World Rabbit Science

World Rabbit Sci. 2013, 21: 85-90
doi:10.4995/wrs.2013.1148

NIRS OF BODY AND TISSUES IN GROWING RABBITS FED DIETS WITH DIFFERENT FAT SOURCES AND SUPPLEMENTED WITH *CURCUMA LONGA*

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Abstract: A portable Near Infrared Reflectance Spectroscopy (NIRS) instrument was applied to 40 growing rabbits to determine body and tissue differences induced by experimental factors. The rabbits were examined at 2 live sites, in 7 warm carcass tissues and in *longissimus dorsi* muscle samples prepared in ethanol. For this purpose, the method was applied in a bi-factorial experiment concerning the dietary oil source (O) (maize *vs.* palm oil) and *Curcuma longa* (C) supplementation (0 and 3 g/kg, respectively). Significant chemical differences emerged for palmitic, oleic and linoleic acids in the *longissimus dorsi* muscle due to the O factor and for linolenic acid due to the C factor. The NIRS spectra and chemical analyses were elaborated by the Partial Least Squares (PLS) method, and the rsquares in cross-validation (R^2cv) were retained as measure of the unoriented differentiation between the levels of the planned factor for each landmark and fatty acid (FA) profile. Multivariate PLS analysis of the FA muscular fat showed that the O factor induced strong differentiation (R^2cv: 0.96), while less influence (0.33) was observed for the C factor. The model based on the NIRS radiation of the landmarks clearly shows the O factor effects, not only in the perirenal (0.90) and scapular (0.85) fats, but also in the belly (0.76), liver (0.73) and hind legs (0.72). Whereas the C effects were only expressed in the live animals (ears: 0.66 and abdominal wall: 0.58) and in *post-mortem* (liver: 0.60). It was concluded that a preliminary NIRS scan of the carcass and of live rabbits can point out the presence of intrinsic experimental effects concerning the lipid metabolism of polyunsaturated FA of the n-6 series (O factor) and n-3 series (C factor).

Key Words: NIRS, rabbits, tissues, dietary oil, *Curcuma longa.*

INTRODUCTION

Rapid analysis systems are becoming more popular throughout the world because they are non polluting, non destructive and cheaper when large volumes are examined. Near Infrared Reflectance Spectroscopy (NIRS) has often been used as a rapid servo-chemical tool. In rabbit research, it has been used for the prediction of the nutritional parameters of feedstuffs (Xiccato *et al.*, 2003; Meineri *et al.*, 2009) as well for the composition of the hind-leg meat (Pla *et al.*, 2004; Bázár *et al.*, 2007) the determination of intramuscular fat content in rabbit selection programmes (Zomeño *et al.,* 2011) and composition of the fatty acids (FA) related to an organic system of production (Pla *et al.*, 2007). A discriminating approach has already been adopted in the experimental use of NIRS to ascertain some ontogenetic, technological or nutritional factors (Masoero *et al.*, 2003, 2004, 2008a), but also to trace foods (Berzaghi and Riovanto, 2009).

The preparation of animal tissue samples to be scanned by NIRS is also a crucial point. The target is intact raw or minced samples, provided they are preserved by vacuum and/or freezing and thawing; freeze drying preparations are very discriminating within each lot, but only just repeatable between experiments (Masoero *et al.*, 2007a). Attempts

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http://dx.doi.org/10.4995/wrs.2013.1148

have been made to develop an easy sample preparation by immersing pieces in absolute ethanol, which dehydrates, coagulates and fixes the protein (Masoero *et al.*, 2002, 2004, 2007a; Dalle Zotte *et al.*, 2007).

Since the advent of fibre optics in portable NIRS instruments, the direct scanning of live tissues or carcasses has become feasible. This technique was applied by Masoero *et al.* (2008b) in a trial with low protein diets.

The present paper has the aim of investigating to what extent rapid NIRS analyses can point out relevant knowledge on the planned factors. For this purpose, the method was applied in a bi-factorial dietary experiment described in a companion paper by Peiretti *et al.* (2011). This trial described how the use of diets containing 4% maize oil *vs.* palm oil increased the polyunsaturated FA (PUFA) content of rabbit meat, and the addition of *Curcuma longa* (*C. longa*) enhanced the PUFA n-3 content improving the nutritional value of the rabbit meat.

