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Carotenoids and tocopherol in plasma and fat to authenticate forage feeding in cattle

M. Blanco[1], F. Molino, G. Ripoll, I. Casasús and M. Joy

[1]Unidad de Tecnología en Producción Animal, CITA de Aragón
Avda de Montañana 930, 50059 Zaragoza, (Spain)
*e-mail: mblanco@aragon.es

Abstract. Forage-feeding in cattle carcasses can be authenticated using an estimator of carotenoid pigment concentration (SUM). Therefore, it is suggested that plasmatic carotenoids and α-tocopherol could be used to authenticate it *in vivo*. Plasmatic concentrations of carotenoids and α-tocopherol, fat colour and SUM were studied in concentrate-fed bulls (C) and two groups of steers until slaughter (500 kg). Both groups of steers were fed a total mixed ration (50% lucerne hay + 10% straw+ 40% corn) during 159 days (winter) and grazed on a mountain meadow plus 1.8 kg DM corn/d for 85 days. Thereafter, one group was finished on a meadow (G) plus 1.8 kg DM corn/d for 75 days and the other group was finished by means of a total mixed ration during 55 days (finishing period) (TMR). During winter, G and TMR steers had greater plasmatic carotenoids concentrations than C bulls ($P < 0.001$). Conversely, C bulls had greater plasmatic α-tocopherol concentration than both groups of steers on day 29 ($P < 0.001$) and 55 ($P < 0.05$) but similar thereafter. During the finishing period, G steers had greater plasmatic β-carotene, lutein and α-tocopherol concentrations than TMR steers ($P < 0.001$). Fat colour of G steers had lower lightness but greater yellowness and SUM than C bulls. Steers finished with TMR had intermediate values for lightness and yellowness. Plasmatic carotenoids content and SUM were useful to detect differences in diet.

Keywords. Authenticate – Forage – Cattle – Carotenoids.

Caroténoïdes et tocopherol dans le plasma et les tissus adipeux pour authentifier l'alimentation fourragère chez le bovin

Résumé. *Une alimentation à base de fourrages peut être authentifiée par l'estimation de la concentration en caroténoïdes (SUM) dans les tissus adipeux de la carcasse, mais la concentration plasmatique en caroténoïdes pourrait l'authentifier* in vivo*. Les concentrations en caroténoïdes et tocopherol dans le plasma, ainsi que la couleur et le SUM des tissus adipeux ont été étudiés sur des taurillons alimentés avec des rations à base de concentré (C) et sur deux groupes de bouvillons alimentés en hiver avec une ration mixte complète (50% luzerne foin + 10% paille + 40% maïs grain: 159 jours), puis au pâturage. Après 85 jours de pâture les bouvillons ont été, soit finis au pâturage pendant 75 jours (G), soit ont reçu la ration mixte complète pendant 55 jours (TMR). Au pâturage, tous les bovins ont reçu 1,8 kg MS maïs/j. Pendant l'hiver, les bouvillons G et TMR ont eu des concentrations en caroténoïdes plus élevées que les taurillons. À l'inverse, les taurillons C ont eu une teneur plus élevée en α-tocopherol que les bouvillons les jours 29 ($P < 0,001$) et 55 ($P < 0,05$), mais similaire par la suite. Pendant la finition, le groupe G a eu des concentrations en caroténoïdes et α-tocopherol plus élevées que le groupe TMR ($P < 0,001$). Le tissu adipeux des bouvillons G a été moins lumineux et plus jaune que celui des taureaux C, alors que les TMR ont eu des valeurs intermédiaires. En conclusion les teneurs plasmatiques en caroténoïdes et SUM peuvent servir à détecter des différences du régime alimentaire.*

Mots-clés. *Authentification – Fourrages – Bovins – Caroténoïdes.*

I – Introduction

In dry mountain areas, forage has been introduced in the diets at the expense of concentrates mainly due to the increase of cereal prices and to satisfy the societal demands regarding environmental and ethical concerns about food production (Bernués *et al.*, 2011). Nowadays, consumers associ-

ate healthy beef with grazed cattle, fed with natural feedstuffs and raised outdoors (Verbeke *et al.*, 2010) and there is an increasing interest in guaranteeing the traceability of these production systems. The authentication of forage feeding in meat from ruminants can be achieved by measuring carotenoid pigments in plasma and fat deposits. Carcasses form forage-fed cattle can be accurately traced by measuring subcutaneous fat colour (Dunne *et al.*, 2009, Blanco *et al.*, 2011). In order to detect forage feeding *in vivo*, carotenoids content in serum have been investigated (Serrano *et al.*, 2006). Our objective was to study the evolution of carotenoids and α-tocopherol during the fattening period to check the feasibility of their use to trace forage feeding in beef cattle.

