

Influence of feed ration size on somatic and muscle growth in landlocked dwarf and farmed Atlantic salmon *Salmo salar*

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ABSTRACT

We examined the possible adaptation of the dwarf Bleke population of Atlantic salmon *Salmo salar* from Lake Byglandsfjord in southern Norway to limited food resources. The growth performance and muscle development in juvenile Bleke and farmed *S. salar* under satiated or restricted (50%) feeding were examined for 10 months, starting 3 weeks after first-feeding stage. Four-thousand fish were divided into four replicated groups and random samples of 16–40 fish per group were measured six times during the experiment. The two strains showed no significant difference in mean body mass when fed restricted ration, but

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the individual variation was considerably higher in the farmed fish. Both Bleke and farmed *S. salar* grew significantly faster when fed to satiation, but the farmed *S. salar* showed much higher gain in mass and were three times heavier (201.5 g v. 66.7 g) and possessed twice as many fast muscle fibres (179682 v. 84779) compared with landlocked *S. salar* after 10 months. Farmed fish fed full ration displayed both hypertrophic and hyperplastic muscle growth, while the increased growth in Bleke *S. salar* was entirely associated with a larger fibre diameter. The landlocked Bleke strain has apparently adapted to low food availability by minimising the metabolic costs of maintenance and growth through reduced dominance hierarchies and by an increase in average muscle fibre diameter relative to the ancestral condition.

KEYWORDS

Adaptation, dwarfism, feed restriction, hypertrophy, landlocked salmon, muscle fibre,

1 | INTRODUCTION

Wild European populations of Atlantic salmon *Salmo salar* L. 1758 are commonly anadromous and return to their native freshwater streams to spawn after 1, 2 or 3 years' growth at sea. Additional complexity to the lifecycle is provided by male parr that sexually mature in freshwater and attempt to fertilise the eggs of sea-run females using sneaky mating behaviour (Hutchings & Myers, 1988;; Hutchings & Jones, 1998; Letcher & Gries, 2003). Non-anadromous populations of *S. salar* are common in parts of North America but are also found in a few localities in Europe (Berg, 1985). Landlocked *S. salar* complete their life

cycle in freshwater above impassable waterfalls formed after postglacial rebound. The Bleke population in the oligotrophic Lake Byglandsfjord of southern Norway was isolated from sea migration *c.* 9000 years ago and shows reduced osmoregulatory capacity when moved to seawater (Dahl, 1928; Lande, 1973; Berg, 1985; Nilsen *et al.*, 2003). This dwarf *S. salar* strain is resource restricted, feeding mainly on zooplankton such that it only reaches a body size of *c.* 150 g and 30 cm after 5–6 years. Female Bleke *S. salar* become sexually mature at 4–5 years and spawn in December with a fecundity of *c.* 250 eggs (Barlaup *et al.*, 2005). In comparison, farmed *S. salar* of the same age would be expected to reach 12–16 kg with a fecundity of *c.* 15,000 eggs (Kittelsen, 1986). *Salmo salar* have been farmed on an industrial scale since the 1970s. Domestication has included genetic selection programmes for increased growth rates realising trait gains of 10–15% per generation, which have been linked to increased appetite and food conversion efficiency (Thodesen *et al.*, 1999; Thodesen & Gjedrem, 2006; Gjedrem, 2010).

Myogenesis is a trait that is closely associated with intraspecific and population differences in body size (Johnston *et al.*, 2003a, 2012). Muscle growth occurs by the continuous production of myotubes that differentiate to form muscle fibres and subsequently expand in length and diameter (Johnston *et al.*, 2011). Individual muscle fibres have a maximum diameter that is limited by diffusional constraints and varies with environmental conditions, chiefly temperature (Johnston *et al.*, 2003b). Using phylogenetic comparative methods maximum body size is a highly significant predictor of species variation in the maximum number of muscle fibres (N_{Fmax} ; Johnston *et al.*, 2003a). N_{Fmax} of Bleke *S. salar* was found to be only 30% and 21% of that found in wild anadromous and farmed *S. salar*, respectively, indicating that fibre recruitment is under strong divergent selection (Johnston *et al.*, 2005). A similar reduction in muscle-fibre number has been observed in post-glacial populations of Icelandic arctic charr *Salvelinus alpinus* (L. 1758) and threespine stickleback

