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Article

The effect of parity number on the growth performance of piglets around weaning

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ABSTRACT - In this study, authors examined the effects of parity number on piglet weight gain around weaning. The research was carried out on a Hunagrian pig farm on the litters of 3 first parity (P1) and 3 second parity (P2) Landrace x Large White sows. The traits examined were individual weight, weight gain, and number of deaths. A total of 90 piglets were individually weighed 9 times. Thus, measurements were taken at 1 day of age, at 10, 20, 26, 28, 29, 30, 36, and 47 days of age. Weaning occured on the 28th day. The difference in weight can be seen as early as 1 day of age between the piglets of the two groups (290 grams). The 1-day average weight of piglets in the P1 group was 1.20 kg, and the average weight of piglets of P2 sows was 1.49 kg (P <0.05). The observed difference remained until the end of the study, it was 2650 grams at 47 days of age. At this time, the mean weight of the piglets of P1 sows was 12.13 kg, while the average weight of the piglets of P2 sows was 14.78 kg (P <0.05). In terms of weight gains, there was a statistically significant difference between the two groups over four periods, including the post weaning periode. During this period, however, weight loss occurred. The weight of the piglets of P1 sows fell to a greater extent. In their case, the average daily weight loss at that time was 185 g. The other group had a weight loss of 80 g over the same period. Several of the piglets (95.2%) of P1 sows lost weight on the day after weaning while 73.5% of the piglets of P2 sows showed weight loss on this day. Based on the results it can be said that piglets of P1 sows are more sensitive to weaning than piglets of P2 sows. At the last measurement time point, there was no statistically significant difference between the performances of the two groups. There is a moderate association (R^2=0.22) between the weight gains measured in the pre- and post-weaning period, with the weight of individuals gaining more in the pre-weaning period falling to a greater extent after weaning. There was a higher mortality rate among the piglets of the P2 sows. The weak piglets died in the first 4 weeks of the trial except for 1 piglet.

Keywords: weaning, first parity sow, second parity sow, piglet weight gain

INTRODUCTION

In Hungary and Europe the 28-day weaning is common in farm conditions. According to Hungarian legislation, “Piglets may be weaned before the age of 28 days only if the health or welfare of the sow or piglets is endangered.” FVM regulation 32/1999 (III.31.). The main reason for this is to maintain and increase production to reach the weaned pig weight of 200 kg per sow per year. In the case of the 18-23 day weaning, the average weight of the weaned piglets is 6.5 kg, so 30.7 piglets per sow per year are needed to achieve the previously mentioned production target. In the case of weaning at 28-30 days of age, the

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average weaning weight is 7.5 kg to 8 kg, so 25-26.6 piglets per sow are required to reach 200 kg of weaned pig weight per year. The advantage of the previous weaning of 21 days for sows is that it significantly shortens the time required for their re-breeding, thus improving the sow's rotation and increasing the number of fatteners raised by one sow. The disadvantage, however, is that if the weaning is made without taking into account the physiological status of the piglets, the improvement listed above will not be achieved, and the piglets will have difficulty surviving the weaning (*Barceló*, 2009; *Horn et al.*, 2011).

In the week before the weaning, the average daily weight gain is 300 g / day with a 28-day weaning, and this can reduce to 200 g / day as a result of the weaning. Since there is a positive correlation between the growth rate in the first few weeks after the weaning and the subsequent growth intensity, it is important to maintain the growth rate even after the weaning. Research shows that piglets that are heavier at weaning have a higher performance than their lighter contemporaries (*AHDB*, 2010). According to some sources, weaning weight is a much more accurate indicator of post-weaning growth than birth weight or age. However, experiments on the effects of birth weight have shown that piglets born with a body weight of less than 1 kg have very little chance of making it till the weaning, and 86% of individuals born with a weight below 0.8 kg did not survive the weaning (*Gondret et al.*, 2005). For optimal performance in both the rearing and fattening periods, the ideal weaning weight should be between 7.5 and 8.0 kg on average, and not more than 10% of individuals should weigh less than 6 kg (*Gondret et al.*, 2005; *Smith et al.*, 2007).

Feeding at the time of weaning

Enhancing intestinal development, pre-weaning growth, and achieving the best possible weaning weight can be accomplished with supplementary feed, which is initially a milk replacer and then a prestarter. It is important that the composition and nutrient content of the milk replacer be similar to that of sow's milk. The earlier the weaning is made, the more important the quality of the feed offered to the piglets. The milk replacer has a crude protein content of at least 25-26%. The energy component is cereals, but since the starch decomposition of the piglets in the first two weeks is only small, the milk replacer feeds contain cereal seeds extracted by a hydrothermal process. The energy concentration of these feeds can be increased with feed oil or feed fat. Already in the first week, the milk replacer can be placed in the pen so that the piglets can get to know the solid feed as soon as possible.

In the pre-weaning period, piglets are already fed prestarter feed as a supplement. Partly because the sow's milk production no longer covers the litter's need for nutrients. Another reason is that this can help to ensure the proper secretion of the required enzymes, and because they can get used to this feed before the weaning, thus partly facilitating their transition after the weaning. Prestarter feed is easily digestible, contains 20-21% protein, essential amino acids, minerals, vitamins, flavors and aroma and more and more organic acids.

Comparison of piglets of first parity sows and multiparous sows

Piglets of first parity (P1) sows are born with less weight (*Hendrix et al.*, 1978; *Tantaasuparuk et al.*, 2001) and also weigh less when weaned (*Burkey et al.*, 2008; *Holyoake*, 2006) than piglets of multiparous sows. Their weight gain also lags behind that of piglets of second-parity sows. Furthermore, it can be said that the mortality rate is higher among the piglets of the P1 sows. These can be explained, on the one hand, by the fact that the body of primiparous sows is still developing and is able to produce less milk than sows that have already farrowed. Also, the transport of immune substances to the colostrum is less efficient, so the weaker immune system of piglets of the P1 sows can be traced back to this (*Vila*, 2013; *Gadd*, 2011).

The aim of the study was to determine the extent to which weaning influences the weight gain of piglets in the immediate post-weaning period, and to compare the growth of piglets of first parity and of second parity sows during the period of weaning.

MATERIAL AND METHOD

The research was carried out at a Hungarian pig farm on the litters of 3 first parity (P1) and 3 second parity (P2) Landrace x Large White sows. The growth of piglets in the litters was monitored by continuous, individual weight measurement. Litter equalization was performed at the farm, so the number of piglets per litter for each sow was 15. During the first measurement, the 1-day weight of the piglets were measured and individually marked. Subsequent measurements occurred at 10, 20, 26, 28, 29, 30, 36, and 47 days of age, in each case between 11 a.m. and 2 p.m. The piglets were placed one by one in a plastic crate and put on a digital scale accurate to 0.05 kg.

