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GENETICS AND MOLECULAR BIOLOGY

Interest in the serum color as an indirect criterion of selection of digestive efficiency in chickens

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ABSTRACT Improving the digestive efficiency of birds is becoming increasingly important with the diversification of feedstuffs used in poultry diets. Compared with time-consuming chemical analyses that were previously used to measure digestive efficiency, near-infrared spectroscopy has been a great advance as it was fast and thus allowed measurements to be taken from a large number of animals, as required for genetic studies. However, it still implies to rear the birds in cages to collect feces, which is questionable in terms of welfare. The purpose of this study was thus to establish whether the serum color could be used as a biomarker of digestive efficiency that would be easy and fast to measure on floor-reared animals. We first compared the serum color of 2 lines of chickens divergently selected for high or low digestive efficiency when fed with a wheat-based diet. Digestive efficiency was assessed by nitrogen-corrected apparent metabolizable energy. Color was assessed by the absorbance of the serum between 300 and 572 nm. Color differed between the 2 lines between 430 and 572 nm, which corresponds to the absorption zone of carotenoids such as lutein and zeaxanthin. In a second step, we estimated the heritability of serum color measurements and their genetic correlations with digestive efficiency. Taking these parameters into account, in our experimental conditions the best trait among those tested that can be used as a biomarker of digestive efficiency is serum absorbance at 492 nm, with a heritability estimate of 0.31 ± 0.09 and a genetic correlation with digestive efficiency of 0.84 ± 0.28.

Key words: biomarker, selection, digestive efficiency, colorimetry, chicken

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INTRODUCTION

The feedstuffs used in poultry diets are becoming increasingly diverse. This diversity limits the competition with human food by using by-products of cereals. Moreover, it allows including more local resources in diets and thus limiting dependency on importations, for example, for soybean in Europe. These alternative feedstuffs are, however, often of lower and more variable quality than the traditional corn and soybean diets. Consequently, the digestive efficiency of chickens fed with these new and less optimal diets is becoming a more important component of feed efficiency than before. Improving digestive efficiency, and in turn feed efficiency, is thus a critical element of economic profitability and of the environmental impact of poultry production.

Nitrogen-corrected apparent metabolizable energy (**AMEn**) is commonly used in poultry to assess digestive efficiency. Traditionally, its measure implied a total collection of feces of individually caged birds, followed by time-consuming chemical analysis of energy and nitrogen content of feces. Thanks to the development of near-infrared spectroscopy technology, the feasibility of large-scale and reliable measurements of digestive efficiency has been greatly improved compared with former chemical analyses (Bastianelli et al., 2010). Performing a genetic experiment on this trait was made possible, during which we showed that digestive efficiency was heritable and could be used as a criterion of selection (Mignon-Grasteau et al., 2004). However, the preparation of feces samples from a large number of animals is still time consuming. Moreover, the total collection of feces involves rearing animals in individual cages for the duration of the balance trial, which is questionable in terms of welfare. Having valuable biomarkers of digestive efficiency that are fast to measure and available from floor-reared animals would therefore be interesting to improve the efficiency of selection in real rearing

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conditions and with respect to animal welfare. Previous observations had shown a variability of the serum color between 2 divergent lines of chickens selected for high or low digestive efficiency, but this result had been obtained on a low number of animals (Beauclercq et al., 2019). The goal of this study was first to confirm on a larger number of animals the phenotypic link between the serum color and digestive efficiency, and second to estimate the genetic parameters of the serum color to establish whether it could be a valuable indirect criterion of selection of digestive efficiency.

MATERIALS AND METHODS

Animals

All animal care and experimental procedures reported in this paper were in accordance with French and European regulations concerning animal experimentation. The experimental unit in which the birds were kept is registered with the French Ministry of Agriculture under license number C-37-175-1 for animal experimentation. All the procedures applied to the birds during this experiment are covered by agreements 00885.02 and 2015121516442084–3202 delivered by the French Ministry of Research after approval by the ethics committee C2EA-19.