Gasterosteus aculeatus L. 1758 (Johnston *et al.*, 2012). Theoretically, the energy cost of maintaining a negative membrane potential is proportional the surface-to-volume ratio of the individual muscle fibres because active ion pumping is required to counteract passive leak of ions across the muscle sarcolemma. According to the optimum-size hypothesis, divergent selection acts on fibre recruitment to produce the mix of fibre sizes that minimises the routine energy costs of maintaining ionic homeostasis (Johnston *et al.*, 2004). The maximum diameter of fast muscle fibres in 4 year-old Bleke *S. salar* was 118 μm , which is similar to that of immature farmed *S. salar* of the same body size (Johnston *et al.*, 2005). This suggests the possibility that resource-limited Bleke *S. salar* could grow to larger size under optimal feeding conditions even with the reduced number of muscle fibres. In the present study, we therefore compared somatic and muscle growth in farmed and Bleke *S. salar* either fed to satiation or a restricted (50%) ration.

2 | MATERIAL AND METHODS

2.1 | Ethical considerations

The experiment protocol was authorised by the University's Ethical Review Committee and the feeding study was performed in strict accordance with the Norwegian Welfare Act to secure fish welfare. The personnel involved had undergone training approved by the Norwegian Food Safety Authority. Tagging, sampling and sacrifice of the fish were performed under anaesthetics using metacain according to the supplier's instructions. As the wild Bleke *S. salar* population is threatened, only fish raised at Syrtveit fishery station were used in this study.

2.2 | Fish samples

The Bleke *S. salar* examined were offspring of four females and two males caught in Lake Byglandsfjord, southern Norway and kept at the local Syrtveit fishery station. The farmed *S. salar* were generated from seven females and two males of the SalmoBreed strain (www.salmobreed.no), which had been selected for high growth rates for seven generations. This late-spawning farmed strain was chosen to match the spawning time of Bleke, thus ensuring concomitant development of the offspring from the two strains. The Bleke eggs were fertilised in early December 2005 and incubated at *c.* 2°C at Syrtveit fishery station, while the farmed eggs were fertilised 2 weeks later and incubated at *c.* 3°C at Erfjord brood stock station in south-western Norway. The Bleke and farmed *S. salar* eggs were transport to Akvaforsk research station, Sunndalsøra, in April 2006 and incubated at 7.0–7.2°C until hatching. The majority of the eggs hatched at 422.5 (Bleke) and 424.5 (SalmoBreed) day degrees (D°; number of days multiplied by temperature degrees in °C) and all eggs hatched within 3 days in late April.

2.3 | Feed rations

Four-thousand fish of the landlocked Bleke and farmed SalmoBreed strains were divided equally among eight 500 l indoor fibreglass kept at 7.5-7.9°C in continuous light. Oxygen levels were measured 2–3 times weekly and did not drop below 80% saturation. The fish were fed commercial *S. salar* feed (Nutra 0.5-3.0 mm, Skretting; www.skretting.com) 3–4 times h⁻¹ diurnally using automated feeders developed in-house. Start feeding was initiated on 6th June (Bleke) and 8th June (SalmoBreed) at 662 and at 683D°, respectively and all groups were fed full ration for 3 weeks prior to the experimental study. The trial lasted from

29th June 2006 to 9th May 2007 and replicated groups of landlocked and farmed *S. salar* kept at 11.5–12.4°C were either fed to satiation or fed a restricted ration of 50% satiation level. The satiation ration was 10–20% above commercial *S. salar* feed ration (Skretting V3 feeding table). Daily feed ration was recalculated at 2 week intervals throughout the study by batch weighing 50–100 fish from each tank and the restricted ration was corrected for differences in body mass between the groups. The mortality in the eight tanks during the experiment ranged from 0.6 and 3.4 % recorded in Bleke and farmed salmon, respectively, under restricted feeding.

