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Comparison of refractometer and biuret reaction as measurement methods for serum total protein concentration in Warmblood foals

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Summary: This study compared two measurement methods to assess serum total protein (TP) concentration (refractometer and biuret reaction) in Warmblood foals on a farm with occurrence of equine proliferative enteropathy (EPE). The aim was to investigate the reliability of TP measurement by an automatic temperature compensated handheld refractometer in foals and its use as a diagnostic monitoring tool, for example in the context of a *Lawsonia* monitoring programme. In addition, the correlation between TP and albumin was investigated as well as the influence of the outdoor temperature on the measurement methods. Serum TP values by refractometry and by biuret reaction (n = 772) were compared in healthy and sick foals (n = 744), aged one to eight months. Additionally, the correlation between TP and albumin concentrations was analysed. The study results were assigned to one of two groups, group A or B. The samples from group A (outdoor temperatures ≤ 29 °C) were taken over several weeks, whereas the samples of group B (outdoor temperature 33 °C) were all collected on one hot day. In both groups, the measurements were compared based on one single sample per foal. In group A (outdoor temperatures ≤ 29 °C), serum TP median value by refractometer was 61 g/L (25 % and 75 % percentile: 58–64 g/L) and 59.8 g/L (25 % and 75 % percentile: 57–62.6 g/L) by biuret reaction. The TP values of both measurement methods, refractometry and biuret reaction, correlated positively with each other (r = 0.79). There was no significant difference (p = 0.98) between the two methods. Albumin values correlated positively with TP values in this group (refractometer: r = 0.19 and biuret reaction: r = 0.27). In group B (outdoor temperature 33 °C), serum TP median value by refractometry was 52 g/L (25 % and 75 % percentile: 50–55 g/L) and 59.9 g/L (25 % and 75 % percentile: 57.2–62 g/L) by biuret. The TP values of both methods correlated positively with each other (r = 0.87). In this group, TP values measured by refractometry were significantly lower than by biuret reaction (p < .0001). Albumin values correlated positively with TP values (refractometer: r = 0.14 and biuret reaction: r = 0.18). Comparing TP values with a pooled reference range for foals aged one to nine months (50–73 g/L), in group A (outdoor temperatures ≤ 29 °C), 98.4 % (1370/1392) of the values from both measurement methods were within the reference range, while 1.6 % (22/1392) were outside. 0.9 % (12/1392) of the values were in the range commonly observed in foals with *Lawsonia intracellularis* infection (< 50 g/L). In group B (outdoor temperature 33 °C), 87.5 % (133/152) of the values were within the reference range and 12.5 % (19/152) were outside. 12.5 % (19/152) of the values were in the range seen in foals with *Lawsonia intracellularis* infection (< 50 g/L). Group B (outdoor temperature of 33 °C) differed from group A (outdoor temperatures ≤ 29 °C) by noticeable deviating refractometrically measured TP values from chemically measured TP values. In general, the results of this study showed no significant difference between the two measurement methods. However, significant deviations occurred at outdoor temperature of 33 °C. Therefore, when using the refractometer, it seems to be important that samples are taken at moderate outside temperatures (≤ 29 °C) or analysed promptly or stored refrigerated. Since only a small number of TP values was < 50 g/L, a statement of the comparability of the two applied measuring methods in the measuring range of interest for *Lawsonia intracellularis* is limited.

Keywords: albumin, biuret reaction, foals, *Lawsonia intracellularis*, refractometer, serum total protein

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Introduction

The measurement of total protein (TP) concentration is an important method for the detection of sick patients in veterinary medicine (*Tothova* et al. 2016). The assessment of TP concentration plays an important role in the diagnosis of equine proliferative enteropathy (EPE), which is caused by the bacterium *Lawsonia intracellularis* (*Lawson* and *Gebhart* 2000, *Pusterla* and *Gebhart* 2013). In a recent study, a reference value of TP concentration of ≤ 48 g/L was determined to serve as an index for the diagnosis of clinically suspected EPE foals (*Ueno* et al. 2019). As the disease EPE usually leads to hypoproteinaemia (< 50 g/L) caused by hypoalbuminemia, it is important to measure TP concentration when this disease is being mon-

itored on a breeding farm or suspected in a patient (*Pusterla* and *Gebhart* 2013).

TP concentration can be measured in various fluids such as serum, plasma and urine (*George* and *O'Neill* 2001, *Wolf* et al. 1962). Different methods of measurement exist, the most commonly used are refractometry and biuret reaction (*Kaneko* 1997). The biuret reaction, being the validated method, was used as the reference method in the current study.