MATERIALS AND METHODS

Animals and sampling

Forty weaned crossbred rabbits, aged 9 wk, with a mean body weight of 1512 g, were randomly assigned to 4 groups of 10 (5 male and 5 female rabbits each) with equal initial weight variability. The animals were housed individually under standard conditions at a temperature of 22±2°C in wire cages at a height of 90 cm from the concrete floor. Four diets, which were reported in Peiretti *et al.* (2011) were administered: iso-nitrogenous (187 g crude protein/kg dry matter (DM)), iso-ethereal (59 g ether extract/kg DM), iso-energetic (12 MJ digestible energy/kg DM), and aniso-unsaturated formulated with 4% maize oil (M, more unsaturated) and 4% palm oil (P, more saturated), without (No C) or with 3 g/kg *C. longa* supplementation (C). The experimental period lasted 30 d, with a previous 14 d adaptation period. The *in vivo* NIRS scans of all rabbits were carried out on the day before slaughtering. The NIRS scans were applied at 7 sites of the warm carcasses. After chilling and dissection, a sample of 25 g of the *longissimus dorsi* (LD) muscle was immersed in a 35 mL volume of ethanol 95%.

Chemical analysis

FA were determined by gas chromatography of the lipids extracted from the LD muscle and from perirenal fat (for details see Peiretti *et al.*, 2011).

NIRS

The NIRS scans of the live body and tissues were conducted using a Model LSP 350-2500P LabSpec-Pro portable spectrophotometer (ASD, Analytical Spectral Devices Inc., Boulder, CO), which was equipped to collect spectra from 350 to 2500 nm. The probe was an A122100 ASD Model high-intensity reflectance probe that served as an external light source (2900°K colour temperature quartz halogen light) to illuminate the object of interest. This probe can be used to collect reflectance spectra over an area as large as 25 mm in diameter. The reflected light was collected through a 1-m long 04-14766 ASD Model fibre optic jump cable that consisted of a bundle of 44 fibres (200-lumen each). A set of 20 spectra was collected in a 3" scan and averaged. *In vivo* spectroscopy was conducted on the shoulder and on the internal side of the ear. *Post-mortem* scans were performed, during the dissection of the warm carcass, on 7 sites (liver, belly, perirenal and scapular fat, hind legs, loins and eyes). After 2 d of marinating, the LD muscle was extracted and aerated for 2 h before scanning.

The 2151 absorbance points of the NIR spectra were mathematically pre-treated as Standard Normal Deviates with Detrend, then first-derived (first derivative) and smoothed (1,4,4,1). The modified Partial Least Squares (PLS) method of the WinISI v.1.50 (Infrasoft International, Port Matilda, PA, USA) software was used over the whole spectra; a cross-validation system assessed the optimal number of latent variables that should be included in the equations; one round allowed the elimination of outliers (t>2.5; H>10; Fearn, 1997; Barker and Rayens, 2003). Chemometrics was also applied to the set of FA obtained from the chemical analysis, which were elaborated by the PLS method, without any pre-treatment. Each FA laboratory value was fitted to the pertinent NIR spectra by means of the PLS method.

Table 1: Matrix of the bi-factorial design.

	Dietary factors		Coding for chemometric models		
No.	Oil	*C. longa*	Groups	Oil	*C. longa*
10	M-Maize	No *C. longa*	1-M	1	1
10	M-Maize	*with C. longa*	2-M-*C*	1	2
10	P-Palm	No *C. longa*	3-P	2	1
10	P-Palm	*with C. longa*	4-P-*C*	2	2

Models

A univariate bi-factor linear model was fitted to each FA in order to estimate the experimental effects pertaining to the 2 factors (R^2model). As shown in Table 1 the effects oil (n=20) and *C. longa* (n=20) factors in a multivariate approach were fitted to fixed integer values (1 or 2) according to their levels; these results were considered as R^2 in cross-validation mode (R^2cv). In general, a very accurate NIRS equation may discriminate the single record, ignoring any tissue composition. However, if the NIRS may accurately estimate the FA composition, the differentiation will be oriented to distinguish the influence of dietary factors according to their specific constituents.

