

## Analysis of amino acid composition of sheep colostrum by near-infrared spectroscopy

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**ABSTRACT:** This paper deals with changes in the basic composition of sheep colostrum within the first 72 hours after parturition on the one hand and with the possibility of determining the major components of sheep colostrum by near-infrared spectroscopy on the other. Levels of essential, nonessential and total amino acids in sheep colostrum were determined by near-infrared reflectance spectroscopy (NIRS). For each component, sets of 90 samples were used to calibrate the instrument by means of a modified partial least-squares regression. The values of correlation coefficients ( $r$ ) were as follows: 0.979 for Thr; 0.954 for Val; 0.968 for Leu; 0.918 for Ile; 0.946 for Lys; 0.908 for Arg; 0.845 for His; 0.999 for Trp; 0.915 for Phe; 0.909 for Met; 0.939 for Cys; 0.911 for  $\Sigma$ Met + Cys; 0.933 for Tyr; 0.945 for Asp; 0.935 for Glu; 0.986 for Ser; 0.985 for Pro; 0.957 for Gly; 0.949 for Ala; 0.940 for  $\Sigma$ EAA; 0.958 for  $\Sigma$ NEAA and 0.977 for  $\Sigma$ AA. Partial least-squares (PLS) regression was used to develop calibration models for examined samples of sheep colostrum. When using the NIRS method, the following correlation coefficients were calculated: Thr (0.959), Val (0.912), Leu (0.936), Ile (0.855), Lys (0.903), Arg (0.853), His (0.717), Trp (0.667), Phe (0.854), Met (0.867), Cys (0.895),  $\Sigma$ Met + Cys (0.868), Tyr (0.886), Asp (0.910), Glu (0.882), Ser (0.968), Pro (0.968), Gly (0.923), Ala (0.916),  $\Sigma$ EAA (0.901),  $\Sigma$ NEAA (0.923) and  $\Sigma$ AA (0.943). Calibration was tested using the same set of samples. NIRS results were compared with reference data and no significant differences between them were found ( $P = 0.05$ ). Calibration and validation models were constructed in the same way. Results of this study indicate that NIR spectroscopy can be used for a rapid analysis of amino acid contents in sheep colostrum.

**Keywords:** near-infrared spectroscopy; sheep colostrum; amino acids

Traditional methods of evaluation of the quality of milk and its major components are relatively slow and rather expensive. Near-infrared spectroscopy of foodstuffs is a new analytical method. The advantages of NIRS involve above all higher rapidity, simultaneous, non-destructive measurement of a number of milk constituents and a great potential for on-line analysis. This method was used to estimate the content of various constituents in both homogenized non-homogenized milk samples (Sato *et al.*, 1987; Rodriguez-Otero *et al.*, 1997; Ru and Glatz, 2000). Tsenkova *et al.* (1999, 2000) found the highest correlation coefficients for fat, lactose and total protein in raw, non-homogenized milk. Our paper deals with the determination of the major compo-

nents (dry matter, protein, fat, lactose and pH) in non-homogenized sheep colostrum. Kukačková *et al.* (2000) used a fibre optic probe to analyse raw milk. Jankovská and Šustová (2003) estimated major components in cow milk by NIRS (total solids, fat, protein, casein, urea nitrogen, lactose, and somatic cells). Contents of basic components (dry matter, crude protein, fat, lactose and pH) of sheep colostrum within the first 72 hours *post partum* as estimated by classical analytical methods and NIRS were described by Gajdůšek *et al.* (2003) and Šustová *et al.* (2004), respectively.

The aim of this study was to evaluate changes taking place in contents of amino acids in sheep colostrum within the first 72 hours after the parturition.

## MATERIAL AND METHODS

Changes in contents of amino acids were studied on an organic farm in Valašská Bystřice (Czech Republic) in the period of January–March 2002. Experiments involved 10 crossbred (F<sub>111</sub>) East-Frisian × Improved Wallachian sheep (EF87IW) with the average live body weight 45 kg. All animals were on the 2nd and/or higher lactation.

Sheep received a diet consisting of ground wheat (0.3 kg), fodder beet (1.0 kg) and good quality hay offered *ad libitum*. The feeding ration also involved a mineral supplement (0.05 kg per head). The feeding dose contained 9.5 MJ NEL, 139 g PDI, 243 g of crude protein, 1 908 g of dry matter, 460 g of fibre and 7.5 g Ca, 6.2 g P, 4.0 g Mg and 2.8 g Na.