Refractometry is an optical measurement method that provides refractive indices; the angle of refraction between an aqueous solution and air is measured. Since the 1960s, handheld refractometers have been used in veterinary medicine as a standard instrument for determining protein concentration in various liquids (*George* 2001). In contrast, the biuret reaction is based on a colour reaction for the detection of peptide bonds. For this, dissolved biuret and copper sulphate in an alkaline, aqueous environment are used. The reaction of the divalent copper with the peptide bonds of the proteins results in a violet colour change, a characteristic feature of the biuret reaction. The protein concentration is finally measured photometrically (*Fischer* and *Stressler* 2018, *Gornall* et al. 1949).

The advantage of the refractometer is the small amount of sample needed (about 0.1 ml), the quick and easy handling, the simple evaluation of the sample and the cost efficiency (*Sutton* 1976). Various studies have compared these two described methods. The agreement between the two methods partly depends on the particular animal species studied (*George* 2001).

To the best of the authors' knowledge, no previous work has compared TP concentrations between the two methods specifically in foals in a large sample size in context of *Lawsonia intracellularis*. The aim of the current study was to investigate the reliability of the refractometer in measuring serum TP in foals compared to the biuret reaction. Additionally, the correlation of TP and albumin was considered as well as the influence of outdoor temperatures on the measurement methods.

Materials and Methods

Study farm

The study took place at a Warmblood breeding farm in Germany. The mares and their foals lived in groups of 10 to 25 pairs. At the age of five and a half to six months, the foals were weaned from their dams and kept in large groups of weanlings.

Foals

Overall, 772 serum samples from 744 foals, including 378 fillies and 359 colts, aged one to eight months, were analysed (one month: n = 2, two months: n = 6, three months: n = 123, four months: n = 220, five months: n = 222, six months: n = 130, seven months: n = 33, eight months: n = 1). Sex, age and disease status were unknown for seven foals. All study foals were part of the *Lawsonia intracellularis* monitoring programme during the foaling season 2019. At the time of sampling, 324 of these foals were sick. Amongst the sick foals, the following diseases were reported: pulmonary findings consistent with pneumonia (two or more consolidations visualised by ultrasonographic examination of the lungs), suspected strangles (enlarged mandibular and/or retropharyngeal lymph nodes and/or abscesses in various places on the head, neck and chest), haematoma, peripheral and/or internal abscess, peripheral oedema, diarrhoea, injuries, fractures, lameness, fever (≥ 40 °C) over several days, leucocytosis (≥ 30,000 G/L) for at least three days in a row.

Method: Sampling and measurement of serum TP

The foals were sampled as part of the *Lawsonia* monitoring programme between August and October 2019. The samplings were assigned to individual calendar weeks (CW) (CW 33 to 40). CW 35 was considered separately as group B (outdoor temperature 33 °C), as there were obvious differences in TP values between refractometry and biuret reaction compared to the values of the other CW examined. Figure 1 shows the TP values of the two measurement methods at the different sampling situations. All samples of group B (outdoor temperature 33 °C) were collected and measured refractometrically on the same day with an outside temperature of 33.2 °C. In comparison, the outside temperatures on the collection days of group A (outdoor temperatures ≤ 29 °C) were between 16.8 °C and 28.9 °C (*Deutscher Wetterdienst* 2021). Thus, the samples from the other examined CW (CW 33, 34, 36–40) formed group A (outdoor temperatures ≤ 29 °C).

Group A (outdoor temperatures ≤ 29 °C) comprised 696 serum samples from 696 foals. Group B (outdoor temperature 33 °C) consisted of 76 serum samples from 76 foals. 28 of these foals were additionally sampled at a different time in group A (outdoor temperatures ≤ 29 °C). Except for these 28

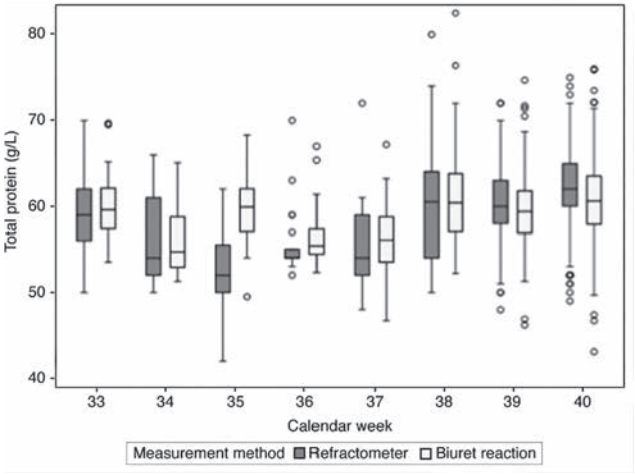

Fig 1 Total protein in serum of foals (n = 772) at eight measurement points (calendar week) in 2019 using the refractometer and biuret method. | *Totalprotein im Serum von Fohlen (n = 772) an acht Messpunkten (Kalenderwoche) im Jahr 2019 unter Anwendung des Refraktometers und der Biuret-Methode.*

foals, one sample per foal was analysed in each group (A and B).

