

Prediction of sheep milk chemical composition using pH, electrical conductivity and refractive index

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Milk quality traits

- **Milk quality traits (fat, protein, lactose and total solids content)**
 - **Directly linked to the processing performance of sheep milk**
- **Improvement of milk quality traits → Dairy industry's demand**
- **Milk quality traits are expensive and difficult to measure**
 - **Need laboratories with trained staff**
 - **Normally done by organized farmers' networks and cooperatives**
 - **Logistics for the transferring of samples**
 - **Considerable capital investment**

The situation in Greece



- **Farmers in Greece**
 - **Do not have easy access to these labs**
 - **Do not consider breeding for milk quality because it is expensive and difficult to measure**

The idea...

- **Can we use other physical traits to select for milk quality?**
 - **Other trait might include pH, milk electrical conductivity, refractive index**
- **Utilize 1) low-cost, 2) readily-used and 3) suitable for on-farm use, analyzers**

Objective

To assess if pH, electrical conductivity and refractive index of sheep milk can predict its fat, protein, lactose and total solids content

Animals and measurements

- 308 Frizarta ewes from two semi-intensive flocks
- Composite milk samples in 50 ml capacity tubes
- Milk electrical conductivity (MEC) and pH were measured (pH/conductivity meter (EZDO 7200, GMM Technoworld®))
- Refractive index (RI) was measured with a portable Brix (0-25 °Bx) (RHW-25ATC-BE, Hong Han Technologies)
- Milk quality traits were measured using MilkoScan™, Foss



Statistics

SPSS

Multiple stepwise regression analysis

$$\text{predicted variable} = \text{intercept} + \text{pH} + \text{MEC} + \text{RI} + e$$

the predicted variables were milk quality traits (fat, protein, lactose and total solids content)

e = random error

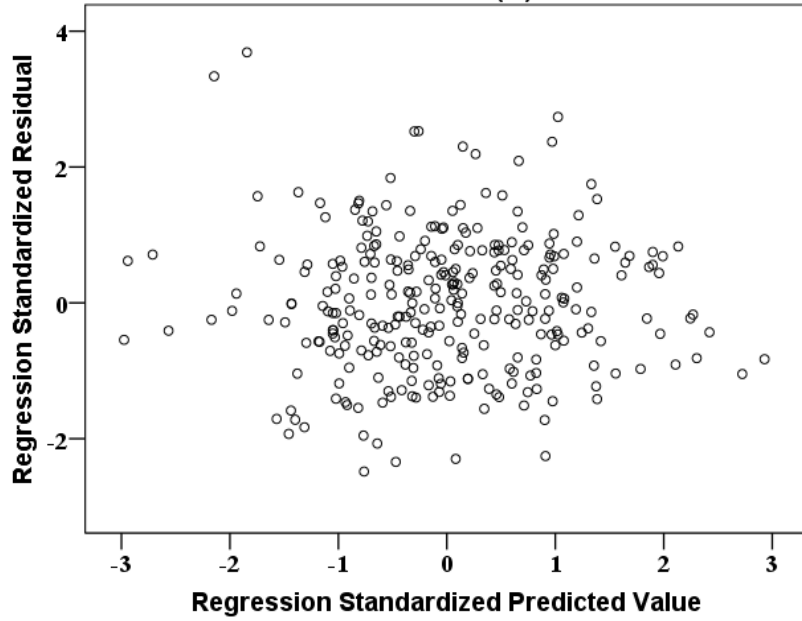
Assumptions testing passed

Assumptions of normality, linearity and homoscedasticity:

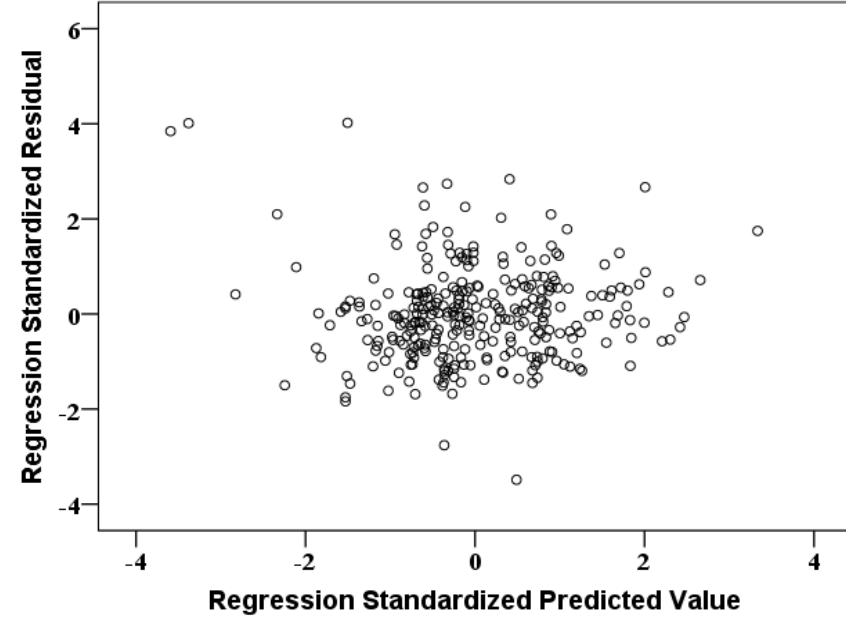
- **Plots of standardized residuals against standardized predicted values**
- **Probability-probability plots (p-p plots) of regression standardized residuals**
- **Variance inflation factor (VIF) was calculated for each predictor to ensure there was no violation of the assumption of multicollinearity**

Plots of standardized residuals against standardized predicted values

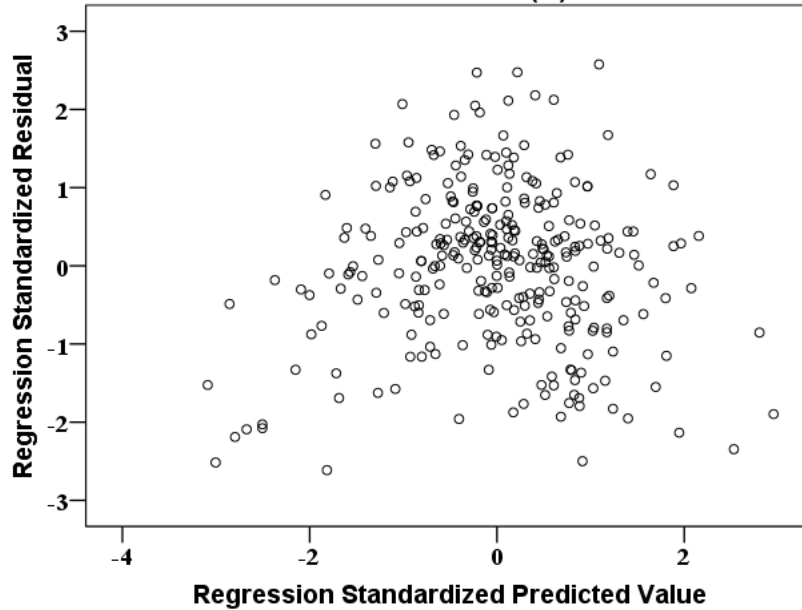
Fat concentration (%)



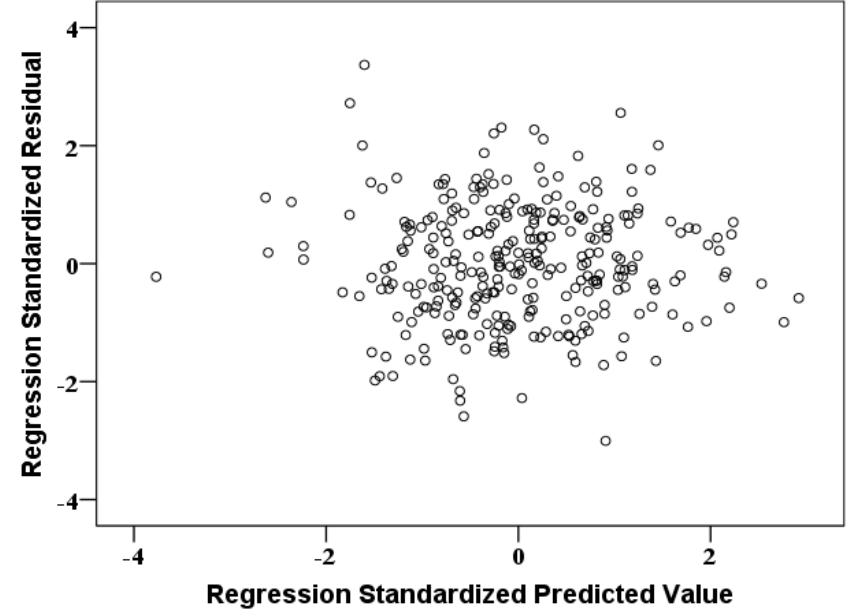
Protein concentration (%)