Experimental animals did not show any health problems during the whole study period.

Colostrum samples were taken at intervals of 2; 12; 24; 36; 48 and 72 hrs after parturition; after sampling, colostrum was homogenized in batches of ca. 50–100 ml and the homogenized samples were frozen thereafter.

Contents of amino acids in colostrum samples were simultaneously estimated using the classical chromatographic analysis and NIRS. Kráčmar *et al.* (2004) followed dynamics of changes in the content of amino acids in the same samples of sheep colostrum within the first 72 h *post partum*.

Colostrum samples for amino acid determination were adjusted using acidic and oxidative acidic hydrolysis HCl ( $c = 6 \text{ mol/dm}^3$ ). Tryptophan (TRP) was estimated after alkaline hydrolysis with LiOH ( $c = 4.2 \text{ mol/dm}^3$ ). The chromatographic analysis of sample hydrolysates was performed in the analyser AAA 400 (manufacturer INGOS Prague, CR) and

Table 1. Parameters of the regression function  $y'_i = a + bx_i$  for the calibration model

Amino acids	<i>a</i>	<i>bx<sub>i</sub></i>	S.E.	CV (%)	<i>r</i>	
Thr	17.9660	+ 0.9576	0.37	0.8	0.979	**
Val	47.5010	+ 0.9107	0.60	1.1	0.954	
Leu	46.4580	+ 0.9375	0.74	1.0	0.968	
Ile	57.7060	+ 0.8417	0.61	1.7	0.918	
Lys	87.3410	+ 0.8955	1.03	1.2	0.946	
Arg	30.5280	+ 0.8251	0.28	1.6	0.908	**
His	52.1120	+ 0.7147	0.24	1.3	0.845	**
Trp	1.0005	– 0.0002	0.21	0.1	0.999	
Phe	72.7790	+ 0.8370	1.10	2.5	0.915	
Met	35.7410	+ 0.8281	0.33	1.6	0.909	*
Cys	8.8517	+ 0.8829	0.12	1.6	0.939	***
ΣMet + Cys	49.5270	+ 0.8311	0.50	1.7	0.911	
ΣEAA	0.5147	+ 0.8849	0.64	0.1	0.940	
Tyr	56.4370	+ 0.8698	0.70	1.6	0.933	
Asp	87.0030	+ 0.8927	1.18	1.5	0.945	
Glu	0.1986	+ 0.8736	0.23	1.5	0.935	***
Ser	0.1986	+ 0.8736	0.40	0.8	0.986	*
Pro	25.3250	+ 0.9705	0.63	0.7	0.985	
Gly	15.7320	+ 0.9166	0.30	1.6	0.957	**
Ala	27.5040	+ 0.9006	0.43	1.6	0.949	*
ΣNEAA	0.3822	+ 0.9182	0.59	1.3	0.958	
ΣAA	0.4214	+ 0.9548	0.85	1.9	0.977	

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$

Table 2. Parameters of the regression function  $y'_i = a + bx_i$  for the validation model

Amino acids	<i>a</i>	<i>bx<sub>i</sub></i>	S.E.	CV (%)	<i>r</i>	
Thr	21.762	+ 0.9442	0.51	1.2	0.960	
Val	63.050	+ 0.9820	0.80	1.5	0.912	
Leu	56.091	+ 0.9323	1.06	1.4	0.936	
Ile	72.210	+ 0.8076	0.81	2.2	0.855	
Lys	115.910	+ 0.8599	1.38	1.7	0.903	
Arg	38.555	+ 0.7863	0.35	2.0	0.853	*
His	69.856	+ 0.6127	0.32	1.8	0.717	*
Trp	0.867	+ 0.5587	4.34	2.2	0.667	
Phe	94.360	+ 0.7778	1.42	3.2	0.854	
Met	45.764	+ 0.7731	0.40	1.9	0.867	*
Cys	10.280	+ 0.8658	0.16	2.1	0.895	***
ΣMet + Cys	60.496	+ 0.7907	0.61	2.1	0.868	
ΣEAA	0.603	+ 0.8662	0.82	1.8	0.901	
Tyr	61.343	+ 0.8600	0.92	2.1	0.886	
Asp	113.000	+ 0.8631	1.50	1.8	0.910	
Glu	0.239	+ 0.8499	0.31	2.0	0.882	**
Ser	20.380	+ 0.9605	0.60	1.1	0.968	
Pro	40.001	+ 0.9588	0.92	1.1	0.968	
Gly	25.302	+ 0.8603	0.40	2.1	0.923	*
Ala	39.962	+ 0.8477	0.55	2.0	0.916	
ΣNEAA	0.435	+ 0.8993	0.79	1.7	0.923	
ΣAA	0.274	+ 0.9782	1.38	3.1	0.943	

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$

using Na-citrate buffers and ninhydrin detection (Official Journal, 1978; Kráčmar *et al.*, 1999; Kráčmar and Liška, 2002).

