

SZENT ISTVÁN UNIVERSITY

**Ultrasound examination of teat anatomy
in Holstein-Friesian cattle breed**

Thesis of Ph.D. dissertation

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1. INTRODUCTION

1.1 Scientific background

Economical milk production depends on the health status of the dairy herds. The profitability of milk production is damaged by a number of diseases, one of which is mastitis, since mastitis can cause a loss of 5-7% of annual sales in a farm (Ózsvári, 2012).

The development of mastitis is supported by a number of housing and feed factors such as poor milking hygiene and a stalemate contaminated with manure (Jánosi et al. 2003) or vitamin A, D3, E, selenium and calcium deficiency (Paulrud, 2005).

In order to reduce the number of mastitis, treating the cows is not enough, but mastitis should also be reduced. To this end, research is increasingly focusing on the development of technologies that can detect mastitis predisposing factors even before the appearance of clinical symptoms.

Ultrasound is a non-invasive diagnostic method that can test all parts of the udder gland and the teat. Ultrasound has been used in earlier studies to study mastitis (Franz et al. 2009) and stenosis (Verkatesan et al. 2016).

The ultrasound imposes many parameters of the teat on the length and thickness of the streak canal (Strapák et al., 2017, Húth 2004, Fasulkov et al., 2014), the thickness of the teat wall (Stádnik et al., 2010), the area of teat end (Húth 2004), the diameter of the pars papillaris (Stojnovič et al., 2012), the thickness of the teat (Klein et al., 2005) was investigated. Changes in the anatomical parts of the teat with the age of the animals (Celik et al., 2018), with its lactation number (Klein et al., 2005, Seker et al., 2009), with lactation (Szencziová et al., 2013), with milk yield (Comalli et al., 1984) or milking (Húth 2004).

In most cases, the microorganisms that cause mastitis reach the udder through the streak canal (Murphy et al. 1988).

This is prevented by the teat's defenses, which are prevented by the keratin plug in the streak canal and the sphincter muscles at the end of the teat (Milne 1977, 1978). Although a number of previous publications have dealt with the size of the streak canal, it has not yet been thoroughly investigated.

The purpose of my research was to study the formulas in the distal part of the teat with ultrasound and to obtain comprehensive knowledge of the functioning of the defense system.

1.2 Objectives

During my research, I set out the following main goals:

1. I would like to determine how the size of the four teat parameters changes during different stages of lactation as a result of milking?
2. Explore the relationship between the production and teat characteristics of cows and the size changes of teat parameters during milking.
3. Find out how the values of each teat parameters are related?
4. Describe the size changes of the teat parameters during the dry period?
5. Determine the effect of excision of cows and heifers on the size of the teat parameters?
6. What is the effect of inflammation of the udder gland on the parameters of the examined teat during the course of the mastitis and after its healing?

2. MATERIALS AND METHODS

My research work was carried out in the dairy cattle farm of, Táncsics Agricultural Co. Ltd. in Nagyalásony in 2016. The Holstein-Friesian cows were freely kept in deep bottomed shed at this Veszprém county farm. The cows were milked twice a day in a 2x24-position milking parlour by a Westfalia-type milking machine (vacuum size: 42 KPa, pulsation ration: 60:40, pulsation rate: 62).

2.1. The procedure of ultrasound examination

I used a SonoScape A6 ultrasound machine and a 5-11 MHZ linear ultrasound transducer. I made ultrasound examination of the teats with the water bath method for the final recognition, that I put 34-36 ° C water into a 200 ml plastic cup and then I put the teat into it. Ultrasound test development in a short time parallel to the outside, like a cup wall. I put Aquasonic Clear ultrasonic gel between the transducer and the cup's wall.

During the ultrasound examination I measured the 4 parameters of the teat, the measurements were carried out on all four teat of the animal.

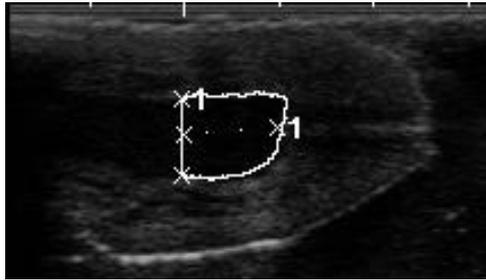
The four parameters of the teat:

- The length of streak canal: distance between the external and internal opening of the streak canal, in mm (picture)



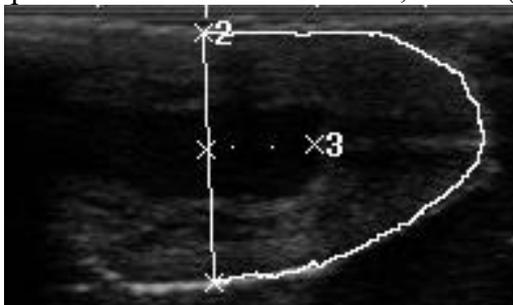
The length of streak canal

- The distal 1 cm area of pars papillaris: up to a height of 1 cm measured proximal to the Fürstenberg rosette, the area of the pars papillaris in cm^2 (picture)



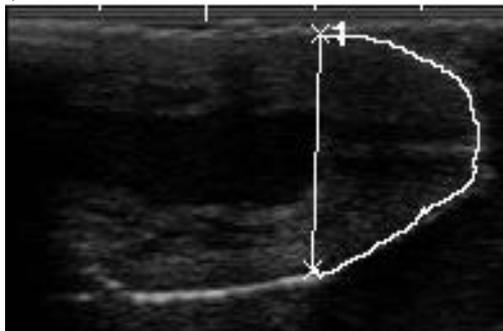
The distal 1 cm area of pars papillaris

- The area of 1 cm end of the teat: total area of the teat was measured from the height of 1 cm from the Fürstenberg rosette proximal to the end of the teat, in cm^2 (picture)



The area of 1 cm end of the teat

- The area of teat end: the area was measured from the height of the Fürtsenberg rosetta to the distal end of the teat, in cm^2 (picture)



The area of teat end

2.2. Single examination of healthy cows

2.2.1. Examining the change in size of teats during milking

In this study, animals were selected in any way that had no mastitis in the 30 days prior to the study. The individuals thus selected were classified into five groups based on their lactation stage.

