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Skin carotenoid levels in lactating dairy cows as measured using multiple spatially resolved reflection spectroscopy

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Research Article

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Abstract

This research communication describes a pilot study measuring skin carotenoid levels of lactating dairy cows. Carotenoids are natural antioxidants, involved in cell communication and immune function, protecting against oxidative stress. They are precursors of vitamin A, important for reproduction efficiency, growth and male fertility. Therefore, easy-to-use, inexpensive methods to measure carotenoids in cattle would provide interesting data for farmers to monitor the health and nutritional status of their herds. In this study, we used a commercially available sensor based on multiple spatially resolved reflection spectroscopy (MSRRS), intended for human use, to measure the carotenoid content in bovine skin in three research herds in France, Ireland and Scotland. Carotenoid levels were measured by applying the sensor to the teat barrel, avoiding pigmented areas of skin. Mean sensor values differed significantly between herds and between diets, with pasture-based animals showing significantly higher carotenoid levels. Our results suggest that MSRRS can be used to accurately to measure skin carotenoids in cows. However, further calibration in bovines is needed to improve of the accuracy of the MSRRS sensor in cattle.

Milk yields in intensive and pasture-based dairy systems have been increasing steadily over the last 70 years (Jones *et al.*, 1994). Mastitis, one of the most common and expensive inflammatory diseases in dairying, is mainly caused by bacterial or fungal infection. Mastitis is associated with an increased somatic cell count (SCC) in milk and is often detected by farm staff *via* identification of milk and udder changes, or by indirect methods including California Milk Test, milk electrical conductivity changes, or lactate dehydrogenase activity (Chagunda *et al.*, 2006; Rainard *et al.*, 2018; Khatun *et al.*, 2019). However, most of these approaches are not suitable, or reliable enough, for early detection (Miekley *et al.*, 2012; Khatun *et al.*, 2019). Early detection can potentially reduce the negative impact of mastitis and reduce antibiotic usage, leading to improved profitability and reducing the risk of antimicrobial resistance.

Oxidative stress, measured in cattle *via* blood and milk biomarkers, occurs in response to an imbalance in the production of highly reactive free radicals, which can damage cells and tissues throughout the body. It is associated with inflammation and chronic diseases such as cataracts and cancer (Rao and Rao, 2007). Antioxidants, such as carotenoids, whether synthesised in the body or obtained from the diet, play a crucial role in the reduction of oxidative stress by neutralising free radicals (Dekkers *et al.*, 1996). Chew and Johnston (1985) showed that vitamin A and carotenoid insufficiency can be directly related to udder infections in dairy cattle, and Chew *et al.* (1982) reported that low plasma concentrations of vitamin A and beta-carotene were associated with increasing severity of mastitis in cows.

In humans, carotenoids measured in skin using multiple spatially resolved reflection spectroscopy (MSRRS) can be used as proxy for multiple factors, including nutritional status and stress level (Darvin *et al.*, 2012), smoking status and body mass index. They are influenced by several factors, including UV exposure and season (Rao and Rao, 2007). As carotenoid levels are associated with udder health and mastitis severity in cattle, and skin carotenoid content in humans can serve as proxy for several different factors, we hypothesised that skin carotenoid levels measured in cows might be a suitable proxy for inflammation associated with mastitis. Therefore, the main aim of this study was to determine whether MSRRS is a suitable tool to accurately measure carotenoid content in the teat skin of dairy cows.

Materials and methods

A portable multiple spatially resolved reflection spectroscopy (MSRRS) sensor (Opsolution, Kassel, Germany) for application on human skin was used to obtain bovine skin carotenoid

measurements. The system comprised of differently positioned LED light sources and detectors with different wavelengths. Light sources covered a spectral range of approximately 350 to 1000 nm in 16 levels. Measurements were taken manually by placing the sensor firmly on the teat skin (targeting a non-pigmented area of the teat barrel), ensuring full sensor coverage. Attempts were made to use the sensor directly on the udder wall but were not successful because the udder hair interfered with the sensor measurements (data not shown). Once fully covered, measurement started automatically and took approximately 15 s. Sensor position, cow identification number and time of measurement were manually recorded. Sensor data was calculated based on the algorithm provided by Opsolution (Germany). Data were quality checked using a score that describes errors in recording (such as the sensor being removed before the measurement was complete). This score goes from 0 to 70, in increments of 10. Measurements with score ≥70 are considered erroneous (based on indications from sensor and analysis algorithm developers) and were, therefore, discarded. The sensor used in this study was calibrated for human application, with sensor values expected to fall between 0 and 15.