The sows were housed in farrowing rooms, one room could accommodate 54 sows. The room had a full plastic slatted floor. The room temperature was 20 ° C, and the additional heaters were put in the pen for the piglets up to 10

days of age. The relative humidity in the room was 60-70% and the air exchange was 1 m^3/ live weight/hour. Routine treatments of piglets such as iron supplementation, castration, tail docking, tooth clipping took place at the age of 3 days. Piglets received ad lib. milk replacer from an automatic system from the age of 3 days and then from the age of 16 days, there is a gradual change to a different type of milk replacer. From the age of 23 days, they received the prestarter feed, and in the nursery they continue to consume this as well. The amount of milk replacer fed is 400-500 g dry matter / 4 weeks/piglet. Nutrient composition of feed is presented in *Table 1.* Weaning of the piglets took place on the 28th day of life and they were placed separately by groups. One group consested of the piglets of first parity (P1) sows, and the other of the piglets of second parity (P2) sows. In the nursery, they received the dry prestarter feed in an automatic feeder for 10 days, then there was a changeover to piglet starter feed at 16 days of age. They did not receive Zinc-oxide supplementation. Nipple drinkers were placed separately. There was also a complete plastic slatted floor in the rearing rooms. 30-35 piglets were placed per pen. The sex ratio was approximately 50% female and 50% male. The temperature was reduced weekly, from 30 ° C to 28 ° C, then to 26 ° C, and finally to 25 ° C. All piglets were vaccinated against Circo virus and Mycoplasma hyopneumoniae at 35 days of age.

Table 1.
Nutrient composition of feed

	Milk replacer	Prestarter	Starter	Lactating sow feed
Crude Protein %	22	16.5	17.7	17.5
Crude Fat %	20	6	4.6	4.7
Crude Ash %	7	4.4	4.8	6.5
Crude Fibre %	0.04	2.7	3.7	5.6

The feeding of sows was adjusted to the needs of the individuals as the lactation progressed, as shown in *Table 2*.

Table 2.
Feed ration of the sows

	Weekly system – days of the week						
	Monday*	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
The week of farrowing	1.5	1.5	3	3	3	3	3
The 1st week after farrowing	4.5	4.5	4.5	4.5	6	6	6
The 2nd week after farrowing	6	6	7.5	7.5	7.5	7.5	7.5
The 3rd week after farrowing	9	9	9	4.5	4.5**		

**the day of farrowing; *the day of weaning

Statistical analyzis: to compare the weights of piglets of P1 and P2 sows at the different measurement points, GLM was used. Differences in weight gains between groups at the measurement pionts was carried out using covariance analyzis, where parity of the sow was the categorical factor and initial weight of piglets at the observed period was the covariate factor. Grouping of piglets based on weight gain/loss or stagnation after weaning was also done subtracting the weight on the day after weaning from the weight on the day of weaning. Linear regression analyzis was used to assess the association between weight gains of periods directly before and after weaning or the last observed period, that is between the periode of days 26-28 and that of days 28-29 and 36-47. Difference in mortality rate between the groups was analysed using Chi-square test. For all statictical procedures the SAS 9.1.4 software was used.

RESULTS AND DISCUSSION

The weights of the piglets of the two groups were averaged at each measurement time point (*Figure 1.*). Based on the calculated average weights, it can be said that the weights of the piglets of the P2 sows were significantly higher than the weights of the piglets of the P1 sows at each time point. This difference at day 1. was 290 grams. The 1-day average weight of piglets in the P1 sows was 1.20 kg, and the average weight of piglets in the P2 sows was 1.49 kg ($P < 0.05$). Thus, our results are in accordance with the findings of *Hendrix et al.* (1978); *Tantasuparuk et al.* (2001) on birth weight. As the age progresses, however, the gap shows a gradual increase. At 10 days of age, 950 grams, at 20 days of age, 1600 grams is the difference between the weights of piglets of P1 and P2 sows based on group averages. Then, there is a statistically significant difference between the weights measured at 26, 28, and 29 days of life. The difference between the weights of the piglets in the two groups at 26 days is 2010 grams and at 28, 29 days 2020 grams, which is not negligible from a practical point of view. The findings of *Burkey et al.* (2008) and *Holyoake et al.* (2006) regarding weaning weight also agree with our results. From the age of 30 days, there is again an increase in the weight difference. The difference is 2120 grams at 30 days of age, 2430 grams at 36 days of age, and 2650 grams at 47 days of age. Thus, the largest difference can be measured at day 47 of life between the mean weights of the two groups. At this time, the mean weight of the piglets of the P1 sows was 12.13 kg, while the mean weight of the piglets of P2 sows was 14.78 kg ($P < 0.05$). Throughout the observed period, piglets of P2 sows weighed 21-33% more with lower value being at day 47 (21%), the highest at day 10 (33%).

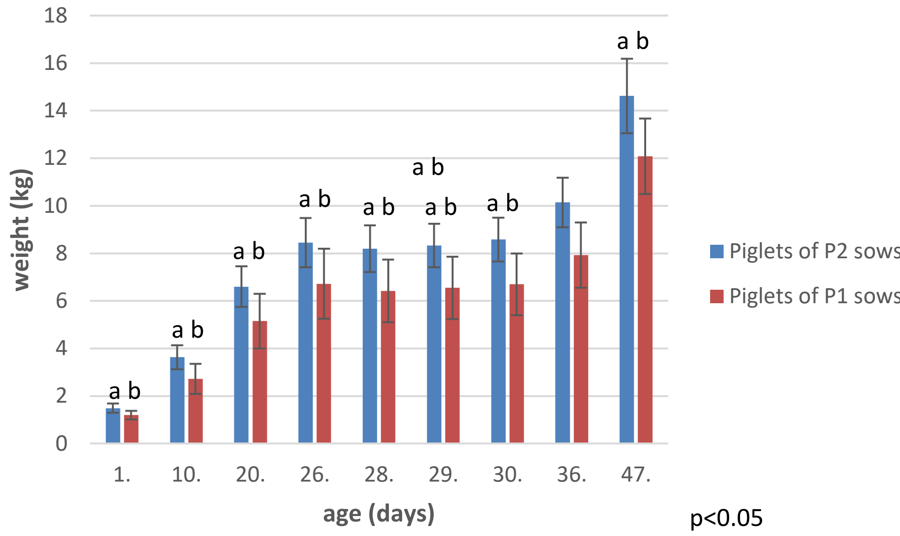

Figure 1: The average weight of piglets of P1 and P2 sows

Results on daily weight gain are showed in *Figure 2.* There was a difference in daily weight gain from day 1 to day 10 between piglets in the two groups. The average daily weight gain of the piglets of P2 sows was 218 g/day, while the average daily weight gain of that of the P1 sows was 182 g/day. During this period, a significant difference can be detected between the two groups. In the next two life stages, however, the weight gain of the two groups leveled off, with the measured values close to each other. In the case of piglets of P2 sows, the daily weight gain in the two life stages is 268 g/day and 276 g/day, respectively. For P1 sows, 265 g and 280 g/day ($P < 0.05$). At 28 days of age, the day after weaning, there was again a difference between the piglets in the two groups. During this period, however, weight loss occurred. The weight of the piglets of P1 sows dropped to a greater extent. In their case, the average daily weight loss at that time was 185 g/day. The other group had a weight loss of 80 g over the same period. By the next measurement time, which was at 29 days of age, weight gain was observed again. Although there was no statistically significant difference between the two groups, it is important from the point of view of practice that the expected trend is characteristic, the weight gain of piglets of P2 sows is higher. At the 30th and 36th day of life, piglets of P1 sows continued to grow with a lower average daily weight gain ($P < 0.05$). At the last measurement time point, piglets at 47 days of age, there was no

statistically significant difference between the performances of the two groups. According to the data obtained at the age of 47 days, the average daily growth of piglets of P1 sows is slightly 5 g/day higher than the growth of piglets of P2 sows. *Pineiro et al.* (2019) studied the piglets' growth from birth to finishing phase and found that growth superiority of piglets from P2 sows was maintained for the overall period. The development of digestive tract in low birth weight piglets is delayed as reported by *Michiels et al.* (2012), that is also reflected in our data (*Figure 2.*)

