Report: Deliverable 3.2 Effects of mother-bonded calf rearing on meat quality in calves

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1. Research question

On farm trials were conducted in Switzerland to test the hypothesis that calves allowed dam suckling (MS) twice a day would benefit with regard to meat quality compared to twice a day bucket fed (BF) group mates. Two runs of the feeding trial with German Friesian Cattle were carried out on an organic certified commercial farm between autumn 2018 and summer 2020, of which 8 MS and 11 BF calves were slaughtered.

Since marketing broke down due to the Covid pandemic, calves became older than planned and the data basis thus became much more heterogeneous than planned for this important trait: age at slaughtering varied from 128 to 288 days, with mean age at 212 days ± 40 days of standard deviation. However, as age has a great influence on meat quality, we refrained from including meat quality results in a scientific publication. Instead, results obtained under this limitation are presented in this publicly accessible report.

2. Material and Methods

2.1 Animals and study design

Directly after birth calves were randomly assigned by sex and parity status of the mother (primi- or mulitparous) to become either a bucket fed (BF) or a mother suckling (MS) calf. All calves involved in this study were kept with their dam for approx. 7 days post partum. Afterwards they were moved to the group pen, where calves from both feeding groups were housed together in a deep straw littered barn with outdoor run. They were fed ad libitum hay plus carrots twice a day, but no concentrates. All animals had free water access. Twice a day MS calves were separated from their pen mates and allowed to suckle the milk from their mother, which was left over after milking, for approx. 30 minutes in the cow waiting area. Milk intake was regularly assessed by weighing the MS calves before and after milking and was controlled by a feeding scheme for BF calves. BF calves were fed with teat buckets during the same time slots as MS calves. We aimed at achieving comparable milk intake, as we wanted to separate the effect of higher milk intake, usually associated with cow-calf contact rearing systems, from the effect of cow contact. In both groups each calf consumed around 900 kg milk during four months and achieved comparable weight gains during this time (daily weight gain: BF= 807± 42 g/d, MS= 815 ± 46 g/ d).

2.2 Meat sampling and sample size

Fourteen calves were slaughtered in the slaughter facility at their birth farm, while 5 calves (samples No. 3, 4, 5, 8, 9) were slaughtered in a slaughterhouse which was around a 1 h drive from their birth farm.

After slaughtering, carcasses were stored in a refrigerated room at 4°C for 24 hours. Meat samples of approx. 300 g of lean meat were taken from the *Longissimus dorsi* muscle on the left side of the animal above the ribs 5 to 6, 24 hours after slaughtering.

The pH was measured 24 – 25.5 hours after slaughtering using a portable pH-meter with a non-penetrating electrode (InLab Solids, P/N: 51343153, S/N: 7100990). A little whole was cut into the muscle with a scalpel where the pH-meter-electrode was introduced for the measurements. Meat samples were then vacuumed (with an identification plastic label inside the bag), kept refrigerated during transport to FiBL, where they were frozen and stored at -20°C.

After all samples had been obtained and pandemic conditions allowed transport again, the frozen meat samples were sent from Switzerland to Italy in insulated Styropor boxes, 1/3 filled with dry ice. They were transported within 48 hours without any interruption of the cold chain.

In total 19 samples, 11 from bucket fed (BF) and 8 from mother suckling (MS) calves, were obtained.

2.3 Meat sample analysis

2.3.1 Fatty acid analysis

Intramuscular fat was extracted from 10 g of tissue with a mixture of chloroform and methanol (2:1, v/v) and 50 mg of lipids were converted to fatty acid methyl ester (FAME) by base catalyzed transesterification, using 1 mL of sodium methoxide in methanol 0.5 N and 2 mL of hexane containing C19:0 as an internal standard. Fatty acids were separated through a gas-chromatograph (model TRACE GC; Thermo Finnigan, Milan, Italy) in a 100-m high-polar fused silica capillary column (25 mm i.d., 0.25-µm film thickness; SP. 24056; Supelco Inc., Bellefonte, PA) and identified by a flame ionization detector (FID). Gas-chromatography conditions and identification of FAME was performed as reported in Natalello et al. (2019).

