★★ $P < 0.001$; ★★ $P < 0.01$; ★ $P < 0.05$; ● $P < 0.1$; otherwise, the exact P-value
[3]body weight: BW
[4]average Daily Gain: ADG
[a-b] Different superscripts indicate adjusted means that differ between feeding treatments ($P < 0.05$, Tukey's pairwise comparison)

From 12-18 mo of age, AFS$_{12.5}$ heifers had a lower growth rate than AFS$_{14.0}$ and AFS$_{15.5}$ heifers ADG of 712 *vs* 799 and 790 g/d, respectively ($P < 0.001$; Table 4). This is consistent with the effects of the feeding treatment and the distribution of animals among the AFS classes and feeding treatments (Table 2).

AFS had no influence on fertility (Table 4). All heifers had a similar interval from the start of the breeding season to the first service, a similar success at the first service and a similar pregnancy rate by the end of the breeding season, with a similar number of services per animal.

No difference in calf BW (37.9 kg) was observed, despite a difference in the dam's BW at first service and at first calving (AFS$_{12.5}$ heifers were lighter than those in AFS$_{14.0,}$ which were lighter than those in AFS$_{15.5}$, Tables 4 and 6). Consistent with the AFS, AFS$_{12.5}$ heifers calved younger than AFS$_{14.0}$ heifers, which calved younger than AFS$_{15.5}$ heifers (Table 4).

Table 4. Relations between age at first service and growth and reproductive performance of heifers during the rearing period

	Age at first service (AFS)			Model[1]		Significance levels[2]
	$AFS_{12.5}$	$AFS_{14.0}$	$AFS_{15.5}$	R^2_{adj}	RSE	
Number of heifers	58	57	60			
Growth						
BW[3] at birth (kg)	41.5	42.0	40.2	0.02	5.13	0.15
BW at first AI[4] (kg)	373.1[a]	394.3[b]	419.8[c]	0.37	28.49	★★★
ADG[5] 0-6 months (g/d)	1001	960	978	0.03	100.8	●
ADG 6-12 months (g/d)	759[a]	688[b]	698[b]	0.30	117.5	★★
ADG 12-18 months (g/d)	712[a]	799[b]	790[b]	0.07	136.3	★★
Reproduction						
Start of breeding season to first service interval (d)	12.9	13.2	14.3	0.00	5.75	0.42
Pregnancy rate at first service (%)	59	60	67	NA	NA	0.30
Number of services	1.7	1.7	1.9	0.20	0.78	0.25
Pregnant (%)	93	91	98	NA	NA	0.37
Age at first calving (months)	22.3[a]	23.8[b]	24.8[c]	0.52	1.06	★★★
Calf body weight (kg)	37.4	38.6	37.7	0.32	4.02	0.31

[1]*adjusted coefficient of determination: R^2_{adj}; residual standard error: RSE*
[2]*★★★ $P < 0.001$; ★★ $P < 0.01$; ★ $P < 0.05$; ● $P < 0.1$; otherwise, the exact P-value*
[3]*body weight: BW*
[4]*artificial insemination: IA*
[5]*average Daily Gain: ADG.*
[a-b] *Different superscripts indicate adjusted means that differ between feeding treatments ($P < 0.05$, Tukey's pairwise comparison)*

Lactating performance of primiparous cows

BW recorded immediately after calving was lower for ID2 cows than for SD and ID1 cows (501 *vs* 542 and 534 kg, respectively; $P < 0.001$; Table 5; Fig. 2A.), which is consistent with the observation that ID2 heifers first calved younger than SD and ID1 heifers (Table 4). No difference in BCS was observed among the feeding treatments during the first lactation (result not shown). On a 308 d basis, ID2 cows tended to produce less milk than SD and ID1 cows (6920 *vs* 7312 and 7370 kg, respectively; $P < 0.10$; Table 5; Fig. 2C). No difference in mean fat and protein contents was observed among feeding treatments. However, cows that received the ID2 treatment when heifers produced less FPCM than cows that received the SD or ID1 treatments (6482 *vs* 6983 and 6973 kg, respectively; $P < 0.05$). ID2 cows had a lower peak milk yield than SD and ID1 cows (28.7 *vs* 31.3 and 31.9 kg/d, respectively; $P < 0.001$). During the first seven weeks of lactation, ID2 cows were lighter (on average, 38 and 25 kg less than SD and ID1 cows, respectively), and produced less milk (3.1 kg/d less than SD and ID1). This difference decreased during the last part of the period (8-15 weeks); ID2 cows weighed 27 and 17 kg less than SD and ID1 cows, respectively, and produced 2.2 and 2.9 kg/d less milk than SD and ID1 cows respectively.