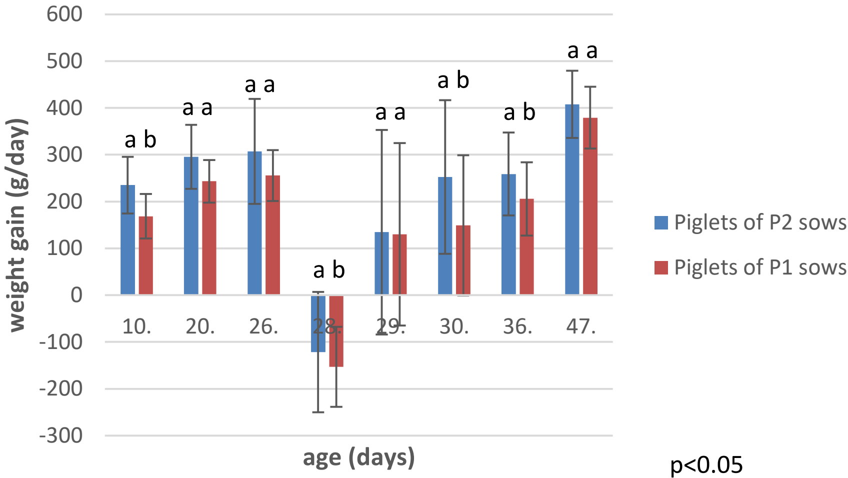

Figure 2: The average daily gain of piglets of P1 and P2 sows

Also the intra-group ratios of weight gain, weight loss, and stagnation in the periods between each measurement time point were evaluated. By the day before the weaning, all individuals in both groups showed growth. Then, during the critical period, the day after weaning, several of the piglets of P1 sows lost weight. In their group, a decrease was observed in 95.2% of the individuals. 73.5% of the piglets of P2 sows showed weight loss on this day. Two days after weaning, we experienced a decline in fewer individuals, and more piglets began to gain weight, but there was still a higher proportion of weight loss among piglets of P1 sows. 3 days after weaning, on day 30 of life, the difference between the two groups of piglets is greater. At this time, 97% of the piglets of P2 sows show an increase. Of the piglets of P1 sows, more than 16% experienced a weight decrease, and the weight of 7% of the individuals did not change

compared to the previous day. For the measurement at 36 days of age, the piglets of the first farrowing sows caught up. Again, all individuals in their group showed growth. This was also seen during the last measurement we performed at 47 days of age in piglets. *Figure 3*. and *4.* show the daily gain of all piglets in P1 and P2 groups. Regardless of group and initial weight weaning had a negative impact on growth, but the rate of growth stabilized at the group level at the last measurement time point.

The intake of supplemental feed for piglets differs between litters and within litters. *Bruininx et al*. (2002) distinguished between eater and non-eater piglets before weaning, and followed their feed intake after weaning. They found that eaters needed less time between weaning and first feed intake than non-eaters. As a result, eater piglets showed higher daily gain in the first 8 days after weaning. General lower feed intake after weaning was reported by *Balogh and Novotniné Dankó* (2013) as well, leading to reduced growth intensity or even weight loss of piglets after weaning.

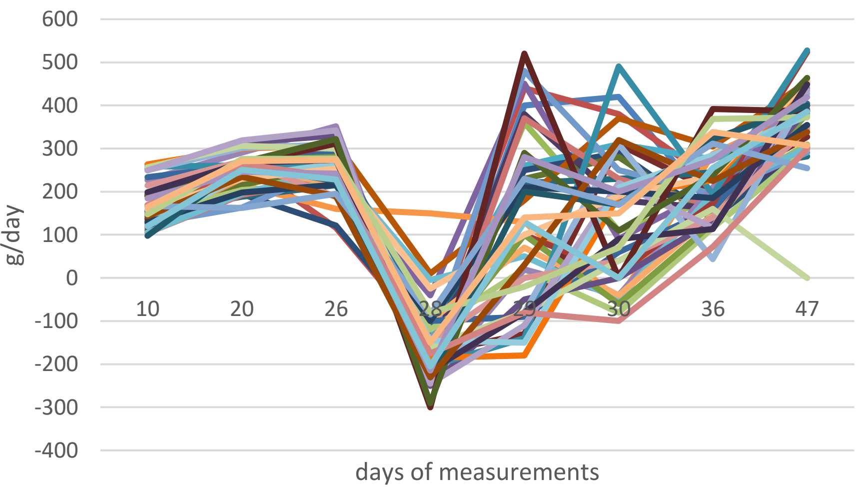

Figure 3: Individual weight gain of piglets of P1 sows

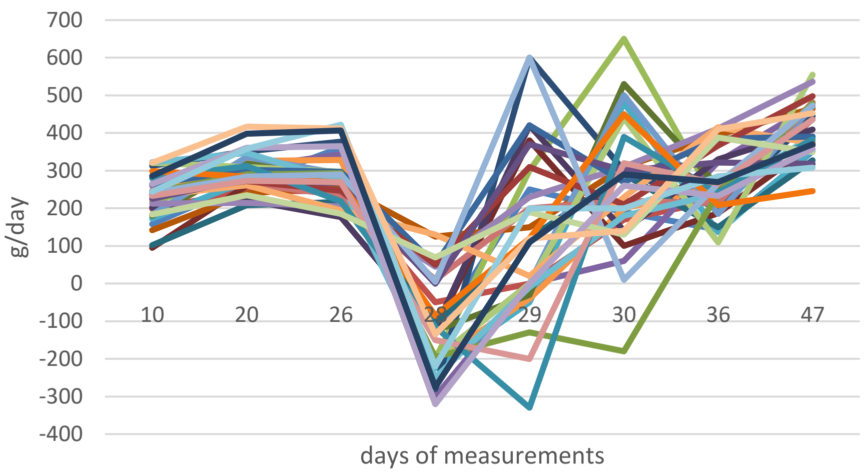

Figure 4: Individual weight gain of piglets of P2 sows

Birth weight is regarded as a parameter which influence the future growth rate of piglets, thus piglets with higher weight are considered to reach slaughter weight earlier. However outstanding pre-weaning weight gain might not necesserily mean better performance right after weaning compared to piglets that showed moderate gain in the farrowing unit. This phenomenon is presented in the next figures (*Figure 5., 6., 7.*). The initial weight gain is a relatively good predictor of later performance of the suckling piglets until weaning (*Figure 5.*)

Association between weight gains of days 20-26 and days 26-28 are shown in *Figure 6.* In this connection, we found that although the association is weak, it is still statistically significant. It can be stated that the post weaning weight gain tended to be lower for piglets that performed better before weaning. One reason could be that a piglet at the top of the litter ranking in the farrowing facility is adequately supplied with milk, thus it will not get used to solid feed early enough. As a result, the weight gain of piglets that grow satisfactorily before weaning will decrease more (*Wiseman et al*., 1998). So in their case, due to reduced feed intake, the decline in weight gain was greater. *Pluske et al.* (2007) observed that piglets which were good eaters in suckling period grew faster after weaning than small or non-eaters. *Mans and Magowan* (2018) confirmed that creep feed consumption in the farrowing unit increases feed intake early after weaning, however they found no effect on the growth after weaning.