All blood samples were taken from the jugular vein using sterile serum tubes of 4 ml and 9 ml and labelled individually. The time intervals between sample collection and refractometric examination varied from one to nine hours. Meanwhile, the sera were stored in a non-climatised room. Subsequently, the 9 ml tubes were centrifuged at 2.5 × 1000 g for 10 minutes on the farm and the serum TP concentration was determined with an automatic temperature compensated handheld refractometer (type HRM 18-T, Krüss) with a measuring range of 0 to 120.0 g/L, measuring accuracy +/- 0.0002 UG. Approximately 0.1 ml serum was pipetted onto the test field of the refractometer. After each measurement, the application surface and its cover glass were cleaned with a dry cloth. The measurements were carried out in a non-climatised room by different persons who were blinded to the disease status of the foals.

The 4 ml tubes were sent uncooled, as is common practice, and uncentrifuged to an external laboratory (LABOKLIN GmbH & Co. KG, Bad Kissingen, Germany) on the same day as sampling. The determination of TP concentration by biuret reaction was performed within 24 hours using a fully automated clinical chemistry device (cobas c701 module, Roche) with a measuring range from 2.0 to 120.0 g/L. Additionally, albumin concentration was analysed by turbidimetry within 24 hours.

Statistical analysis

For statistical evaluation of TP measurement by refractometer and by biuret reaction, a linear mixed model with measurement methods and CW, as well as their interactions as main effects, was used for group A (outdoor temperatures ≤ 29 °C) and the paired t-test for group B (outdoor temperature 33 °C). Descriptive analysis was used to determine how the results of the two methods were related to each other with regard to the reference range (RR). The normal distribution of all data sets was checked with the Shapiro-Wilk test. A p-value of < 0.05 was taken as the limit for statistical significance. The correlation coefficients were calculated according to Pearson. The data were analysed using SAS Software, version 9.4, and SAS Enterprise Guide 7.13 HF8 (SAS Institute Inc, Cary, NC, USA).

Results

TP values and correlation

Group A (outdoor temperatures ≤ 29 °C): Serum TP median value by refractometer was 61 g/L (minimum: 48 g/L, maximum: 80 g/L, 25% and 75% percentile: 58–64 g/L) and by biuret reaction 59.8 g/L (minimum: 43.1 g/L, maximum: 82.5 g/L, 25% and 75% percentile: 57–62.6 g/L). TP values of both measurement methods correlated positively with each other ($r = 0.79$, $p < .0001$). Restricting the analysis to observations, for which at least one of the measurements was < 50 g/L, no statistically significant correlation was found ($r = 0.10$, $p = 0.78$, $n = 10$).

Group B (outdoor temperature 33 °C): Serum TP median value by refractometer was 52 g/L (minimum: 42 g/L, maximum: 62 g/L, 25% and 75% percentile: 50–55 g/L) and 59.9 g/L (minimum: 49.5 g/L, maximum: 68.3 g/L, 25% and 75% percentile: 57.2–62 g/L) by biuret reaction. The TP values of both measurement methods correlated positively with each other ($r = 0.87$, $p < .0001$). The same correlation was found for the observations with at least one of the measurements < 50 g/L, ($r = 0.87$, $p < .0001$, $n = 18$). Only one of the observations showed a TP value < 50 g/L in the biuret reaction.

Comparison of the two measurement methods

Group A (outdoor temperatures ≤ 29 °C): There was no significant difference ($p = 0.98$) in the mean of serum TP between the two methods, refractometer (median value: 61 g/L) and biuret reaction (median value: 59.8 g/L). The TP values measured by refractometry were on average 1 g/L higher than those of the biuret reaction. Overall, 65.1% of the TP values measured by refractometry were higher than the TP values of the biuret method and 33% of the TP values measured by refractometry were lower than the chemically determined values. Deviations between the TP values of the two methods

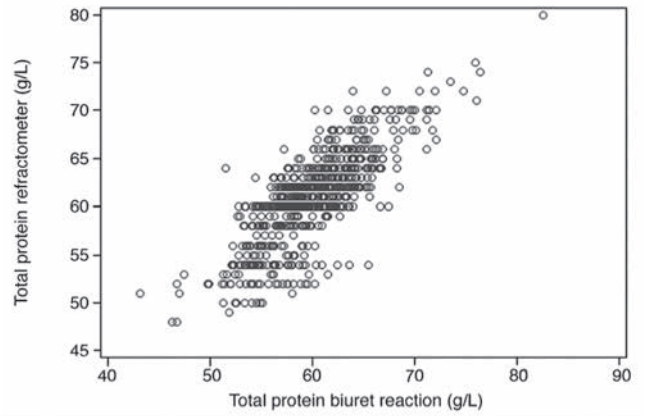

Fig 2 Serum total protein values measured by refractometer and biuret method at outdoor temperatures ≤ 29 °C (n = 696 foals). | *Serum-Totalproteinwerte gemessen mit Refraktometer und Biuret-Methode bei Außentemperaturen ≤ 29 °C (n = 696 Fohlen).*