Groups	Description of the group
1.	Multiparous cows, day after calving (n = 40)
2.	Cows with lactation of 100-150 days (n = 70)
3.	Cows with lactation of 290-358 days (n=58)
4.	Cows on dry, tested at drying time (day 222 of gestation) (n = 40)
5.	Primiparous cows on the day after calving (n = 31)

During my research, I examined how the four examined parameters of the teats change during milking. The examined teat parameters were measured by ultrasound before milking and immediately after milking and 2 hours after milking. On the one hand, I used the values obtained at the three times, and the ratios between the values obtained. When calculating ratios, pre-milking results were taken as baseline and I compared the values obtained immediately after milking and 2 hours after milking.

Calculating the ratios used in my work:

$$\text{- Ratio after milking: } \frac{\text{Value of after milking}}{\text{Value of before milking}}$$

$$\text{- Ratio 2 hours after milking: } \frac{\text{Value of 2 hours after milking}}{\text{Value of before milking}}$$

The statistical processing of the data was performed with the SPSS 18 program. To test the change in size of the teats during milking Paired t-test to compare the values of the teats

during milking between the groups I used Linear Variance Analysis, the Levene Test and the Tukey Test. Comparison of the ratios of the teats between the groups was done by the Kruskal-Wallis test.

2.2.2. Examination of the production characteristics of cows

My work, among other things, was to find out how the different production characteristics of the animals, such as the number of lactations, the number of lactation days, the number of days of pregnancy, and the amount of morning and the daily milk quantity affect the changes in the parameters of the teat. In the correlation analysis of the production characteristics of the animals, I calculated the mean of the measured values of each of the tested teats of the individual animals for the different teat parameters. I used the arithmetic mean for the values of the teat parameters, and the geometric mean for the ratios.

For statistical analysis of the data I used the Spearman and Pearson rank correlation method.

2.2.3. Examination of the own properties of teat

In this part of my research I examined how the three properties of the teat, such as the pigmentation, the location of the udder and ratio of teat affect the changes of the teats parameters during milking.

Based on their pigmentation, the teats were classified into three groups - pigmented, multicolored, completely pigmented.

When examining the location of the udder, I classified the teats with a score of 1-9 based on their location on the udder. The teat on the outside of the udder got a score of 1 while the teat on the inside of the udder had a score of 9.

When determining the ratio of the teat, I divided the length of the teat into the base width of the teat.

For statistical evaluation of the results I used Spearman and Pearson rank correlation calculation method.

2.2.4. Comparison of individual teat parameters

In this part of my study, the four teat parameters were compared for each lactation group with respect to the values measured during milking and the calculated proportions. For this comparison I used the Spearman and Pearson rank correlation method.

2.3. Examination of the dry period of healthy cows

2.3.1. Examining the change in size of the teat parameters during the dry period

In this study, I studied the size changes of cow's teats during the dry period. To do this, I measured the teats five times. The first measurement was done on the 222nd day of gestation, the next three measurements were done on the 229th, 250th and 271th gestation days, while the 5th measurement was done the day after the calving. The first and last measurements were made before the morning milking.

In the course of the statistical analysis, after each Bonferroni correction was made for each teat parameter, all the results obtained at the five measurement times were compared with each result with a Paired t-test.

2.3.2. Analyzing the relationship between the teat parameters measured during the dry period and the relationship between some of the cow's production and teat properties

In this study, what is the relationship between the changes in the teat parameters during the dry period and the cows' production and teat properties. With the integral calculation, the area under the curve linking the values measured at the five test times for each teat parameter was calculated.

To correlate the cows' production characteristics (lactation number, number of lactation days, number of days of gestation, and morning and daily milk yield), the arithmetic mean of the cow's calves' integral of each cow was used. In order to correlate the own properties of the teats (the pigmentation, the location of the udder and the proportion of the teat), I used the integrals of the individual teats separately.

Due to the different length of pregnancy of the examined cows, during the calculation of the integral of the individual teat parameters, I corrected the last values measured at the time of calving to 280 days of gestation.

For statistical evaluation of the results I used Spearman and Pearson rank correlation calculation method.

2.4. Examination of healthy heifers in the last weeks of gestation

2.4.1. Teat parameters are a measure of the size change in the last weeks of pregnancy

In this study, I analyzed the size changes in the last weeks of gestation in the teat of pregnant heifers. Measurements were made on days 250 and 271 of gestation and on the morning of the day after calving.

In the course of the statistical analysis, after each Bonferroni correction was made for each teat parameter, all the results obtained at the three measurement times were compared with each result by the Paired t-test.

2.4.2. Analyzing the relationship between the teat parameters in the last weeks of gestation and some properties of heifers' production and the teat

In this section, I investigate the relationship between the changes in the teat parameters during the last weeks of pregnancy and the characteristics of the heifers' production and the teats. With the integral calculation, the area below the curve measured at the three test times was calculated for each teat parameter.

For the correlation of heifers' production characteristics (morning and daily milk yield) I used the arithmetic mean of the examined cow's teats. In order to correlate the own properties of the teats (the pigmentation, the location of the udder and the proportion of the teat), I used the integrals of the individual teats separately.

Due to the different gestation lengths of the examined heifers, during the calculation of the integral of the individual teat parameters, the last measured values were corrected to 280 days of gestation.

For statistical evaluation of the results I used Spearman and Pearson rank correlation calculation method.

2.5. Comparison of examination of cows and heifers during the last weeks of pregnancy

In this study, the cows and heifers compared the 250 day, 271 day, and the day of after calving values. To do this, I used the Levene test and two-sample t-test. Changes in the individual teat parameters were measured by the average of the four teats.

In addition to the ultrasound values, I compared the integrator parameters of the two groups between 250 and 271 and 271 and 280 days. For this, I also used the Levene test and two-sample t-test.

2.6. Examination of teat infections of udder and healthy udder quarters

In the study of mastitis animals, cows were selected by examining this first effervescent beam with the California Mastitis Test for morning milking. In the case of a positive result, the rate of mastitis was marked with 1, 2 or 3 crosses and in these cows I performed an ultrasound examination of the teat. It is important that the milk of the test animals did not show a positive reaction at the milking before the examination. So all of the cows I tested were fresh mastitis.

After finding the udder infection, I measured the parameters of the udder of the inflammatory udder quadrant and the adjacent healthy neighboring teat by ultrasound. Measurements were made before milking, immediately after milking and 2 hours after milking.

Parental parameters of the sick and healthy udder quarters were compared with a Paired t-test.

Based on the mastitis test, Levene test and Two-sample t-test were used to compare the values and ratios of the udder quarters 2 + and 3+.