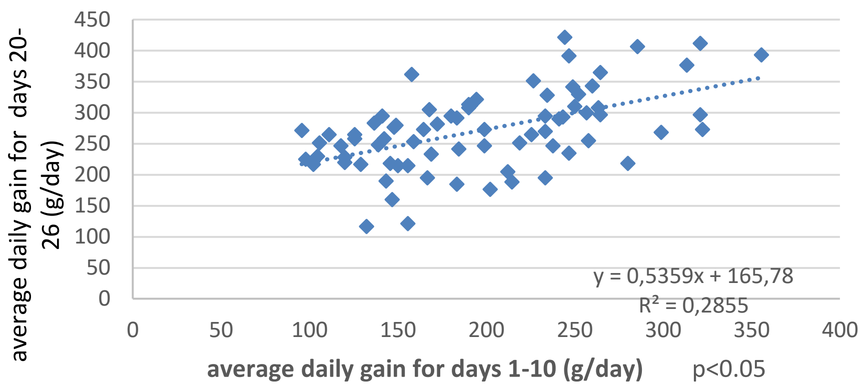

Figure 5: Association between average daily gain for days 1-10 and 20-26

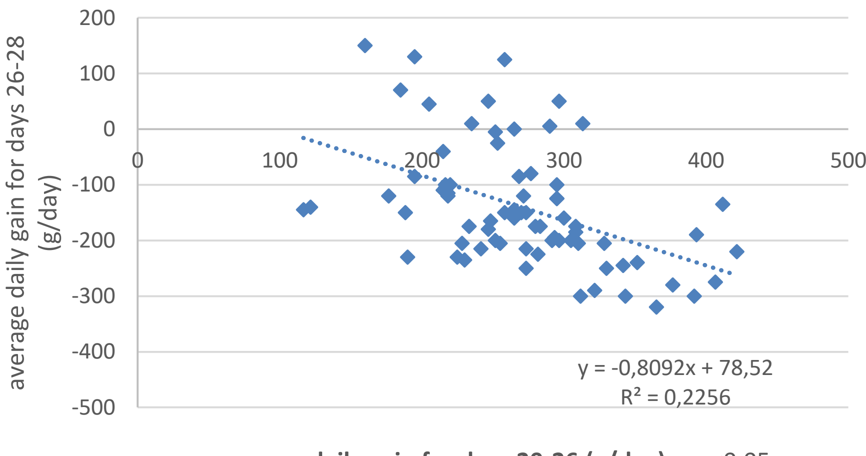

Figure 6: Association between average daily gain for days 20-26 and 26-28

Figure 7. shows the relationship between pre-weaning gain and the gain at the last period. The result is not significant but the trend has reversed, indicating that piglets growing faster in the farrowing unit are coming over difficulties around weaning. It also shows the importance of this phenomenon,

since 20 days after weaning were not sufficient to clearly restore the initial trend.

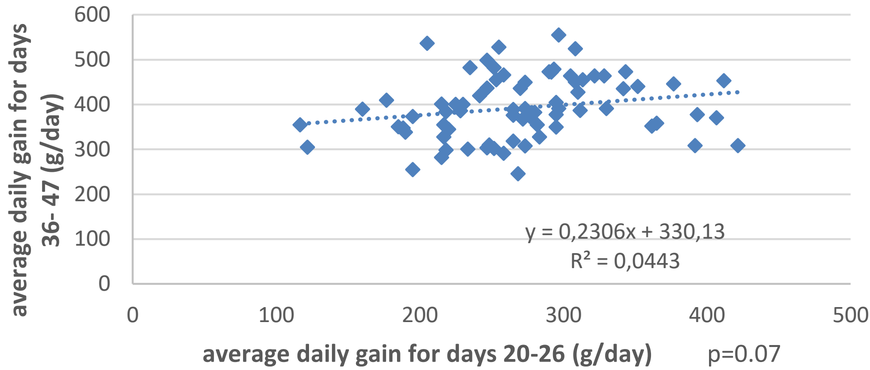

Figure 7: Association between average daily gain for days 20-26 and 36-47

Our results on mortality are presented in *Table 3.* The results obtained do not agree with that of *Holyoake et al.* (2006), as in our study we observed a higher rate of mortality in the piglets of P2 sows (p=0,04), 17,7 % of the P2 piglets died after the first weighing, with an average weight of 1.38 kg. The cause of this high rate of mortality was not determined, but it can be traced back to one sow, of whose piglets 46 % died after the first measurement. Furthermore, it can be said that mortality did not increase after weaning in none of the groups.

Table 3.
Mortality counts and ratios of piglets during the study

Age, days	10.	20.	26.	28.	29.	30.	36.	47.	Total	%
Piglets of P2 sows	8	1	1	1	0	0	0	0	11	24.4
Piglets of P1 sows	2	0	1	0	0	0	0	1	4	8.8

CONCLUSIONS AND RECOMMENDATIONS

The weights of the piglets in the two groups showed statistically significant differences at each measurement time point with free access to the milk replacer and prestarter feed. The weight of piglets of P2 sows was greater in all stages of life we studied.

Weight gain in the piglets of P1 sows was also weaker on days 10 and 30 than in the case of the other group. Furthermore, piglets of P1 sows lost more weight on the day after weaning. In order to reduce the disadvantage of piglets of P1 sows, it would be necessary to separate these piglets from piglets of multiparous sows. Thus, their needs would be better met in terms of both feed and husbandry technology. Furthermore, this would make the stock more homogeneous and there would not be such differences between groups.

For best herd feed efficiency and overall efficient pig production it is also recommended to find the optimal sow herd structure where the proportion of first parity sows does not exceed 20%.

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Review

Study on performance traits of laying hens with crossing the White Leghorn and the Rhode Island breeds

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ABSTRACT - The current steady increase in the global population is accompanied by a dramatic growth in demand for food, whose satisfaction relies heavily on animal protein sources. Poultry farming is already mankind's key source of protein. Changes in consumer habits combined with the negative impacts of climate change are posing new challenges to poultry breeding companies today. Certain traits properties relating to egg production among offspring groups resulting from the crossing of the White Leghorn and the Rhode Island breeds show improvements relative to the corresponding properties of pure-bred offspring. This literature review is aimed at summing up experiments focusing on White Leghorn and Rhode Island crosses in terms of the main traits properties relating to egg production. Experiments involving the crossing of the White Leghorn and Rhode Island breeds tend to occur mainly during pure-bred poultry breeding. Studies discussing the performance of groups of offspring of White Leghorn (WL) and Rhode Island (RI) breeds, without analysing physiological types of experiments, have been few and far between in the past 20 years. Most of the few such studies originate from countries of the third world focusing in most cases on matters of feeding. This is explained, for the most part, by the fact that in the current period of modern poultry farming, only the world's top breeding companies are in possession of high performance Leghorn and Rhode lines and they seldom ever publish scientific papers allowing glimpse into breeders' development or strategic activities of breeders.

