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

Y. Le Cozler [1], J. Jurquet [2], N. Bedere [3*]

[1] PEGASE, INRA, AGROCAMPUS OUEST, 35590, Saint-Gilles, France
[2] Institut de l'Elevage, Monvoisin, 35652 Le Rheu, France
[3] URSE, Ecole Supérieure d'Agricultures, Université de Bretagne Loire, 55 rue Rabelais, Angers, France
[*] Current address: PEGASE, INRA, AGROCAMPUS OUEST, 35590, Saint-Gilles, France

Corresponding author: Yannick Le Cozler. Email: yannick.lecozler@agrocampus-ouest.fr

Abstract

The objective of this study was to investigate the effects of feeding rearing programs aiming a first calving between 20 and 27 months (mo) of age on growth, reproduction and production performance of Holstein cows at nulliparous and primiparous stages. Our hypothesis was that, in a seasonal autumn calving strategy, it was possible for late-born heifers in the season to catch up with the growth of heifers born earlier and be inseminated at the same time-period, at a 370 kg body weight (BW) minimum. This would result a first calving age at about 21 to 22 mo, without impairing their later performance. To answer this question, an experiment was run, involving a total of 217 heifers over 3 years. These heifers were split into 3 groups: the first group received a control feeding treatment (SD), the second one an intensive-plane diet (ID1) from birth to 6 mo, and the last group an intensive-plane diet until 1 year of age. Groups SD and ID1 comprised heifers born from September until end of November; ID2 was composed of heifers born later. The present study showed that late-born heifers (ID2) could catch up with the growth of the other thanks to feeding treatment, although there were still 42 kg lighter than both SD and ID1 ones at first calving. There was no difference in reproductive performance of the heifers between the groups. Once primiparous, the cows reared with the ID2 treatment tended to produce less milk than SD and ID1 (about -400 kg over the lactation), and there were no difference regarding milk quality, feed intake, body condition score, or BW. A classification on age at first service was created *a posteriori* leading to 3 classes with heifers first inseminated at about 12.5 mo ($AFS_{12.5}$), 14.0 mo ($AFS_{14.0}$), 15.5 mo ($AFS_{15.5}$) of age. Heifers in $AFS_{12.5}$ had a faster growth 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 $AFS_{15.5}$ (about -1.5 kg/d) although there was no difference regarding total milk production over the lactation. There was no difference between these groups regarding milk quality, feed intake, body condition score, or BW. All these results support the conclusion that it is possible, through feeding treatment, to help late-born heifers to catch up with the growth of other. This leads to an earlier first calving but do not impair their reproductive and productive performance.

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 calving at 22.5 months (mo) of age or less presented similar performances than those calving at 23.8 mo of age or more.

Introduction

In seasonal calving systems, heifers usually first calve at a young age (around 24 months(mo)) but 1st insemination may be delayed for those born at the end of the calving period if an adequate body weight (BW) is not reached (i.e. 360 to 380 kg for Holstein heifers in French dairy herds; Le Cozler *et al.*, 2008). Increasing the nutrient uptake and thus the growth rate for these late-born heifers is a solution to lower this risk. A high growth rate during rearing is associated with a decreased age at puberty and, consequently, 1st calving may occur as early as 20 to 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 strategy of rearing (group-calving or not), it is, however, necessary for animals to have achieved an adequate body size before calving or milk production potential in the first lactation is compromised (Bach and Ahedo, 2008). Indeed, accelerated growth program for dairy heifers cannot be resumed to puberty attainment. Many authors have studied the impact of growth intensity on further performances (see the literature review of Le Cozler *et al.*, 2008), but if most of them indicated negative impact of too high a growth, some authors indicated limited impact. According to Pirlo *et al.* (1997), reducing age at first calving to 23 to 24 mo was the most profitable procedure, but not less than 22 mo (except in cases of low milk prices and high rearing costs). They concluded that reluctance to decrease age at first calving is generally attributable to the belief that early calving is detrimental to milk yield and longevity. Here, we designed and led an experiment to determine the effects of feeding treatments on growth parameters, reproduction and production performance of Holstein primiparous heifers first calving between 20 and 27 mo of age, in a seasonal calving system. We hypothesised that genetic improvement over the last decades in dairy production resulted in animals that could calved now at an earlier age than 24 mo of age and results from autumn groups calving strategy could be used in a non-grouping strategy. We focused on 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, which resulted in an age at first calving lower than 22 mo.

Material and Methods

General design

A total of 217 Holstein heifers, born during the 2009-10 (n = 65), 2010-11 (n = 73) and 2011-12 (n = 76) calving seasons (September to February), were reared and followed until oestrus synchronisation (12 to 15 mo of age) at the INRA experimental farm of Méjusseaume (Le Rheu, France). Another study based on the same experiment was already published, where rearing procedures and strategies used for the 3 cohorts have been fully detailed for one of them (Abeni *et al.*, 2019). Briefly, calves born between 1st of September and 30th of November were alternately allocated to 1 of 2 nutritional treatments (according to birth order) and fed either a standard diet (SD) or an intensive-plane diet (ID1) from 0 to 6 mo of age. It was expected that, thanks to the feeding intensity chosen, heifers fed SD and ID1 diets would reach 190 to 200 and 220 to 230 kg at 6 mo of age, respectively. Heifers born after 1st of December (ID2) received the same intensive-plane diet as ID1 heifers from 0 to 6 mo of age, to limit the possible confusion effect between age and treatment during this period. Thereafter, a complementary diet was formulated for ID2 heifers in order to reach 380 kg at 1 year of age. The main objective of this latest procedure was to study 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. It was expected that this corresponded to average ages of 15 and 12 mo for (SD and ID1) and for (ID2) heifers, respectively. From end of 1st season of grazing, all heifers were grouped-housed until turning out to pasture season 2. Three weeks before expecting date of calving, heifers entered cows herd and were fed individually a similar total mixed ration (TMR). During lactation, milk was recorded twice a day and animals were weighed every day. Experiment ended week 15 after calving.