2.7. Examination of the teats during mastitis and after healing

In this study, the teats were measured by ultrasound when diagnosing mastitis and when the mastitis test again showed negative results. The comparative test was performed with a Paired t-test.

I also compared the values and ratios of the teat parameters measured after the healing of the 2+ and 3+ udder quarters, for which I used the Levene test and the Two-sample t-test.

3. RESULTS

3.1 Examination of teats of healthy cows

In this part of my research, I examined how the dimensions of the teats of the healthy cows and the dimensional changes caused by milking change as the lactation days progress.

3.1.1. Examination of the streak channel

In pre-milking measurements, it was observed that the multiparous cows had the shortest streak canal ($P < 0.05$). As the lactation days increased, the length of the streak canal increased ($P < 0.05$). Primiparous cows had a significantly lower ($P < 0.05$) length of streak canal than the multiparous cows.

As a result of milking, the length of the streak canal increased significantly ($P < 0.05$) in all five groups. In cows, the length of streak canal increased significantly ($P < 0.05$) as the milking days progressed. The increase in the length of the streak canal of the primiparous cows was significantly ($P < 0.05$) lower than that of the multiparous cows.

In the 2-hour measurement, the length of streak canal increased significantly ($P < 0.05$) by 3% in the 100-150 lactation day group and 5% in the group of primiparous cows. In the 290-358 lactation day group, the post-milking device does not change the size of the streak canal within 2 hours.

At the time of calving, the 6% reduction was so small that the length of the streak canal of the multiparous cows was still significantly ($P < 0.05$) 22% higher than the pre-milking value. As a result of the 4% reduction in the group of the cows at the time of drying, the length of the streak canal was only 2% higher at baseline, so the difference between the two values was no significant ($P > 0.05$).

In the two hours after milking, the cows that were multiparous cows and dipped were significantly shorter ($P < 0.05$) compared to the other two lactation groups in the cows tested. At the same time, primiparous cows' canal length was significantly lower ($P < 0.05$) compared to all cows.

As a result of milking, the length of the streak canal increases significantly ($P < 0.05$) at all lactation groups in cows. In the 2 hours after milking, the size of the streak canal only in the group of the cows at the time of drying can be restored to the extent that it does not differ significantly ($P < 0.05$) from the pre-milking values.

3.1.2. Analyze of size change of the area of pars papillaris

The amount of morning and daily milk in cows decreases ($P < 0.05$) as the lactation progresses. In this context, the size of the area of pars papillaris the cows before milking decreases significantly ($P < 0.05$). The pars papillaris area of multiparous cows were ($P < 0.05$) higher than that of primiparous cows.

The pars papillaris area significantly decreased ($P < 0.05$) in all lactation groups of cows and in the case of heifers. The magnitude of the decrease in size was significant ($P < 0.05$) with increasing lactation days. There was no significant difference ($P > 0.05$) between the fresh calved cows 42% and the heifers 41% milking effect.

Immediately after milking, the pars papillary area of the cows at the time of drying did not differ significantly ($P < 0.05$) from the multiparous cows. The area of pars papillaris of the primiparous cows was significantly lower ($P < 0.05$) from the multiparous cow.

After two hours, the pars papillaris area of each of the five study groups increased. However, the 2-hour rate of cows that were lactating for more than 150 days was significantly

lower ($P < 0.05$) than cows that were lactating for less than 150 days. Thus, in the second half of lactation, the pars papillaris area of the cows is significantly ($P < 0.05$) more effective in returning to the baseline. The pars papillaris area of the primiparous cows also increases in 2 hours, but is still 35% lower than the pre-milking value. The 35% 2-hour rate of heifers does not differ significantly ($P > 0.05$) from the 32% 2-hour ratio of multiparous cows.

Two hours after milking showed that pars papillaris area increased in all groups, but for each group, the 2-hour measured values were still significantly lower ($P < 0.05$) in pre-milking.

3.1.3. Analyze of the size change in the area of the teat end

In the multiparous cows I measured significantly ($P < 0.05$) the smallest ($P < 0.05$) area, which increased ($P < 0.05$) up as the number of lactation days increased. Primiparous cows had a ($P < 0.05$) smaller area of the teat end before milking than the multiparous cows.

The area of teat end was significantly increased ($P < 0.05$) in all examined groups by milking. The extent of area-induced milking increased significantly ($P < 0.05$) as the lactation days progressed. The 21% area growth of primiparous cows was significantly ($P < 0.05$) lower than the 38% increase in multiparous cows.

The area of the primiparous cows after milking was significantly lower ($P < 0.05$) in the multiparous cows.

Only the cows at the time of drying diminished the area of teat end within 2 hours so that it was no significantly ($P > 0.05$) higher than before milking. The area of teat end of the primiparous cows increased by an additional 1% over 2 hours.

Based on the two-hour measurement results, there was no significant difference ($P>0.05$) between the area of the multiparous cows and the group of the cows at the time of drying, and the area of these two groups was significantly ($P<0.05$) smaller in the other two groups of cows. Two. The promiparous cows had significantly ($P<0.05$) smaller than all groups of cows.

3.1.4. Analyze of the size change of the area of 1 cm end of the teat

The measurement before milking the area of 1 cm end of the teat of the cows at the time of drying was significantly larger ($P<0.05$) than cows with less 150 lactation days. The size of the area measured in the heifers was significantly ($P<0.05$) smaller than all lactation groups in the cows.

As a result of milking, the area of 1 cm area of the end of the teats were increased ($P<0.05$) in all groups. Thus, the size of the area increased significantly ($P<0.05$) with increasing number of lactation days. The 1 cm area of the promiparous cows grew significantly ($P<0.05$) a lesser extent than the multiparous cows.

Within two hours after milking, the 1 cm area of the teats of the cows of the 100-150-day lactation group increased by 1%, while the size of the cows in the other groups decreased. Only in the cows at the time of drying and promiparous cows did I see an area depletion of less than two hours that did not differ significantly ($P>0.05$) from the pre-milking value.

The 2-hour measurement value of promiparous cows was significantly lower ($P<0.05$) than in multiparous cows.

3.1.5. Analyze the relationship between the cows' teat parameters and the production characteristics of the cows

The lactation number

In only one of the 60 correlations studied, I found a positive middle strong ($r=0.44$) correlation in the 1 cm end of the teats of the group of 290-358 lactation day before milking. In the other 59 cases I found a very weak ($r < 0.3$) or weak ($r < 0.4$) correlation between the teat parameters and the lactation number.