2.4 | Body growth and muscle-fibre analysis

Body mass (M_T , 0.1 g) and fork length (L_F , 0.1 cm) were measured in fish randomly sampled from all tanks during the first week of August, September and December in 2006 and of February, April and May in 2007 ($n = 16–40$ per treatment, or $n = 8–20$ per tank; Supporting Information Table S1). The lightly anaesthetised (MS-222) fish were returned to the tanks, except for the fish analysed for muscle fibre number and diameter in September 2006 and May 2007 ($n = 8–11$ per group; Table 1), as described by Johnston *et al.* (2004, 2005). Briefly, the fish were over-anaesthetised in MS-222 and killed by a blow to the head. A 0.5 cm thick steak through the trunk muscle on the right-hand side was prepared at $0.7 L_F$ and photographed at high resolution. A series of up to 6 muscle blocks were prepared and frozen in isopentane cooled to freezing in liquid nitrogen to sample the major part of one half of the myotomal cross-section. Frozen sections were cut at 8 μm on a cryostat (Leica Microsystems CM1850; www.leicabiosystems.com), mounted on poly-L-lysine-coated slides and air dried for storage at -80°C . Sections were stained in modified Harris haematoxylin solution (Sigma-Aldrich; www.sigma-aldrich.com) and examined with an Axioskop 2 microscope and a X10

objective (Zeiss; www.zeiss.com). The steak and fast-muscle outlines were photographed and digitised using an AxioCamHRC and Axiovision software (Zeiss). A total of 8–10 randomly selected fields were photographed per block. Fast-muscle fibres were digitised using a standardised square reference grid. Fibres more than 50% within the grid were included and those more than 50% outside the grid excluded. In total, 800–1000 muscle fibres were measured per fish and 800 were randomly selected for further analysis using a computer program. Full details of the software used and the method of estimating muscle fibre number are provided in Johnston *et al.* (1999).

2.5 | Calculations and statistical analysis

The effect of strain and feed ration and their interaction on M_T and L_F , within each of the six sampling dates, was analysed using a single trait linear mixed model (SAS 9.4 computer software, SAS Institute Inc.; www.sas.com) with strain, feed ration and their interaction as fixed effects and tank nested within the interaction effect as a random effect. As variance of M_T increases with the mean, variance in M_T within the different strain and feed ration combinations were different. These heterogeneous variances were accounted for by weighting each M_T observation with the inverse of the variance of M_T within these strain by feed ration combinations using the group = strain × feed ration in the random statement in the mixed model. For M_T , the tank effect accounted for on average 5.5% (varying from 0 to 11.8% for the six sampling dates) of the total variance (tank variance plus residual variance). For L_F the corresponding figures was on average 6.3 % (varying from 0 to 11.9% for the six sampling dates). A similar statistical model was used for muscle fibre number (N_F) as for M_T and L_F , but without the random effect of tank nested within the interaction effect.

The coefficient of variation (CV) for each set of sampling data by strain by feed ration combination was calculated as: $[\text{SD} (\text{mean value})^{-1}]100$. The effect of strain and feed ration and their interaction on the mean CV of M_T (CV_M) for each strain by feed ration by tank combination, within each of the six sampling dates, was analysed using a single-trait linear mixed model with strain, feed ration and their interaction as fixed effects and tank nested within the interaction effect as a random effect. For each of the six sampling dates the strain by feed ration effect was not significantly different from zero ($P > 0.05$) and was therefore omitted from the model. Consequently, CV_M was analysed across the six sampling dates with sampling data as a fixed effect in addition to strain and feed ration effects.

3 | RESULTS

3.1 | Body growth and feed ration

Hatchery-reared offspring of the landlocked Bleke and the farmed SalmoBreed strains of *S. salar* were either fed to satiation or a restricted (50%) ration for 10 months starting 3 weeks after first-feeding stage. The interaction effect of strain by feed ration on M_T and L_F was significant ($P < 0.05$) at each of the six sampling dates. Consequently, both traits are presented as least-squares means for each strain by feed ration combination (Figure 1). No significant difference in M_T and L_F was shown between the two strains under restricted feeding. Both strains had a significantly ($P < 0.01$) higher M_T when fed to satiation until the final measurement (May 07) at which stage Bleke *S. salar* showed no significant difference with the restricted groups. Under satiation feeding the farmed *S. salar* were significantly heavier than Bleke *S. salar* at each sampling point ($P < 0.01$) and three-fold higher M_T was found at the final measurement (201.5 g v. 66.7 g; Figure 1a and Supporting Information

Table S1a). The body growth in the four treatment groups were consistent with the results from the batch weighings, which were performed to recalculate feed rations (data not shown). Farmed *S. salar* fed to satiation were significantly longer than the other groups from September 2006 and possessed a final $L_F = 25.6$ cm compared with $L_F = 18.7$ cm in Bleke *S. salar* on full ration (Figure 1b and Supporting Information Table S1b).