Feeding

Diets were formulated for the different stages of growth according to recommendations and procedures presented by Agabriel and Mechy (2007). They were formulated to reach a targeted average daily gain (ADG) per period, with respect to the initial BW and feeding treatment used. At the end of the pre-experimental phase (0-10 d), heifers were group-housed indoors on cumulated straw bedding. They were fed reconstituted milk replacer made of 135 g milk powder (23.9 % crude protein and 19.0 % fat content) with 865 g water per litre until weaning (about 77 to 84 d of age). They were reared in dynamic groups, individually fed with automatic milk feeding systems (AMFS), with free access to fresh water, straw and hay. Group size varied from 8 to 24 calves per AMFS. Milk was distributed according to either the standard ration routinely used in the experimental herd

Table 1: Ingredients and chemical composition of the experimental diets

Item[1]	TMR1	TMR2	TMR3a	TMR3b	TMR4	TMR5	TMR6	TMR7
Stage of growth, age	(7d to 4 mo)	(4 to 6/8mo)	(9 to 11mo)	(6 to 11mo)	(11 to 15 mo)	(21 to 26 mo)	(21 to 26 mo)	(21 to 35 mo)

					(winter 1)	*(- 21 d prior calving until calving)*	*Calving + 14 d*	*(15 d until end of lactation)*
Feeding Treatment group	All	All	SD, ID1	ID2	All	All		All
Ingredient, %								
Corn silage	47.5	72.0	80.0	80.0	79.0	84.5	52.5	65.0
Soybean meal	-	8.0	20.0	20.0	21.0	9.0	8.0	8.0
18% CP alfafa pellets	5						10	10
Straw						2.5	2.5	2.5
Urea								0.8
Minerals + vitamins								1.0
Concentrate 1[2]	47.5	20.0						
Concentrate 2[3] (kg/head/d)			1.0	2.0	1.0			
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 either on a standard (SD) or increased-plane (ID1 & ID2) feeding treatment; DM: dry matter; PDIE: metabolizable protein supply; PDIN: rumen-degradable nitrogen; UFL: unit of feed for lactation.

[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.

(SD) or an increased 15% milk ration (ID1 & ID2). All calves were fed *ad libitum* total mixed ration 1 (TMR1; Table 1).

From weaning to 6-8 mo of age (i.e. turning out to pasture), 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 concentrate reached 2 kg DM/head/d. No restriction was applied to ID1 & ID2 heifers. From 4 to 6-8 mo of age, TMR2 was distributed *ad libitum* until the maximum daily allowance of concentrate reached 2 kg and 2.5 kg DM/head/d for SD and (ID1 & ID2) heifers, respectively, i.e. a total daily allowance of 10 and 12.5 kg DM/head/d of TMR2 for SD and (ID1 & ID2) heifers respectively. These amounts did not change until turning out to pasture.

Starting from mid-May and mid-June for (SD & ID1) and ID2 heifers, heifers were turned out to pasture and rotationally grazed on a perennial ryegrass sward. After a 5-d transition phase and throughout the pasture season, SD and ID1 groups received a daily supplement of 1 kg DM/heifer of concentrate 2, whereas the ID2 group received 1 kg DM/heifer/d of corn 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 achieve 380 kg at the end of outdoor season (when oestrus synchronisation started), the expected ADG for SD and ID1 heifers was estimated to be around 600 g/d during this period, with a feeding regime based on pasture 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 and TMR3b was used (Table 1). In the pasture area, a permanent headlock barrier (80 places on concrete floor) was used daily to feed SD and ID1 heifers with their concentrate. Heifers were locked for 1 hour while eating, to limit competition on feed intake between heifers. The SD2 group had free access to its ration, and so, heifers were not locked.

At the end of the first pasture season (1st 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% corn silage and 21% soybean meal. They had free access to fresh water, straw and mineral complements. After a 2-week adaptation period, oestrous cycles were synchronised in heifers (see below), and the same procedure of rearing was applied for all heifers. Depending on their date of successful insemination, heifers turned out to pasture (generally in March). They were them all reared in an unique group and received no additional feed but grass, except a mineral and vitamins complementation.

Three weeks before the expected date of calving, all heifers were housed indoors, together with multiparous cows, in a cubicle barn with fresh straw bedding distributed daily. Heifers were fed individually and received a daily TMR5 composed of corn silage (84.5 %), soybean meal (9 %), concentrate (4 %) and straw.