Number of days of gestation and lactation days

Ultrasound-tested teat parameters are very weak ($r < 0.3$) related to the gestation status of the animals and the number of lactation days.

The amount of milk in the morning

In the correlation study of the morning milk quantity, in 90 cases out of the 100 correlations, I received only very weak ($r < 0.3$) and weak ($r < 0.4$) results. In ten cases I got a non-tendentious moderately strong ($r=0.42-0.52$) result.

Amount of daily milk

In the daily milk correlation study, 92% experienced very poor ($r < 0.3$) or weak ($r < 0.4$) results. Moderately strong ($r < 0.6$) measured for the remaining 8% showed no tendency.

3.1.6. Analyze the relationship between the teat parameters of animals and some characteristics of teat

During my studies I found that the length of the streak canal, the area of teat end and the 1 cm end of the teat measured values during milking and the calculated ratios are very weak ($r < 0.3$) or weak ($r < 0.4$) correlated with the pigmentation of the teat, the teat location on the udder and the ratio of the teat. In the pars papillaris area, I found a moderately strong ($r = 0.41-0.44$) correlation with the properties of the teat examined in my case, but their occurrence did not show any tendency.

3.1.7. Analyze the relationship between the individual teat parameters

When examining the relationship between the individual teat parameters, I found that in each lactation group, the length of the streak canal, the area of teat end and the area of 1 cm end of the teat were positive strong ($r > 0.6$) or very strong ($r > 0.8$) correlation in the measured values during milking and the calculated ratios. Based on this, these three teat parameters form an anatomical unit and their size changes uniformly as a result of milking.

In contrast, the distal 1 cm area of pars papillaris of the 88% correlation showed only very weak ($r < 0.3$) or weak ($r < 0.4$) correlation with the other three teat parameters. In the remaining 12% I received only a moderately strong ($r = -0.4-0.58$) non-tendency.

3.2. Examination of the teats of healthy cows during the dry period

3.2.1. Changes in cow's teats' parameters during dry periods

Length of the streak canal

Compared to the last milking before the dry milking, the length of the streak canal was shortened from 10.8 mm to 9.9 mm in one week, which was 8.3% significant ($P < 0.05$). At the 250 day of gestation, I found that the length of the streak canal did not increase significantly ($P > 0.05$) by 4% compared to the 229th day. At 271 days, there was a significant ($P < 0.05$) decrease of 5.8% in length compared to the 250th day. The values measured after calving showed a further non-significant reduction ($P > 0.05$) of 4.1% in the last week of pregnancy.

Overall, the length of the streak canal was reduced ($P < 0.05$) by 13.9% during the whole dry period.

The distal 1 cm area of pars papillaris

Pars papillaris area increased significantly ($P < 0.05$) by 9.1% in one week, then changed direction after three weeks and significantly decreased by 0.13 cm² ($P < 0.05$) by 15.5%. After another three weeks, I found that the pars papillaris area was not significantly ($P > 0.05$), but increased by 7%. This increase in size increased significantly ($P < 0.05$) up to 0.24 cm² until after calving.

Overall, the significantly higher ($P < 0.05$) milk produced by fresh calved cows expands the cavity of the pars papillaris by 30% more than the cows at the time of drying.

The area of teat end

Within one week after the last milking, the area of teat end decreased significantly ($P < 0.05$) by 14.6%. Then its size increased by 6.2% over the next three weeks, but this increase

was not significant ($P>0.05$). Compared to the 250 day of gestation measurement, the area was not significantly reduced ($P>0.05$) by 3.6% to 271 day of gestation. Subsequently, until the post-calving measurement, the area of teat end was not significantly ($P>0.05$) but further reduced by 3%. The final result of the measurement of the teat end was that the value measured at the time of drying decreased significantly ($P<0.05$) by 15.2% during the dry period.

The area of 1 cm end of the teat

The area of the 1 cm end of the teat was significantly reduced ($P<0.05$) by 9.5% in the first week of dry period by 0.22 cm². Then, from this time of measurement to the calving, the size of the area gradually increased. This increase was not significant ($P>0.05$), between day 229 and day 250 (1.2%) and between day 250 and day 271 (1.8%), while until calving, growth was accelerated and significant ($P<0.05$). The area of the 1 cm end of the teat was significantly reduced ($P<0.05$) by 3.3% during the dry period.

3.2.2. The relationship between integrates of the teat parameters during dry period and some of the cow's production and teat properties

The production and teat properties I have studied are very weak ($r<3$) or weak ($r<4$) correlated with the integral of the ultrasound-measured teat parameters during dry period. So, it is not possible to deduce from these properties the extent of the change in size of the teat parameters studied by me during the dry period.

3.3. Examination of healthy heifers in the last weeks of gestation

3.3.1. Changes in teat parameters of heifers during the last weeks of gestation

Length of the streak canal

A non-significant ($P < 0.05$) increase in length of 3.2% was observed between 250th and 271st day of gestation. Then, at calving, I found a significant 15.5% streak canal length reduction ($P < 0.05$) compared to the 271 day result. Compared to the 250 day value, the calving value was significantly lower ($P < 0.05$) by 12.8%.

The distal 1 cm area of pars papillaris

Significant ($P < 0.05$) 27.1% increase in size between the first two measurements was 0.13 cm². Then, a further 36.1% significant ($P < 0.05$) increase in area was found until calving.

The area of teat end

Significant ($P < 0.05$) increase of 0.15 cm² was observed between the first two dates ($P < 0.05$). Then, compared to the value measured on day 271, the area of teat end decreased significantly ($P < 0.05$) by 15.6% until calving. When I compared at day 250, and at the time of calving, I found that the area was reduced non significantly ($P > 0.05$) by 3.7% between the two times.

The area of 1 cm end of the teat

Between days 250 and 271, the 1 cm area of the heifers increased significantly ($P < 0.05$) by 13.2% and then decreased by 1% ($P > 0.05$) until calving. As a result, there was a significant

($P < 0.05$) difference of 12.1% between the results of the 250th day of gestation and postpartum measurements.

3.3.2 Integration of the teat parameters during the last weeks of gestation and the relationship between some of the properties of animal production and teats in heifers

My studies showed that only the integral of the length of the streak canal and the area of 1 cm end of the teat was in the positive moderately strong ($r = 0.41$ and $r = 0.44$) in relation to daily milk production. There was a very weak ($r < 0.3$) or weak ($r < 0.4$) correlation between the pigmentation of the teat, its position on the udder and the ratio of the teat.