The effect of strain and feed ration on CV_M was highly significant across the six sampling dates, but in most cases not significant within sampling date ($P > 0.05$; Figure 2). The CV_M was overall higher for farmed *v.* Bleke *S. salar* and for restricted *v.* satiation fed groups across the six sampling dates. Only the larger individuals of the farmed *S. salar* fed to satiation were lacking the parr marks by the end of the experiment indicating onset of smoltification (Figure 3).

3.2 | Muscle cellularity

Bleke *S. salar* and farmed *S. salar* on restricted feed ration showed no difference in fibre number or diameter (Table 1). When fed to satiation for 10 months, farmed *S. salar* showed a two-fold increase in number of fibres, while the fibre number in Bleke *S. salar* did not differ between the satiation and restricted feeding groups. However, the mean fibre diameter was almost 30% higher in both Bleke *S. salar* and farmed *S. salar* with satiation feeding. The combined hypertrophic and hyperplastic muscle growth in the satiation fed farmed *S. salar* resulted in a total muscle cross section area of 411 mm^2 compared with only 178 mm^2 in the landlocked *S. salar* (Table 1).

4 | DISCUSSION

The gain in mass displayed by Bleke *S. salar* fed restricted ration for 10 months was similar to that found in the wild population, which reach the body size of about 30 g and 15 cm after one year in its natural habitat of Lake Byglandsfjord (Barlaup *et al.*, 2005). The growth potential of this dwarf *S. salar* strain was indicated by a two-fold higher M_T when fed to satiation compared with fish under restricted feeding. Accordingly, landlocked *S. salar* from the Swedish Klarälv River grew faster at 100 % than at 50 % ration and the difference was stronger at high lipid (2.4 fold) than low lipid levels (1.4 fold) after 19 months (Norrgård *et al.*, 2014). The considerably heavier farmed *S. salar* compared with Bleke *S. salar* is consistent with the up to three-fold higher M_T in farmed *S. salar* than wild *S. salar* examined in a hatchery environment (Glover *et al.*, 2009; Solberg *et al.*, 2013). In contrast, farmed and Bleke *S. salar* fed half ration showed no difference in M_T , which agrees with the modest or marginal growth difference between farmed and wild *S. salar* in wild stream environments (Fleming *et al.*, 2000; Skaala *et al.*, 2012) or in a semi-natural environment with restricted food (Solberg *et al.*, 2013). Competition for limited resources is expected to favour the most aggressive and territorial individuals (Ruzzante, 1994). Consistently, the farmed *S. salar* under restricted feeding showed large individual variation in M_T and fin damages were only observed in this group (E. Stubø, pers. comm.). Wild *S. salar* parr were reported to be outcompeted and displaced by the more aggressive farmed *S. salar* in captivity (Einum & Fleming, 1997). Farmed *S. salar* parr were also more aggressive than wild parr when released into the river, but the wild fish fed more effectively by choosing habitats with preferred sizes of food items, while the diet of cultured fish comprised food items and inedible particles avoided by the wild fish (Orlov *et al.*, 2006). Hence, farmed *S. salar* selected for fast growth with surplus food in captivity may be maladapted to survive in the wild (Skaala *et al.*, 2012; Solberg *et al.*, 2013). In contrast, the landlocked Bleke *S. salar* seem to have adapted to the low food availability in the Lake Byglandsfjord by foraging together with brown trout *Salmo*

trutta L. 1758 on pelagic and benthic prey items, respectively (Barlaup *et al.*, 2005). The effective foraging of the dwarf Bleke *S. salar* was supported by the present study showing that farmed *S. salar*, which had been selected for high growth rates during seven generations, did not outgrow the Bleke *S. salar* when fed a restricted ration.