Table 5. Effects of feeding treatment during the rearing period on productive and reproductive performances of primiparous cows

	Feeding Treatment			Model[1]		Significance levels[2]
	SD1	ID1	ID2	R^2_{adj}	RSE	
Number of cows	67	68	24			
Production						
Total milk yield per 308 d (kg)	7312	7370	6920	0.19	706.9	●
Peak milk yield (kg)	31.3[a]	31.9[a]	28.7[b]	0.10	3.50	★★
Mean fat content (g/kg)	37.0	36.5	36.2	0.10	3.66	0.75
Mean protein content (g/kg)	30.2	29.7	29.4	0.02	1.53	0.17
Fat- and protein- corrected milk (kg)	6983[a]	6973[a]	6138[b]	0.26	668.5	★
Conformation						
BW[3] at first calving (kg)	542[a]	534[a]	501[b]	0.10	43.0	★★★
BCS[4] at calving (0-5 scale)	2.45	2.40	2.30	0.33	0.296	0.11
BCS at nadir (0-5 scale)	1.85	1.80	1.75	0.43	0.267	0.47
BCS loss to nadir (0-5 scale)	-0.55	-0.60	-0.60	0.44	0.255	0.81
Cyclicity[5]						
CLA (d)	20.9	24.8	20.1	0.00	0.56	0.23
IOI_1	20.7	23.8	24.9	0.04	14.01	0.47
LUT_1	13.3	13.9	14.9	0.18	10.77	0.88
ILI_1	9.6	11.2	7.7	0.04	11.29	0.55
IOI_{2-4}	23.3	23.6	21.2	0.00	5.91	0.42
LUT_{2-4}	13.8	13.7	12.5	0.39	5.79	0.77
ILI_{2-4}	9.0	10.2	9.0	0.45	4.76	0.54
Normal (%)	65%	59%	53%	NA	NA	0.52
PLP (%)	19%	18%	33%	NA	NA	0.44
Delayed (%)	10%	12%	7%	NA	NA	0.81
Fertility						
Number of services per cow	1.9[a]	2.4[b]	2.2[ab]	0.10	1.27	★
Pregnancy rate (%)	86%	85%	87%	NA	NA	0.92
Calf BW (kg)	38.4	37.8	36.9	0.00	4.84	0.40

[1]*adjusted coefficient of determination: R^2_{adj}; residual standard error: RSE*
[2]*★★★ $P < 0.001$; ★★ $P < 0.01$; ★ $P < 0.05$; ● $P < 0.1$; otherwise, the exact P-value*
[3]*body weight: BW*
[4]*body condition score: BCS*
[5]*commencement of luteal activity: CLA; cycle length: IOI; luteal phase length : LUT; inter-luteal interval : ILI; prolonged luteal phase: PLP*
[a-b] *Different superscripts indicate adjusted means that differ between feeding treatments ($P < 0.05$, Tukey's pairwise comparison)*

The feeding treatment of dairy cows during the rearing period did not affect ovarian cyclicity during the first lactation (Table 5). Mean CLA was 20.4 d, and the first IOI was 20.7 d, with no difference in LUT or ILI among treatments. No difference in the subsequent cycles was observed, with a mean IOI of 23.3 d. The distribution of abnormal patterns of ovarian activity was not significant, although ID2 cows had a lower normal profile rate than ID1 cows, which had a lower normal profile rate than SD cows (53% *vs* 59% *vs* 65%, respectively; Table 5). ID2 cows had an incidence of PLP abnormalities of 33%, while that for ID1 and SD cows was 18% and 19%, respectively (Table 5). Ca. 86% of cows were pregnant at the end of the breeding season, which had no relationship with feeding treatment. Although the difference in cyclicity among feeding treatments did not influence the re-calving rate, ID1 cows required more services for pregnancy to occur than SD cows (2.4 *vs* 1.9, respectively; $P < 0.05$; Table 5). The number of services required to achieve pregnancy was ca. 2.2 for ID2 cows. Feeding treatment had no influence on subsequent calf BW.