The data used in this study come from chickens of the 21th generation of a divergent selection experiment for high (D+) or low (D-) digestive efficiency. The initial population is a commercial medium-growing broiler, reaching 2 kg at 7 weeks. During the first 8 generations, the birds were fed a difficult-to-digest diet that included 55% of Rialto wheat (Mignon-Grasteau et al., 2004), a hard and viscous wheat variety enhancing between-animal differences in digestive efficiency. Breeders were selected for digestive efficiency using the AMEn measured during a balance trial at 3 weeks, age that has been chosen because it was in the middle of the rearing period. The 2 lines were then reproduced without selection for AMEn during the 12 following generations. At generation 20, the selection process was restarted with the same criterion.

Measures

Birds from generation 21 (N = 192 D+, 192 D-) were produced in 2 successive hatches. Diet composition is given in Table 1. Crude protein content of the diet was 20%, and AMEn was 3,000 kcal kg^{-1} DM. Animals were reared on the floor from hatch to 13 D and then transferred to individual cages for a balance trial. The balance trial was done between 21 and 24 D. The birds were weighed at 21 and 24 D (body weight [**BW**] at 21 D [**BW21**] and BW24), and individual feed intake was recorded between 21 and 24 D (**FI21_24**). A total collection of feces was done during this time, following the method proposed by Bourdillon et al. (1990). Feces were freeze dried and analyzed with near-infrared spectroscopy to obtain AMEn values as described in Bastianelli et al. (2010).

Table 1. Composition of diet and elementary statistics on AMEn at 3 weeks for D+ and D− lines (mean ± standard deviation).

Ingredient	Percentage
Corn	4.31
Wheat	51.40
Rye	5.00
Soybean oil	3.00
Palm oil	3.00
Soybean cake 48	28.87
Calcium carbonate	1.14
Bicalcic phosphate	1.99
Salt	0.30
Vitamins and minerals	0.40
DL methionine	0.26
HCl lysine	0.21
Threonine	0.07
Anticoccidial	0.05

Abbreviation: AMEn, nitrogen-corrected apparent metabolizable energy.

At the end of the balance trial (24 D), the animals were blood sampled at the occipital sinus to measure the serum color. Blood was collected at 9 am, after 13 h of feed deprivation, to obtain data at a comparable basal physiological stage, as it had been previously shown that transit time varies largely between D+ and D- birds (Rougière and Carré, 2009). Serum was prepared by keeping the blood at room temperature for 15 min until coagulation and then centrifuged (3,000 *g* for 10 min). Samples of 200 μL of serum from the birds were transferred to a transparent 96-well plate (Greiner Bio-One, Kremsmünster, Austria), and their absorption spectra were acquired using an Infinite M200 spectrophotometer (Tecan, Männedorf, Switzerland) between 342 and 572 nm (2 nm steps).

Phenotypic and Genetic Analyses

Elementary statistics on digestive efficiency, BW, feed intake, and serum color were obtained by an analysis of variance using the GLM procedure from SAS© 9.4 (SAS Institute, Cary, NC). Model [1] was used for AMEn, BW, and feed intake data:

$$y_{ijklm} = \mu + L_i + H_j + C_k + S_l + e_{ijklm} \quad [1]$$

where y_{ijklm} is AMEn, feed intake between 21 and 24 D or BW at 21 or 24 D of animal m, μ the general intercept, L_i the fixed effect of line i (i = D+, D−), H_j the fixed effect of hatch j (N = 2), C_k the fixed effect of rearing cell k (N = 2), S_l the fixed effect of sex l (N = 2), and e_{ijklm} the residual pertaining to animal m.

We used the model [2] to analyze the absorbance of serum every 2 nm between 342 and 572 nm:

$$y_{ijklmn} = \mu + L_i + H_j + C_k + P(H)_{jl} + S_m + e_{ijklmn} \quad [2]$$

where y_{ijklmn} is absorbance of serum for animal n, μ the general intercept, L_i the fixed effect of line i (i = D+, D-), H_j the fixed effect of hatch j (N = 2), C_k the fixed effect of

Table 2. Least square means (±SE) of digestive efficiency (AMEn), feed intake between 21 and 24 D (FI21_24), and body weight at 21 and 24 D (BW21 and BW24).