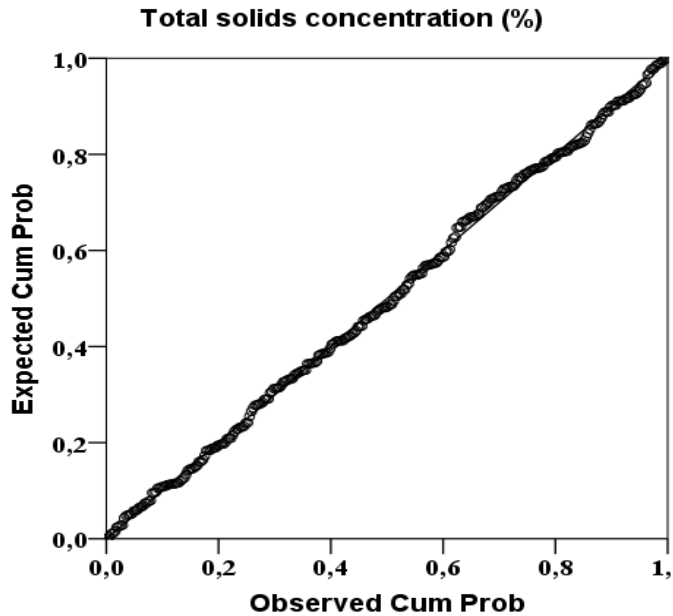
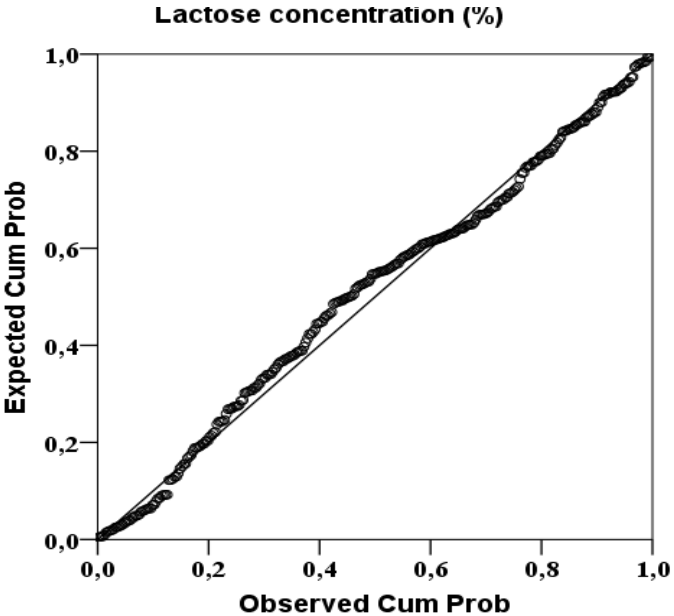
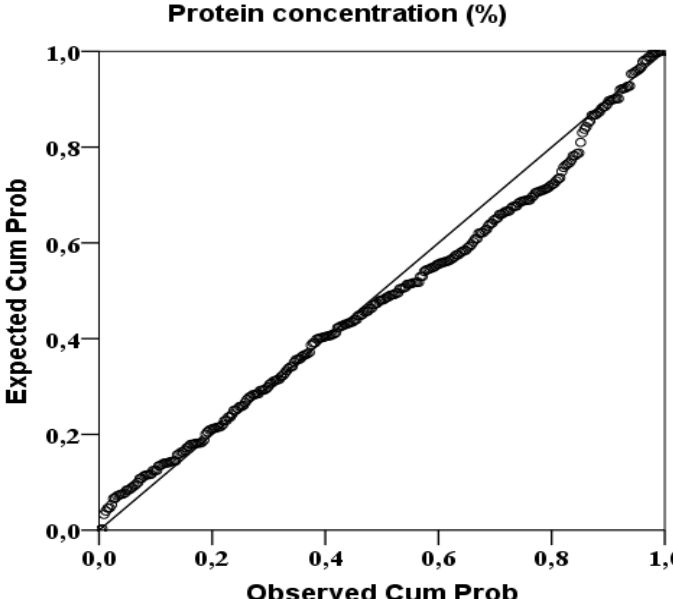
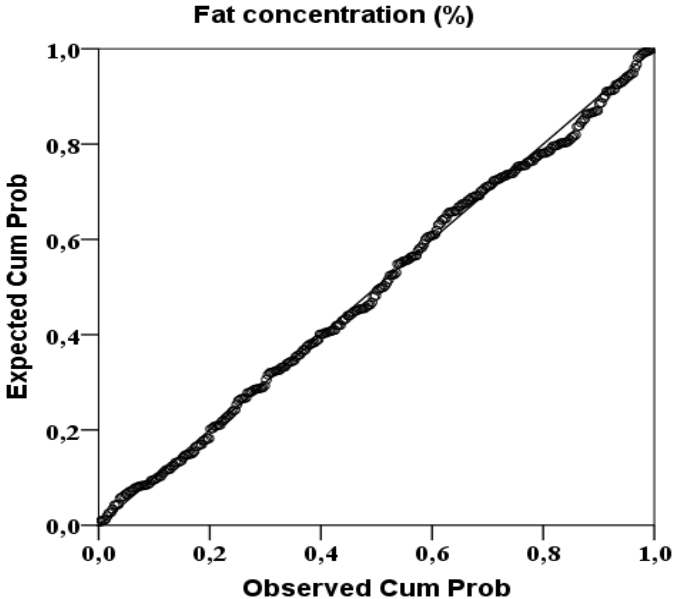
Lactose concentration (%)