The polygenic nature of body growth was demonstrated by genome mapping multiple quantitative trait loci (QTL) for M_T and L_F in *S. salar* using F2 families from founding generations consisting of Bleke males and farmed females (Baranski *et al.*, 2010). Studying the endocrine mechanisms underlying the faster growth in domesticated fish, Fleming *et al.* (2002) measured significantly higher pituitary and plasma growth hormone (GH) levels in the seventh-generation of farmed *S. salar* compared with the wild principal founder population. GH also plays a role in the development of seawater tolerance in salmonids the reduced osmoregulatory capacity of Bleke *S. salar* was found to be associated with the lack of increased plasma GH and gill GH receptor mRNA levels as found in smoltifying anadromous *S. salar* (Nilsen *et al.*, 2008). Parallel evolution of dwarfism in Icelandic *S. alpinus* has been accompanied by modification in the expression of growth-related genes, including *igfbp4* coding for the insulin-like growth-factor binding protein 4 (Macqueen *et al.* 2011). IGF-1 and myostatin are conserved stimulatory and inhibitory factors regulating myoblast proliferation and muscle growth (Garikipati & Rodgers, 2012; Gabillard *et al.*, 2013; Li *et al.*, 2014; Retamales *et al.*, 2015). Low genetic variation of the myostatin gene paralogue *ssa-mstn1b* in Bleke *S. salar* compared with farmed *S. salar* was found by analysing an internal polymorphic microsatellite (Østbye *et al.* 2007), while several private alleles of lysyl oxidase required to initiate muscle cross-link formation was reported in Bleke *S. salar* (Consuegra & Johnston, 2008). Relative low genetic variation was shown in the landlocked Namsblank *S. salar* population (Sandlund *et al.*, 2014), but genome sequences from a single dwarf fish revealed no loss of coding

regions suggesting that fine-scale (epi)genetic alterations and population genetic processes underlie adaption to the landlocked habit (Hauge *et al.*, 2016). In support of this idea, we recently reported that effects of genetic background and embryonic temperature on muscle development in *S. salar* were associated with altered DNA methylation and gene expression of the myogenic regulatory factor myogenin (Burgerhout *et al.*, 2017).

Muscle enlargement in teleosts with indeterminate growth, such as salmonids, occurs by both hypertrophy and hyperplasia during a large part of post-hatching life (Rowlerson & Veggetti 2001; Johnston *et al.*, 2011). Large muscle-fibre size is metabolically advantageous and dwarfism in landlocked populations of salmonid and *G. aculeatus* has been associated with a reduction in fibre numbers compared with anadromous populations (Johnston *et al.*, 2004, 2012; Jimenez *et al.*, 2013). Our study showed similar muscle-fibre number in Bleke and farmed *S. salar* fed a restricted ration, whereas the fibre number in farmed *S. salar* was more than twice that of Bleke *S. salar* after 10 months on full ration. The increased growth of the Bleke *S. salar* fed to satiation was entirely due to fibre hypertrophy. It should be noted that had the trial gone on longer, fibre number would have been expected to increase further based on results for mature adults (Johnston *et al.*, 2005). However, $N_{F_{max}}$ in Bleke *S. salar* is nevertheless only 21% of that of adult farmed *S. salar* (Johnston *et al.*, 2005). Resource limitation in the landlocked Lake Byglandsfjord *S. salar* is probably the primary cause of dwarfism, which resulted in divergent selection to reduce fibre number and increase fibre diameter relative to the ancestral condition. In addition, dominance hierarchies also seemed to be reduced contributing to a further reduction in the metabolic costs of maintenance and growth. These adaptive traits are apparently maintained during conditions of surplus food suggesting the involvement of genetic or epigenetic mechanisms.

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CONTRIBUTIONS

O.A. and I.A.J. designed the study. O.A. was responsible for the experiment. V.V. performed the laboratory analyses. I.A.J. and J.E.D. analysed the data. O.A. wrote the manuscript with contributions from I.A.J. and J.E.D.