Trait	N	Probability associated with fixed effect of			Sex	LS means ± SE	
		Line	Hatch	Rearing cell[1]		D+	D-
AMEn (kcal.kg^{-1} DM)	382	<0.0001	0.0004	<0.0001	0.2066	3,089 ± 10.7	2,982 ± 10.7
FI21_24 (g)	382	<0.0001	0.0001	0.0010	<0.0001	161 ± 2.6	189 ± 2.6
BW21 (g)	382	0.0506	0.0129	0.9281	0.2576	338 ± 2.9	345 ± 2.9
BW24 (g)	381	0.9148	0.3591	0.0002	<0.0001	445 ± 3.4	446 ± 3.5

Abbreviations: AMEn, nitrogen-corrected apparent metabolizable energy; LS, least square; SE, standard error.
[1]Cell in which animals are reared.

rearing cell k (N = 2), $P(H)_{jl}$ the fixed effect of plate l (N = 2 by hatch) within hatch j, S_m the fixed effect of sex m (N = 2), and e_{ijklmn} the residual pertaining to animal n.

For genetic analyses, we included all available AMEn, BW, and feed intake data collected during each generation of selection of D+ and D-lines (generations 1 to 8 and generations 20 to 21), that is, 4,626, 5,068, 4,609, and 417 animals for AMEn, BW at 23 D, feed intake between 21 and 24 D, and the serum color, respectively. AMEn, BW, and feed intake data came from the selection experiment itself (around 200 birds per line and per generation) and from an additional experiment on growth at generation 4 in the same conditions than for the selection experiment. Serum color data came from the present experiment and from a preliminary study performed at generation 20 in the same conditions. Data came from D+ birds for 45 to 47% depending on the trait. Genetic analyses were performed using VCE6.0 (Neumaier and Groeneveld, 1998; Groeneveld et al., 2010). We applied an animal model including the fixed effects of hatch by generation (N = 28), rearing cell (N = 8), and sex. For the serum color, a fixed effect of plate (N = 6) was also included. The pedigree file included a total of 6,376 animals. Taking into account the large number of traits and the high correlation between absorbance variables at neighboring wavelengths, we made a series of analyses including each AMEn, BW at 23 D, and absorbance of serum at 2 different wavelengths. All possible combinations between the 2 different wavelengths were tested, leading to a total of 276 analyses. The results presented below are the mean values of genetic parameters and of their standard errors obtained for each separate analysis.

RESULTS AND DISCUSSION

Phenotypic Results

Feed intake was 17% higher in D- than in D+ birds, and BW was similar between the 2 lines. AMEn was 107 kcal kg^{-1} DM higher in D+ than in D- birds (Table 2). The results are consistent with those obtained in previous studies in the same lines.

The serum color was significantly different between the 2 lines from 376 to 572 nm (Figure 1A). This includes the range of differences (430-516 nm) previously observed by Beauclercq et al. (2019) on the same lines. As in the study by Beauclercq et al. (2019), the most significant differences were found at 490-492 nm (Figure 1B), which corresponds to the region of carotenoid absorption, especially lutein and zeaxanthin (Rodriguez-Amaya, 2001), which are the major carotenoids present in cereal grains, particularly wheat (Abrar et al., 2015). Lutein and zeaxanthin are better absorbed by chickens than other carotenoids present in this spectral zone, such as β-carotene, astaxanthin, or cryptoxanthin (Surai et al., 2001). Moreover, serum lipidomic analysis by mass spectrometry has confirmed that the feature assigned to lutein and zeaxanthin was the most differential between the D+ and D-lines, with a fold change of 4,63 in favor of D+ line (Beauclercq et al., 2019). Altogether, these results support the hypothesis that this peak of absorbance at 490-492 nm is due to a higher concentration of lutein and zeaxanthin pigments in the serum of D+ birds in relation with their higher DE.

Genetic Parameters of Serum Color

Heritability estimates of AMEn, FI21_24, and BW24 were moderate to high (0.36 ± 0.02, 0.35 ± 0.03, and 0.50 ± 0.04, respectively). The genetic correlation between AMEn and BW24 was not significant (0.09 ± 0.06). The genetic correlation between feed intake and AMEn or BW24 was higher (−0.64 ± 0.04 and 0.48 ± 0.05, respectively). The estimates are consistent with previous estimates obtained from these lines (Mignon-Grasteau et al., 2004).