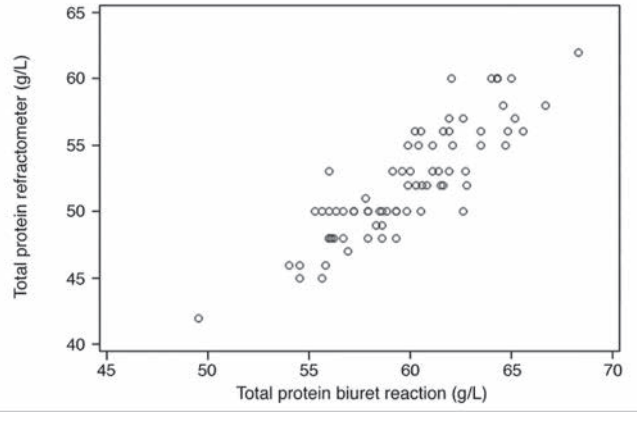

Fig 3 Serum total protein values measured by refractometer and biuret method at outdoor temperature of 33 °C (n = 76 foals). | *Serum-Totalproteinwerte gemessen mit Refraktometer und Biuret-Methode bei Außentemperaturen von 33 °C (n = 76 Fohlen).*

ranged from -12.5 g/L to 11.4 g/L. In 1.9 % of the results, the TP value of both methods was identical. Figure 2 shows the TP values of the two methods of all foals from this group.

Group B (outdoor temperature 33 °C): The results of refractometry (median value: 52 g/L) and biuret reaction (median value: 59.9 g/L) differed from each other ($p < .0001$). All TP values measured by refractometer were on average 7.6 g/L lower, with variations from 2 g/L up to 12.6 g/L. Figure 3 illustrates the TP values of the two methods of all foals from this group.

Reference range (RR) (50–73 g/L)

Group A (outdoor temperatures ≤ 29 °C): Comparing the TP values of this study with the pooled RR of foals aged one to nine months (50–73 g/L), 99 % (689/696) of the refractometric measured results were within the RR and 1 % (7/696) were out of range (*Bauer* et al. 1985). Looking at biuret reaction, 97.8 % (681/696) of the values were within the range and 2.2 % (15/696) were outside. In total, 98.4 % (1370/1392) of the TP values of both measuring methods were within the RR and 1.6 % (22/1392) were outside. The mean deviation in the values determined by refractometry and by biuret method was 1.8 g/L, while the TP value of one method was outside and that of the other within the RR.

Table 1 Serum total protein values of healthy and sick foals (n = 689) aged from one to eight months. The number of foals of the respective age group taken into account into the calculation of the values is given in brackets. Indicated are the median value and the respective value range below. | *Serum-Totalproteinwerte von gesunden und kranken Fohlen (n = 689) im Alter von einem bis acht Monaten. In Klammern steht die bei der Berechnung der Werte berücksichtigte Anzahl an Fohlen der jeweiligen Altersgruppe. Angegeben sind der Medianwert und darunter der jeweilige Wertebereich.*

	Total protein (g/L)		Albumin (g/L)
Age	Refractometer	Chemical (Biuret)	Chemical (turbidimetry)
1 month (n = 2)	62 54–70	62.9 56.3–69.5	32.7 30.1–35.2
2 months (n = 6)	54 50–62	57.1 51.4–59.1	31.3 29.2–31.8
3 months (n = 123)	62 50–75	60.5 49.7–76.4	30.8 23–35.9
4 months (n = 220)	62 48–80	60.4 46.2–82.5	31.1 21.4–37.3
5 months (n = 222)	62 50–73	59.5 43.1–73.5	31.7 22.9–39.8
6 months (n = 99)	59 49–68	59.5 46.9–69.7	32 21.4–36.5
7 months (n = 16)	55.5 50–69	58.4 51.3–67.5	32 27.7–34.9
8 months (n = 1)	59 59	56.2 56.2	30.1 30.1

45.5 % (10/22) of these values were simultaneously outside the RR with both methods, the chemically measured values were on average 0.5 g/L lower. 28.6 % (2/7) of the values measured by refractometry were outside the RR, while the biuret values were within the range. Similar to this, 66.7 % (10/15) of the TP values measured by biuret reaction were outside the RR, while the values measured by refractometry were within the RR. In total, 0.7 % (10/1392) of all TP values of both methods were > 73 g/L and 0.9 % (12/1392) were < 50 g/L. At values < 50 g/L, the biuret values were on average 3 g/L lower than the values measured by refractometry. Table 1 shows the median values determined in this group and the range of serum values measured in relation to the age of the foals.