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Figure legends

FIGURE 1 (a) Least-square mean (+ SE) of body mass (M_T) and (b) fork length (L_F) of juvenile landlocked Bleke and farmed *Salmo salar* fed full (100%) or restricted (50%) ration within each sampling date. Different letters at each sampling date indicate significant differences ($P < 0.05$).

FIGURE 2 Differences in mean (+ SE) coefficient of variation of body mass (CV_M) between farmed and landlocked *Salmo salar* strains and between restricted (50%) and full (100%) ration at each sampling date and across the six sampling dates (overall).

FIGURE 3 Typical specimens of (a) landlocked Bleke and (b) farmed *Salmo salar* on 50% rations, (c) landlocked Bleke and (d) farmed *S. salar* on 100% rations.

TABLE 1 Muscle growth in landlocked Bleke and farmed *Salmo salar* on restricted (50 %) or full (100 %) feed ration.

Different letters indicate significant difference: $P < 0.05$ (September 2006) and $P < 0.001$

Strain	Ratio n (%)	Number of fish	TCA (mean \pm SE, mm ²)	Diameter (mean \pm SE, μ m)	Fibre number
September 2006					
Landlocked	50	8	17 \pm 1	35.0 \pm 0.5	14315 \pm 470 ^a
Farmed	50	8	16 \pm 1	33.4 \pm 0.3	14595 \pm 1120 ^a
Landlocked	10	8	22 \pm 1	35.0 \pm 0.6	17627 \pm 1138 ^a
d	0				
Farmed	10	8	31 \pm 2	36.4 \pm 0.4	23190 \pm 1623 ^b
	0				
May 2007					
	50	10	113 \pm 12	37.0 \pm 1.0	82007 \pm 5590 ^a
Landlocked					
d					
Farmed	50	9	131 \pm 11	37.5 \pm 1.0	90749 \pm 7963 ^a
	10	11	178 \pm 11	47.8 \pm 2.0	84779 \pm 9187 ^a
Landlocked					
d	0				
Farmed	10	10	411 \pm 15	48.0 \pm 1.0	179682 \pm 11926 ^b
	0				

(May 2007)

TCA, Total cross section area.