Heritability estimates of serum color (Table 3) were low and not significant at the lowest (342 to 452 nm) and highest (512 to 572 nm) wavelengths. Estimates between 462 and 502 nm were moderate (between 0.24 and 0.31) and significantly different from 0, with a maximum heritability at 492 nm. These estimates are close to the heritability of carotenoid concentration in 8-week-old broilers (0.20 to 0.32) obtained in prior studies (Stone et al., 1971; Washburn and Ruff, 1978). The genetic correlations between AMEn and serum color were the highest (0.76 to 0.97) for wavelengths between 392 and 502 nm. By contrast, in the same zone, genetic correlations between serum color and BW and feed intake were moderate and not significant, except for

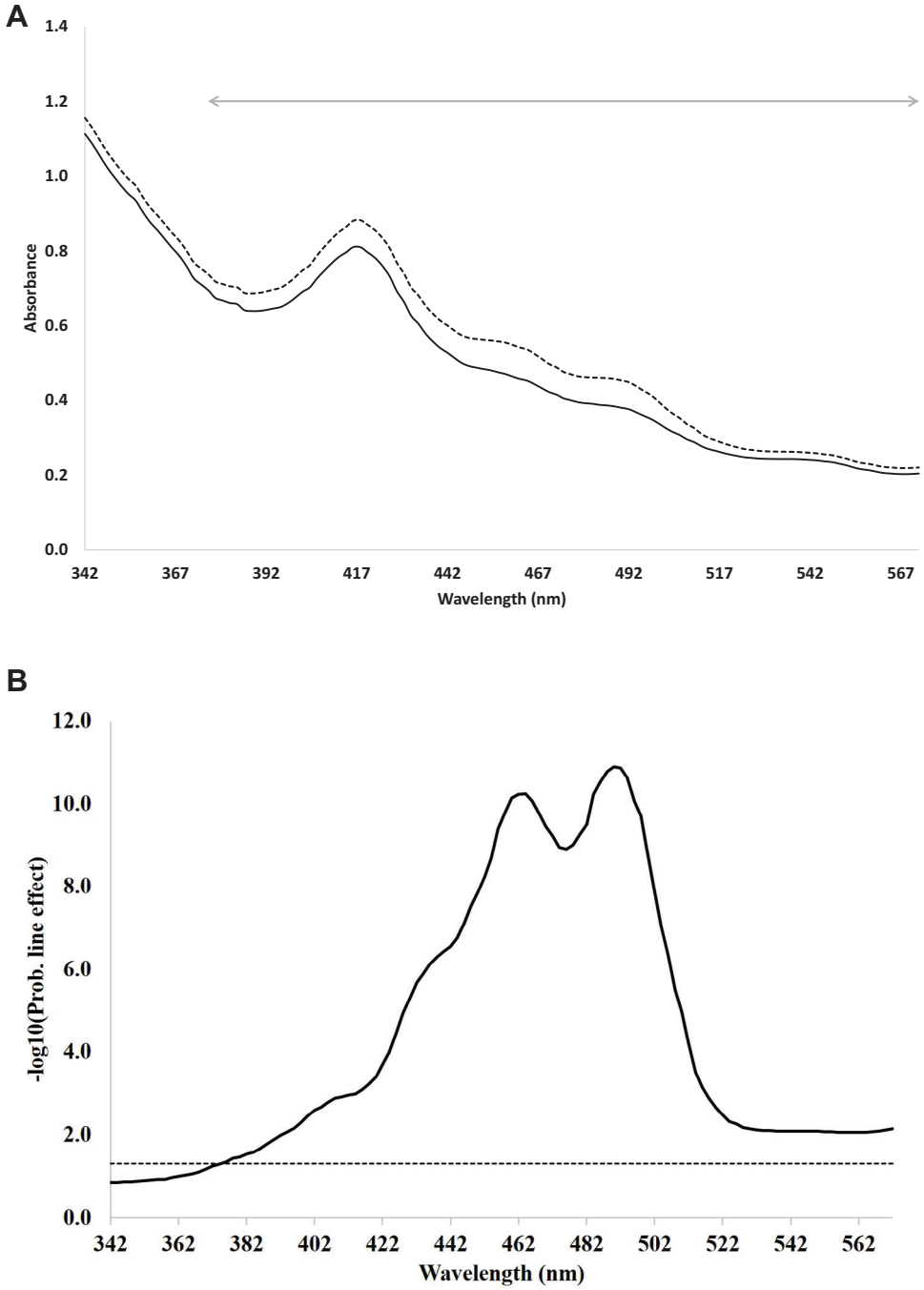

Figure 1. A. Absorbance of serum between 342 and 572 nm for D+ (dashed line) and D− (solid line) birds. The arrow indicates the zone for which the difference between the 2 lines is significant ($P < 0.05$). B. Significance of line effect [-log10(P value)]. The dashed line indicates the threshold corresponding to a P value of 0.05.