Total solids concentration (%)



Probability-probability plots (p-p plots) of regression standardized residuals



Validation

Prediction models were validated using:

- **Bootstrapping - 1,000 bootstrap samples were repeatedly resampled, with replacement**
- **Split-half cross validation**
- **Regression coefficients, standard errors, 95% confidence intervals and p-values for the overall model and for the predictors were calculated**
- **Statistical significance was set at $P \leq 0.05$**

Descriptives

Physical properties of sheep milk and milk quality traits

| Variable | Mean | Std. Dev. |
|--------------------------|------|-----------|
| pH | 6.7 | 0.14 |
| Conductivity (ms/cm) | 4.0 | 0.36 |
| Refractive index (°Bx) | 13.4 | 0.80 |
| Fat content (%) | 5.5 | 1.33 |
| Protein content (%) | 5.4 | 0.49 |
| Lactose content (%) | 4.7 | 0.26 |
| Total solids content (%) | 16.5 | 1.51 |

Milk fat

Coefficients

| | Unstandardized coefficients | | | Collinearity statistics |
|------------------|-----------------------------|------|-------|-------------------------|
| Predictor | <i>B</i> | S.E. | P | VIF* |
| Intercept | 3.96 | 3.63 | 0.276 | |
| Refractive index | 0.77 | 0.09 | 0.000 | 1.03 |
| pH | -1.30 | 0.49 | 0.000 | 1.03 |

Bootstrap for Coefficients

| Predictor | <i>B</i> | S.E. | P |
|------------------|----------|------|-------|
| Intercept | 3.96 | 3.73 | 0.304 |
| Refractive index | 0.77 | 0.08 | 0.001 |
| pH | -1.30 | 0.53 | 0.014 |

Milk protein

Coefficients

| | Unstandardized coefficients | | | Collinearity statistics |
|------------------|-----------------------------|------|-------|-------------------------|
| Predictor | <i>B</i> | S.E. | P | VIF* |
| Intercept | -3.27 | 0.35 | 0.000 | |
| Refractive index | 0.58 | 0.02 | 0.000 | 1.24 |
| Conductivity | 0.24 | 0.04 | 0.000 | 1.24 |

Bootstrap for Coefficients

| Predictor | <i>B</i> | S.E. | P |
|------------------|----------|------|-------|
| Intercept | -3.27 | 0.55 | 0.001 |
| Refractive index | 0.58 | 0.03 | 0.001 |
| Conductivity | 0.24 | 0.06 | 0.001 |

Milk lactose

Coefficients

| | Unstandardized coefficients | | | Collinearity statistics |
|------------------|-----------------------------|------|-------|-------------------------|
| Predictor | <i>B</i> | S.E. | P | VIF* |
| Intercept | 8.41 | 0.32 | 0.000 | |
| Refractive index | -0.13 | 0.02 | 0.000 | 1.24 |
| Conductivity | -0.50 | 0.04 | 0.000 | 1.24 |