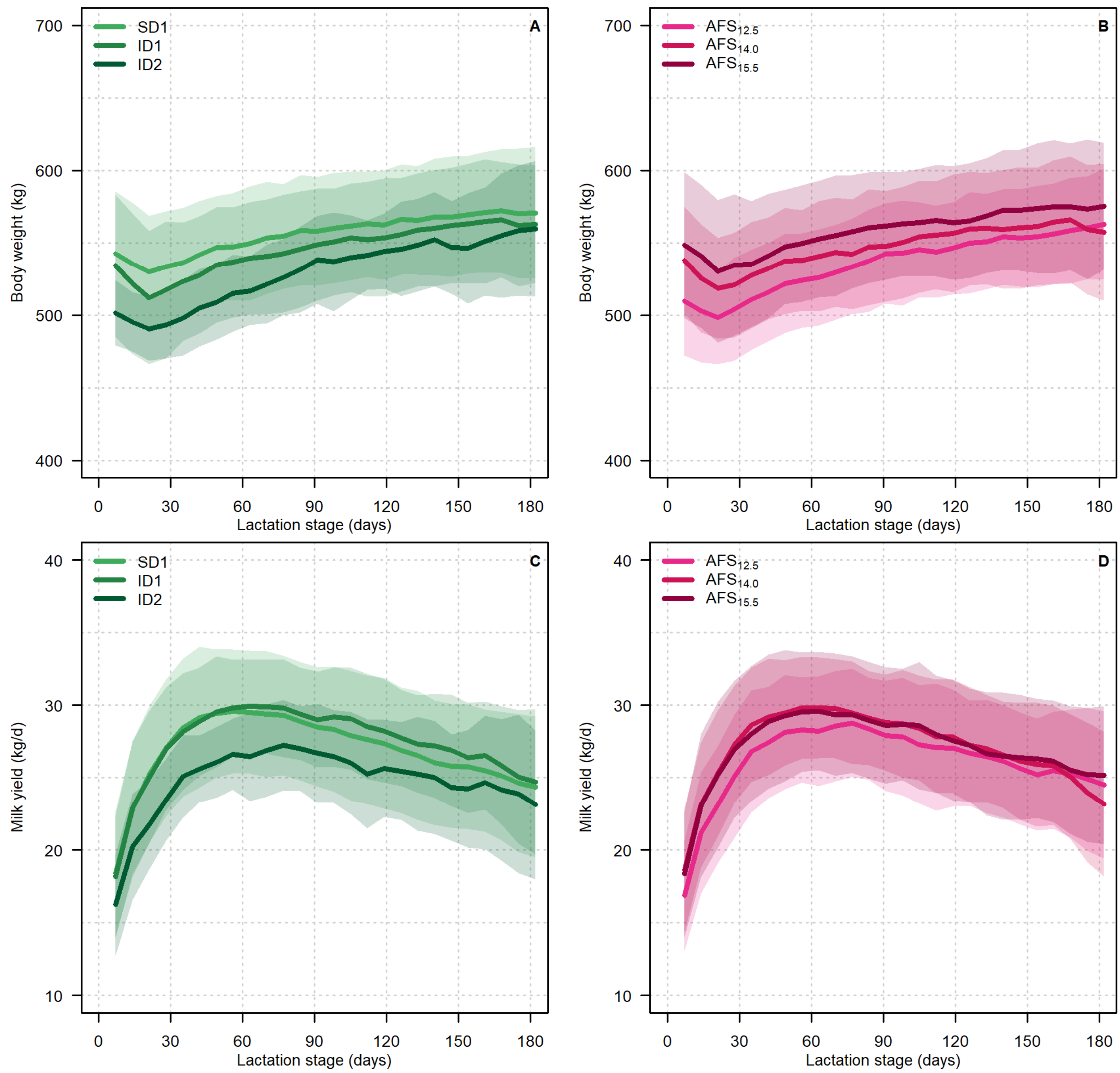

Figure 2. (A and B) body weight and (C and D) milk yield of primiparous cows during lactation by (A and C) feeding treatment (SD, ID1, ID2: animals fed a standard (SD) or increased-plane (ID1 & ID2) feeding treatment) and (B and D) class of age at first service (AFS). Shaded areas are the dispersions of the data around the means (± one standard deviation).