the correlation between feed intake and serum color at 492 nm (−0.45 ± 0.22, $P = 0.04$). Taking into account heritability and genetic correlation estimates, the serum absorbance at 492 nm would be the most efficient indirect criterion of selection for AMEn among the different wavelengths tested and would lead to an indirect response estimated at 78% of the response of AMEn to direct selection (Figure 2). Taking into account the ease of measurement of serum color by comparison to the current measurement of AMEn, its measurement could be performed on more numerous candidates. This makes it possible to apply a higher intensity of selection and to counteract the loss of genetic progress compared with the direct selection on AMEn.

As our birds were healthy and not affected by pathogens such as coccidia that can change the serum color, our hypothesis is that the genetic correlation between AMEn and serum color is due to an indirect effect of selecting for AMEn on lipid digestibility, and thus, carotenoid absorption. It has been shown that D+ birds have a much better coefficient of digestive use of lipids than D- birds (Garcia et al., 2007). This better aptitude

Table 3. Genetic parameters (±standard errors) for serum color.

Absorbance at wavelength	Heritability	Genetic correlation with AMEn	BW24	FI21_24
342 nm	0.08 ± 0.07	0.52 ± 0.61	0.75 ± 0.26	0.17 ± 0.27
352 nm	0.09 ± 0.07	0.54 ± 0.46	0.73 ± 0.25	0.18 ± 0.41
362 nm	0.10 ± 0.09	0.58 ± 0.45	0.69 ± 0.28	0.18 ± 0.24
372 nm	0.10 ± 0.09	0.62 ± 0.44	0.70 ± 0.27	0.14 ± 0.25
382 nm	0.10 ± 0.14	0.68 ± 0.46	0.66 ± 0.45	0.08 ± 0.26
392 nm	0.11 ± 0.08	0.78 ± 0.32	0.57 ± 0.25	−0.01 ± 0.23
402 nm	0.13 ± 0.07	0.92 ± 0.16	0.43 ± 0.27	−0.16 ± 0.32
412 nm	0.12 ± 0.08	0.97 ± 0.15	0.31 ± 0.48	−0.27 ± 0.25
422 nm	0.13 ± 0.07	0.97 ± 0.10	0.25 ± 0.28	−0.35 ± 0.22
432 nm	0.18 ± 0.08	0.90 ± 0.18	0.32 ± 0.24	−0.38 ± 0.26
442 nm	0.20 ± 0.16	0.83 ± 0.48	0.36 ± 0.37	−0.36 ± 0.27
452 nm	0.26 ± 0.18	0.85 ± 0.41	0.33 ± 0.47	−0.40 ± 0.25
462 nm	0.28 ± 0.10	0.81 ± 0.23	0.30 ± 0.24	−0.45 ± 0.23
472 nm	0.27 ± 0.09	0.82 ± 0.22	0.32 ± 0.20	−0.41 ± 0.24
482 nm	0.28 ± 0.09	0.82 ± 0.23	0.32 ± 0.22	−0.41 ± 0.27
492 nm	0.31 ± 0.09	0.84 ± 0.28	0.29 ± 0.27	−0.45 ± 0.22
502 nm	0.24 ± 0.12	0.76 ± 0.30	0.37 ± 0.24	−0.33 ± 0.25
512 nm	0.16 ± 0.08	0.65 ± 0.54	0.51 ± 0.25	−0.11 ± 0.27
522 nm	0.13 ± 0.16	0.55 ± 0.44	0.58 ± 0.36	0.04 ± 0.17
532 nm	0.11 ± 0.07	0.60 ± 0.43	0.61 ± 0.26	0.07 ± 0.18
542 nm	0.11 ± 0.09	0.71 ± 0.72	0.60 ± 0.30	0.05 ± 0.25
552 nm	0.11 ± 0.07	0.69 ± 0.36	0.61 ± 0.26	0.06 ± 0.21
562 nm	0.11 ± 0.07	0.61 ± 0.41	0.62 ± 0.26	0.09 ± 0.23
572 nm	0.11 ± 0.07	0.75 ± 0.29	0.60 ± 0.24	0.06 ± 0.25