Bootstrap for Coefficients

| Predictor | <i>B</i> | S.E. | P |
|------------------|----------|------|-------|
| Intercept | 8.41 | 0.39 | 0.001 |
| Refractive index | -0.13 | 0.02 | 0.001 |
| Conductivity | 0.50 | 0.05 | 0.001 |

Milk total solids

Coefficients

| | Unstandardized coefficients | | | Collinearity statistics |
|------------------|-----------------------------|------|-------|-------------------------|
| Predictor | <i>B</i> | S.E. | P | VIF* |
| Intercept | 10.73 | 3.34 | 0.001 | |
| Refractive index | 1.16 | 0.09 | 0.000 | 1.24 |
| Conductivity | -0.59 | 0.20 | 0.004 | 1.35 |
| pH | -1.10 | 0.47 | 0.019 | 1.12 |

Bootstrap for Coefficients

| Predictor | <i>B</i> | S.E. | P |
|------------------|----------|------|-------|
| Intercept | 10.73 | 3.34 | 0.003 |
| Refractive index | 1.16 | 0.09 | 0.001 |
| Conductivity | -0.59 | 0.20 | 0.006 |
| pH | -1.10 | 0.48 | 0.023 |

Split-half cross validation

| | | Full data set | Split 1 | Split 2 |
|--------------|--|---------------|--------------|--------------|
| Fat | ANOVA significance ($P \leq 0.05$) | ≤ 0.001 | ≤ 0.001 | ≤ 0.001 |
| | R^2 | 0.250 | 0.349 | 0.158 |
| | Significant coefficients in the model* | RI*, pH | RI, pH | RI |
| Protein | ANOVA significance ($P \leq 0.05$) | ≤ 0.001 | ≤ 0.001 | ≤ 0.001 |
| | R^2 | 0.779 | 0.794 | 0.765 |
| | Significant coefficients in the model | RI, MEC** | RI, MEC | RI, MEC |
| Lactose | ANOVA significance ($P \leq 0.05$) | ≤ 0.001 | ≤ 0.001 | ≤ 0.001 |
| | R^2 | 0.379 | 0.409 | 0.354 |
| | Significant coefficients in the model | RI, MEC | RI, MEC | RI, MEC |
| Total solids | ANOVA significance ($P \leq 0.05$) | ≤ 0.001 | ≤ 0.001 | ≤ 0.001 |
| | R^2 | 0.511 | 0.581 | 0.443 |
| | Significant coefficients in the model | RI, MEC, pH | RI, MEC, pH | RI, MEC |

* $P \leq 0.05$

Results

Prediction equations of milk quality traits:

$$\text{Fat} = 3.96 + 0.77 \times \text{RI} - 1.30 \times \text{pH}$$

$$\text{Protein} = -3.27 + 0.58 \times \text{RI} + 0.24 \times \text{MEC}$$

$$\text{Lactose} = 8.41 - 0.13 \times \text{RI} - 0.5 \times \text{MEC}$$

$$\text{Total solids} = 10.73 + 1.16 \times \text{RI} - 0.59 \times \text{MEC} - 1.1 \times \text{pH}$$

Example

A milk sample with

- ✓ Refractive index = 14.0 °Bx
- ✓ pH = 6.7
- ✓ Electrical conductivity = 4.0 ms/cm

... is expected to have

- Fat = 6.0 %
- Protein = 5.8 %
- Lactose = 4.6 %
- Total Solids = 17.2 %



Conclusions

- **We can predict milk protein, lactose and total solids content using refractive index and milk electrical conductivity in sheep's milk**
- **Milk quality trait prediction models will help farmers and breeders breed the sheep that produce the best quality milk**
- **External validation of the models using milk produced from different**
 - a) dairy sheep breeds**
 - b) farming systems**
 - c) time-points within the lactation****will make the predictions more applicable**

Acknowledgements

- **The Laboratory of Milk Quality Control of the Hellenic Milk and Meat Organization (ELOGAK, Epirus Department)**
- **Vasilios Konstantinou and the farmers Ioannis Tolis and Panagiotis Pselis from the Agricultural Cooperative of Western Greece are greatly acknowledged for their collaboration and contribution to the study**

A group of white goats are gathered in a wooden pen, eating hay from a trough. The goats are of various ages and some have blue markings on their backs. The pen is made of light-colored wood and is filled with hay. The text "Thank you for your attention..." is overlaid in the center of the image in a bold, yellow font with a black outline.

**Thank you for your
attention...**

Conclusions

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