From calving to 14 d post-calving, TMR6 was composed of corn silage (52.5 %), soya bean meal (8 %), concentrate (25 %), dehydrated lucerne (1 %), mineral/vitamin complement (150 g, with 7% P, 22% Ca and 4% Mg), urea and straw (Table 1).

From day 14 after calving, cows individually received TMR7, composed of corn silage (65 %), soybean meal (8 %), concentrate (15 %), dehydrated lucerne (1 %), urea and completed with mineral/vitamin complement (7% P; 22% Ca and 4% Mg). During lactation, all heifers were fed *ad libitum*, based on a 10% refusal at least per day. Feed was distributed twice a day (09.00 and 16.30), and refusals collected every morning, before distribution of fresh TMR.

During the entire experiment, all heifers and cows housed indoor had free access to fresh water.

Age at first service

A classification on age at first service (AFS) was created *a posteriori* in order to better understand which factors are leading to different AFS and how future performance can be related to AFS.

Three classes were made so that the number of animal in each of them is balanced (Table 2).

Table 2: Description of the classes of age at first service

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

[1] *Mean age at first calving with standard deviations in parentheses*

Oestrus synchronisation

All heifers were inseminated after oestrus synchronization during winter 2 of rearing, so that calving should occur at around two years of age. At the end of November, for nearly half of the heifers, oestrus was synchronized using a progestin ear implant (Norgestomet®, Intervet, Angers, France) in conjunction with an intramuscular oestrogen injection (Crestar®, Intervet, Angers, France) without consideration of ovarian activity. A second synchronization was performed three weeks later for the rest of the heifers. After nine days of treatment, the ear implant was removed. Heifers generally exhibited signs of oestrus within 24 to 96 h and were then inseminated on detected oestrus. In case of failure to conceive, heifers exhibiting further signs of oestrus were inseminated until the end of the reproductive season (April). Ultrasonography was conducted 42 d after insemination on average in order to diagnose pregnancy. Non-gestating heifers were then removed from the rest of the experiment.

Measurements and registration

Heifers were weighed every 14 d from birth to weaning, every 21 d from weaning until turning out to pasture, and every 28 d until the end of the experiment. Interpolations were performed in order to compare BW of heifers at similar stage of growth. Average daily gains were then calculated. Heifer health and care information was recorded during the experiment. Their body condition score (BCS) was recorded 3 weeks before the expected date of calving and then, once a month. The method and scale developed by Bazin *et al.* (1984) that ranged from 0 to 5, was used. BCS was scored by 3 trained technicians and their records were averaged.

To monitor morphological traits during rearing and first lactation, five measurements were recorded: heart girth (HG), chest depth (CD), wither height (WH), hip width (HW) and backside width (BaW). A tape measure was used to measure HG, while a height gauge was used for the other measurements. These measurements were recorded only for the 2 firsts cohorts (2009-10 and 2010-11), results are presented on complementary data 1 and figures 1 & 2. Presentations are based on a classification on age at first service (AFC), created *a posteriori* (not presented and discussed in present paper).

Daily feed intake was calculated individually as the difference between daily feed allowance and refusals. Refusals were collected every day at 7.00 and weighed. The composition of refusal and allowance were presumed to be the same. Dry matter (DM) for silage was determined 5 times per week, while DM of the pellets was determined once a week. Feed composition was estimated based on average samples for corn silage, straw, soybean, and concentrate. No such information was available for fresh grass (see Table 1).

Milk content analysis

Milk production was automatically recorded at each milking (i.e. twice daily). During 6 successive milkings (Tuesday to Thursday), milk samples were collected and analysed from each cow, to determine fat and protein contents (Milkoscan, Foss Electric, DK-3400 Hillerod, Denmark).

Milk progesterone analysis

From calving to either 2 weeks after the service inducing pregnancy or to 5 weeks after the end of the breeding season (i.e. July), morning milk samples were collected on Monday, Wednesday and Friday, and stored at -20°C for progesterone determination by commercial ELISA kits (Milk Progesterone ELISA, Ridgeway Science Ltd., England). The coefficients of variation between assays for ELISA on 5 ng/ml control samples ranged between 8 % and 14 % among experimental years.

Determination of Luteal Activity

Two progesterone (P4) milk concentration thresholds were defined as in Petersson *et al.* (2006) 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 qualified as follows: negative (< threshold 1), positive (> threshold 2) and intermediate. In short, rises of P4 milk concentrations were considered to be induced by corpus luteum activity if at least 2 consecutive values were not negative and at least one positive. Drops in P4 milk concentrations were considered to result from luteolysis of the corpus luteum when at least 1 value became negative. Theses definitions enabled to identify and distinguish luteal phases from inter-luteal phases.

Qualification of Progesterone Profiles

For each luteal phase, physiological intervals were computed: 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 lasted longer than 25 days. Ovulation was considered to be delayed if inter luteal interval is longer than 12 days. Based on these definitions, P4 profiles were classified as (i) normal, (ii) PLP profile (if at least one PLP was observed), (iii) delayed (D; if CLA > 60 days), (iv) interrupted (I; if at least one ovulation of rank > 2 was delayed) or (v) disordered (Z; if luteal activity appeared irregular but could not be included in any abnormality class).

