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A comparison of several methods of protein determination in pig blood plasma.

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INTRODUCTION

Chemical composition of blood and blood plasma of slaughter livestock including total protein contents and proportion in both globulins and albumins as well as fibrynogen and haemoproteis and blood plasma physicochemical indices /pH,viscosity,specific gravity,fat emulsifying capacity,gelling temperature etc./ are mainly species dependent.However,content of total,crude protein of industrially manufactured blood plasma may depends also on:variation in construction of centrifuge used,operational conditions during centrifugation such as:blood temperature,rotor velocity,distances between plates,filling rate of blood and to appropriate extend also on operator skillness and accuracy of job performed etc.All of the above mentioned biophysicochemical and technical factors might affect protein content in blood plasma processed./11,13,14,15/.Effective control and regulation of the technological process needs required purpose oriented methods of protein determination.The adjustement of plasma production process deviations, influencing quantity of protein in blood plasma i.e.so called inter-manufacturing control of processing requires quick /fast/ and simultaneously sufficiently precise analytical method of protein determination.The analytical techniques of protein determination at present available are quite a numerous./1,2,3,4,5,7,9,10,12/.

Aiming at selection of the most suitable analytical techniques for the inter-manufacturing control of blood plasma protein content we have coparatively assessed seven methods of protein determination in industrially produced blood plasma using determination of protein according to the Kjeldahl procedure as a reference method.

MATERIAL and METHODS

The experimental material consisted of 45 batches,each of 3.0 litres,of swine blood plasma, freshly processed incommercial conditions during January-April,1985 at plasma manufacturing plant of the local slaughterhouse.The determination of total,crude protein in each out of 45 batches of swine blood plasma was complted within 4-7 hours on the day of plasma collecting at local slaughterhouse.The protein content was determined quadruply for each of 7 methods of protein determination assessed for the purpose of selectin of the fastest and simultaneously most reliable one for the inter-manufacturing control of protein content in plasma.The following methods were assessed:

1.Determination of protein content by Kjeldahl method - reference method.

A commonly applied Kjeldahl method was used.5 ml of plasma was digested in 200 ml flask with 25 ml of concentrated H_2SO_4 /d=1.84/ and 1 g of potassium sulphate /60%/ and copper sulphate /40%/ catalizator.After digestion and neutralization with 33% NaOH the ammonia was determined by absorption in 4.0% boric acid and titrated with 0.05 N H_2SO_4 using Tashiro indicator./Polish Standard PN-75/A-04018/.

2.Densimetric determination of protein content./6/.

Attempt was made to find the correlation between specific gravity of plasma and protein content.The specific gravity of plasma was determined at 20°C using 100 ml picnometer fitted with thermometer.

3.Refractometric determination of protein content./6/.

Index of refraction for plasma was determined at 20°C using laboratory refractometer RL - 1 coupled with a thermostate and correlation between indexes of refraction read with accuracy of 10^{-4} and protein content was calculated.

4.Modified method of refractometric determination of protein content./6/.

For determination of protein content in plasma a difference between index of refraction value determined for fresh plasma at 20°C and index of refraction value for the filtrate of plasma after precipitation of protein with 20% trichloracetic acid added.Erlemeyer flask was filled up with 20 ml of plasma and 5 ml of 20% solution of trichloracetic acid was added.The supernatant after protein precipitation was filtered throughout a filter paper and the index of refraction for filtrate was determined at 20°C.Difference between indexes of refraction for plasma and filtrate was calculated and used for calculation of the correlation coefficient of protein content in plasma.

5.Spectrophotometric determination of protein content according to Ahokosa./6/.

The method is based on determination of the difference in absorbance of protein solution in 0.9% NaCl at 215 and 225 nm.Into 100 ml of measuring flask 1 ml of plasma was pipeted and flask was filled-up to 100 ml with 0.9% NaCl.After mixing 1 ml of plasma solution was pipeted into test-tube and 10 ml of 0.9% NaCl was added and again well mixed.Quarc cuvette of 1 cm was than filled-up with plasma protein solution.The absorbance was determined at 215 nm /wave number approx.46.6/ and at 225 nm /wave number approx.44.5/ using spectrophotometer SPECORD UV VIS.The difference between absorbance at 215 nm and 225 nm was used for calculation of the correlation coefficient for protein content in blood plasma.

6.Spectrophotometric determination of protein content according to Toma and Nakai./12/.