3.4. Comparing healthy cows and heifers to teat parameters in the last weeks of gestation

3.4.1. Comparison of teat parameter changes in healthy cows and heifers in the last weeks of gestation

All four teat parameters of cows at day 250th and day after calving were significantly higher ($P < 0.05$) at heifers.

Length of the streak canal

In the 271st day, the cows showed a significant ($P < 0.05$) length decrease of 5.8% compared to the 250th day, and a non significant ($P > 0.05$) increase of 3.2% in the heifers. On day 271, the size of the two groups was so close to each other that the difference between them was not significant ($P > 0.05$). Until calving, the cows' streak canal length was not significantly ($P > 0.05$), but was further reduced by 4.1%. In the heifers, the direction of the change in size changed and showed a significant ($P < 0.05$) decrease of 15.5% compared to day 271. In the last

month of gestation, the length of streak canal decreased significantly ($P < 0,05$) by 9.7% in cows and 12.8% in heifers.

The distal 1 cm area of pars papillaris

This teat parameter increased in both groups between the day 250 and day 271 – it was a 7% non significant ($P > 0,05$) for the cows and 27.1% significant ($P > 0,05$) for the heifers. On day 271, compared to the two groups, the pars papillaris area of the heifers was significantly smaller ($P < 0,05$). Until calving, the size of both groups (cows: 31.6%, heifers: 36.1%) increased significantly ($P < 0,05$) and there was still a significant difference ($P < 0,05$) between the groups at the time of calving. During the whole period the pars papillaris area increased significantly ($P < 0,05$) by 40,9% in cows and 72,9% in heifers.

The area of teat end

The cows did not have a significant ($P > 0,05$) decrease of 3.7%, while in the heifers the size of the area increased significantly ($P < 0,05$) by 14% between days 250 and 271. Thus, on day 271 the dimensions of the two groups approached each other to such an extent that the difference of 0.1 cm² between them was not significant ($P > 0,05$). Then the area of both groups became smaller until calving, the heifers significantly ($P < 0,05$) by 15.6%, but the cows were not significant ($P > 0,05$) by 3%. Between the 250th day of gestation and the time of calving, the area of the cows was significantly reduced by 6.6% ($P < 0,05$), but the area of the heifers was not significant ($P > 0,05$) by only 3.7%.

The area of 1 cm end of the teat

From the 250th to the 271st day, the area of 1 cm end of the teat in the cows increased not significantly ($P > 0,05$) by 1.8%, and significantly ($P < 0,05$) in the heifers by 13.2%. On day 271 there was a significant ($P < 0,05$) size difference between the values of the two groups. Subsequently, the cow area increased significantly ($P < 0,05$) by 3.8% until calving. The heifers,

however, did not decrease significantly ($P>0.05$) by 1%. There was a significant difference ($P<0.05$) between the two groups at the time of calving. The area of 1 cm end of the teat grew by 5.6% in the cows and 12.1% in the heifers during the whole period examined.

3.4.2 Integration of the teat parameters during the last weeks of gestation and the relationship between the cows and heifers

I examined the integral of the teats parameters between days 250 and 271 and between day 271 and day 280. And these periods were compared separately between the two groups. On the basis of the examinations, for both of the two periods, I got significantly higher values ($P<0.05$) in the group of cows.

3.5. Comparative examination of teat parameters of the teat with mastitis and healthy teat next to it

3.5.1. Comparison of sick and healthy udder quarters

The results of my study show that there are no significant ($P<0.05$) differences in the values and ratios of the teat parameters of the sick udder quarters and the adjacent healthy udder quarters.

As the results of the examination of the sick cows, I performed the comparison of the individual teats in the case of healthy cows. I have examined whether there is a significant difference between the individual teats in the case of healthy cows. In this study I compared the right and left teats with the front and separate hindquarters in healthy cows. This was done in all five healthy groups. The result was that there was no significant difference ($P>0.05$) between the teat parameters of the right and left teats in all groups of healthy animals.

3.5.2. Comparison of sick udder quarters parameters and ratio of patient's own quarter by degree of mastitis

On the basis of the mastitis test, the values and proportions of the udder quarters 2+ and 3+ were compared. There was no significant difference ($P>0.05$) between any of the two udder inflammation parameters in either of the udder infections. This shows that the extent of mastitis does not affect the size of the teats and the change in size at the time of milking.

3.6. Comparative study of the teat parameters of udder quarter infections at the time of the disease and after its recovery

3.6.1. Comparison of sick and healed udder quarters with respect to teat parameter values and ratios at milking

In the distal 1 cm area of pars papillaris, I found a significant difference ($P<0.05$) at pre-milking. In the case of the disease, the teats had an average of 11.7% higher than after the healing. In all other cases, there was no significant difference ($P>0.05$) between the two studies.

3.6.2. Comparison of values and ratio of teat parameters of healed udder quarters by mastitis

I compared the values and ratios of the teat parameters measured after the healing of the udder quarters 2+ and 3+. After healing, the pars papillary pre-milking and 2-hour measurements showed that the distal 1 cm area of pars papillaris was significantly ($P<0.05$) smaller in the udder quarters that had a inflammation of 2+ degree. For all other measurements, I found no significant difference ($P>0.05$) between the two mastitis levels.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1. Examining the change in size of the teat parameters of healthy animals as a result of milking

Anti-infectivity through the streak canal is protected by the keratin plug in the streak canal and the sphincter muscle surrounding the streak canal (Húth, 2004, Paulrud, 2005). As a result of milking, the streak canal opens up the possibility of pathogens entering the milk canals. The animal organization is trying to restore this natural protection system as soon as possible. In this part of my study, I studied how the parameters of the teats of animals at different stages of lactation change during milking and how they can restore this protection system.

During my study, I found that the length of streak canal, the area of teat end and the the area of 1 cm end of the teat increased significantly ($P < 0.05$) as the lactation days progress by twice daily milking. In all lactation groups, the size of these three teat parameters increased significantly ($P < 0.05$) due to milking. The extent of size increase was significantly reduced ($P < 0.05$) as the lactation days progressed. Through milking opened and elongated, the pathogens can easily enter the udder through the streak canal.