The study was carried out at three different sites, namely INRAE Le Pin, France, TEAGASC Moorepark, Ireland and SRUC Dumfries, Scotland. These sites had different production systems (grazing *vs.* loose housing) and breeds, and a different number of teats per cow were measured by each partner, due to the exploratory nature of the study (four in INRAE, two in TEAGASC and SRUC: Table 1). Additional animal data were collected including diet, breed, body weight, body condition score (BCS), stage of lactation (days in milk, DIM), milk yield and parity. A total of 3657 samples were taken from Holstein cows, 1185 from Normande cows and 408 from crossbreeds. Animals were grouped based on DIM to reflect nutritional energy balance (EB) differences, where between 1 and 84 DIM was considered negative EB, between 85 and 245 DIM was considered neutral and above 245 DIM was considered positive. Animals were body condition scored by direct physical assessment whilst standing, performed by one observer utilising a 21-point scale based on NIRD dairy cow condition scoring (Mulvany, 1977), with quarter point scores. Across all sites there was one case of clinical mastitis, and no other diseases documented. Data from the animal with mastitis were disregarded as there were no reliable comparisons with other, similarly affected cows. Mastitis incidence for each site was 33, 13 and 37 cases per 100 cows per year for INRAE, TEAGASC and SRUC respectively.

Statistical analysis was carried out using JMP® 14.0, (SAS Institute, Cary, North Carolina, USA) comparing differences between groups and SAS (SAS Institute, Cary, North Carolina, USA) software using generalised linear mixed models (GLMM) with fixed effects breed, feed, BCS and DIM, and the sensor value as response variable estimating the restricted maximum likelihood.

Results

Site specific data describing the sampled animals by DIM, NEB, BCS, average sensor score and measurement quality with standard deviation are given in Table 1. The quality of measurements differed across sites. At SRUC, 75% of measurements achieved the two best quality levels, 0 and 10 with an average of 8.4. At INRAE, 75% of measurements were between 0 and 30, providing an average value of 13.6. At TEAGASC 78% of the measurements

Table 1. Site specific data describing the sampled animals by days in milk (DIM), nutritional energy balance (NEB), body condition score (BCS), average sensor score and measurement quality with standard deviation

Site	Breeds	Management/diet	#Animals	#Samples	DIM	NEB[a]	BCS	Sensor score[b]	Sensor quality score	Milk yield (kg/year)
INRAE	Normande	Grazing	21	1186	78.2 ± 35.2	Negative; neutral	2.7 (1.5–4.8)	13.0 ± 3.5	13.6 ± 18.0	6.500
	Holstein-Friesian	Grazing	10	691						9.000
	Holstein-Friesian	Housed – silage[c]	10	546						10.000
			41	2423						
TEAGASC	crossbreds of Holstein-Friesian, Jersey, Norwegian Red and Normande	Grazing + 2 kg concentrate	28	743	211.0 ± 19.2	Neutral	3.1 (3.0–3.5)	8.2 ± 2.4	15.1 ± 18.5	6.300
SRUC	Holstein-Friesian	Housed – forage + concentrate mix	32	2080	183.7 ± 78.5	Negative; neutral; positive	2.2 (1.5–2.8)	10.7 ± 2.0	8.4 ± 15.7	10.000

[a]Nutritional energy balance; negative: DIM < 84; neutral: 85 < DIM < 245; positive: DIM > 245.
[b]Measurements taken prior to afternoon milking at INRAE and SRUC and prior to daily milking at TEAGASC.
[c]Dry matter intake 20.3 kg.

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were between 0 and 30, and the average value was 15.1. Mean sensor value decreased as measurement quality decreased from 0 to 40. The qualities 50 and 60, totalling 4% of all readings did not follow this trend. The sensor used in this study was calibrated for human application, with values expected to fall between 0 and 15. Since sensor values measured in bovine teats in the present study ranged from 1.2 to 22.7, the carotenoid content was extrapolated based on the human model developed by Opsolution.