Calculations and statistical analysis

All data handling and statistical analyses were performed in R using either the *lm* procedure for ANOVA or the *glm* for logistic regressions (R Core Team, 2019). Quantitative traits (i.e. growth, ages, live weight, milk production, body condition score, CLA and 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 was the mean of the variable of interest, $year_i$ was the fixed effect of the experimental year (i=1, 2 or 3), either AFS_j that was the fixed effect of age at first service (j= 12.5, 14.0 or 15.5 mo) or T_j that was the fixed effect of the feeding treatment (j= SD, ID1 or ID2) was included in the model, e_{ij} was the random residual effect.

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 mean and the fixed effects $year_i$, AFS_j or T_j are the same as described above. In the case of reproductive performance of heifers, the covariate PRI_{ij} that describes the effect of the interval from the removal of the last progesterone-releasing implant to the insemination was included. This covariate was not needed for performance of cows because only heifers are synchronized.

Effects were declared highly significant at P<0.001, significant at P<0.05 and as a trend at P<0.10.

Results

There were initially 217 heifers enrolled in the experiment, out of which 175 successfully calved. The 42 remaining animal either died during rearing (7), were culled because of injuries (6), or were not pregnant within the breeding period considered for the present study (29).

Growth and reproductive performance of heifers

The average BW at birth was 41 kg (±5.2) and was balanced across the groups (i.e. not associated to neither feeding treatment nor AFS).

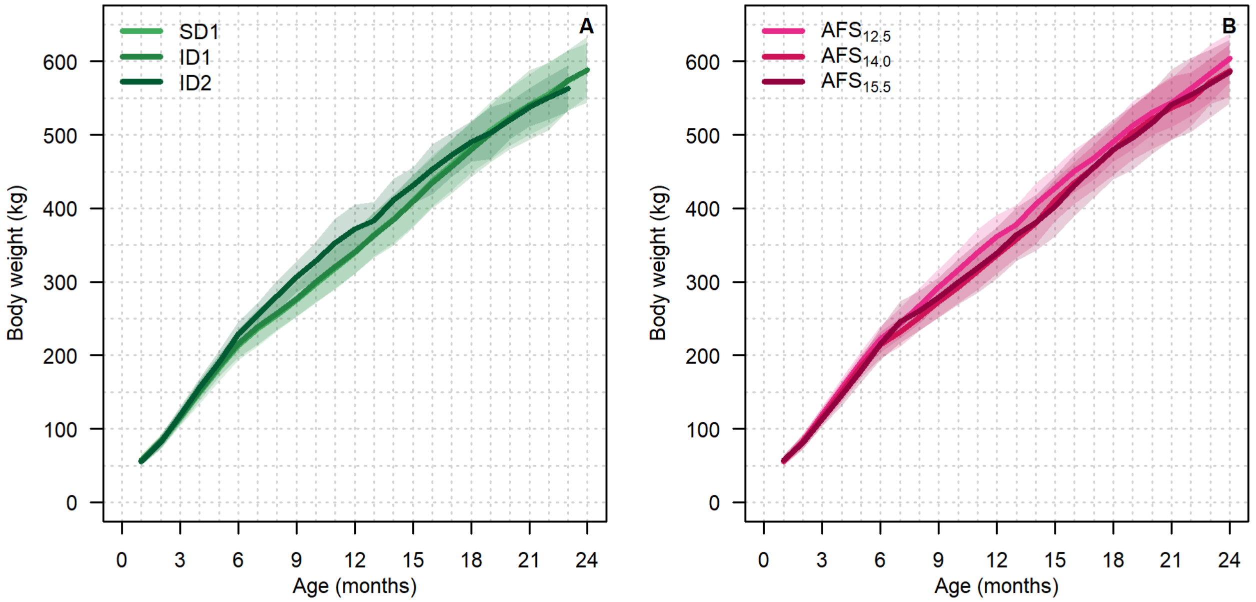

Figure 1: Body weight of the heifers during the rearing period, according to the feeding treatment (A) and classes of age at first service (AFS; B).

Feeding treatment had limited effect on growth during milking phase and heifers reached 117 kg (±11.8) at 3 mo of age (just after weaning). From weaning to 6 mo of age, heifers in the ID2 group were heavier than these in both the SD and ID1 treatments (229 kg *vs* 213 kg and 217 kg at 6 mo of age respectively; $P < 0.001$; Figure 1.A). The highest ADG was found for ID2 heifers from 0 to 6 mo (1046 *vs* 958 and 976 g/d for ID2, SD and ID1 respectively; $P < 0.001$). This difference was still significant from 6 to 12 mo of age (789, 703 and 699 g/d for ID2, SD and ID1 heifers, respectively). However, from 12 to 18 mo of age, ADG was significantly reduced for ID2 heifers in comparison of SD and ID1 heifers (660 *vs* 800 and 774 g/d respectively).

Reproductive performance was not affected by the feeding treatment (Table 3), although the number of service tended to be lower for ID2 heifers than for SD and ID1 heifers (1.5 vs 1.9 and 1.8, respectively). Indeed, cows in the 3 feeding treatment showed a similar interval from the start of the breeding season to the 1st service (13.5 d), similar success at 1st service (about 62% of pregnant heifers) and similar pregnancy rate by the end of the breeding season (94%).