2.3.2 Meat oxidative stability

Oxidative stability was evaluated in fresh meat over aerobic storage as reported by Natalello et al. (2020). In brief, three 2-cm-thick slices were placed in polystyrene trays, over-wrapped with 2 layers of domestic cling film and stored at 4 °C in dark for 0 (after 2 hours of blooming), 4 and 7 days. At the end of the respective storage time, one of the 3 slices was used to determine color parameters and then frozen pending lipid oxidation analysis.

Color stability of raw meat was measured by a Minolta CM 2022 spectrophotometer (d/8° geometry; Minolta Co. Ltd. Osaka, Japan) set to operate in the specular components excluded (SCE) mode and to measure with the illuminant A and 10° standard observer. Three measurements were taken on the meat surface and the mean value was calculated. The colour descriptors L* (lightness), a* (redness), b* (yellowness), C (saturation) and h_{ab} (hue angle) were measured in the CIE L* a* b* colour space.

Lipid oxidation was determined in meat slices by measuring the 2-thiobarbituric acid reactive substances (TBARS) at the end of each storage time, as reported in Natalello et al. (2020) with some modifications. In brief, meat samples (2.5 g) were homogenized with 12.5 mL of distilled water using a Heidolph Diax 900 tissue homogenizer (Heidolph ElektroGmbH & Co. KG, Kelheim, Germany) operating at 9500 rpm. During the homogenization, samples were put in a water/ice bath. Subsequently, 12.5 mL of 10% (w/v) trichloroacetic acid (TCA) was added to precipitate proteins, after which samples were filtered through Whatman No. 1 filter paper. The clear filtrate (4 mL) was added to 1 mL of 0.06M aqueous thiobarbituric acid into pyrex-glass tubes. The tubes were incubated in a water bath at 80 °C for 90 min and the

absorbance of each sample was read at 532 nm using a Shimadzu UV/vis spectrophotometer (UV-1601; Shimadzu Corporation, Milan, Italy). A calibration curve was prepared with TEP (1,1,3,3,-tetraethoxypropane) in distilled water at concentrations ranging from 5 to 65 nmol/4 mL and results were expressed as mg of malondialdehyde (MDA) per kg of meat.

2.3.3 Myoglobin analysis

Myoglobin concentration was determined as described by Krzywicki (1982). Briefly, muscle samples were homogenized with phosphate buffer, centrifuged at 6800 × g at 4 °C and filtration through Whatman 541 paper. The filtered supernatant was scanned in a UV/VIS spectrophotometer (UV-1601; Shimadzu Co., Milan, Italy) and the absorbance at 525 nm was used to calculate Mb concentration, expressed as mg/g of fresh tissue.

2.4 Statistical analysis

One-way analyses of variance were performed for pH, fat, fatty acids and myoglobin as dependent variables with the following model:

$$\mathbf{y_{ijkl} = \mu + T_i + Sex_j + Trial_k + ParityStatusMother_l + ageAtSlaughter + e_{ijkl},}$$

where: y_{ijkl} is the dependent trait (pH, fat, fatty acids or myoglobin); μ is the overall mean; T_i is the treatment (i= "bucket"; "mother"), Sex_j (j=male, female),$Trial_k$ (k=first run, second run), $ParityStatusMother_l$ (l=primiparous, multiparous), ageAtSlaughter (covariate in days); e_{ijkl} is the residual error

Oxidative stability data (colour and lipid oxidation over time of storage) were analysed with the following mixed model to test the effects of the treatment (T), of the day of storage (D) and of the T × D interaction as fixed factors:

$$\mathbf{Y_{ijklm} = \mu + T_i + D_j + I_k + (T \times D)_{ij} + Sex_k + Trial_l + ParityStatusMother_m + ageAtSlaughter + e_{ijkl},}$$

where y_{ijklm} is the dependent trait (colour or lipid oxidation); μ is the overall mean; T_i the fixed effect of treatment (i="bucket"; "mother"); D_j the fixed effect of day of storage (j= 0, 3, 7 days for colour and 0, 7 for lipid oxidation); $I_k(T)$ is the random effect of the individual animal; $(T\times D)_{ij}$ is the interaction between treatment and day of storage; Sex_k (k=male, female) ,$Trial_l$ (l= first run, second run), $ParityStatusMother_m$ (m=primiparous, multiparous), ageAtSlaughter (covariate in days); e_{ijklm} is the residual error.