This is confirmed by Húth's (2004) studies, according to which the increase in the length of the streak canal can increase the risk of mastitis, which is supported by the rising somatic cell count. My studies showed that after two hours, only the teat parameters of the cows at the end of lactation had developed to such an extent that it did not differ significantly ($P > 0.05$) from the pre-milking value. So, in the case of cows in the previous stages of lactation, two hours was not enough for the regeneration of the teat defense system. The development of mastitis is influenced by several factors, but the possibility of infection through the streak canal is lower in cows at the end of lactation than in cows in the early stages of lactation. This fact

draws attention to the fact that the disinfection of post-milking teats is of great importance at all stages of lactation, but especially at the beginning of lactation.

In the study of lactation groups, I found that only the teat parameters of the animals at the end of lactation can return to baseline within two hours after milking. Based on the above relationships, I suggest that we measure how quickly the teats can regenerate after milking at certain stages of lactation. In addition, the ultrasonic examination of the teat parameters is incorporated into the workflow of the battery so that it can monitor the current state of the teat defenses in the flock with regular checks.

The area of pars papillaris measured before milking decreased significantly ($P < 0.05$) with increasing number of lactation days; As a result of milking, pars papillaris area is significantly reduced ($P < 0.05$) in all lactation groups. Significantly ($P < 0.05$) less milk producing cows had a significantly lower ($P < 0.05$) area of pars papillaris during milking. In all lactation groups, it was observed that two hours after milking did not produce enough milk to dilute the pars papillaris so that its two-hour size was still significantly lower ($P < 0.05$) than before milking.

All four teat parameters of the heifers were significantly ($P < 0.05$) lower than the cows at the same lactation stage at all time points. However, the size changes experienced during milking no longer show such a uniform picture. As a result of milking, the heifers and the fresh calved cows' length of streak canal, the area of teat end and the area of 1 cm end of the teat increased significantly ($P < 0.05$), and this increase in size was significantly ($P < 0.05$) higher in cows than in the heifers. In the case of deviations of these three teat parameters from the pre-milking values two hours after milking, the cows and heifers did not differ significantly ($P > 0.05$). The size of the change in size of the pars papillaris area was not significantly different ($P > 0.05$)

between the fresh calved cows and the heifers. Despite the fact that the cows have significantly ($P < 0.05$) 6.2 liters more average daily milk production.

In the study of healthy animals, the values of each lactation group and the extent of the change in size were compared. During my research, I have found that we can infer the state of the defenses of the udder, the change in size during milking and the extent of regeneration from the extent of the size changes. I suggest that when examining the size changes of any of the teat parameters, do not investigate how many mm or cm^2 of the given parameter have been changed, but examine how much this change was.

4.2. Correlation study of the animal's own production and own properties of the teats

In the correlation study conducted for each lactation group, only 1.2% had non-tendentious moderate ($r > 0.4$) correlations. In the vast majority of studies, there was only a very weak ($r < 0.3$) or weak ($r < 0.4$) correlation between the lactation number of the animals, the number of days of gestation, the number of days of lactation, the amount of morning and the daily amount of milk and between the values and proportions of teat parameters measured by ultrasound during milking. Similar results were very poor ($r < 0.3$) or weak ($r < 0.4$) for pigmentation, positioning on the udder, and the ratio of the teat. Thus, it can be said that these properties of the production and the teats do not affect the dimensions of the teats and their changes in size during milking. Changes in the teat's defense system during milking are independent of the above-mentioned properties.

4.3. The relationship between the individual teat parameters in the different lactation groups

Three teat parameters are the length of the streak canal, the area of teat end and the area of 1 cm end of the teat are strong ($r > 0.6$) or very strong ($r > 0.8$) to each other changes in size. In the 88% of cases, the area of pars papillaris was very weak ($r < 0.3$) or weak ($r < 0.4$) in relation to the other three parameters of the teat. I have determined that the function of the defense system at the end of the teats is not influenced by the size and size of the pars papillaris area.

4.4. Examination of healthy cows during dry period

The results of ultrasound examination of the teat parameters of healthy cows show that by the time of lactation twice the length of the streak canal and the area of teat end were increased significantly ($P < 0.05$) however the distal 1 cm area of pars papillaris significantly decreased ($P < 0.05$).

Early stage of dry period

The chance of developing new mastitis is the highest in the first 1-2 weeks of dry period (Hurley, 2010). In the early stages of dry period, a number of factors contribute to the development of mastitis:

- When milking ceases, milk does not leave from the milk canals, and it is still produced in the first 2-4 days of the dry period, and the size the cavity of the udder is further increased.
- In the accumulated milk, bacteria multiply well.
- There is a milk leak through the open streak canal that increases the chance of infection.

My research results show that during the first week of dry period, the teat parameters of healthy cows have undergone significant changes. The length of the streak canal was 8.3%, the area of the teat end decreased by 14.6%, and the area of 1 cm end

of the teat decreased by 9.5%, while the pars papillaris area of grew by 9%. These dimensional changes show that the streak canal and the area of teat end which are part of the udder defense system significantly regenerated during the first week of dry period in a healthy cow, thus minimizing the possibility of contamination through the streak canal.

This is confirmed by a previous study by Húth (2004), according to which the risk of developing mastitis increases with the increase in the length of the streak canal. Thus, udder infections occurring during the first week of dry period significantly increase the risk of infection through the streak canal if the length of the streak canal does not shorten significantly during this time ($P < 0.05$). Therefore, effective antibiotic therapy after the last milking prior to dry-setting is important in preventing the development of mastitis in the early involution of the udder. Milk production is still taking place until the first 2-4 days of dry period (Hurley, 2010), and because milk ceases, milk produced is not removed from the milk canals, so the retained milk expands the milk canals, including the the distal 1 cm area of pars papillaris. In the first week of dry period, the size of the the distal 1 cm area of pars papillaris is significantly increased ($P < 0.05$).

The middle section of dry period

In the middle of the dry period (about 25 days), the involution of the udder is completed (Capuco et al. 1999). At this stage of dry period, the risk of developing mastitis is minimized by a number of factors:

- The streak canal is closed at this time and no milk leak is observed.
- The amount of milk in the cavity of the udder is significantly reduced, so the size of the cavity system is reduced to 9.5% of the values measured in the lactating cow.
- The composition of the fluid present in the cavity system of the udder is less conducive to the growth of pathogens.