AFS influenced BW at calving, and was lower for $AFS_{12.5}$ than for $AFS_{14.0}$ and $AFS_{15.5}$ cows (509 *vs* 539 and 549 kg, respectively, P < 0.001; Table 6; Fig. 2B). BCS at calving was higher for $AFS_{15.5}$ than for $AFS_{12.5}$ and $AFS_{14.0}$ cows (2.45 *vs* 2.35 and 2.35, respectively; P < 0.05). After calving, BCS did not differ between AFS classes. On a 308 d basis, no difference in milk yield, composition or FPCM was observed. Only peak milk yield tended to be lower for $AFS_{12.5}$ cows (30.2 kg) than for $AFS_{14.0}$ and $AFS_{15.5}$ cows (31.6 and 31.7 kg, respectively; Fig. 2D; Table 6).

AFS influenced fertility characteristics little. For ovarian cyclicity, all three AFS classes had similar CLA, with similar cycle lengths, except for $AFS_{15.5}$ cows, which tended to have longer ILI from the second to fourth cycle than $AFS_{12.5}$ and $AFS_{14.0}$ cows (Table 6). $AFS_{14.0}$ cows had a lower incidence of PLP than $AFS_{12.5}$ and $AFS_{15.5}$ cows (8% *vs* 29% and 23%, respectively; P < 0.05; Table 6). AFS did not influence fertility: all classes had similar number of services (2.2, on average), and an average of 86% of the cows in each class were pregnant at the end of the breeding season. Subsequent calf BW was heavier for $AFS_{14.0}$ cows than for $AFS_{12.5}$ and $AFS_{15.5}$ cows (+2 kg; P < 0.05; Table 6). Feed intake did not differ among feeding treatments or among AFS classes (17 kg DM/d), even when it was corrected per kg of BW (Fig. 3).

Morphological trait analysis based on age at first calving (AFC) cohorts 2009-10 and 2010-11 (Supplementary Fig.1) indicated that young cows at first calving (mean age of 21 mo, n = 30; AFC_{21}) were lighter than those that first calved at a mean age of 23.5 mo (n = 39; $AFC_{23.5}$) or 25 mo (n = 36; AFC_{25}; 498 *vs* 528 and 563 kg, respectively; P < 0.05) and also had smaller morphological traits. For example, WH was 137.4, 139.1 and 140.4 cm for AFC_{21}, $AFC_{23.5}$ and AFC_{25}, respectively; P < 0.05). However, at a given age (e.g. 25 mo), no difference among the three AFC treatments was observed (140.7, 140.4 and 142.0 mm, respectively).

Discussion

The present study indicates that reducing the age of first service to ca. 12 mo and, consequently, age at first calving to 22 mo or less, influenced the performance of primiparous Holstein cows little. Several authors have shown that setting age at first calving of heifers at 23-26 mo of age increases longevity and maximises economic returns (Bach 2011; Wathes *et al.,* 2014; Boulton *et al.,* 2017). The early rearing period is key to reaching this target, as sub-optimal nutrition delays the onset of puberty, adversely affects skeletal growth and increases the risk of dystocia at first calving (Ettema and Santos 2004). Poor growth is the main reason for culling heifers prior to calving (Esslemont and Kossaibati 1997). Pre-weaning growth in dairy heifers is generally associated with the performance of first lactation (Khan *et al.* 2011; Soberon *et al.,* 2012). Some studies reported that pre-weaning differences caused by different feeding regimes were not statistically significant as calves aged (Morrison *et al.*

2009; Quigley *et al.* 2006). This may be explained in part by a compensatory increase in growth when the feed allowance (e.g. level, energy, protein) is no longer limited after a period of restriction.