After such preliminary test, nutritional studies could be made about the chemical consequences of the dietary factors applied in the experimental protocols and expressed in organs or tissues. Because PLS regression is a one-way method, in this bi-factorial trial a separate analyses of the 2 factors (oil and *Curcuma*) were carried on, and the relative importance was measured by the ratio of their R^2cv values, expressed at the landmarks.

RESULTS AND DISCUSSION

The focal point of the experiment was the modification of the FA profiles induced by the aniso-unsaturated diets. The differences in the oil groups pertaining the muscle FA (Peiretti *et al*., 2011) are summarized in Table 2 on the basis of the univariate model. The maize oil decreased the palmitic (–12%) and oleic acids (–19%) and increased linoleic acid (+50%), while C increased the linolenic acid contents by 19%. All these FA, except linolenic acid, were successfully fitted from NIRS spectra of the perirenal fat (Table 2: R^2cv 0.72/0.93) and a narrow set of wavelengths (1159 and 1211 nm) explained the main relationships with the muscle FA. None of the other tissues were as efficient in the prediction of some muscle FA, and the best performance was obtained for the belly site for linoleic acid prediction (0.68). In fact, when a multivariate PLS approach of FA (Table 3) was adopted, the O factor was expressed in the acidic profile with very high R^2cv values (0.96/0.97) in both the intramuscular and perirenal fats. In contrast, the differentiation of the *C. longa* supplement on FA profiles was much lower in the muscular FA (0.33) and reduced (0.66) in the perirenal FA.

Table 2: Univariate analyses of fatty acid (FA) profile and NIRS calibrations. Significant differences of the single FA between the oil type and *Curcuma longa* factors, and fitting performances on each single FA by using the NIRS spectra of landmarks.

		Significant fatty acids of groups			NIRS fitting of FA from various landmarks (R^2cv)			
Fatty acids	R^2 model	Maize	Palm	*P*-values	Perirenal fat	Belly	Liver	Live ear
Palmitic acid	0.58	27.5	31.2	<0.0001	0.72	0.30	0.14	0.08
Oleic acid	0.83	24.7	30.7	<0.0001	0.89	0.56	0.60	0.58
Linoleic acid	0.83	28.0	18.7	<0.0001	0.93	0.68	0.41	0.51
		No *C. longa*	*C. longa*					
Linolenic acid	0.36	2.4	2.9	0.0003	0.11	0.00	0.24	0.10

R^2: rsquare of the univariate analysis of single FA. R^2cv: rsquare in cross-validation model of the Partial Least Squares (PLS) equation for the unoriented differentiation between the oil type or *Curcuma longa* factors.

Table 3: Unoriented differentiation coefficients (R^2cv) between the oil type (O) and *Curcuma longa* (C) factors by the fatty acids (FA) profile and NIRS examination of landmarks.

System of analysis	Landmark	O R^2cv	C R^2cv	O/C ratio
Multivariate PLS of chemical FA				
	FA perirenal fat	0.97	0.66	1.5
	FA muscular fat	0.96	0.33	2.9
NIRS of carcass tissues				
	Perirenal-fat	0.90	0.51	1.8
	Scapular-fat	0.85	0	-
	Hind legs	0.72	0.33	2.2
	Belly	0.76	0.45	1.7
	Liver	0.73	0.60	1.2
	Loins	0.66	0.52	1.3
	Eyes	0.55	0.48	1.2
NIRS of LD on ethanol				
	LD muscle	0.43	0	-
NIRS of live body				
	Live-Loins	0.53	0.58	0.9
	Live-Ears	0.44	0.66	0.7
	mean	0.71	0.43	1.7
	±sd	0.19	0.22	

R^2cv: Rsquare in cross-validation model of the Partial Least Squares (PLS) equation for the unoriented differentiation between the oil type or *Curcuma longa* factors. LD: *longissimus dorsi.* O/C ratio: the ratio of R^2cv oil type factor on the corresponding value of the *Curcuma longa* factor.

The model based on the NIRS radiation of the landmarks clearly perceived the O factor effects, not only in the perirenal (0.90) and scapular (0.85) fats, but also in the belly (0.76), liver (0.73) and hind legs (0.72). Conversely, the C effects were expressed in particular in the liver (0.60), where the O/C ratio was 1.2; similar values were achieved in the eyes (1.2) and loins (1.3), a sign of modifications of the non-fat parts by *C. longa*.