The streak canal and the teat end which are part of the defensive system of the teat showed no significant ($P>0.05$) size increase (4 and 6.2%) from the first week of dry period to the end of the involution of the udder. Thus, in the case of healthy cows, the regeneration of these two teat parameters occurs early in the involution. The distal 1 cm area of pars papillaris became significantly ($P<0.05$) 15.5% lower in this period of dry period. In the second half of the involution, macrophage activity is increased in the udder, during which the remnants of milk are removed from the milk canals (Hurley, 2010). My test result is Capuco et al. (1999) also confirms that the cavity system of the udder decreases to 9.5% of the value measured during lactation by the end of the involution.

The final stage of dry period

In the pre-calving period, especially when colostrum production begins, the risk of udder infection increases again (Hurley, 2010). This danger is caused by several factors:

- In the udder, the production of colostrum gets bigger, which increases the size of the milk.
- Periodic removal of milk from milk canals to calving does not begin.
- As the pressure increases in the udder, the milk flow starts through the streak canal.

In the final stage of dry period, when the udder prepares for the next lactation, the length of the streak canal passage is further reduced by 9.9%, while the area of the teat end decreases by 6.6% ($P<0.05$). In healthy cows, the risk of infection through the streak canal and the risk of mastitis thus formed is minimized at the end of the dry period by significantly reducing the length of the streak canal ($P<0.05$). As the milk yields the milk out of the milk canals (Hurley, 2010), the distal 1 cm area of pars papillaris grew from the middle of dry period, and this increase was very high at 31.6% a week before calving.

The streak canal and the teat end which are part of defensive system of the udder can regenerate adequately during the dry period, so the possibility of infection through the streak canal and the risk of mastitis is minimal.

On the basis of the above, I suggest that ultrasound examination on the farms should be continuously monitored the teat parameters during dry period. With this method you can easily get information on whether the teats are sufficiently regenerated during dry period. If the regeneration is less or fail, then the cause should be discovered as soon as possible.

Considering that the integration of the teats parameters is very weak ($r < 0.3$) or weak ($r < 0.4$) correlates with the cow's production and the teats' own properties, it is not possible to deduce from this data the regeneration of the teats.

4.5. Examination of healthy heifers in the last weeks of pregnancy

Pars papillars area gradually increased significantly ($P < 0.05$) from the 250th day of gestation to the time of calving due to elongation and milk production. However, the area of 1 cm end of the teat, with a significant ($P < 0.05$) increase in size between 250 and 271 days, practically reached the full size at the beginning of the first lactation by day 271 of gestation.

The length of the streak canal is only significantly reduced ($P < 0.05$) by 15.5% in the last week of pregnancy.

However, the change in size of the area of teat end shows that in the last month of pregnancy, the size of the area increases by 14% in the first three weeks, and this area decreases by 15.6% in a week from 271 days. Thus, after the calving, the area of the teat end returned to the value measured on day 250.

The streak canal changed its size between days 250 and 271 of gestation irrespective of the teat end. In the last month of gestation, I suggest to investigate the cause of the

change in size at the teat end of the heifers with Doppler ultrasound with more detailed histological sampling.

The amount of milk produced after calving and the properties of the teats are not relevant to the integration of the four teat parameters in the last weeks of pregnancy. So it is not possible to infer from these properties to what extent the teat has changed in the last weeks of pregnancy.

4.6. Comparison of changes in cows 'and heifers' teat parameters in the last weeks of gestation

When sexual maturity reaches, the udder of heifers start to grow and develop. This growth is intensified during pregnancy (Nishimura et al. 2010). In the last month of gestation, the rate of growth of the udder is further increased due to milk production (Ford et al. 1999). However, the size of the udder of the cows increased during the previous lactations, and in the first half of the dry period the size of the udder during the involution is somewhat reduced, but the growth is again observed.

During the last month of gestation, the following changes were made in the size change of the teat parameters during the treatment of heifers and cows:

- By the fact that the cows' milk canals have already expanded during previous lactation, their pars papillaris area was significantly ($P < 0.05$) higher than that of the heifers during the entire period of grafting. However, the extent of the change in size was just about the same between the two groups, just one week before the calving. Cows increased by 31.6% and heifers by 36%. In the early stages of grafting, the 27% increase in heifers was significantly ($P < 0.05$) higher than the 7% increase in cows. This result shows that in cows only in the week before calving, the area of the pars papillaris grows significantly ($P < 0.05$), while in the case of heifers this significant ($P < 0.05$) increase in size. can be observed throughout the entire period. This can be explained by the fact that the udder of

the heifers are still developing, so the increase in size due to the development of the udder is also added to the increase in size caused by milk production.

- The area of 1 cm end of the teat in the cows did not show any significant increase in size in the second half of the dry period. In contrast, the area of 1 cm end of the teat of the heifers increased significantly ($P < 0.05$) to 13.2% by the 271th gestation day and did not show any significant change thereafter. The increase in the size of the teat of the heifers depends on the fact that their udder shows significant growth during this period of pregnancy.
- The length of the streak canal and the area of the teat end of the cow in the last month of gestation showed a continuous decrease in size. I recommend the Doppler ultrasonic examination of these two teat parameters with histological sampling to investigate the size changes in heifers in the length of the streak canal and the end of the teat.
- In the last weeks of pregnancy, the cows' integration was significantly ($P < 0.05$) higher in all four teat parameters than in the heifers. This is because the cows' teats have grown during previous lactations, while the teats of the heifers are still developing.

4.7. Comparison of sick and healthy udder quarters with respect to teat parameter values and ratios at milking

In the course of studying the literature, I have found that the tests of the teat parameters of the healthy and the sick udder have shown different results. There were authors who found no significant difference ($P > 0.05$) between the length of the streak canal between the sick and the healthy udder quarters (Hamana et al. 1993). While Klein et al. (2005) found significant ($P < 0.001$) longer streak canal in healthy udder quarters than in the sick quarter. These authors compared the udder quarters of sick cows with udder quarters of healthy cows. In contrast, I compared the animal's healthy and sick udder quarters.

I started my study to see if there are differences between the different lactation groups of healthy animals between the left and right teats parameters, separately for the front and separately for the hindquarters. The result obtained showed that there was no significant difference ($P>0.05$) between the udder quarters of the two sides. The results of healthy cows are also supported by the results of other authors who also found no significant difference ($P>0.05$) between the udder quarters of healthy cows with regard to the length of the streak canal, the width of the teat end, the thickness of the teat wall and the diameter of the pars papillaris (Weiss et al. 2004, Celic et al., 2008, Stojnovič et al., 2012). Thus, it can be stated that the size and size changes of the teat of udder quarters of healthy cows do not differ significantly ($P>0.05$).