Table 6. Effects of the class of age at first service (AFS) on the productive and reproductive performance of primiparous cows

	Age at first service (AFS)			Model[1]		Significance levels[2]
	$AFS_{12.5}$	$AFS_{14.0}$	$AFS_{15.5}$	R^2_{adj}	RSE	
Number of cows	51	50	58			
Production						
Total milk yield per 308 d (kg)	7229	7236	7370	0.15	721.7	0.68
Peak milk yield (kg)	30.2	31.6	31.7	0.04	3.59	●
Mean fat content (g/kg)	36.2	36.9	36.8	0.10	3.65	0.66
Mean protein content (g/kg)	29.8	29.9	29.9	0.00	1.56	0.93
Fat- and protein- corrected milk (kg)	6800	6891	7000	0.26	688.4	0.51
Conformation						
BW[3] at first calving (kg)	509[a]	539[b]	549[b]	0.14	41.9	★★★
BCS[4] at calving (0-5 scale)	2.35[a]	2.35[a]	2.45[b]	0.34	0.295	0.05
BCS at nadir (0-5 scale)	1.75	1.8	1.85	0.44	0.264	0.13
BCS loss to nadir (0-5 scale)	-0.60	-0.60	-0.55	0.44	0.254	0.41
Cyclicity[5]						
CLA (d)	20.2	23.6	23.7	0.00	0.56	0.39
IOI_1	25.0	19.8	23.2	0.04	13.96	0.31
LUT_1	13.9	12.3	14.9	0.19	10.73	0.57
ILI_1	10.7	8.7	10.7	0.04	11.32	0.68
IOI_{2-4}	23.0	22.3	24.1	0.00	5.92	0.45
LUT_{2-4}	14.5	13.6	12.7	0.39	5.75	0.44
ILI_{2-4}	8.8	8.8	11.1	0.48	4.67	●
Normal (%)	58	68	56	NA	NA	0.55
PLP (%)	29	8	23	NA	NA	★
Delayed (%)	5	13	14	NA	NA	0.23
Fertility						
Number of services per cow	1.9	2.4	2.2	0.08	1.28	0.16
Pregnancy rate (%)	86%	88%	84%	NA	NA	0.90
Calf BW (kg)	37.2[a]	39.3[b]	37.3[a]	0.04	4.77	★

[1]*adjusted coefficient of determination: R^2_{adj}; residual standard error: RSE*
[2]*★★★ P < 0.001; ★★ P < 0.01; ★ P < 0.05; ● P < 0.1; otherwise, the exact P-value*
[3]*body weight: BW*
[4]*body condition score: BCS*
[5]*commencement of luteal activity: CLA; cycle length: IOI; luteal phase length : LUT; inter-luteal interval : ILI; prolonged luteal phase: PLP*
[a-b] *Different superscripts indicate adjusted means that differ between feeding treatments (P < 0.05, Tukey's pairwise comparison)*

The differences in feed allowance resulted in differences in development and size at 6 and 12 mo of age but had little effect on BW at weaning. In a study by Johnson et al (2019), two treatment groups before weaning had significant differences in pre-weaning performance that persisted up to 6 mo. In our study, the high feed allowance before weaning, without restricting the TMR for control heifers, probably explains the lack of difference in BW observed at weaning. According to Morrison et al.