There was no difference in calf BW (37.9 kg) despite a difference in their dam's BW at both 1st service and 1st calving (ID2 heifers were lighter than both SD and ID1, Table 3 and Table 5). Heifers in the ID2 treatment calved at a younger age than those in the SD and ID1 treatments (about 2 months earlier, $P<0.001$; Table 3).

Heifers inseminated at a young age (12.5 mo of age on average; $AFS_{12.5}$) tended to have a higher growth rate than heifers inseminated at either 14.0 ($AFS_{14.0}$) or 15.5 ($AFS_{15.5}$) mo of age, from 0 to 6 mo of age (1001 *vs* 960 and 978 g/d; P 0<0.10; Table 4). This difference became more important from 6 to 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 3: Effect of the 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 records	74	72	29			
Growth						
BW at birth (kg)	41.2	41.7	41.1	0.00	5.19	0.85
BW at 1st AI (kg)	400.7^a	398.5^a	378.1^b	0.14	33.29	★★
ADG 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 1st service interval (d)	13.9	12.8	14.0	0.00	5.76	0.46
Success at 1st service (%)	64	58	66	NA	NA	0.64
Number of service	1.9	1.8	1.5	0.21	0.78	●
Pregnant (%)	95	96	90	NA	NA	0.67
Age at 1st calving (months)	24.0^a	23.9^a	21.9^b	0.32	1.26	★★★
Calf body weight (kg)	38.4	37.6	37.2	0.32	4.02	0.37

[1] *adjusted coefficient of determination: R^2_{adj}; and residual standard error: RSE.*

[2] *★★★ $P<0.001$; ★★ $P<0.01$; ★ $P<0.05$; ● $P<0.1$; the exact P-value otherwise*

[a-b] *Different superscripts point out adjusted means that are different between feeding treatments ($P<0.05$, Tukey's pairwise comparison)*

Table 4: Effect of the feeding treatment on the 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 records	58	57	60			
Growth						
BW at birth (kg)	41.5	42.0	40.2	0.02	5.13	0.15
BW at 1st AI (kg)	373.1^a	394.3^b	419.8^c	0.37	28.49	★★★
ADG 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	★★
Fertility						
Start of breeding season to 1st service interval (d)	12.9	13.2	14.3	0.00	5.75	0.42
Success at 1st service (%)	59	60	67	NA	NA	0.30
Number of service	1.7	1.7	1.9	0.20	0.78	0.25
Pregnant (%)	93	91	98	NA	NA	0.37
Age at 1st 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}; and residual standard error: RSE.*

[2] *★★★ $P<0.001$; ★★ $P<0.01$; ★ $P<0.05$; ● $P<0.1$; the exact P-value otherwise*

[a-b] *Different superscripts point out adjusted means that are different between feeding treatments ($P<0.05$, Tukey's pairwise comparison)*

Table 4; Figure 1.B). Growth was reduced for $AFS_{12.5}$ animals from 12 to 18 mo of age, with an ADG of 712 g/d, compared to 799 and 790 g/d for $AFS_{14.0}$ and $AFS_{15.5}$ ($P < 0.001$; Table 4). This is consistent with the effects of feeding treatment observed, and with the distribution of animals among the classes of AFS and feeding treatments (Table 2).

Fertility was not affected by age at first service (Table 4): all heifers showed a similar interval from the start of the breeding season to the 1st service, a similar success rate at 1st service and pregnancy rate by the end of the breeding season, with a similar number of service per animal.

There was no difference in calf BW (37.9 kg) despite a difference in their dam's BW at 1st service and at 1st calving ($AFS_{12.5}$ heifers were lighter than the ones in $AFS_{14.0}$ themselves lighter than the one in $AFS_{15.5}$, Table 4 and Table 6). Consistent with the age at 1st service, heifers in the $AFS_{12.5}$ group calved younger than those in the $AFS_{14.0}$ who calved at a younger age than those in the $AFS_{15.5}$ group (Table 4).

Lactating performance of primiparous cows

BW recorded immediately after calving was lower for ID2 cows compared to SD and ID1 cows (501 *vs* 542 and 534 kg; $P < 0.001$; Table 5; Figure 2.A.), which is consistent with the fact that ID2 heifers first calved at a younger age than SD and ID1 heifers (Table 4). No difference between the feeding treatments was noticed in BCS during the first lactation. On a 308 d basis, ID2 cows tended to produce less milk than SD and ID1 cows (6920 *vs* 7312 and 7370 kg; $P < 0.10$; Table 5; Figure 2.C.). There was no difference between feeding treatments regarding average fat and protein contents. Milk yield peak was reduced for ID2 cows compared to both SD and ID1 ones (28.7 *vs* 31.3 and 31.9 kg; $P < 0.001$). During the first 7 weeks of lactation, ID2 cows were lighter (on average -38 kg compared to SD and -25 kg compared to ID1); and produced less milk (-3.1 kg/d compared to both SD and ID1). This difference was already shrunk during the last part of the period (from 8 to 15 weeks), ID2 cows weighed 27 kg and 17 kg less than SD and HD1 cows respectively; and produced 2.2 kg/d and 2.9 kg/d of milk less than SD and HD1 cows respectively.