Keywords: laying hen, egg, White Leghorn, Rhode Island, cross-breeding

INTRODUCTION

The global population has doubled in the past fifty years, having reached 7.8 billion by now (*UN*, 2021). Projections show that the total population on Earth will grow to reach 9.8 billion by 2050 (*UN*, 2017). The ongoing population increase and the rise of living standards is accompanied by a like increase in demand for food, the satisfaction of which relies heavily on animal protein sources. In Addition to these, the climate change will affect agriculture through higher temperatures, elevated carbon dioxide (CO_2) concentration, precipitation changes, increased weeds, pests and disease pressure. Global mean surface temperature is projected to rise in a range from 1.8°C to 4.0°C by 2100. Such changes will have more or less severe impacts on all components of food security: food production and availability, stability of food supplies, access to food and food utilization (*FAO*, 2009).

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Poultry farming is already the single most important source of protein for people; in the form of the highly popular poultry meat, of which a total of 112.99 million tonnes, and table eggs, of which 69.79 million tonnes (that is, 1320 billion eggs) was produced in 2014 (*Witzke et al.,* 2017**,** *Windhorst,* 2018).

The favourable biological properties chicken as a species have contributed to the increase in the consumption of poultry meat and eggs, as detailed below: *(1)* high reproduction rate; *(2)* short generation interval; *(3)* excellent nutrient transformation; *(4)* highly effective adaptability; *(5)* genetic properties enabling the production of hybrids; *(6)* cheap transportation of hatching eggs and day-old-chicks; *(7)* excellent utilisation of space etc. (*Horn*, 2000).

THE CURRENT SITUATION IN TERMS OF TABLE EGG PRODUCTION

The importance of chicken egg production and consumption is clearly indicated by an *FAO* (2010) forecast of a 1.1% annual growth rate for the period between 2010 and 2020, in view of which the global chicken egg output is likely to exceed 70 million a year by 2020. This output volume has already been reached, as according to the latest – June 2021 – FAO data the world's total egg production was up at 83.5 million tonnes (or some 1579 billion eggs) in 2019 already.

The total global demand for protein of animal origin is expected (*Mulder,* 2018) to increase between 2017 and 2037 by some 35%, of which the global egg production will increase by 1.6% per year.

The current approx. 77 million tonnes global chicken egg production can be examined in essentially two categories: half of the total output comes from light-bodied Leghorn type hybrids producing white-shelled eggs, while the other half is produced by medium-heavy hybrids laying brown-shelled eggs. Breeder companies in Europe (including Hungary), Africa and the Far-East prefer the breeding of hybrids laying brown-shelled eggs to cater for consumer demand, their share equalling up to 80% (*Cavero et al.,* 2012).

Preisinger (2016) argues that this is a definite disadvantage in Europe because Leghorn hens laying white-shelled eggs perform better in non-caged farming than breeds laying brown-shelled eggs. Demand for brown coloured eggs has been changing in Europe as well recently, in response to which traders are increasingly looking for typically “tint” or “tinted” (or “cream” and “beige”) coloured eggs. From a professional perspective this is all the more exciting, since the four wild chicken (junglefowl) species lay cream or Isabel colour eggs, which is starkly different from the deep brown colour which is generally held to be the ancient “natural” egg colour (*Sütő and Szász*, 2013). A

number of major traditional breeder companies (e.g. Babcock, Shaver, Tokai) had hybrids laying cream coloured eggs already back in the 1970s and 1980s, but now demand for this kind of colour seems to be reviving and on the increase again, because Hungarian TETRA's competitors have been coming out with such genotypes one after another (e.g.: H&N, CORAL Tinted Eggs Layers, Hy-Line Sonia, Dominant Tinted, etc.).

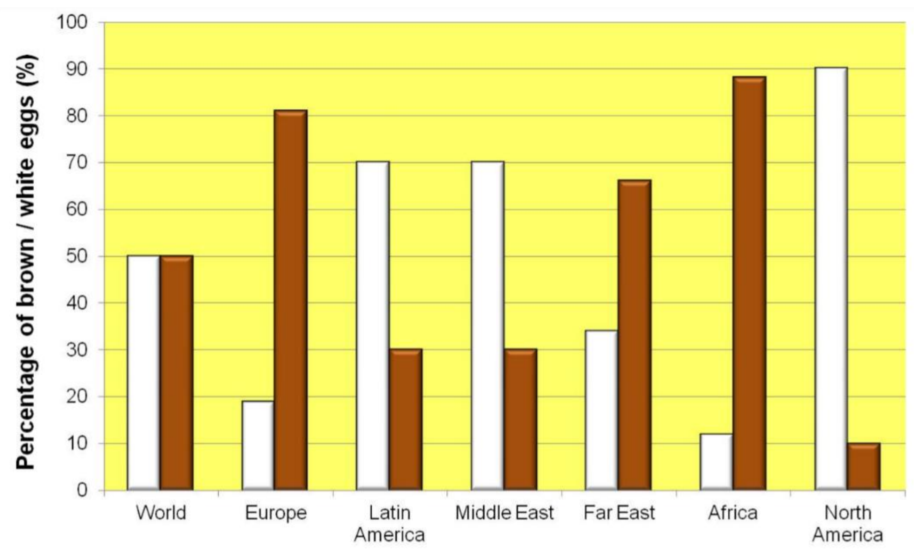

Figure 1: Estimated percentage of brown and white eggs worldwide
(Source: Attractive Eggshell Color as a Breeding Goal, *Link*)

THE TWO INITIAL TYPES

The Leghorn breed was created back in the first half of the 19th century in the United States of America, by cross-breeding the Italian unimproved chicken with the Wyandotte and Minorca breeds. Its name came from the name of the Italian town of Livorno where the majority of the birds transported to America were loaded on ships in around 1835. The Leghorn was the first chicken breed selected exclusively for maximising its egg production capacity, neglecting a variety of appearance related features of lesser importance for production. This breed comes in more than twenty varieties, each having the same type of build, except for the colour of the plumage and the form of the comb. The most economically important of these is the White Leghorn (WL) with a single comb

because each of the Leghorn type hybrids – laying white-shelled eggs – has been bred from this breed through heterosis breeding (*Sütő and Szász*, 2013).

The Rhode Island breed was created in the mid-19th century, also in the United States of America. Its breeding started from a red-coloured unimproved chicken variety living on Rhode Island which was then cross-bred with a number of other varieties (Cochin, Red Malay and Yellow Shanghae) to improve its meat forms. Later on, brown Leghorn roosters were used for cross-breeding in order to increase their egg production. Breeders gave preference to bright cherry-red (that is, dark red) coloured specimens, significantly impeding, particularly in Europe, the progress of improvement in terms of the most important traits. Two colour variants of the breed are of relevance today the red (genetically: gold) Rhode Island Red (RIR) and the white-plumed (genetically: silver) Rhode Island White (RIW) types. The breed's economic significance lies primarily in the fact that breeders use many different lines of the breed for breeding medium-heavy bodied laying hybrids producing brown-shelled eggs (*Sütő and Szász*, 2013), though there is less and less reason for referring to them as "medium-heavy bodied".