Group B (outdoor temperature 33 °C): Comparing the TP values of this study with the pooled RR of foals aged one to nine months (50–73 g/L), 76.3 % (58/76) of the refractometric measured results were inside and 23.7 % (18/76) were outside the RR. Looking at biuret, 98.7 % (75/76) of the TP values were within the RR and 1.3 % (1/76) were outside. 87.5 % (133/152) of the values of both measuring methods were within the range and 12.5 % (19/152) were outside. In total, 10.5 % (2/19) of the values were simultaneously outside the RR with both methods. The chemically measured value was 7.5 g/L higher than the value by refractometry. 94.4 % (17/18) of these values measured by refractometry were outside the RR, while the chemically measured values were within. No chemically measured value was outside the RR, while the refractometrically measured value was within. Overall, no value of all TP values of both methods was > 73 g/L and 12.5 % (19/152) were < 50 g/L. At values < 50 g/L, the biuret values were on average 9.1 g/L higher than the values measured by refractometry. Table 2 shows the median values determined in this group and the range of serum values measured in relation to the age of the foals.

Albumin

Group A (outdoor temperatures ≤ 29 °C): The median value of albumin was 31.3 g/L (minimum: 21.4 g/L, maximum: 39.8 g/L, 25 % and 75 % percentile: 29.7–33 g/L). Albumin was positively correlated with the TP values of refractometer ($r = 0.19$) and of biuret method ($r = 0.27$).

Group B (outdoor temperature 33 °C): The median value of albumin was 28.4 g/L (minimum: 24.9 g/L, maximum: 32.4 g/L, 25 % and 75 % percentile: 27.4–29.3 g/L). Albumin was positively correlated with the TP values of refractometer ($r = 0.14$) and of biuret analysis ($r = 0.18$).

Outdoor temperatures

The maximum daily outdoor temperatures in the calendar week (CW) (Monday until Friday) in the year 2019 were as follows during sampling: 25.2 °C (CW 33), 28.9 °C (CW 34), 33.2 °C (CW 35), 22.9 °C (CW 36), 21.6 °C (CW 37), 17 °C (CW 38), 22.8 °C (CW 39) and 16.8 °C (CW 40) (*Deutscher Wetterdienst* 2021).

Discussion

The main question addressed in the current study was whether the use of the refractometer is a reliable method for measuring serum TP in foals. In group A (outdoor temperatures ≤ 29 °C), the hypothesis was confirmed that TP values measured by refractometry were not significantly different from those measured by biuret reaction, one of the most commonly used methods (*Kaneko* 1997). The refractometrically measured TP values were on average 1 g/L higher than the results by biuret method. Although there was no significant difference between the two measurement methods at outdoor temperatures ≤ 29 °C, the fact that there were variations in the TP value of both methods from-12.5 g/L to 11.4 g/L in group A (outdoor temperatures ≤ 29 °C) should not be disregarded. Previous studies that did not specifically examine foals, but horses of different ages, especially adult animals, concluded that the refractometry is a reliable measurement method (*Arfuso* et al. 2018, *Sutton* 1976). In the study of *Benoist* (2001), who took samples from horses of all ages, serum TP values measured by refractometry were on average 7 g/L lower than the biuret results. In another study, TP values of equine plasma measured by refractometry were about 12% lower than values of the biuret analysis (*Lackhoff* and *Walden* 1984). Studies of the two measurement methods in other animal species such as dogs (plasma), cats (plasma) and cattle (serum) also showed no major differences; here, too, the TP values were sometimes higher (plasma) or slightly lower (serum) when measured by refractometry (*Briend-Marchal* et al. 2005, *McSherry* and *Al-Baker* 1976). However, striking differences between refractometer and biuret were found in avians (*George* 2001). In various studies, TP values measured by refractometry were lower than those measured chemically in contrast to our results (*Benoist* 2001, *McSherry* and *Al-Baker* 1976). Altogether, the current TP values confirmed that refractometry may well be applicable as a diagnostic tool, for example as part of a monitoring programme for *Lawsonia intracellularis*.