The feeding treatment of dairy cows during the rearing period did not affect ovarian cyclicity during the 1st lactation (Table 5). On average, the CLA was about 20.4 d, the first IOI was about 20.7 d with no difference among treatments concerning the LUT and the ILI. There was no difference concerning the subsequent cycles neither, with an IOI of 23.3 d on average. The distribution of abnormal pattern of ovarian activity was not significant, although the ID2 cows showed a lower proportion of normal profile than ID1 cows, that had themselves a lower proportion of normal profiles than SD cows (53% *vs* 59% *vs* 65% respectively; Table 5). ID2 cows had an incidence of 33% of PLP abnormalities, while the incidence in ID1 and SD cows were 18 and 19% respectively (Table 5). About 86% of the cows were pregnant at the end of the breeding season, with no relationship with feeding treatment. Although the difference in cyclicity between feeding treatment did not impair the re-calving rate, ID1 cows needed more inseminations to be pregnant than SD cows (2.4 *vs* 1.9; $P < 0.05$; Table 5). The number of services needed to achieve pregnancy was about 2.2 for the ID2 cows. Subsequent calf BW was not affected by the feeding treatment.

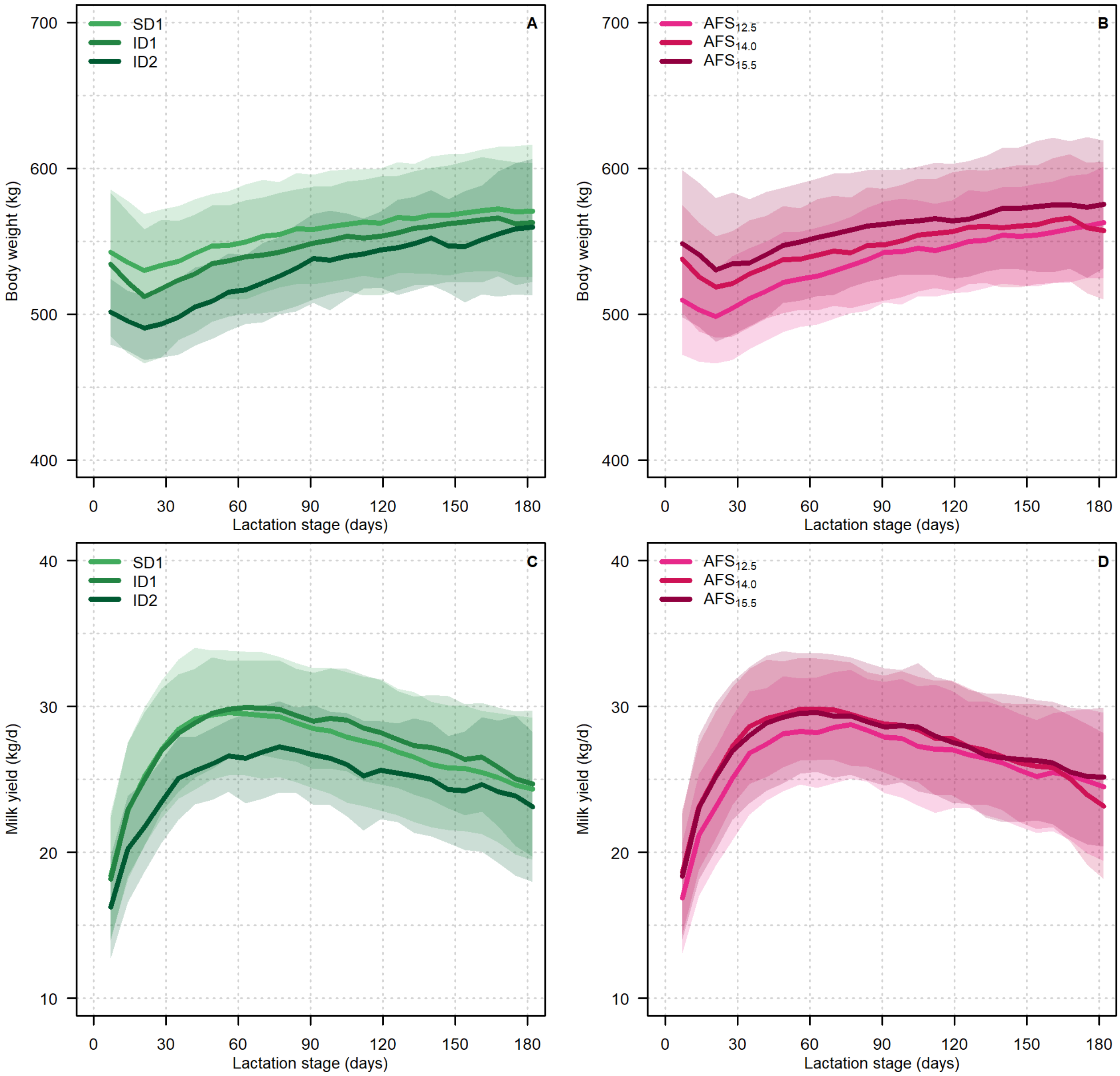

Figure 2: Body weight of the primiparous cows during lactation, according to the feeding treatment (A) and classes of age at first service (B); and milk yield of the primiparous cows during lactation, according to the feeding treatment (C) and classes of age at first service (D).