A DISCUSSION OF THE CROSSING OF WHITE LEGHORN WITH RHODE ISLAND

The White Leghorn and the Rhode Island lines are commercially used in layer production worldwide. The two basic layer breeds differ from another in many of their properties, which was also the basis of the distinction between the two large groups of layer hybrids. It is clear however, that consumer markets were divided definitely in terms of their preferences regarding the eggshell colour (white or brown), and therefore it is rather only in the pure breeding phase that one can find cross-bred types created for experimental purposes. The idea is justified by considerations such as size of bodyweight that cross-breeding enables a reduction in the live weight of layer hens in comparison to Rhode type hybrids, along with an improvement in the feed conversion ratio, which improves the chances – in regions where brown-shelled eggs are preferred (such as in Europe) – for mitigating the adverse effects of today's global trend of climate change (including better heat tolerance, smaller environmental footprint). Crosses between RIR and WL produce tint eggs, which constitute higher and higher market shares in parts of Asia, and especially China. According to the statistics of China Animal Agriculture Association, tint eggs constitutes averagely 61% of the eggs from domestic breeds and 24% from the imported breeds in recent 4 year (*Adamu et. al.,* 2020).

Sexual maturity

The laying hens are sensitive to light and changes in day length. When hens are reared only under natural light conditions, they reach sexual maturity at different ages depending on latitude and season. Age at sexual maturity has a direct influence on laying performance. One of the factors contributing to the differences in egg production. The age at first egg used as a measure of age at sexual maturity. The two pure breeds differed widely with respect to this characteristic. For the White Leghorns the average age at first egg was between 172.4-188 days and for the Rhode Island Reds 247.6-255 days (*Warren,* 1930; *Glazener et al.* (1952). *Warren* (1930) reported that WL♂ x RIR♀ offspring reached sexual maturity than reciprocal-crossed ones (175.9 days), and nearly at the same age as pure breed, the White Leghorn birds. The number of days to sexual maturity was largest in the case of the Rhode Island Red pure breed. *Knox and Olsen* (1938) found during their experiments that the WL♂ x RIR♀ offspring underperformed the control pure breed specimens at age of sexual maturity. Because the pure-bred White Leghorn (WL) breed reached earlier (192 days) the sexual maturity than WL♂ x RIR♀ cross-breeds (211 days). *Dudley* (1944) got similar results as Warren. In his studies compared the performances of the White Leghorn (WL) and Rhode Island Red (RIR) breeds as well as their cross-bred and reciprocal cross-bred offspring. The crossbred hens reached more quickly sexual maturity than pure breeds. The pure-breed Rhode Island Reds breed took longer to mature than any other type of progeny (211.9 days). The WL♂ x RIR♀ reached the sexual maturity the earliest (186.1 days), earlier than pure-breed WL♂ x WL♀ (190,4 days) and RIR♂ x WL♀ (199.3). *Podchalwar et al.* (2013) found too that the RIR♂ x WL♀ offspring reached sexual maturity in less time.

Crossbreeding for egg production

The improvement in poultry performance for egg production during the last three-quarters of the 20th century has been tremendous: from 176 eggs per hen per year in 1925 to 309 eggs per hen per year in 1998 (*Decuypere et al.,* 2003).

Warren (1930) found that the hybrids from the cross WL♂ x RIR♀, were the best producers. This crossbred reached more eggs (214.6 eggs) than purebred White Leghorn (211.6 eggs). The hybrids from the reciprocal cross, RIR♂ x WL♀ cross had an average production of 13 fewer eggs (198.7 eggs) than the White Leghorns. The Rhode Island Reds were the lowest producers (168.9

eggs). *Te Hennepe* (1937) reported that in the Lancashire International Laying-hen Test in 1936-1937 among various cross-breeds the WL x RIR offspring produced nearly twenty eggs less than White Leghorn and Rhode Island Red. *Knox and Olsen* (1938) crossed White Leghorn (WL) roosters with Rhode Island Red (RIR) hens and compared this crossbred for example single comb White Leghorn. They found that the crossbred offspring had fewer eggs (145.4 eggs) than the control pure-bred Single Combs White Leghorns (201.3 eggs). *Knox* (1939) reported one year later that the performance of cross-bred lines fell short of pure-breeds in terms of egg production and other growth parameters. *Dudley* (1944) reported in his studies that the averages of annual egg production for the cross-breeds were slightly higher than those for the pure-breds. Also the hybrids from the cross WL♂ x RIR♀ produced the most of eggs (204.5 eggs). Then the RIR♂ x WL♀ reciprocal crossbreds reached more eggs (197 eggs) than purebred White Leghorn (194.4 eggs) and Rhode Island Red (196.8 eggs). *Ambar et al.* (1999) compared offspring produced by the crossing of birds native to tropical climates and exotic breeds. In terms of egg production WL♂ x RIR♀ came in 3th 54.71 % eggs production), while the RIR♂ x WL♀ cross-breed took the 5th position (57.89% eggs production). *Adamu et al.* (2020) in their study, resource populations of Rhode Island Red (RIR) and White Leghorn (WL) pure-bred chickens were reciprocally crossed to generate 4 distinct groups. They reported that White Leghorn and the hybrids commenced laying earlier than RIR pullets and egg production traits were favorable in the crossbreds compared with purebreds.

Egg weight

The egg weight is one of the important performance traits. There are several factors what influencing the resulting egg weight: genetics, health conditions and nutrition. An important factor is the genetics. By the egg size and weight are different the two main types. White Leghorn hens produce leighter egg than Rhode Island hens. The weight of the egg can be further increased by crossing. That reported *Warren* (1930) in his studies. *Warren* (1930) crossed White Leghorn (WL) roosters with Rhode Island Red (RIR) hens and compared this crossbred with purebred White Leghorn and Rhode Island, where the average egg-weight of the offspring of both cross-breeds was identical to those of the two pure-line breeds. *Knox and Olsen* (1938) found the WL x RIR crossbred had a better average egg weight than the purebred White Leghorn. *Ambar et al.* (1999) reported too that by the crossing increased weight of the egg.

Adamu et al. (2020) reported that heterosis for egg number and clutch size was moderate in WL♂ × RIR♀ but low in RIR♂ × WL♀ hens.