On the other hand, differences between both methods were evident on a day with higher outdoor temperature (group B (outdoor temperature 33 °C)). All refractometrically measured TP values were on average 7.6 g/L lower than the ones measured by biuret reaction. The lower TP values with the refractometry could be due to the higher outdoor temperature (33 °C) on the day of sampling and measurement. However, this is only a hypothesis of the authors and might be investigated in the future in standardised stepwise increasing environmental temperature. The TP results of refractometry were similar to those of *Benoist* (2001), where the refractometrically measured TP values were on average 7 g/L lower than the chemically measured values. In the current study, all samples of group B (outdoor temperature 33 °C) were taken and measured on the same day by refractometry. It was noticeable that on the sampling and measurement day of group B (outdoor temperature 33 °C), the maximum outdoor temperature was higher (33.2 °C) compared to the sampling and measurement days of group A (outdoor temperatures ≤ 29 °C) (maximum temperature 28.9 °C) (*Deutscher Wetterdienst* 2021). The temperature of the measured liquid influences the refractive index of the refractometer and a high temperature of the applied medium reduces the density to be measured (*Chadha* et al. 2001, *Vandeputte* et al. 2011). Thus, it is possible that TP values measured by refractometry were falsely too low at high outdoor temperature (33 °C). According to the manufacturer, the refractometer used in the current study performs temperature compensation in the range of 15 to 35 °C. Therefore, the increased temperature should have been automatically adjusted by the refractometer. The daily maximum temperature measured at the nearest weather station was 33.2 °C on the sampling day. However, it cannot be ruled out that the actual outdoor temperature at the sampling location was higher and thus the upper temperature limit (35 °C) of the refractometer for temperature compensation was exceeded. Another possible explanation is that due to the time factor of sampling all foals, the samples were not examined immediately after collection, which may have resulted in additional heating in the sun. *Arfuso* et al. (2018) has found that there was no significant difference in the results of serum TP determination by refractometry when the samples were analysed 30 minutes after collection or stored refrigerated and analysed 24 or 48 hours after collection. Therefore, especially, in case of outdoor temperatures > 29 °C, it is recommended to examine the samples immediately, to keep them refrigerated or to test them by biuret reaction to obtain reliable TP results.

One aspect that needs to be questioned is the use of the measurement methods in the clinically more critical measurement ranges, that is outside the RR. As the reference values for foals vary in the first weeks and months of life, the current study was focussed on a pooled RR (50–73 g/L) for foals aged from one to nine months (*Bauer* et al. 1985, *Brommer* et al. 2001, *Harvey* et al. 1984, *Sato* et al. 1979). Looking at the TP values (n = 10) where at least the TP value of one measurement method is < 50 g/L, in group A (outdoor temperatures ≤ 29 °C), a deviating mean value of 3 g/L was determined between the values of both methods. TP values < 50 g/L are particularly interesting in supporting a diagnosis of *Lawsonia intracellularis* enteritis (*Pusterla* et al. 2014). Generally, the results from group A (outdoor temperatures ≤ 29 °C) illustrated that the refractometry is a reliable method even for values below the range. However, in this work, the total TP values < 50 g/L measured both, refractometrically and via biuret reaction, were small in this group (n = 12).

Table 2 Serum total protein values of healthy and sick foals (n = 76) aged from six to seven months at high outdoor temperatures (33 °C). The number of foals of the respective age group taken into account into the calculation of the values is given in brackets. Indicated are the median value and the respective value range below. | *Serum-Totalproteinwerte von gesunden und kranken Fohlen (n = 76) bei hohen Außentemperaturen (33 °C) im Alter von sechs bis sieben Monaten. In Klammern steht die bei der Berechnung der Werte berücksichtigte Anzahl an Fohlen der jeweiligen Altersgruppe. Angegeben sind der Medianwert und darunter der jeweilige Wertebereich.*

	Total protein (g/L)		Albumin (g/L)
Age	Refractometer	Chemical (Biuret)	Chemical (turbidimetry)
6 months (n = 51)	52 42–62	60.2 49.5–68.3	28.5 24.9–32.4
7 months (n = 25)	50 45–60	58.6 55.6–65.2	28.3 25.4–31.8

In contrast, for samples of group B (outdoor temperature 33 °C) measured by refractometry with results < 50 g/L (n = 18), it must be considered that the increased outdoor temperature could have led to altered TP values. Thus, they might be falsely outside the RR, which was reflected in a deviation of 9.1 g/L in the TP mean of both methods in this group. Another indication for the assumption of temperature influence on the measurement of refractometer is the small number of values < 50 g/L (n = 1) by biuret method. Consequently, the warmer outdoor temperature in this group would affect results of refractometry and would mean that refractometry is not as reliable as expected under certain circumstances. In order to make a more precise statement for the reliability of the refractometry in connection with EPE, further examinations with TP results < 50 g/L are necessary.