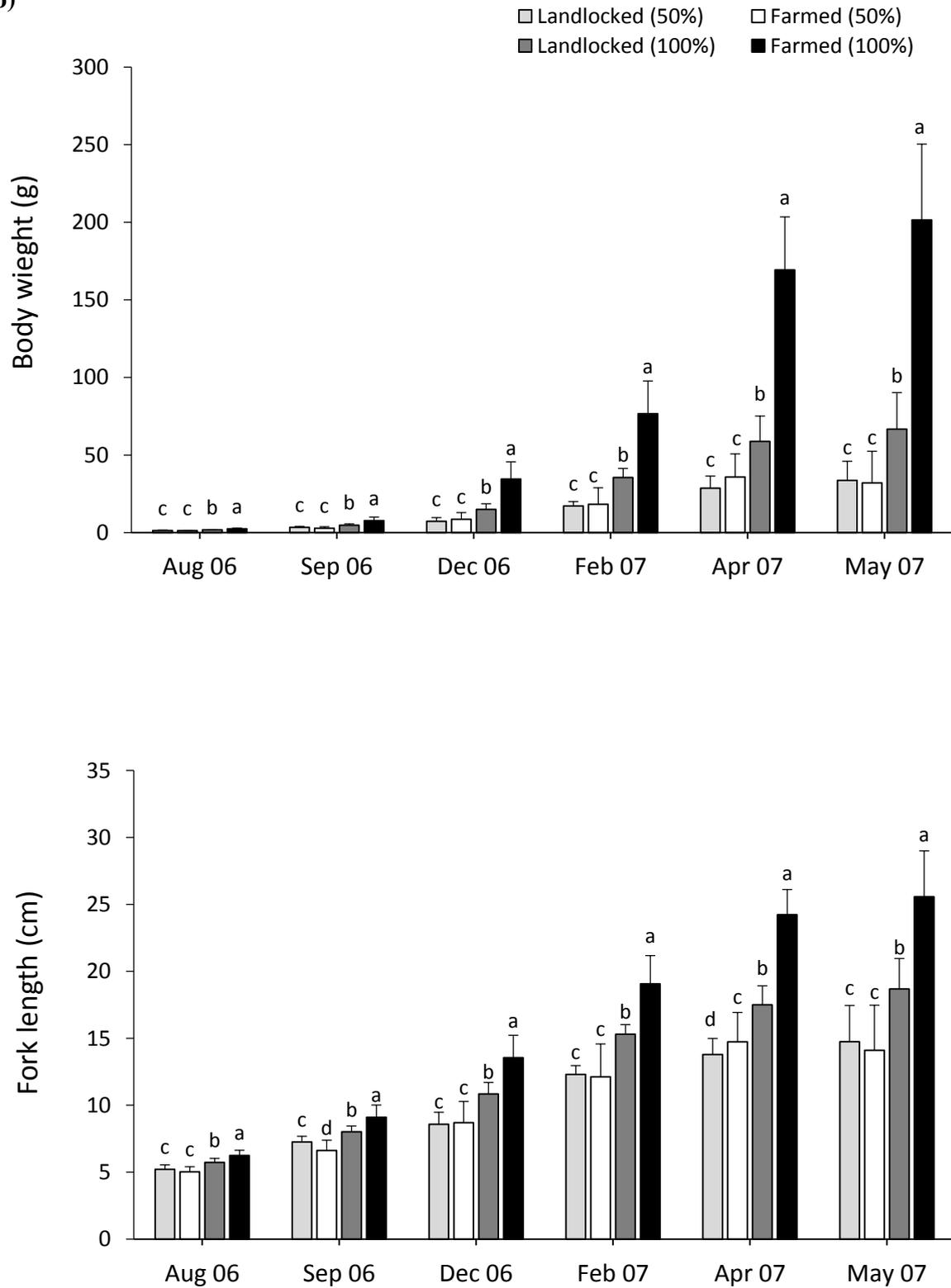
Figure 1**(a)****(b)**

Figure 2

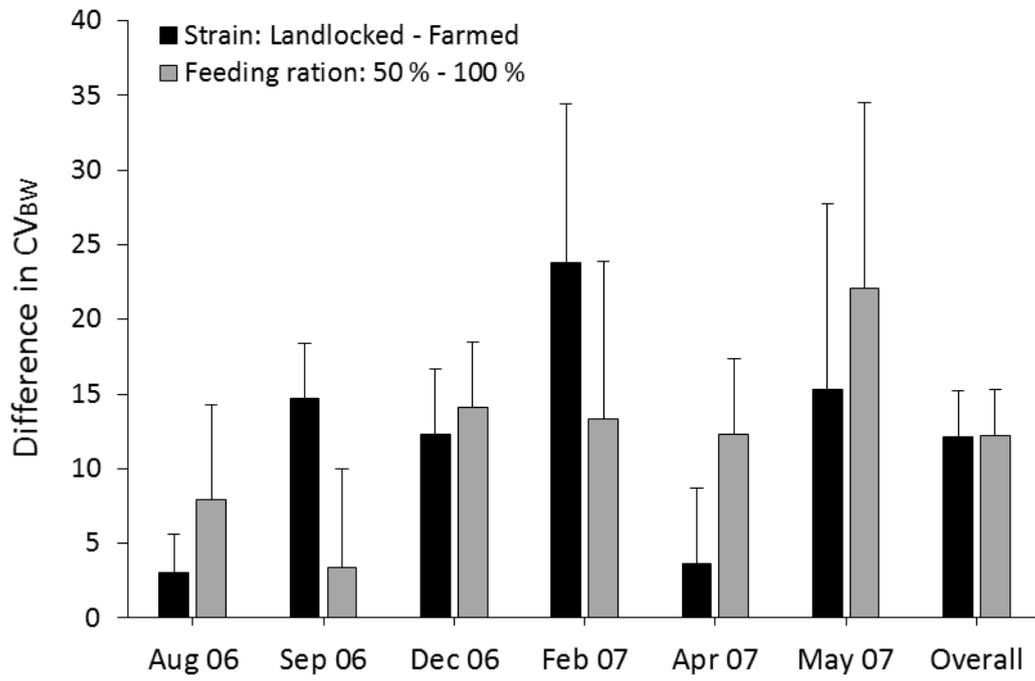


Figure 3



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