Table 1: Comparing the traits of different laying hens lines

Genotypes (Male x Female)	Age at sexual maturity (days)	Egg weight (g)	Egg production (egg, %*)	References
RIR x RIR	247.6	54.3	168.9	*Warren, D. C. (1930)*
WL x RIR	175.9	54.6	214.6	
RIR x WL	206.4	54.4	198.7	
WL x WL	172.4	51.2	211.6	
RIR x RIR	-	-	-	*Knox, C. W. - Olsen, M. W. (1938)*
WL x RIR	211.0	56.4	145.4	
RIR x WL	-	-	-	
WL x WL	192.0	54.7	201.3	
RIR x RIR	210.9	-	196.8	*Dudley, F. J. (1944)*
WL x RIR	186.1	-	204.5	
RIR x WL	199.3	-	197.0	
WL x WL	190.4	-	194.4	
RIR x RIR	255.0	59.2	130 (six months)	*Glazener et al. (1952)*
WL x RIR	-	-	-	
RIR x WL	-	-	-	
WL x WL	188.0	57.5	112 (six months)	
RIR x RIR	-	-	-	*Ambar et al. (1999)*
WL x RIR	-	55.1	54.71*	
RIR x WL	-	57.9	57.89*	
WL x WL	-	-	-	

WL = White Leghorn; WL♂ x RIR♀ = White Leghorn male by Rhode Island Red female; RIR = Rhode Island Red; RIR♂ x WL♀ = Rhode Island Red male by White Leghorn female

CONCLUSIONS

The conclusion we have drawn from literature is that in addition to meeting consumer demand (for creme-coloured eggs), the crossing of the White Leghorn (WL) and the Rhode Island Red (RIR) lines produced favourable results in a number of traits. The WL x RIR offspring took less time to reach sexual maturity and in several studies the egg production of the offspring exceeded that of the pure line groups. The cross-bred combinations outperformed the parents in terms of lower mortality rates as well. Crossing did not have much of an impact on egg quality parameters. Broodiness, however, were found to increase in cross-bred groups relative to pure line offspring groups. Examples of cross-breeding with White Leghorn (WL) and Rhode Island (RI) breeds for purposes of experiments were found only in the pure-breeding stage and there is only a very limited number of publications in the past 20 years covering such

tests for experimental purposes. Studying such types of cross-breeding combinations, however, have become topical, and professionally exciting, again with the aim of mitigating the current climate change effects and in order to fully satisfy consumer demand.

We are confident that there will be significant professional interest in the new experimental report on the characteristics of the offspring produced by crossing the Leghorn and Rhode lines in different housing systems.

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Article

The *in vivo* crude protein digestibility of soybean species cultivated in Hungary

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ABSTRACT - The purpose of the trial was to use the mobile nylon bag technique to determine the crude protein digestibility and calculate the digestible protein yield of soybean varieties cultivated in Hungary. The trial was carried out with 10, double cannulated (duodenal- and PVTC-cannula) hybrid barrows with initial live weights of 40±3.5 kg. The experimental basal diets were formulated on a corn-wheat-barley-soybean basis according to the requierement of growing pigs (Tybirk, 2015). A total of 20 soybean varieties were tested in this experiment. After simulating gastric digestion the nylon bags were inserted into the duodenum of ten barrows through simple duodenal T-cannulae. Ten bags were administered to each pig daily. A total of 200 (10 samples/soybean variety) bags were inserted over a 4-day period. The mean, standard deviation, minimum and maximum values of the crude protein digestibility of soybean samples were calculated. The protein yields per hectare according to the crop yield and the protein content values and also the digestible protein yield values were calculated. To examine the relationship between crop yield and crude protein yield and also between crop yield and digestible crude protein yield regression analysis were used. The overall results of this experiment indicate that the average crude protein digestibility of the tested soybean varieties was 76.0%, with an absolute difference of 17.3% between the best and the least digestible varieties. ES Mentor variety reached the best digestible crude protein yield, with 1305.4 kg/ha. The variety with the lowest digestible crude protein yield was Boglár, with 752.3 kg/ha. The difference in digestible crude protein yield between these two varieties was 173.5%. In the correlation analysis between digestible crude protein content and yield for soybean varieties our results show that there is no correlation between the two factors. However, Aires, Prestopro, and ES Mentor should be highlighted among the varieties with above trend line results, as they have the best yield (4020 kg/ha; 4100 kg/ha, 4510 kg/ha) and digestible crude protein content (31%, 30.3%, and 28.9%). ES Mentor produced the fourth best digestible crude protein content (28.9%) with the best yield (4510 kg/ha).

Keywords: soybean, digestibility, nylon bag, yield

INTRODUCTION

In Hungary, soybean are the most important protein source for the production of feed mixes. In the EU, the nutritional importance of soybean meal is shown by a calculation of protein use: livestock production uses 43 million tones of protein per year (*Popp et al.,* 2015). One of the most important feed ingredients are cereals, which, although not considered as a source of protein, account for 40% of the protein content of compound feed. Soybean meal accounts for 34%

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of the protein supply, rapeseed meal 11%, sunflower meal 7% and other oilseeds 2%. Its extracted meal plays an important role in the mix of all intensive livestock species and is essential for the amino acid supply of animals, especially in poultry and pig meat production.

Economical, environmentally friendly and sustainable pork production is unthinkable without a balanced animal feed. It has long been known that feed costs account for around 70-75% of the cost of pig meat production. Therefore, to maintain economical production, it is necessary to develop and apply feeding technologies based on the digestible nutrient content of feed components. In this way, they are able to provide appropriate nutrients to the needs of the animals in order to enable them to reach their genetic potential.

The years 2020 and 2021 brought unaccustomed challenges for both the livestock sectors and the feed industry. For some farms, survival has already been a major challenge, due to the already present market and climate change related difficulties, enhanced or coupled with the african swine fever (ASF) and the emerging pandemic situation. Despite these setbacks, the yields of corn and sunflower exceeded those of recent years. However, soybean yields were decreased compared to the data monitored during 2019. The evolving situation has caused a steady increase in the price of autumn-sown crops, resulting a sharp rise in the purchase price of feed materials for pork production, with doubled sunflower prices compared to the previous year (*Gregosits*, 2021). In Hungary there are about 60 cultivated soybean varieties with difference crude protein content and yield values. But there is no information about the digestible crude protein digestible of these varieties. Due to the drastic increase in feed prices, the use of soybean varieties with the highest digestible crude protein content as feedstocks is inevitable in the feed industry, to improve the efficiency of economic production.

Aims

The purpose of the trial was to use the mobile nylon bag technique to determine the crude protein digestibility and calculate the digestible protein yield of soybean varieties cultivated in Hungary. Using the protein digestibility and the available yield results our further objective was to rank the tested species according to the digestible protein yield.

MATERIAL AND METHOD

The trial was carried out with 10, double cannulated (duodenal- and PVTC cannula) hybrid (DanBred) barrows with initial live weights of 40±3.5 kg. During the entire experimental period the animals were kept in special individual

pens (2 m^2/animal). The room temperature and relative humidity were regulated in accordance with the requirements of growing pigs (*Tybirk,* 2015). During the trial animals received a coarse meal diet ad libitum. Water was offered *ad libitum* via automatic drinkers.

Before the start of the trial (ethitcal permission number: SOI/31/00659-14/2018) we implanted a duodenal T-cannula to the proximal part of duodenum and a PVTC-cannula onto the ileocecal valve (*Sauer et al.,* 1982; *Steiner et al.,* 2011, *Van Leeuwen et al.,* 1991). After the surgery the animals had a 14 day long regeneration period before the trial.

The basal diets were formulated on a corn-wheat-barley-soybean basis according to the requierement of growing pigs (*Tybirk,* 2015). The composition and nutrient content of the basal diet are summarized in *Table 1*.