Models were estimated in R version 3.6.3 (2020-02-29, R Core Team 2020).The differences between least square means of the fixed effects were verified by Tukey tests using the «emmeans» package (version 1.4.5, Lenth, 2020). We checked normal distribution of the residuals of all models by visually inspecting residual plots. Statistical significance was assumed at $P<0.05$, with tendency between $P>0.05$ and $P<0.10$.

3. Results and conclusions:

The pH measured 24 hours after slaughtering did not differ in meat from calves of different feeding regimes (BF= 6.15 ± 0.14, MS= 6.09 ± 0.16, P= 0.7515). We also could not identify any difference between meat of BF and MS calves regarding colour, lipid oxidation (Table 1) or fatty acid composition (Table 2).

Table 1: Effect of feeding regime, storage time and its interaction on colour and lipid oxidation in meat samples from bucket fed (BF, n=11) versus mother suckling (MS, n=8) calves

	Feeding (F)		Day of storage (D)				P-Value[3]		
	BF	MS	0	3	7	SEM[2]	F	D	FxD
Colour descriptors									
L* (lightness)	50.3	49.9	52.1^{a}	48.5^{ab}	49.5^{b}	1.594	0.504	0.0183	0.149
a* (redness)	13.8	15.9	18.5^{a}	15.2^{b}	10.9^{c}	0.984	0.260	<0.001	0.839
b* (yellowness)	14.3^{b}	16.6^{a}	17.8^{a}	15.3^{b}	13.3^{c}	0.8923	0.449	<0.001	0.238
C (saturation)	20.0	23.1	25.7^{a}	21.6^{b}	17.2^{c}	1.267	0.327	<0.001	0.532
h_{ab} (hue angle)	46.6	47.0	44.1^{b}	45.2^{b}	51.3^{a}	0.603	0.481	<0.001	0.165
Lipid oxidation[1]									
TBARS mg/kg	1.61	1.95	0.22	-	3.33	0.524	0.916	<0.001	0.505

[1]TBARS= thiobarbituric acid reactive substances, [2]SEM= standard error of the mean of factor day, [3] P value of the F-Test (Type 3) for the respective factor or interaction

Myoglobin content (mg/g of muscle) was slightly higher in bucket compared to mother fed calves (LSM: 0.79 ± 0.05 BF vs. 0.65 ± 0.05, P= 0.0683, figure 1).
The by tendency higher content in bucket fed calves has to be interpreted with care. In order to verify this effect a more homogenous age at slaughtering (as originally planned, but inhibited by Covid) would be needed.

From our results we conclude that meat quality does not benefit from mother contact alone, when milk intake is comparable between calves with and without mother contact.

Figure 1: Myoglobin (mg/g of musle) in meat of claves fed by bucket or mother

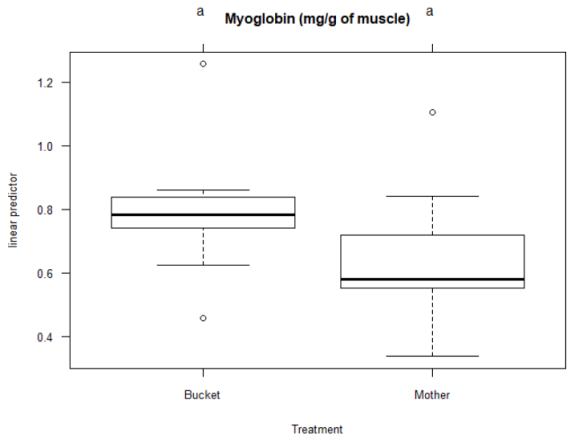

Table 2: Intramuscular fat content (g/100 g of meat), Fatty acid profiles (g/100g of total fatty acids) in meat samples from bucket fed (n=11) versus mother suckling (n=8) calves