Abbreviations: AMEn, nitrogen-corrected apparent metabolizable energy; BW24, body weight at 24 D; FI21_24, feed intake between 21 and 24 D.

to digest lipids is partly due to a higher concentration of conjugated bile acids used to create micelles in which carotenoids are emulsified to be absorbed in the intestine (Surai et al., 2001; Garcia et al., 2007). In our case, the efficient formation of micelles is especially important as we are using a high–fiber content diet, which makes the contact between the micelles and the epithelial membrane more difficult (Faure et al., 1999). Moreover, the first step of carotenoid absorption is its separation from the feed matrix under the action of digestive enzymes, which are more active at low gastric pH (Surai et al., 2001). As pH in the gizzard is lower in the D+

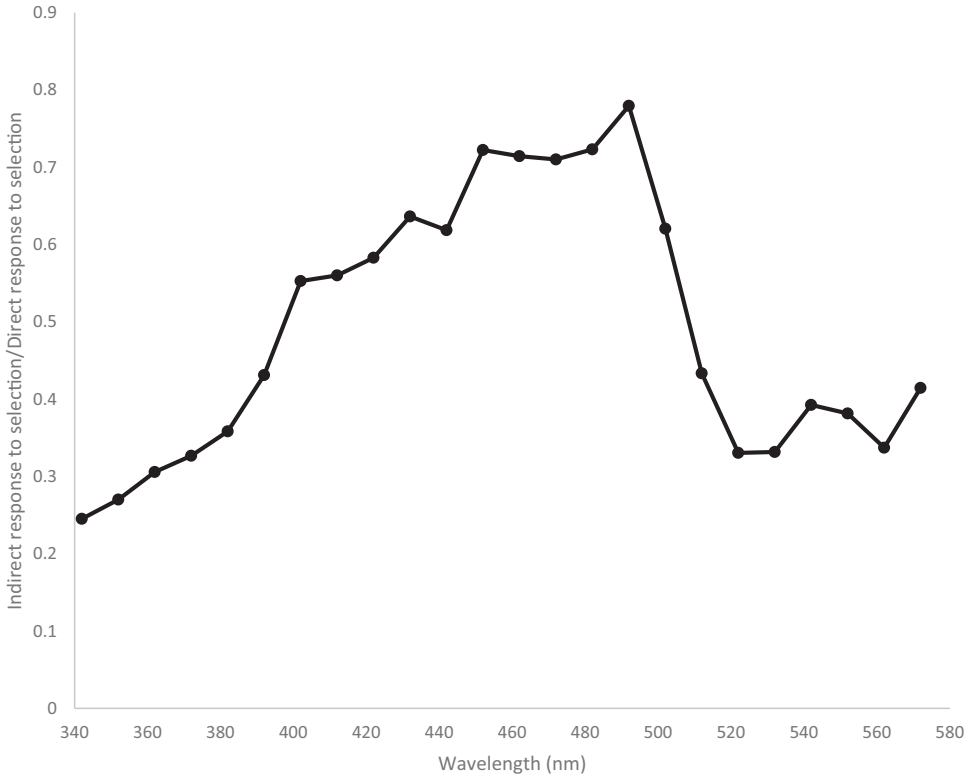

Figure 2. Ratio of the expected response of nitrogen-corrected apparent metabolizable energy (AMEn) to indirect selection of serum color to the expected response of AMEn to direct selection.

line than in the D- line, this first step of carotenoid absorption is also probably more efficient in the D+ line than in the D- line (de Verdal et al., 2013).

In our conditions, we showed that serum absorbance at 492 nm would be a valuable indirect criterion for selecting digestive efficiency. These results now have to be expanded to evaluate the interest of this new biomarker of feed digestibility in other nutritional and genetic conditions.

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