BW at calving was affected by AFS and was lower for $AFS_{12.5}$ than for $AFS_{14.0}$ and for $AFS_{15.5}$ cows (509 kg *vs* 539 kg and 549 kg respectively, P< 0.001; Table 6; Figure 2.B.). BCS at calving was significantly higher for $AFS_{15.5}$ heifers in comparison of BCS of $AFS_{12.5}$, $AFS_{14.0}$ (2.45 *vs* 2.35, respectively; P<0.05). After calving, BCS did not differ between groups of heifers. On a 308 d basis, there was no difference in milk yield or composition. Only milk yield pic tended to be reduced for

$AFS_{12.5}$ (30.2 kg), in comparison of milk yield of $AFS_{14.0}$ and $AFS_{15.5}$ (31.6 and 31.7 respectively; Figure 2.D.; Table 6).

Table 6: Effect of the class of age at first calving 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 records	51	50	58			
Production						
Total milk yield over 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	●
Average Fat Content (g/kg)	36.2	36.9	36.8	0.10	3.65	0.66
Average Protein Content (g/kg)	29.8	29.9	29.9	0.00	1.56	0.93
Conformation						
BW at 1st calving (kg)	509a	539b	549b	0.14	41.9	★★★
BCS at calving (0-5 scale)	2.35a	2.35a	2.45b	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						
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 service per cow	1.9	2.4	2.2	0.08	1.28	0.16
Pregnant (%)	86%	88%	84%	NA	NA	0.90
Calf body weight (kg)	37.2a	39.3b	37.3a	0.04	4.77	★

[1] *adjusted coefficient of determination: R^2_{adj}; and residual standard error: RSE.*

[2] *★★★ P<0.001; ★★ P<0.01; ★ P<0.05; ● P<0.1; the exact P-value otherwise*

$^{a-b}$ *Different superscripts point out adjusted means that are different between feeding treatments (P<0.05, Tukey's pairwise comparison)*

Fertility of cows was very little affected by AFS. Concerning ovarian cyclicity, all 3 groups of AFS showed a similar CLA, with similar cycles length, except for cows in $AFS_{15.5}$ that tended to show longer ILI between the 2nd to 4th cycle, than cows in $AFS_{12.5}$ and $AFS_{14.0}$ (Table 6) Cows in the $AFS_{14.0}$ group showed a lower incidence of PLP than cows in $AFS_{12.5}$ and $AFS_{15.5}$ (8% *vs* 29% and 23% respectively; P < 0.05; Table 6). Fertility was not affected by AFS neither: all groups showed a similar number of inseminations (2.2 on average) and 86% of the cows were pregnant at the end of the breeding season.

Subsequent calf BW was heavier for cows in the $AFS_{14.0}$ group compared to cows in $AFS_{12.5}$ and $AFS_{15.5}$ (+2kg; $P < 0.05$; Table 6).

Feed intake was not different neither between feeding treatment, nor between AFS groups (17 kg DM/d; Appendix 1). Morphological traits analysis based on age at first calving cohorts 2009-10 and 2010-11 (supplementary files 1) indicated that young cows at first calving (21 mo of age on average, n= 30; AFC_{21}) were not only lighter compared to heifers first calving at average age of 23.5 (n = 39; $AFC_{23.5}$) or 25 (n = 36; AFC_{25}) mo of age (498 vs 528 and 563 kg respectively, $P < 0.05$) but they also presented reduced morphological traits. For example, HW 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 similar age (25 mo for example), no such difference was noticed between treatments (140.7, 140.4 and 142 mm).

Discussion

Present experiment indicates that reducing age at first service down to around 1 year of age, and consequently, age at first calving down to 22 mo of age or less had limited impact on performance of Holstein primiparous cows. Several authors have shown that calving down heifers between 23-26 months of age increases longevity and maximises economic returns (Bach 2011; Wathes *et al.,* 2014; Boulton *et al.,* 2017). The early rearing period is a key period to achieve this target, as sub-optimal nutrition delays the onset of puberty and adversely affects skeletal growth and increases the risk of dystocia at first calving (Ettema and Santos 2004). Poor growth is also one of the main reasons for culling heifers prior to calving (Esslemont and Kossaibati 1997). Pre-weaning growth in dairy heifers has generally been associated with performance in first lactation (Khan *et al.* 2011; Soberon *et al.,* 2012). Some studies reported, however, that pre-weaning differences associated with different feeding regimes were no longer statistically significant as calves aged (Morrison *et al.* 2009; Quigley *et al.* 2006). This may in part be explained by compensatory increase in growth for animals when feed allowance (level, energy, protein) is not limited after a period of restriction.