About 90 samples of sheep colostrum were analysed for calibration and validation of the calibration performed. A wavelength scanning instrument FT NIR Antaris (f. ThermoNicolet, USA) was used with a scanning range from 4 000 to 10 000  $\text{cm}^{-1}$  and with 100 scans in reflectance mode. Samples of milk were warmed to 40°C, agitated, cooled to the temperature of 20°C and transferred to Petri dishes. The measured area was spaced by a metallic mirror. Each sample was analysed three times and the average spectrum was used for calibration. The whole spectrum area was tested. The same samples were employed for

full cross validation by software FT NIR Reference Analysis.

All results were evaluated by the statistical analysis of variance (ANOVA). Correlation matrices and regression functions were calculated according to Snedecor and Cochran (1967) when using the statistical package Microsoft® Excel 2000 and Unistat 5.1.

## RESULTS AND DISCUSSION

The purpose of this study was to evaluate the possibility of estimating total essential, total non-essential and total amino acids by NIRS method. Calibration was performed using the PLS (partial least-squares) algorithm.

Table 3. Parameters of concentrations of amino acids in sheep colostrum as estimated by NIRS. Reference values and their mutual comparison by paired *T*-test

Amino acids	<i>n</i>	$\bar{x}$ NIR (mg/100 ml)	IREF (mg/100 ml)	<i>d</i>	S.D.
Thr	84	424.02	424.01	0.01	0.33
Val	81	532.04	532.04	0.00	0.61
Leu	87	743.34	743.34	0.00	0.73
Ile	90	364.65	364.65	0.00	0.61
Lys	87	836.01	836.01	0.00	1.03
Arg	90	174.49	174.49	0.00	0.27
His	87	182.83	182.77	0.06	0.24
Trp	87	1.97	1.97	0.00	0.02
Phe	87	446.29	446.32	–0.03	1.09
Met	84	207.88	207.88	0.00	0.30
Cys	87	75.62	75.62	0.00	0.10
ΣMet + Cys	87	293.29	293.29	0.00	0.50
Tyr	84	433.44	433.43	0.01	0.70
Asp	90	810.83	810.83	0.00	1.17
Glu	90	1 572.30	1 572.17	0.13	0.22
Ser	87	527.75	527.75	0.00	0.39
Pro	87	857.72	857.72	0.00	0.62
Gly	90	190.65	188.54	2.12	0.30 **
Ala	90	276.78	276.78	0.00	0.43
ΣEAA	90	4 470.20	4 470.20	0.00	0.63
ΣNEAA	90	4 670.97	4 670.93	0.04	0.58
ΣAA	90	9 322.50	9 322.86	–0.36	0.84

$\bar{x}$ NIR = mean of the NIR values;  $\bar{x}$ REF = mean of the reference values (Kráčmar *et al.*, 2004)

*d* = difference in the mean of NIR and reference values

\*\**P* < 0.01

The results are summarized in Tables 1–3. Table 1 presents parameters of the regression function  $y'_i = a + bx_i$  and basic statistical data for calibration models of individual essential and nonessential amino acids, sum of Cys + Met (ΣMet + Cys), sum of essential amino acids (ΣEAA), sum of nonessential amino acids (ΣNEAA) and sum of total amino acids (ΣAA). The construction of calibration model requires the use of a computer equipped with chemo-metric software and also a programme enabling the operation of the instrument and the collection of recorded spectra. The principle of calibration is to obtain spectra of amino acids and to

compare the obtained spectral data with results of classical chemical analysis.

The calibration model indicates that all amino acids showed a very high correlation ( $r = 0.9<$ ); the only exception was His with high correlation ( $r = 0.845$ ). Statistically significant differences are described in Table 1. Homogeneity of measured values was also characterised by very low variation coefficients (0.1–2.5%).