Conclusion

The evaluation of TP values in the current study showed that the automatic temperature compensated handheld refractometer is a reliable alternative to the chemical measurement of serum TP in foals at outdoor temperatures ≤ 29 °C. However, there were significant differences between the two measurement methods at outdoor temperature of 33 °C. When using the refractometer, it seems generally important that samples are taken at moderate outdoor temperatures (≤ 29 °C) or promptly analysed or refrigerated shortly after collection to avoid prolonged exposure to heat. If this is not possible, the authors recommend that the sera be measured by biuret reaction. For a more precise assessment of the measurement agreement between the two methods in the diagnostic range for *Lawsonia intracellularis*, it is useful to evaluate especially values < 50 g/L in a further study.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Erweiterte Zusammenfassung

Vergleich der beiden Messmethoden Refraktometer und Biuret-Reaktion für Serum-Totalprotein bei Fohlen

In der Pferdemedizin ist die Messung des Totalproteins (TP) bei vielen unterschiedlichen Erkrankungen ein wichtiger diagnostischer Laborparameter. Er ist ein einfach und schnell zu ermittelnder Wert, der bereits erste Hinweise auf die aktuelle Krankheitssituation des Pferdes geben kann. Eine dieser Krankheiten ist die equine proliferative Enteropathie (EPE), die beim Fohlen durch das Bakterium *Lawsonia intracellularis* verursacht wird und zu einer Hypoproteinämie (< 50 g/L) führt. Die TP-Bestimmung eignet sich somit als ein wichtiges Diagnostikum bei der Überwachung von *Lawsonia intracellularis* in der Fohlenpopulation eines Aufzuchtbetriebes. In dieser Studie wurden die beiden am häufigsten verwendeten Messmethoden (Refraktometrie und Biuret-Reaktion) anhand der Messung der Gesamtprotein-Konzentration im Serum von Fohlen auf einem Warmblutgestüt mit nachgewiesenem Vorkommen von EPE miteinander verglichen. Das Ziel der Studie war es herauszufinden, ob die schnellere und kostengünstigere Bestimmung von TP mittels Refraktometer vergleichbare Ergebnisse erzielt, wie die im Labor durchgeführte Biuret-Reaktion, um somit das Refraktometer als zuverlässiges Diagnostikum, zum Beispiel im Rahmen eines *Lawsonien*-Monitorings, anzuwenden. Weitere untersuchte Aspekte waren die Korrelationen zwischen den TP-Werten beider Messverfahren und dem Albuminwert sowie der Einfluss der Außentemperaturen auf die angewandten Messmethoden. In dieser Arbeit wurden die TP-Ergebnisse der beiden genannten Methoden anhand von insgesamt 772 Serumproben von 744 gesunden und kranken Fohlen im Alter von einem bis acht Monaten miteinander verglichen. Die untersuchten Proben stammten aus einem auf dem Gestüt durchgeführten *Lawsonien*-Monitoring der Monate August bis Oktober 2019. Um die TP- und Albuminkonzentration zu ermitteln, wurde eine Blutprobe aus der Vena jugularis entnommen. Die Probenentnahmen wurden einzelnen Kalenderwochen (Kalenderwochen 33 bis 40) zugeordnet. Bei der Analyse der Probenergebnisse fiel auf, dass während eines bestimmten Beprobungszeitraums (Kalenderwoche 35) die refraktometrisch gemessenen TP-Werte im Vergleich zu denen der anderen untersuchten Zeitpunkte deutlich unter den Werten lagen, die mittels Biuret-Reaktion erzielt wurden. Um die Auswertung des Vergleichs beider Messmethoden nicht zu beeinflussen, wurden die Proben aus der Kalenderwoche 35 als eigene Gruppe betrachtet (Gruppe B (Außentemperatur 33 °C)). Die Proben aus den anderen Beprobungswochen (Kalenderwochen 33, 34 und 36 bis 40) bildeten die Gruppe A (Außentemperatur ≤ 29 °C). Die Gruppe A (Außentemperatur ≤ 29 °C) umfasste 696 Serumproben von 696 gesunden und kranken Fohlen. Die Proben wurden über einen Zeitraum von acht Wochen genommen. Gruppe B (Außentemperatur 33 °C) umfasste 76 Serumproben von 76 gesunden und kranken Fohlen. Die Probenentnahme der Fohlen aus Gruppe B (Außentemperatur 33 °C) erfolgte an einem gemeinsamen Tag, an welchem die maximale Außentemperatur (33,2 °C) im Vergleich zu den anderen Probeentnahmetagen (16,8 °C bis 28,9 °C) höher war. In jeder Gruppe wurden die zwei Messmethoden jeweils anhand des TP-Wertes einer Serumprobe pro Fohlen miteinander verglichen. Von einer kleinen Anzahl an Fohlen (n = 28) wurde je eine Probe in beiden Gruppen untersucht, die in unterschiedlichen Kalenderwochen entnommen wurden. Die Untersuchung der Seren erfolgte jeweils am Tag der Probenentnahme mit einem automatisch temperaturkompensierten Handrefraktometer in einem nicht klimatisierten Raum. Für die Biuret-Analyse wurden die Proben nach der Blutentnahme an ein externes Labor geschickt, in dem die Serum-Gesamtproteinkonzentration mittels Biuret-Methode und die Albuminkonzentration mittels Turbidimetrie innerhalb von 24 Stunden nach der Probenentnahme gemessen wurden.