The difference in feed allowance resulted in different development and size at 6 and 12 mo of age, but it has limited effect on BW at weaning. In a study of Johnson et al (2019), the two treatment groups before weaning induced significant differences in size in pre-weaning performance and this persisted until six months. In our case, the high level of feed allowance before weaning, without restriction of total mixes ration for control heifers, probably explain that no difference on BW at weaning was observed. Usually, the amount of milk until weaning is low in most practices: about 4 to 6 L/day of whole milk, or 400 to 600 g of milk replacer (MR) until weaning at 42-56 days of age (Morrison *et al.* 2009). According to Jasper and Weary (2002), *ad libitum milk* intake is around 12 L/day of whole milk, and in present study, it varied around 9 L/d per heifer until 11 weeks of age. The development and BW of animals at 6 mo of age were high (111 cm heart girth and 220 kg body weight, for example), which fits well with recommendation for optimal age at first calving at 24 mo of age or less. In a study of Ettema and Santos (2004) on importance of age and BW at first calving for Holstein heifers, only 2.7% of dairy farms achieve the recommended targets and, therefore, this leads to economic losses. Total nutrient intake, source of energy and protein content of the diet have additive effects on how calves partition nutrients into tissue (Van Amburgh and Drackley 2005). During milking phase, calves benefit when MRs contain more protein and less fat, achieving higher levels of skeletal growth (Hill *et al.,* 2010). Providing greater quantities of MR therefore improves both growth and feed efficiency (Bartlett *et al.,* 2006). Increased nutrient intake is also associated with increased plasma IGF1 (Smith *et al.,* 2002; Bartlett *et al.,* 2006) which in part regulates the subsequent growth rate (Hammon *et al.,* 2002; Brickell *et al.,* 2009a).

The effect of intensive growth during rearing have been presented and discussed in several papers (Le Cozler et al. 2008), and as already presented in these papers, increasing growth rate resulted in earlier puberty in (Abeni *et al.*, 2019). However, authors do not agree on the impact on milk performance. Indeed, while some authors noticed a negative impact on milk production, other did not. Abeni *et al.* (2000) and Van Amburgh *et al.* (1998) concluded that calving earlier than 23 mo of age is associated with lower milk yields and lower milk fat content, although, it also leads to a higher milk protein content. They concluded as well that earlier calving leads to reduced reproduction performance. In a more recent study, Krpálková *et al.* (2014) did not observe effect of age at first calving on milk yields, except on milk yield in the first 100 d of first lactation and even found that the highest milk yield in the second lactation, third lactation for an age at first calving lower than 699 days. In the present paper, a negative impact was noticed at the start of lactation 1 only. In their study, Van De Stroet *et al.* (2016) also observed that pre-weaning growth has associations with milk yield in later life, but the differences in milk yield were most apparent during early and peak lactation. In particular, higher calf growth rates were not significantly associated with future milk yield but were associated with higher BW in lactating cows and higher odds of survival to first lactation. When milk lactation was corrected to BW, no difference was found in milk yield or composition, regardless of rearing treatment.

Decreasing age at first calving is an effective way to decrease the length of non-productive days during rearing and first calving at around 24 months of age appears to be is optimal for profitable production (Mourits *et al.,* 1999b; Ettema and Santos; 2004; Shamay *et al.*, 2005). In a metanalysis based on results from 100 herds, Mohd Nor *et al.* (2013) estimated that heifers having a first calving age of 24 mo produced, on average, 7 164 kg of milk per 305 d, and calving 1 mo earlier gave 143 kg less milk on a 305 d lactation length basis. In present study, we also noticed that young heifers produced less milk during the first part of lactation, but the total milk yield over 305 d was not different. However, it could be noticed that despite no difference from the statistical point of view, the difference was very similar when age at first calving decreased from 24.8 to 23.8 mo of age: 134 kg less on a 305 d basis.

In present study, fertility was not affected by age at first service. In a previous paper on puberty attainment based on 2011-12 cohort, we noticed that most heifers reached puberty before oestrus synchronisation, at an average age of 10.3 ± 2.2 mo (6.2 to 14.4 mo), averaging 296 ± 40 kg (224 to 369 kg) BW (Abeni *et al.*, 2019). It occurred one mo earlier for ID2 heifers than in SD and ID1 heifers. Puberty onset at 9 to 10 mo of age or less meant that 3 or 4 oestrous cycles occurred before insemination, which is generally consistent with good fertility results in many species (Lin *et al.*, 1986; Byerley *et al.*, 1987; Robinson, 1990; Le Cozler *et al.*, 1999). Regardless of calving strategy, lowering the age at puberty and, consequently, the age at first insemination means that it is an efficient way to shorten the non-productive period before calving. However, as suggested by Meyer *et al.* (2006), it might reduce pre-pubertal mammary gland development by shortening the allometric phase of mammary gland growth and, in some cases, impair further milk production. Similar to fertility in heifers, fertility in primiparous cows during first lactation was not affected with age at first calving. Wathes *et al.* (2008) reported that optimal fertility and maintenance of maximum performance in the first lactation were reached at the calving age of 24 to 25 mo, although heifers that calved at the age of 22 to 23 mo were the best in overall performance and longevity over 5 year, partly because heifers with good fertility also had a high level of fertility as cows.

Finally, In the present paper, we also noticed that for a similar feed allowance, early calving heifers ate a similar amount of feed, produced less milk and at the end, were able to catch the difference in BW and development. All these results indicate that, as already reported by Krpálková *et al*. (2014), the objective of a rearing period leading to an age at first calving less than 23 mo of age in Holstein heifers proves to be a suitable option for successful rearing of heifers with optimal subsequent production and reproduction in a herd with suitable management.

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Declaration of interest

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

Ethics statement

Experimental work has been conducted in accordance with French national legislation on the use of animals for research. Protocol received agreement (00944-02) from French Ethical Committee n°7.

Software and data repository resources

None of the data were deposited in an official repository.

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