Into 100 ml of 0.1 N citric acid 2 ml of plasma was pipeted and homogenized for 3 min.1 ml of homogenate was than pipeted into test-tube and 7 ml of 8 m urea in 2 N NaOH was added /480.8 g

of urea and 80 g of NaOH was dissolved in 1000 ml of distilled water/.The absorbance of sample was measured against control solution of 1 ml of o.1 N citric acid and 7 ml of urea in NaOH at pre-determined for the plasma protein solution maximum of absorbance i.e.at 241 nm /wave number 41.4/ using quarc cuvette and spectrophotometer SPECORG UV VIS Carl Zeiss Jena.The correlation coefficient for protein content was than calculated.

7.Spectrophotometric determination of protein content according to Gabor./4/.

Into homogenizer filled-up with 100 ml of 0.1 N NaOH 4 ml of plasma was pipeted and homogenized for 3 min. 2 ml of homogenate was than pipeted into test-tube and 5 ml of 97% acetic acid /glacial acetic acid/ was added and after 1 min. 1 ml of chloroforme was added and the test - tube content well mixed.The absorbance of a mixture was measured at 280 nm /wave number 36.0/ against control solution of 2 ml 0.1 N NaOH,5 ml of glacial acetic acid and 1 ml of chloroforme using 1 cm quarc cuvette and spectrophotometer SPECORD UV VIS.The maximum of absorbance for plasma protein solution was pre-determined in range of 200 - 300 nm.The readings of absorbance was used for calculation of the correlation coefficient of protein content in blood plasma.

RESULTS and DISCUSSION

Assessment of various methods of protein content determination in pig blood plasma and their suitability for inter-operational control was based and involved the calculation of:means,Sd, variability and dispersibility of the results and calculation of a linear regression coefficients.As a objective criteria of the methods applied for protein estimation in blood plasma in comparison with standard Kjeldahl method the sensitivity,precission and accuracy as well as correlation coefficients and their significance were calculated.All statistical calculations was made using a microcomputer ZX SPECTRUM and a standard statistical methods./8/.

The results of protein determination in blood plasma reflecting both direct measured data and figures /values/ of protein contents calculated using regression equations for 6 assessed methods with Kjeldahl method being a reference one are collected and presented in Table 1.

An average content of protein in pig blood plasma determined by Kjeldahl method was 5.84% ± 0.631 ranging from 4.48% to 7.17% and was characterized with relatively great coefficient of variation i.e.V% = 10.80 which was however lower than calculated for direct data of spectrophotometric methods according to Toma and Nakai and Gabor.The smallest coefficient of variation was established for immediate readings of specific gravity /densimetric method/ and both of refractometric methods,while for method by Ahokosa was nearly similar to calculated for Kjeldahl method.Direct data of absorbance of the methods according to Toma and Nakai and Gabor shows greater coefficient of variation.It should be however underlined that after re-calculation of the immediate findings for percentage of protein content using regession equations the coefficient of variation is practically similar as calculated for Kjeldahl method.

The results of protein determination in blood plasma by five assessed methods,except for the estimation according to Toma and Nakai /r=0.894/ shows an acceptable satisfactory correlation with results determined by Kjeldahl method ranging from r=0.952 to 0.902 being in average r = 0.920.The highest correlation coefficient with results determined by Kjeldahl method shows analytical findings according to Ahokosa /r=0.952/ and unmodified refractometry method /r=0.925/ while the lowest was calculated for spectrophotometric method according to Toma and Nakai.

The sensitivity of protein determination in blood plasma by method according to Ahokosa was fourfold smaller in comparison with Kjeldahl method while the best one and even better than for Kjeldahl method was found for densimetric method /0.02%/.The other assessed methods regardimg their sensitivity were similar to the standard Kjeldahl method.

The precission of the methods assessed was calculated according to Bartlet test./ 8/.Table 3. The precission of densimetric method is significantly better than Kjeldahl method /lowest mean dispersion of results/ while the precission of protein determination by Kjeldahl and both refractometric methods are similar.The spectrophotometric methods are significantly less precise in comparison to Kjeldahl method.Assuming that the accuracy of Kjeldahl method is 0 /zero/i.e.that determined quantity of protein in plasma by this method reflects real /effective/ amount of protein the accuracy of method by Ahokosa is the best one /±0.157%/ while for the method by Toma and Nakai shows the highest mean deviation /±0.243%/.