II – Materials and methods

1. Animals

This study was conducted in La Garcipollera Research Station, located in the Spanish Pyrenees (Spain, 42º37' N, 0º30'W; 945 m a.s.l.) and in CITA Research Centre, located in the Ebro Valley (41° 43' N, 0° 48' W; 225 m a.s.l.). During the winter housing period, a group of 8 intact young bulls (C) received commercial concentrates plus straw until they reached the target slaughter weight (500 kg) and 2 groups of steers received a total mixed ration (50% lucerne hay + 10% straw + 40% corn) until mid-April. Both groups of steers rotationally grazed together in valley meadows and were supplemented with 1.8 kg DM corn/day/head during 85 days. Thereafter, one group of steers (G; n = 8) remained on the meadows during 75 days with 1.8 kg DM corn/head/day until they reached the target slaughter weight and the second one (TMR; n = 8) was finished for 55 days with the same total mixed ration fed during the winter period.

2. Sampling

Samples of the different feedstuffs were collected fortnightly. They were immediately frozen and freeze-dried. The animals were bled monthly except for the first two months of the grazing season and the first month of the finishing period, when they were bled weekly.

Fat cover was scored on a 15-point scale at 24 hours *post-mortem*. Subcutaneous fat colour at the 10th rib was measured with a Minolta CM-2006d spectrophotometer. The estimator of fat carotenoid pigments content (SUM) was calculated as proposed by Prache and Theriez (1999).

3. Analyses of carotenoids and tocopherol concentrations

The contents of β-carotene, lutein and α-tocopherol were determined by HPLC following the procedures of Chauveau-Duriot *et al.* (2010). The analyses were modified for the determination in plasma as described by Molino *et al.* (2012). The dry residues were dissolved in 0.5 ml of acetonitrile–dichloromethane–methanol (75–10–15). HPLC was run on a HPLC 1100 Agilent equipped with a photodiode array detector. β-carotene and lutein were detected at 450 nm, and α-tocopherol at 295 nm.

III – Results and discussion

Average carotenoids and α-tocopherol contents of the feedstuffs used in the experiment are detailed in Table 1. Pasture contents remained unchanged during the grazing season (data not shown). However, Dunne *et al.* (2009) reported that carotenoids in pasture are affected by season. In the current study, the meadow was rotationally grazed, thus, the stage of maturity of consumed pasture was probably constant throughout the grazing season.

Both groups of steers had greater plasmatic β-carotene and lutein concentrations than C bulls throughout the winter feeding period, reflecting differences in carotenoid intakes (Serrano *et al.*, 2006). However, both groups of steers had lower plasmatic α-tocopherol concentration than C bulls on day 29 and 55 ($P < 0.05$) (Fig. 1), which is a result of the greater content of α-tocopherol in the concentrate. When the steers were turned out to pasture, the plasmatic carotenoids and α-tocopherol concentrations increased in both groups of steers, which had similar plasmatic concentrations during the first 85 days of the grazing season.

Table 1. β-carotene, lutein and α-tocopherol concentrations in the feedstuffs

	Lucerne hay	Straw	Concentrate	Pasture	Corn
β-carotene, mg/kg DM	1.3 ± 0.42	n.d.	n.d.	370.7 ± 12.27	n.d.
Lutein, mg/kg DM	9.0 ± 0.32	0.6 ± 0.02	n.d.	232.1 ± 2.17	1.4 ± 0.05
α-tocopherol, mg/kg DM	2.1 ± 0.14	2.1 ± 0.10	6†	117.2 ± 13.75	1.3 ± 0.46

† Incorporated in the concentrate as 10 mg/kg of tocopheryl acetate n.d.: not detected.