	Treatment (T)		SEM	p-value
	bucket	mother		
Intramuscular fat (g/100 g of meat)	1.70	1.25	0.481	0.3754
Fatty acid (g/100 g of total fatty acids)				
C10:0	0.07	0.07	0.005	0.9641
C12:0	0.28	0.27	0.060	0.8737
C13:0	0.05	0.05	0.009	0.9773
C14:0 iso	0.08	0.08	0.011	0.8286
C14:0	4.85	4.53	0.793	0.6887
C15:0 iso	0.21	0.20	0.024	0.5322
C15:0 anteiso	0.30	0.30	0.036	0.9450
C14:1 c9	0.73	0.69	0.119	0.7688
C15:0	0.72	0.73	0.097	0.9055
C16:0 iso	0.23	0.23	0.020	0.8501
C16:0	23.9	23.4	1.422	0.7146
C17:0 iso	0.45	0.39	0.039	0.1329
C16:1 t9	0.16	0.15	0.032	0.7802
C16:1 c7	0.26	0.24	0.018	0.3758
C17:0 anteiso	0.52	0.47	0.049	0.3610
C16:1 c9	2.23	1.94	0.207	0.1902
C17:0	1.13	1.06	0.095	0.4471
C17:1 t10	0.01	0.03	0.015	0.4198
C18:0	12.8	12.1	0.715	0.3900
C18:1 t6+t7+t8	0.08	0.08	0.012	0.6702
C18:1 t9	0.20	0.20	0.023	0.7301
C18:1 t10	0.08	0.08	0.016	0.8614
C18:1 t11	1.98	1.94	0.027	0.8729
C18:1 c6	0.35	0.36	0.048	0.9180
C18:1 c9	23.8	22.7	1.319	0.4610
C18:1 c11	1.22	1.23	0.186	0.9785
C18:1 c12	0.12	0.10	0.021	0.3874
C18:1 c13	0.21	0.18	0.027	0.3245
C18:2 c9c12	4.65	5.52	1.475	0.5637
C20:0	0.12	0.12	0.013	0.8005
C18:3 c6,9,12	0.03	0.03	0.007	0.3950
C20:1 c11	0.11	0.09	0.018	0.5462
C18:3 c9,12,15	1.69	2.12	0.377	0.2692
C18:2 c9t11	1.00	1.03	0.126	0.8354

C21:0	0.05	0.05	0.006	0.6063
C20:2 c11,14	0.07	0.07	0.026	0.9829
C22:0	0.02	0.03	0.009	0.5736
C20:3 n-6	0.31	0.40	0.127	0.4635
C20:3 n-3	0.04	0.05	0.015	0.4657
C20:4 n-6	1.32	1.62	0.500	0.5552
C24:0	0.95	1.24	0.410	0.4854
C24:1 c9	0.05	0.05	0.023	0.8581
C22:5 n-6	0.88	1.21	0.383	0.3963
C22:5 n-3	0.12	0.15	0.041	0.4917
∑Saturated FA	43.0	41.8	2.079	0.5771
∑Monounsaturated FA	31.5	30.1	1.612	0.3959
∑Polyunsaturated FA	10.1	12.2	2.871	0.4699
∑Odd- Branched chain FA	3.75	3.53	0.323	0.5176
∑n-6 PUFA	7.24	8.86	2.487	0.5264
∑n-3 PUFA	1.84	2.31	0.425	0.2855
n-6/n-3 PUFA ratio	3.79	3.59	0.416	0.6415

References:

Krzywicki, K. (1982). The determination of haem pigments in meat. Meat Science, 7(1), 29-36.

Lenth, R., 2020. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.4.5. https://CRAN.R-project.org/package=emmeans

Natalello, A., Luciano, G., Morbidini, L., Valenti, B., Pauselli, M., Frutos, P., Bionid, L., Rufino-Moya, P.J., Lanza, M., Priolo, A. (2019). Effect of feeding pomegranate byproduct on fatty acid composition of ruminal digesta, liver, and muscle in lambs. Journal of Agricultural and Food Chemistry, 67(16), 4472-4482. https://doi.org/10.1021/acs.jafc.9b00307

Natalello, A., Priolo, A., Valenti, B., Codini, M., Mattioli, S., Pauselli, M., Puccio, M., Lanza, M., Stergiadis, S., Luciano, G. (2020). Dietary pomegranate by-product improves oxidative stability of lamb meat. Meat Science, 162, 108037. https://doi.org/10.1016/j.meatsci.2019.108037

R Core Team, 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria. https://www.R-project.org/

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