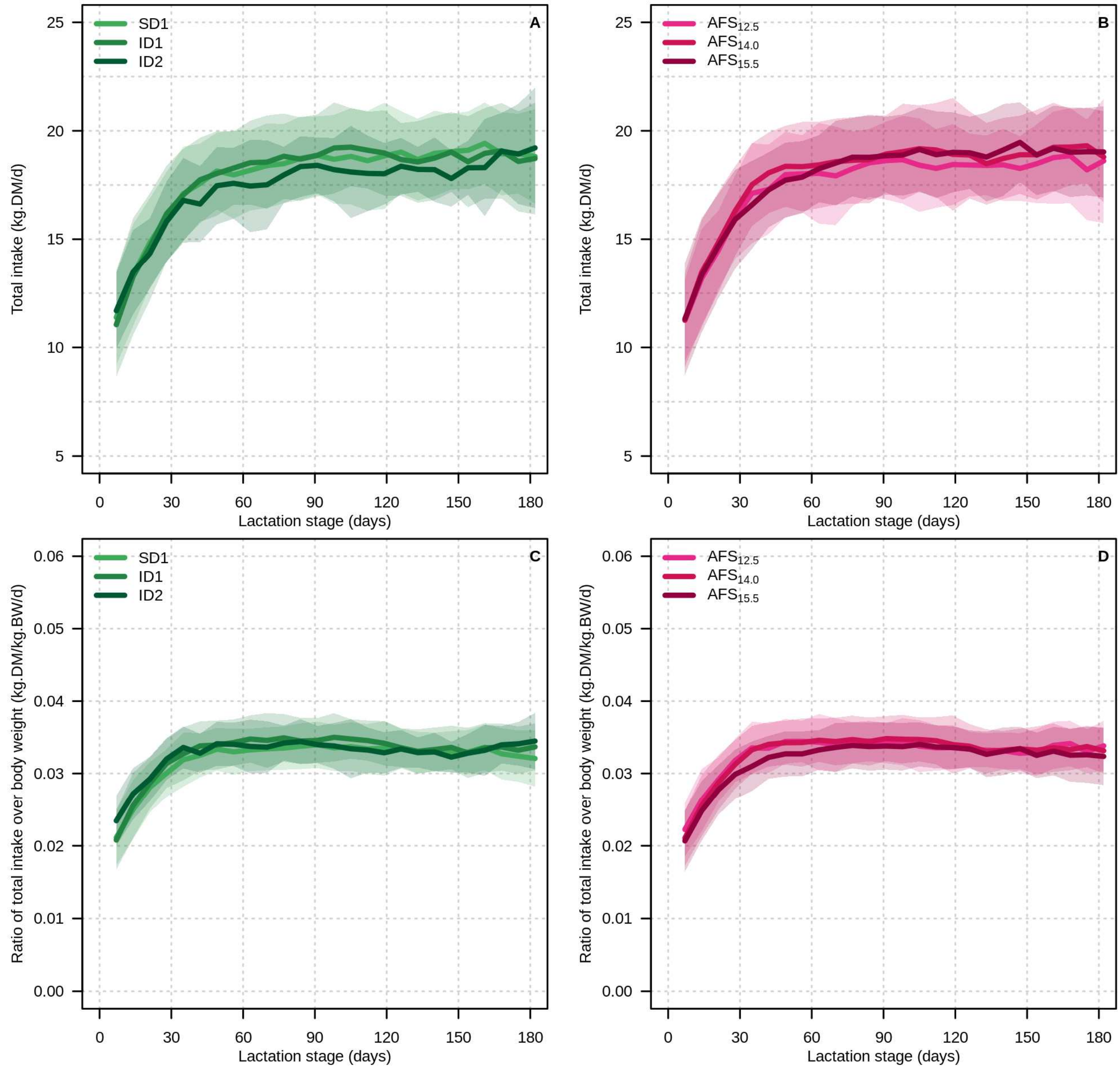

Figure 3. (A and B) daily dry matter intake and (C and D) daily ratio of dry matter intake over body weight of primiparous cows during lactation by (A and C) feeding treatment (SD, ID1, ID2: animals fed a standard (SD) or increased-plane (ID1 & ID2) feeding treatment) and (B and D) class of age at first service (AFS). Shaded areas are the dispersions of the data around the means (± one standard deviation).

(2009), on most commercial farms, a small amount of milk (4-6 L/day of whole milk or 400-600 g of milk replacer (MR) is offered until weaning at 42-56 days of age. According to Jasper and Weary (2002), *ad libitum* milk intake is ca. 12 L/day of whole milk, and intake in the present study was ca. 9 L/d per heifer until 11 weeks of age. The development and BW of animals at 6 mo were high (e.g. 111 cm HG and 220 kg BW), which fits well with recommendations for an optimal age at first calving at 24 mo of age or less. In a study by Ettema and Santos (2004) on the importance of age and BW at first calving for Holstein heifers, only 2.7% of dairy farms reached the recommended target BW, which resulted in economic losses. Total nutrient intake, energy source and protein content in the diet have a cumulative effect on how calves partition nutrients into tissue (Van Amburgh and Drackley 2005). During the milking phase, calves benefit when MRs contain more protein and less fat, and reach higher levels of skeletal growth (Hill *et al.,* 2010). Therefore, providing more MR improves growth and feed efficiency (Bartlett *et al.,* 2006). Increased nutrient intake is also associated with increased plasma levels of insulin-like growth factor 1 (Smith *et al.,* 2002; Bartlett *et al.,* 2006), which in part regulates the subsequent growth rate (Hammon *et al.,* 2002; Brickell *et al.,* 2009a).

