APPENDIX 2 REPRESENTATIVE SAMPLES FROM MILK AND OTHER MILK DERIVATIVES FOR MILK ANALYSER'S CALIBRATION

1. General

The samples used for analyser's calibration have to be representative for the corresponding milk type and have to be with known quality parameters: fat in percentage, SNF in percentage, density, lactose in percentage, total protein in percentage and salts in percentage. Changes in the analyzed parameters in the samples have, if possible, to cover the whole measuring range – i.e. used samples to be with low, middle and high content of the analyzed components.
The exact value of the parameters is decisive for correct and accurate calibration, because if the parameters are not set correctly during calibration the same parameter will not be measured correctly.

2. Necessary quality parameters values determination

For more precise determination of above listed quality parameters of the milk and its derivatives is advisable they to be examined in an authorized laboratories, using the corresponding arbitration methods for this purpose.

2.1. Laboratory methods

2.1.1. Determination of fat content

Determination of fat content in the milk and its derivatives (cream, whey, buttered milk) is one of the most important analyses in the dairy production and milk processing. According this parameter the payment schemes are made and it is observed from the point of view correct production process and the basic economy balances are made with its help.

A/ Röse-Gottlieb method
The fat content is determined using the gravimetric method, fat extraction from ammonia-alcohol milk solution using diethyl and petroleum ether, evaporation of the solvent and weighting the residuum.

B/ Gerber method
The proteins in the milk and dairy products are dissolved with sulphuric acid with definite concentration in butyrometer and the fat is separated under the influence of amyl alcohol, heating and centrifuging in a form of dense,

transparent layer. The volume of this layer is measured in the divided part of the butyrometer.

This is quick, easy method with sufficient accuracy. We recommend it for usage. For more detailed description see *Appendix Methods*.

2.1.2. Milk density determination

A/ With picnometer and Mor-Vestval scales
This is the most exact method for determination of milk and its derivatives' density.

B/ with aerometer (lacto-density-meter)
Compared with the above method this is quick and easy readable with satisfactory accuracy. We recommend it. For more detailed description see Appendix Methods.
During the lactation period and under the influence of different zoo engineering factors the density of the different milk kinds varies in the following bounds:

Milk kind	Minimum	Maximum	Average
Cow	1,027	1,033	1,030
Buffalo	1,026	1,032	1,029
Goat	1,027	1,033	1,030
Sheep	1,031	1,040	1,034

2.1.3. Determination of total proteins

A/ Kjeldahl method
Heating with concentrated sulphuric acid in the presence of catalyst mineralizes a definite volume of the milk sample. The liberated ammonium combines with the sulphuric acid and forms ammonium sulphate. After adding surplus soda caustic ammonium is liberated. When distilled it combines with the boronic acid. The quantity of the combined ammonium is determined by titration with acid with determined titer. From the combined with the ammonium acid the initial nitrogen content is determined, and also the proteins in the milk.

B/ Titration with formalin
Formalin, added to the milk, combines with the amino group in the protein's molecule and forms methyl groups, which have no alkaline reaction. Milk acidity increases by the liberated carboxylic groups, which are titrated with soda caustic solution. The used volume soda caustic is proportional to the protein content in the milk.

2.1.4. Determination of casein content in the milk

A/ Kjeldahl method
The total nitrogen content in the milk is determined. Casein is precipitated with acetic acid (acetate buffer) and is filtrated. The content of nitrogen in the filtrate is determined. Casein content is the difference between the two results for nitrogen using the Kjeldahl's method.

B/ Titration with formalin
More details for this method – see *Appendix Methods*.

2.1.5. Determination of salts in milk.

For the salts in milk and its derivatives is judged by its ashes content. Milk dries, becomes carbonized and turns to ashes till constant mass. The ashes received are calculated in percentage.

2.1.6. Determination of solids in milk

Solids describe the content of fats, proteins, carbohydrates and salts. Its value may be used for determination of each of these parameters in case of known other values.
Salts are determined by drying till constant mass – see Appendix *Methods*.

Express methods by using another milk analysers

It is possible another device to be used for determination of some of the quality parameters of milk and its derivatives samples, intended for calibration, but it has to be noted that it is possible incorrect values to be received, that's why it is necessary to be completely sure in the accuracy of their readings.

Usage of Milkoscan and other milk analysers based on the infrared measurement principle.
By using it the fat, lactose and protein content may be determined. Problem may arise with determination of salts and SNF. This is due to the impossibility of the infrared method to determine the solids and in order to receive the solids in the sample their meaning is accepted as a constant.