After examining the udder quarters of healthy cows, I also tested the mastitis cows. Before examining the sick cows, I expected that the sick udder quadrant would be more sensitive, painful and, as a result of milking, the size change of the teat would deviate from the adjacent healthy udder quarters. In contrast to the expected results, I found that the sick and healthy udder quadrant had no significant difference ($P>0.05$). I found that the teat parameters of the udder quadrant did not differ from those of the neighboring udder quarters as in the case of healthy cows.

On the day before the mastitis, the amount of milk produced significantly decreased by 48.7% ($P<0.05$) due to mastitis. Thus, the inflammation of an udder quarters not only reduces the milk production of the affected udder quarters, but also reduces the milk production of the other three udder quarters. Before the ultrasound examination, the udder was physically examined and in all these studies I found that the sick udder quarters had a warmer, more painful tact. The cow was touched by hand, and the cow showed pain, kicked to my hand. The sick teat had a tighter, tighter tactile than the healthy.

From the above, I have determined that the physical examination of the teat makes it clear that the teat of the udder quarters is more sensitive and painful. Thus, inflammation of the gland of the udder makes the teat more sensitive not only to manual touch, but also to the mechanical effect caused by the vacuum of the milking machine. As a result of the pain caused by the inflammation, not only the parameters of the sick teats change, but also the same changes in the parameters of the neighboring teats. Therefore, there are no significant ($P>0.05$) differences between the teat parameters of the sick and the healthy udder quarters.

No significant ($P<0.05$) difference was found between the 2+ and 3+ degree of the mastitis in the teat parameters of udder quarters. With this in mind, I have found that the degree of inflammation in the udder quarter does not affect the size and size changes at the time of milking.

4.8. Comparison of teat parameters measured in the presence of mastitis and after healing of mastitis

After curing, cows produced 79.6% ($P<0.05$) more milk than patients. Knowing the milk production data, I expected that the significantly ($P<0.05$) increased milk production would result in a greater area of pars papillaris in the cows. In contrast, it was observed that when the disease occurred, the area of pars papillaris was significantly ($P<0.05$) larger than after healing. This is due to the fact that milk is coagulating in the mammary glands, so in both parts of the cisterna lactis during mastitis, and this coagulated milk extends the pars papillaris better in the presence of inflammation than the higher milk quantity after healing.

Examining the degree of mastitis is a definite finding of the disease the teat parameters were not significant ($P>0.05$)

different between the 2+ and 3+ degree of mastitis. However, after the mastitis was healed, the distal 1 cm area of pars papillaris of the 2+ cows was significantly lower ($P<0.05$) than the 3+ cows before the milking and 2 hours after milking. In the light of these results, I found that after the healing of more severe mastitis, the pars papillaris area before milking is less diminished than for mild mastitis. Thus, regeneration of this teat parameter occurs more slowly after healing of mastitis. In addition, the severity of mastitis also affects the regeneration of dimensional changes caused by milking, so that in the more severe mastitis cured quarters, the pars papillaris area can return to its original value to a lesser extent ($P<0.05$) two hours after milking than cows with milder mastitis.

The size and size change of the streak canal and teat end were not affected by neither the presence of mastitis or its healing.

5. NEW SCIENTIFIC RESULTS

1. I proved that the length of the teat canal, the area of the teat end and the 1 cm area of the teat after milking increase more in the case of multiparous cows ($P < 0.05$) than in the primiparous cows. there was no difference in hourly measurement rates ($P > 0.05$).
2. I first described that there is a very weak ($r < 0.3$) or weak ($r = 0.3-0.4$) correlation between the integrant parameters measured during the dry period and the cows' production and teat properties between.
3. In healthy cows, the length of the teat canal and teat end decreases until day 7 of dryness ($P < 0.05$) and the values easured at that time were not greater ($P > 0.05$) than those measured after calving. Thus, the streak canal and teat end forming the defensive system of the teat are completely regenerated during the first week of dryness.
4. I found that between 250 and 271 days of gestation, the size of the pars papillary of heifers undergoes a larger size change ($P < 0.021$) than the pars papillary of previously lactated cows. However, there was no difference ($P > 0.05$) in the size of the pars papillaries of the two groups in the one week prior to parturition.
5. I proved that the area of the pars papillary after healing was higher at pre-milking and at 2 hours ($P = 0.047$ and $P = 0.024$) in 3+ than at 2+. Thus, after more severe inflammation, the pars papillary is less able to regenerate.

6. PUBLICATIONS RELATED TO THE TOPIC OF THE THESIS

Publications in scientific journals

- T. Tóth, Zs. Abonyi-Tóth, F. Pajor, R. Kocsis, A. Juhász A, J. Tőzsér P. Póti (2019)** Changes in the size of two ultrasound-examined teat parameters during the dry period in dairy cows. *Acta Veterinaria Hungarica* 67 (3), pp. 456–462,
- T. Tóth, Zs. Abonyi-Tóth, R. Kocsis, F. Pajor, P. Póti, J. Tőzsér (2018):** Size changes in some ultrasound examined teat parameters during milking in different lactation stages. *Magyar Állatorvosok Lapja* Vol. 140.11. 663-668 (2018)
- T. Tóth, R. Kocsis, F. Pajor, P. Póti, J. Tőzsér (2018):** Anatomy and ultrasonography of the bovine udder and teat, *AWETH* Vol 14.2 (2018)
- T. Tóth, R. Kocsis, F. Pajor, P. Póti, J. Tőzsér (2018):** Ultrasonography of the mammary gland and teat in cattle A review, *AWETH* Vol 14.2 (2018)
- T. Tóth, P. Póti, J. Tőzsér (2017):** Results of repeated ultrasound measurements of four teat parameters in Holstein-Friesian breed, *AWETH* Vol 13.1

Conference posters

- T. Tóth, J. Tőzsér, F. Pajor, P. Póti (2018)** Evaluation of Holstein-Frisian cows' certain teat parameters at diminution and after calving. XXXVII. Óvár Scientific Days, ISBN 978-615-5837-14-2. p188
- T. Tóth, J. Tőzsér, F. Pajor, P. Póti (2018)** Evaluation of Holstein-Frisian cows' certain teat parameters during milking. XXXVII. Óvár Scientific Days, ISBN 978-615-5837-14-2. p189