Several studies discuss effects of intensive growth during rearing (Le Cozler et al. 2008), and that an increase in growth rate resulted in earlier puberty (Abeni *et al*., 2019). However, authors do not agree on the influence of earlier calving on milk performance: some observed a negative influence, while others did not. Abeni *et al.* (2000) and Van Amburgh *et al.* (1998) concluded that calving earlier than 23 mo is associated with lower milk yields and lower milk fat content; however, it also results in a higher milk protein content. They also concluded that earlier calving results in a decrease in reproductive performance. In a more recent study, Krpálková *et al.* (2014) observed that age at first calving had no influence on milk yields of primiparous cows, except for those during the first 100 d of lactation. They also observed the highest milk yield for the second and third lactation of heifers that first calved at 23 mo of age. In the present study, a negative influence was observed only at the start of the first lactation, but not for all of it. No data were available for later lactations. Van De Stroet *et al.* (2016) observed that primiparous cows that had consumed more starter feed as calves tended to have higher peak milk yields during first lactation than those that had consumed less. However, higher calf growth rates were not significantly related to future milk yield, but were related to higher BW of lactating cows and higher odds of surviving to first lactation. When lactation was corrected for BW, no difference in milk yield or composition was observed, regardless of the feeding strategy during the rearing period.

Decreasing the age of first calving is an effective way to decrease the length of the non-productive period during rearing. First calving at ca. 24 mo appears optimal for profitable production (Mourits *et al.,* 1999b; Ettema and Santos; 2004; Shamay *et al*., 2005). In a meta-analysis of results of 100 herds, Mohd Nor *et al.* (2013) estimated that heifers that first calved at 24 mo produced a mean of 7 164 kg of milk per 305 d, and calving one mo earlier resulted in 143 kg less milk per 305 d. In the present study, younger heifers produced less milk during the first part of lactation, but the total milk yield per 305 d did not differ. The decrease in milk yield was similar (134 kg less per 305 d), albeit not significantly different, when age at first calving decreased from 24.8 to 23.8 mo of age.

Age at first service had no effect on fertility. In a previous study on puberty attainment in the 2011-12 cohort, we observed that most heifers reached puberty before oestrus synchronisation, at a mean age of 10.3 ± 2.2 mo (6.2-14.4 mo) and a mean BW of 296 ± 40 kg (224-369 kg; Abeni *et al*., 2019). ID2 heifers reached puberty one month earlier than SD and ID1 heifers. The onset of puberty at 9-10 mo or less meant that 3 or 4 oestrous cycles occurred before insemination, which is generally consistent with acceptable fertility results in many domestic species (Lin *et al.*, 1986; Byerley *et al.*, 1987; Robinson, 1990; Le Cozler *et al.*, 1999). Regardless of calving strategy, decreasing the age of puberty and, consequently, the age of first service, is an effective way to shorten the non-productive period before calving. As Meyer *et al.* (2006) suggested, however, could reduce pre-pubertal mammary gland development by shortening the allometric phase of mammary gland growth and, in some cases, impair future milk production. Like its lack of effect on fertility in heifers, age at first calving did not influence fertility of primiparous cows during first lactation. Wathes *et al.* (2008) reported that fertility was optimised and maximum performance was maintained during first lactation when heifers first calved at 24-25 mo, although those that first calved at 22-23 mo had the best overall performance and longevity over 5 years, in partly because heifers with high fertility maintained high fertility as cows.

We also observed that at a similar feed allowance, early-calving heifers ate a similar amount of feed, produced less milk and ultimately were able to catch up in BW and development. As Krpalkova *et al.* (2014) reported, our results indicate that a feeding-rearing program that aims for first calving at less than 23 mo of age is a suitable option for successfully rearing Holstein heifers with optimal subsequent production and reproduction in a herd with suitable management. However, future studies are required to explore performances during the second and later lactations, as well as animal longevity.

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Declaration of interest

The authors declare that the research was conducted in the absence of commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics statement

Experimental work was conducted in accordance with French national legislation on the use of animals for research. Protocol agreement no. 00944-02 was received from French Ethical Committee n0. 7.

Software and data repository resources

None of the data were deposited in an official repository.

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This certificate confirms that the revised manuscript entitled **“Effects of feeding treatment on growth rate and performance of primiparous Holstein dairy heifers”** by Yannick Le Cozler et al. was edited and proofread for correct English-language content by two native speakers of American English.

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