In Gruppe A (Außentemperatur ≤ 29 °C) ergaben sich folgende Serum-TP-Werte mittels Refraktometer: Messwerte 48–80 g/L, Medianwert 61 g/L, 25 %- und 75 %-Perzentile: 58–64 g/L. Die Ergebnisse der Biuret-Reaktion betrugen in dieser Gruppe: Messwerte 43,1–82,5 g/L, Medianwert 59,8 g/L, 25 %- und 75 %-Perzentile: 57–62,6 g/L. In dieser Gruppe gab es keinen signifikanten Unterschied (p = 0,98) zwischen den beiden Messmethoden und es lag eine positive Korrelation vor (r = 0,79). Außerdem korrelierten die Albuminwerte positiv mit den TP-Werten aus beiden Messverfahren (Refraktometer: r = 0,19 und Biuret-Reaktion: r = 0,27). Im Hinblick auf Beobachtungen, bei denen mindestens einer der Messwerte < 50 g/L war, wurde keine statistisch signifikante Korrelation festgestellt (r = 0,10, p = 0,78). In Gruppe B (Außentemperatur 33 °C) zeigten die refraktometrischen Untersuchungen folgende Werte: Messwerte 42–62 g/L, Medianwert 52 g/L, 25 %- und 75 %-Perzentile: 50–55 g/L. Bei der Anwendung der Biuret-Methode ergaben sich folgende TP-Werte: Messwerte 49,5–68,3 g/L, Medianwert 59,9 g/L, 25 %- und 75 %-Perzentile: 57,2–62 g/L. Die refraktometrisch gemessenen TP-Werte waren in dieser Gruppe im Durchschnitt jeweils um 7,6 g/L niedriger als die Werte der Biuret-Reaktion. Somit waren die refraktometrisch gemessenen TP-Werte signifikant niedriger als bei der Biuret-Methode (p < .0001), dennoch lag eine positive Korrelation vor (r = 0,87). Auch bei hohen Außentemperaturen korrelierten die Albuminwerte positiv mit den TP-Werten aus beiden Messverfahren (Refraktometer: r = 0,14 und Biuret-Reaktion: r = 0,18). Bei den Werten < 50 g/L gab es Unterschiede zwischen beiden Methoden (p < .0001), jedoch eine positive Korrelation (r = 0,87). Verglich man die TP-Werte mit einem altersübergreifenden Referenzbereich von Fohlen im Alter von einem bis neun Monaten (50–73 g/L), so lagen in Gruppe A (Außentemperatur ≤ 29 °C) 98,4 % (1370/1392) der TP-Werte beider Messmethoden innerhalb des Referenzbereichs und 1,6 % (22/1392) außerhalb des Normbereichs, 45,5 % (10/22) davon gleichzeitig mit beiden Methoden. Lediglich 0,9 % (12/1392) aller TP-Werte aus beiden Methoden waren < 50 g/L. In Gruppe B (Außentemperatur 33 °C) befanden sich 87,5 % (133/152) der TP-Werte innerhalb des Referenzbereichs und 12,5 % (19/152) außerhalb, 10,5 % (2/19) davon gleichzeitig mit beiden Methoden. 12,5 % (19/152) der TP-Werte aus beiden Methoden lagen < 50 g/L.

Die Auswertung der TP-Werte in der aktuellen Studie zeigte zunächst, dass es eine gute Übereinstimmung der TP-Werte mit beiden Methoden gab. Somit ist die Refraktometrie eine mögliche Alternative zur chemischen Messung von Serum-TP bei Fohlen. Allerdings gab es bei Außentemperaturen von 33 °C signifikante Unterschiede zwischen den Messmethoden. Somit ist bei der Verwendung des Refraktometers generell darauf zu achten, dass die Proben bei moderaten Außentemperaturen (≤ 29 °C) genommen oder zeitnah analysiert oder gekühlt gelagert werden, um längere Hitzeeinwirkung zu vermeiden. Wenn dies nicht möglich ist, empfehlen die Autoren, die Seren mittels Biuret-Reaktion zu messen. Da in dieser Arbeit nur eine geringe Anzahl an TP-Werten < 50 g/L lag, ist eine Aussage zur Vergleichbarkeit der beiden angewandten Messmethoden für Proben, die sich in dem für *Lawsonia intracellularis* interessanten Messbereich befinden, nur eingeschränkt möglich. Diese Studie hat jedoch gezeigt, dass die Refraktometrie eine geeignete Methode zur Messung von TP bei Außentemperaturen ≤ 29 °C darstellt.

Schlüsselwörter: Albumin, Biuret-Reaktion, Fohlen, *Lawsonia intracellularis*, Refraktometer, Serum-Totalprotein