Sensor values for teats averaged 13.0 ± 3.5, 8.2 ± 2.4 and 10.7 ± 2.0 at INRAE, TEAGASC and SRUC, respectively. Within the INRAE group, sensor values differed significantly due to diet and breed. Grass-fed Holsteins showed significantly higher sensor values (by 1.9 ± 0.3) than silage-fed Holsteins (13.8 *vs.* 11.8), whereas grass-fed Normande showed significantly higher values than grass-fed Holstein (14.9 *vs.* 13.8). The INRAE group included 2 breeds (Normande and Holstein) and 2 diets (grazing and silage-fed), TEAGASC included a mix of breeds (Holstein Friesian, Jersey, Norwegian Red and Normande mix) and SRUC comprised only Holstein Friesian. Both TEAGASC and SRUC used one diet each, grazing plus concentrate and forage-concentrate mix, respectively. INRAE cattle had a wide range of BCS values, averaging 2.7 (1.5–4.75), whereas in TEAGASC and SRUC these averaged 3.1 (3–3.5) and 2.2 (1.5–2.75), respectively. The statistical analysis showed that BCS was not significantly correlated with sensor values at any of the sites.

The INRAE group included relatively early lactation animals (DIM = 78 ± 35) with negative and neutral EB whilst all TEAGASC cows were in later lactation (DIM = 211 ± 19) and had a neutral EB, and the SRUC herd ranged across all EB categories (DIM = 183 ± 78). SRUC animals with a positive EB had significantly higher sensor values (by 1 point) than the neutral and negative groups ($P < 0.001$). There was no significant difference between neutral and negative groups, either at INRAE or SRUC.

Discussion

The sensor used in this study was developed for human use and its calibration algorithms developed for human skin. Quality of measurements was high across all sites, with approximately 75% of sensor measurements being allocated to one of the first four quality categories, suggesting that measurements based on MSRSS on the teat barrel of dairy cattle were accurate. The extrapolation of values by Opsolution using the human calibration model will have influenced measurements in this pilot study. For future work, sensors should be validated for use in cattle, preferably by direct comparison with blood carotenoid levels.

Carotenoid concentration in humans is associated with multiple factors, including diet and health (Darvin *et al.*, 2016), and is indicative of vegetable intake and associated with biomarkers of circulatory diseases and metabolic syndromes (Matsumoto *et al.*, 2020). The most interesting findings in our study were that we were able to capture differences between feed and breed using the sensor. The higher sensor values, indicative of higher carotenoid concentrations, noted in grazing Holstein animals (INRAE) may be explained by diet composition. This variation is in agreement with Prache *et al.* (2003) who were able to distinguish grass-fed from stall-fed lambs using plasma carotenoid concentration and suggested that carotenoids are suitable biomarker for nutritional status in ruminants.

Grass-fed cows at TEAGASC had a significantly lower sensor value than those at INRAE, despite the average BCS of the Irish cattle being 0.2 units higher than those at INRAE. Both sites have a similar annual milk yield and only EB differed. The INRAE group had a wider range of BCS values than the other sites, which could be due to variation in breeds and diets. Time of year and climate may have caused qualitative differences in the grass, which affected carotenoid availability, with INRAE measurements collected in April and TEAGASC in September. In spring, grass is rich in carotenoids, but this progressively decreases as it dries and pigments photodegrade (Nozière *et al.*, 2006).

Calderon *et al.* (2007) found correlation between days to parturition and carotenoid content in blood plasma in cattle. In this study, the higher sensor values noted in SRUC animals closest to parturition (positive NEB) supports these findings. Breed also impacted sensor values reported in this study. INRAE grass-fed animals were purebred Normande and Holstein breeds, whereas TEAGASC cows were crossbreeds. The different sensor values noted between sites could be as a result of variation in fat deposits between breeds and animals. Carotenoid enrichment differs between fat deposits and even at identical carotenoid intake levels, fat carotenoid signature intensity can vary (Macari *et al.*, 2017).

In conclusion, this study suggests that it is possible to utilise a commercially available MSRRS sensor intended for human use to identify variation in bovine skin carotenoid levels. However, limitations such as skin pigmentation must be considered. Significant differences in the sensor measurement were associated with feed and breed. Further application of MSRRS sensors in cattle requires proper calibration in combination with blood sampling. Calibration and exploration of data from animals identified with clinical mastitis is necessary to understand whether this sensor can be used for disease-specific approaches to on-farm health monitoring.

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