Table 2 presents the values used for the construction of validation model. Reliability of this calibration model can be evaluated by means of cross validation. The principle of this test is as fol-

lows: a particular sample (or a group of samples) is eliminated from the calibration set and all other samples are used as a calibration and validation set of samples. The obtained results are evaluated and the whole procedure is thereafter repeated with all other samples. Validation of contents of calibrated and tested amino acids revealed that Trp showed a significant correlation coefficient ( $r = 0.667$ ) while correlation coefficients for Ile, Arg, Phe, Met, Cys, Tyr, Glu and  $\Sigma$ Met + Cys were highly significant. Correlation coefficients for all other amino acids,  $\Sigma$ EAA,  $\Sigma$ NEAA and  $\Sigma$ AA were very highly significant. Statistically significant differences are described in Table 2.

A comparison of concentrations of amino acids in sheep colostrum estimated by NIRS with reference values (i.e. estimation of amino acids after acid hydrolysis, elution of the sample with sodium-citrate buffers and detection with ninhydrin using the apparatus AAA 400; Kráčmar *et al.*, 2004) revealed that when using NIRS (Table 3), contents of Phe and  $\Sigma$ AA were lower 0.03 and 0.36 mg/100 ml of colostrum, resp., while levels of Thr and Tyr were slightly higher (0.01 mg/100 ml). For  $\Sigma$ NEAA, His and Glu the corresponding levels were 0.04; 0.06 and 0.13 mg/100 ml, respectively. The highest difference was found for Gly (2.12 mg/100 ml;  $P < 0.01$ ), it is impossible to use NIRS method to determine this amino acid.

The results of estimation of amino acid levels in sheep colostrum have not been published yet. Šustová *et al.* (2004) analysed the basic composition of sheep colostrum by NIRS and found relatively high correlation coefficients of calibration for DM (0.994), crude and net protein (0.985 and 0.983, resp.) and fat (0.972). Jankovská and Šustová (2003) also concluded that NIRS is a suitable method for a rapid analysis of milk composition. They found the following values of correlation coefficients of calibration: for total solids 0.928, fat 0.961, protein 0.985, casein 0.932, urea nitrogen 0.906, lactose 0.931, and somatic cells 0.872. Tsenkova *et al.* (1999, 2000) and Ru and Glatz (2000) obtained similar results for non-homogenized milk. Kukačková *et al.* (2000) found the best calibration results for the prediction of total solids ( $r = 0.975$ ), fat ( $r = 0.967$ ), and protein ( $r = 0.965$ ).

## CONCLUSION

The results of the reference values of samples and of the calculated values of NIR were statistically

analysed by ANOVA test in UNISTAT. Statistically significant differences between the reference values and the calculated values of NIR were not found (except Gly, Table 3). The calibration models for amino acids of sheep colostrum are applicable to speed up its analysis.

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## ABSTRAKT

### Analýza aminokyselinového složení ovčího mleziva pomocí blízké infračervené spektroskopie

Práce řeší změny aminokyselinového složení ovčího mleziva v průběhu 2–72 hodin po porodu a možnost použití blízké infračervené spektroskopie pro jejich stanovení. Dynamika a změny obsahu aminokyselin byly sledovány u 10 kříženek ( $F_{111}$ ) plemene východofrízská ovce × zušlechtěná valaška (EF87IV) na druhé a vyšší laktaci. Korelační koeficienty pro kalibrační model byly zjištěny: Thr 0,979; Val 0,954; Leu 0,968; Ile 0,918; Lys 0,946; Arg 0,908; His 0,845; Trp 0,999; Phe 0,915; Met 0,909; Cys 0,939;  $\Sigma$ Met + Cys 0,911; Tyr 0,933; Asp 0,945; Glu 0,935; Ser 0,986; Pro 0,985; Gly 0,957; Ala 0,949;  $\Sigma$ EAK 0,940;  $\Sigma$ NEAK 0,958 a 0,977 pro  $\Sigma$ AK. Korelační koeficienty aminokyselin pro validační model činily Thr 0,959; Val 0,912; Leu 0,936; Ile 0,855; Lys 0,903; Arg 0,853; His 0,717; Trp 0,667; Phe 0,854; Met 0,867; Cys 0,895;  $\Sigma$ Met + Cys 0,868; Tyr 0,886; Asp 0,910; Glu 0,882; Ser 0,968; Pro 0,968; Gly 0,923; Ala 0,916;  $\Sigma$ EAK 0,901;  $\Sigma$ NEAK 0,923 a  $\Sigma$ AK 0,943. Konstruovaný kalibrační model plně odpovídá validovaným hodnotám.

**Klíčová slova:** blízká infračervená spektroskopie; ovčí mlezivo; aminokyseliny

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