As it was expected inrelation to the time consumption of the Kjeldahl method the quickest /fastes/ are the refractometric determination of protein content in blood plasma and it require only approx.6.5 - 7.0% of time consumed by Kjeldahl method.Densimetric determination of protein could be completed within approx.12% of the time required for Kjeldahl method while three assessed spectrophotometric methods could be accoplished during 29-30% of time consumed by Kjeldahl method.It seems also worthwhile to mention that uncomparably cheaper to other assessed methods including Kjeldahl method are the physicochemical methods of protein determination in blood plasma i.e.densimetric and both refractometric methods.

CONCLUSSIONS

1.The great variations of the protein content between batches of industrially produced swine blood plasma seems fully to justify the requirement for inter-operational control of protein amount in plasma as well as justify the necessity of selection of suitable,fast analytical technique which will fulfill of the purpose in mind.

2.In commercial conditions of blood plasma manufacturing with acceptable precission and accuracy the inter-operational control of protein content in plasma could be accoplished using refractometric and/or densimetric methods for protein determination.

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TABLE 1.

Direct measured data and protein content calculated using regression equations for seven methods of protein determination in swine blood plasma./n = 45 batches x 4 replications/.

Trait	METHOD No.						
	1	2	3	4	5	6	7
Mean /$\bar{x}$/	5.84	1.0311[1]	1.3483[2]	0.0078[2]	0.420[3]	0.880[3]	0.730[3]
Protein %	5.84	5.83	5.83	5.83	5.84	5.82	5.85
Sd.	0.631	0.003	0.00135	0.00121	0.0410	0.1165	0.0910
Sd.protein %	0.631	0.570	0.587	0.574	0.602	0.564	0.578
R[4]	2.69	0.0125	0.0056	0.0052	0.190	0.50	0.380
R protein %	2.69	2.36	2.42	2.46	2.53	2.43	2.43
V %	10.80	0.30	0.10	0.516	9.50	15.30	14.90
V% protein %	10.80	9.78	10.07	9.84	10.31	9.69	9.88

1 = d_{20} ; 2 = n_{20} ; 3 = absorbance ; 4 = range

TABLE 2.

Selected characteristics of various methods of protein content determination in swine blood plasma. / n = 45 batches x 4 replications/.

Method No.	Regression equations percent of protein	Correlation coefficient	METHOD Sensitivity %	Precission	Accuracy %
1.	reference	reference	0.04	0.075	reference
2.	$188.666 \times d_{20} - 188.699$	0.902	0.02	0.020	$\pm$ 0.205
3.	$473.864 \times n_{20} - 575.736$	0.925	0.05	0.055	$\pm$ 0.193
4.	$473.864 \times \Delta n_{20} + 2.139$	0.909	0.06	0.060	$\pm$ 0.224
5.	$14.649 \times \Delta A - 0.297$	0.952	0.15	0.136	$\pm$ 0.157
6.	$4.841 \times A + 1.560$	0.894	0.05	0.141	$\pm$ 0.243
7.	$6.311 \times A + 1.227$	0.910	0.06	0.121	$\pm$ 0.201

d_{20} = specific gravity ; n_{20} = index of refraction ; A = absorbance ;
ΔA = difference in absorbance ; Δn_{20} = difference in index of refraction

TABLE 3.

Assessment of the precission of various methods of protein determination in swine blood plasma by Bertlet test

Bertlet test /method/	Precission S	Variation S^2	S^2_{mean}	C	Q	X^2	Signif. differ.
Kjeldahl	0.075	5.63×10^{-3}					
Densimetric	0.020	0.40×10^{-3}					
Refractometric	0.055	3.03×10^{-3}					
Refractometric mod.	0.060	3.60×10^{-3}	9.38×10^{-3}	1.0086	168.45	12.59	Signif.
Ahokosa	0.136	0.0185					
Toma and Nakai	0.141	0.0199					
Gabor	0.121	0.0146					
Ahokosa	0.136	0.0185					
Toma and Nakai	0.141	0.0199	0.0177	1.0101	1.079	5.99	Not Signif.
Gabor	0.121	0.0146					
Kjeldahl	0.075	5.63×10^{-3}					
Densimetric	0.020	0.40×10^{-3}					
Refractometric	0.055	3.03×10^{-3}	3.16×10^{-3}	1.0095	61.67	7.82	Signif.
Refractometric mod.	0.060	3.60×10^{-3}					
Kjeldahl	0.075	5.63×10^{-3}					
Refractometric	0.055	3.03×10^{-3}	4.08×10^{-3}	1.0101	4.58	5.99	Not Signif.
Refractometric mod.	0.060	3.60×10^{-3}					

Q = empiric value of Bertlet test ; C = parameter which depends on quantity of methods and samples