The muscle ethanol prepared specimens scanned by NIRS only recognised differentiation for the O factor, but at a low level (0.43), which showed that the *C. longa* supplement did not affect the coagulated muscle tissue radiation. In fact, the oxidative stability measured by means of thiobarbituric acid reactive substances (TBARS) remained stable in the treatments (Peiretti *et al.*, 2011) .

In general, the best performance rates obtained with the NIRS instruments can be found in dry and fatty matrices, since water creates scattering noise: for this reason, when the studied factors substantially modify the FA profile, the rapid test gives good results. In this experiment, the hypothesised modifications induced by means of the aniso-unsaturated diets were *a priori* more consistent than those hypothetically linked to the *C. longa* supplement. In fact, a differentiation degree of 0.96, obtained from the multivariate analysis of the muscular FAs, was anticipated and predicted as 0.90 from a simple NIRS of the perirenal fat. These results are in complete agreement with an experiment conducted on false flax (*Camelina sativa*) included at 0, 10 and 15% levels, in which the multivariate elaboration of muscle FAs discriminated the groups of rabbits at a 0.98 value, while the NIRS of the perirenal fat predicted the differentiation at 0.90 (Masoero *et al.*, 2007b).

According to the NIRS live examination, the relative relevance of the factor was reversed, thus the O/C ratio was much lower: 0.9 for the loins and 0.7 for the ears, since the O factor was again under-appreciated (0.53 and 0.44, respectively), while the C effect was over-appreciated (0.58 and 0.66, respectively). This feature can be related to possible modifications of the epidermal tissue, which is clearly expressed in the internal side of the ear. In fact, the supplementation with *C. longa* had only specifically increased the linolenic acid by 19%, a key compound which can undergo desaturation and give rise to favourable precursors of icosanoids. Numerous studies have shown that curcumin, a non-water-soluble polyphenol, has antioxidant and anti-inflammatory properties (Chainani-Wu *et al.*, 2003), and prevent or reduce the effects of aflatoxin in chicks fed aflatoxin-contaminated diets (Yarru *et al.*, 2009).

In normal healthy dogs supplemented with lysozyme, Masoero *et al.* (2008c) repeating some NIRS measures of the coat have predicted some positive physiological effects (plasmatic protein, creatinine, blood urea and chloride were lowered) and quality of the coat was improved; this result was probably due to the excellent fit of NIRS for the amino acids, which dominate the composition of the coat (De la Haba *et al.*, 2006).

As far as the main aim of the experiment is concerned, we believe that *C. longa* can have different effects on the NIR radiation of the different measurement sites.

In a previous experiment (Masoero *et al.*, 2008a), Chia (*Salvia hispanica*) seeds were introduced at 0, 10 and 15% into aniso-unsaturated diets. The reference pattern, which resulted from a multivariate set of 81 variables, was fixed at a 0.85 value. In that case, *in vivo* NIRS scanning of loins predicted the same clustering for the 3 groups, but at a level of 0.32 which is equivalent to the clustering based on the TBARS determined 3 times (0.26).

It should be pointed out that NIRS R^2 values that are not suitable for individual prediction may be able to predict the unoriented differentiation of the intrinsic and global averages of groups. This preliminary knowledge could be profitably concretised in progressive investigations.

CONCLUSIONS

This study on NIRS body scanning of live animals and on the carcass has proved that rapid methods can anticipate the presence of some real intrinsic experimental dietary effects concerning oil sources and *C. longa* supplementation. The hypothesised effects promoted by the 2 oil sources, unsaturated at a different degree, effectively involved differentiation in the muscle contents of palmitic, oleic and linoleic acids. *C. longa* supplementation, which raised the linolenic acid contents in the muscle, also modified the intrinsic spectral characteristics of the epithelia and tissues in a pathway that is not related to PUFA n-3, but to other biological compounds, which need to be further investigated. To improve the experiments with *C. longa* powder, it is suggested that diets enriched in oil or fat could be used to promote the curcumin bioavailability, so the powder or extract should be solubilised in polar solvents, like fats, before mealing and pelleting.

New portable NIRS devices could be profitably used to test discriminant dietary effects on rabbit meat.

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