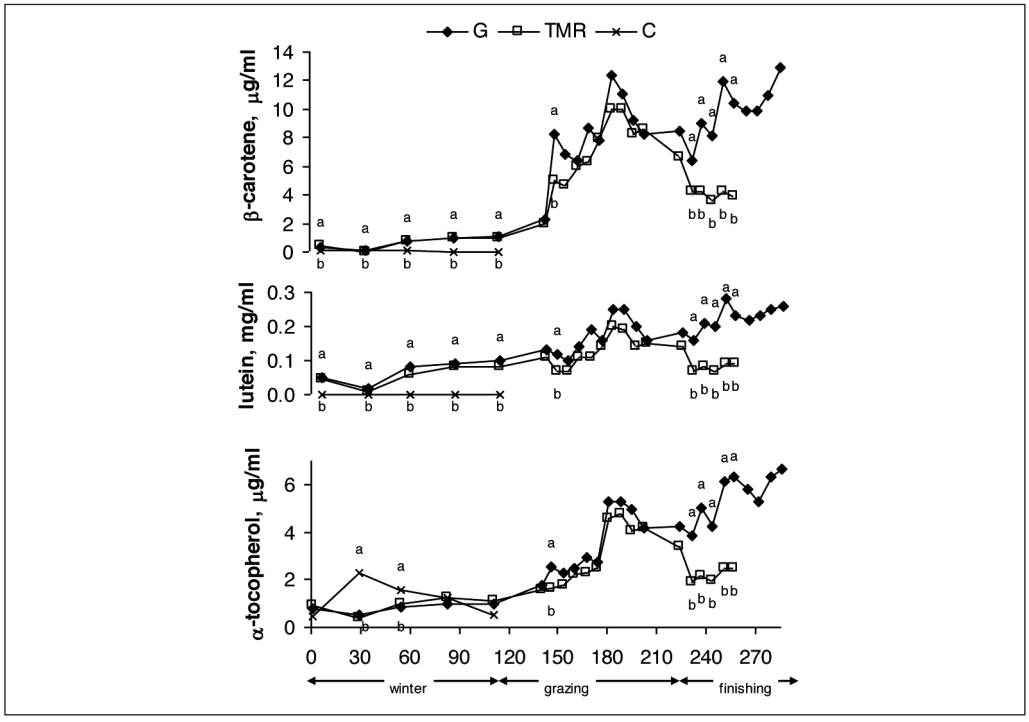

Fig. 1. Plasmatic β-carotene and lutein concentrations according to the management strategy throughout the experimental period. G: grazing steers; TMR: indoors finished steers; C: concentrate-fed bulls. Within a date, means with different letters differ at P<0.05.

As carotenoids and α-tocopherol contents in pasture did not change through the grazing season, the increase in the plasmatic concentration would reflect an increase in forage intake. During finishing, G steers had greater plasmatic carotenoids and α-tocopherol concentrations than TMR steers, as consequence of the sharp decrease observed when steers started eating the total mixed ration, based on dry-preserved forages and grain.

Fat of both groups of steers had greater yellowness, redness and SUM than that of C bulls since yellowness is caused by carotenoids of green forage (Dunne *et al.*, 2009). Regarding fat colour differences between both groups of steers, the 55 day finishing period on a low carotenoid content diet after grazing only affected fat yellowness and Chroma (Table 2). These results agree partially with those reported by Blanco *et al.* (2011) and Serrano *et al.* (2006). In both studies, a low carotenoid diet (concentrates or hay) reduced fat yellowness and SUM after 58 and 150 days, respectively. However, as carotenoids deposition in fat depends on the amount of fat accumulated during finishing (Dunne *et al.*, 2009), when SUM was covariated with subcutaneous fat score, G steers had greater SUM than TMR steers (368 vs. 325, P = 0.03).

Table 2. Effect of the management strategy on the subcutaneous fat colour and estimator of carotenoid pigments content in fat (SUM)

	G	TMR	C	s.e.m.	Pr > F
Lightness (L*)	68.3b	71.2ab	72.8a	1.9	0.03
Redness (a*)	2.8a	2.7a	1.4b	0.6	0.02
Yellowness (b*)	18.1a	16.3b	9.2c	0.9	0.001
Chroma (C*)	18.4a	16.5b	9.4c	0.9	0.001
SUM	353a	334a	140b	26.2	0.001
Covariated with fatness score	368a	327b	133c	21.1	0.001

G: grazing steers; TMR: indoors finished steers; C: concentrate-fed bulls. Within a parameter, means with different letters differ at P<0.05.

IV – Conclusions

Subcutaneous fat colour and SUM are useful to trace forage feeding in carcass. Both β-carotene and lutein in plasma were useful to detect differences in diets in growing cattle. However, plasmatic α-tocopherol concentration is not useful to trace forage-feeding because it is usually added to commercial concentrates.

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