Table 1

The composition and calculated nutrient content of the basal diet

Ingredients	**g/kg**	**Nutrients**	**g/kg**
Corn	302.95	Dry matter	885.6
Soybean meal	160.0	DE (MJ/kg)**	13.8
Wheat	250.0	Crude protein	150.3
Barley	250.0	Crude fat	26.4
Vegetable oil*	4.0	Crude fiber	32.5
MCP	9.2	Crude ash	25.0
Limestone	10.3	Lysine	9.2
NaCl	3.6	Methionine+Cystine	5.2
Lysine-HCL	3.6	Threonine	6.0
DL-Methionine	0.4	Thryptophan	1.9
L-Threonine	0.8	Calcium	6.4
L-Tryptophan	0.2	Phosphorus	5.3
Premix 0,5%***	5.0		
Total	**1000.0**		

* Sunflower oil; ** Calculated value; *** 1 kg premix contain: Vit. A: 1,750,000 IU, Vit. D3: 350,000 IU, Vit. E: 8,750 mg, Vit. K3: 350 mg, Vit. B1: 262.5 mg, Vit. B2: 875 mg, Vit. B3: 2,100 mg, Vit. B6: 700 mg, Vit. B12: 4,375 mg, Biotin: 21 mg, Folic acid: 105.07 mg, Cholin: 24,000 mg, Fe: 19,175 mg, Zn: 20,001 mg, Mn: 6,488.3 mg, Cu: 2,225 mg, Co: 6.5 mg, I: 65 mg, Se: 67.75 mg.

The crude protein digestibility of the relevant soybean varieties cultivated in Hungary (n=39) were determined by an *in vitro* method in a pre-experiment. According to these results 20 varieties with the best crude protein digestibilty

value were chosen to test by *in vivo* method. During the trial the *in vivo* crude protein digestibility of the samples were determined by mobile nylon bag technique (*Sauer et al.,* 1982, *Quiao et al.,* 2004, *Steiner et al.,* 2011). Feed was ground through a 1 mm screen and 1 g samples (10 samples/soybean species) were enclosed in 25 × 40-mm monofilament nylon bags (50-µm mesh). At first step the samples were pre-digested by an *in vitro* gastric-digestion method (*Babinszky et al.,* 1990, *Cone,* 1993, *Boisen et al.,* 1995). The bags were grouped in blocks of 10 and placed in a 1000 ml beaker containing 500 ml of a solution made up of deionized water, 0.01 N HCl and one g of purified activated pepsin powder. The beaker was then placed into a shaking water bath (65 oscillations/min) and incubated for 4 h at 40 °C to simulate gastric digestion. After incubation, the bags were removed from the beaker, washed with deionized water and frozen (-20 °C) in small plastic bags until required. In the second step prior to insertion, nylon bags were removed from the freezer and thawed for 5 min in a 37.8 °C water bath. The nylon bags were inserted through the duodenum of ten barrows through simple duodenal T-cannulae 30 minutes after the morning feeding. Two bags were introduced 30 minutes apart (i.e. two bags 30 minutes after the morning feeding and further pair of bags in every 30 minutes). Ten bags were administered to 5 pig daily. A total of 200 (10 samples/soybean variety) bags were inserted over a 4-day period.

Bags were collected via PVTC-cannulas wich were opened 2.5 hours later than the first inserting. The collected samples were stored at −80°C until analysis.

The crude protein content of the original and the collected soybean samples were determined in accordance with the *AOAC* (1989) recommendations.

The apparent crude protein digestibility of soybean samples was calculated according to the protein content of the original samples and the protein contect of the collected samples as the following:

$$\text{CP digestibility (\%)} = \frac{CPin - CPout}{CPin} x100$$

CPin = CP in original sample (g)

CPout = CP in collected sample (g)

Means and standard deviations for digestible crude protein were calculated using Microsoft Excel (*Microsoft,* 2016). The protein yields per hectar according to the crop yield and the proteint content values and also the digestible protein yield values were determined.

Regression analysis was carried out to examine the relationship between crop yield and crude protein yield and crop yield and digestible crude protein yield. The univariate linear regression modell was performed as the follows: Y = aX + b.

Y = CP conetent (g/kg) or digestible CP content (g/kg)

X = crop yield (t/ha)

RESULTS AND DISCUSSION

The crude ptotein content, crude protein digestibility and the digestible crude protein content data of the 20 soybean varieties are shown in *Table 2*. The results show that the Bólyi 1117 soybean variety had the highest apparent crude protein digestibility (84.3%), while the lowest value was observed in Bólyi 612 (67.0%), 20.5% less than the best performing variety Bólyi 1117. The average in vivo crude protein digestibility of the tested soybean varieties is 76.0%, with an absolute difference of 17.3% between the best and the least digestible varieties. In our study 60% of the soybean varieties had the apparent crude protein digestibility between 74.0% and 82.0%. *Boisen et al.* (1995) and *Cone et al.* (1993) determined higher apparent crude protein diegestibility (78.1% and 82.1 to 83.8%) than our results, but it is not relevant to compare these results because they studied different varieties from different cultivation area.

The distribution of in vivo protein digestibility data for the studied soybean varieties is shown in Figure 1. 15-15% of the samples were rated in the in vivo crude protein digestibility interval of 66-74%. 60% of the tested samples fell within the range from 74% to 82%. 10% of the samples can be taken in the in vivo crude protein digestibility interval of 84 ± 2,0%.

Table 2
The crude protein content, the crude protein digestibility and the digestible crude protein content of different soybean varieties (%)

Variety	Crude protein content	Crude protein digestibility	Digestible crude protein content
Bólyi 1117	35.1	84.3	29.6
Suedina	38.2	82.4	31.5
Aires	37.9	81.8	31.0
Bólyi 27	38.3	79.7	30.5
ES Gladiator	36.5	78.8	28.8
Pannónia Kincse	35.0	78.8	27.6
Seka	35.8	78.6	28.1
ES Advisor	37.0	78.0	28.9
Boglár	34.6	77.7	26.9
Prestopro	39.6	76.5	30.3
Bahia	34.0	76.4	26.0
Speda	40.5	76.4	30.9
ES Mentor	38.2	75.8	28.9
Sponzor	35.1	74.6	26.2
ES Mediator	37.7	73.8	27.8
ES Comandor	38.1	72.2	27.5
Stumpa	34.4	71.9	24.7
Borbála	38.9	68.2	26.5
Navigator	37.1	67.4	25.0
Bólyi 612	35.2	67.0	23.6
mean	**36.9**	**76.0**	**28.0**
St. deviation	*1.9*	*4.8*	*2.3*
difference	*6.5*	*17.3*	*7.9*
minimum	*34.0*	*67.0*	*23.6*
maximum	*40.5*	*84.3*	*31.5*

In *Table 3* the crop yields and the digestible crude protein yields of soybean varieties per hectare are presented. Based on our results, the ES Mentor variety reached the best digestible crude protein yield, with 1305 kg/ha. The variety with the lowest digestible crude protein yield was Boglár, with 752 kg/ha. The difference in digestible crude protein yield between these two varieties was 173.5%.

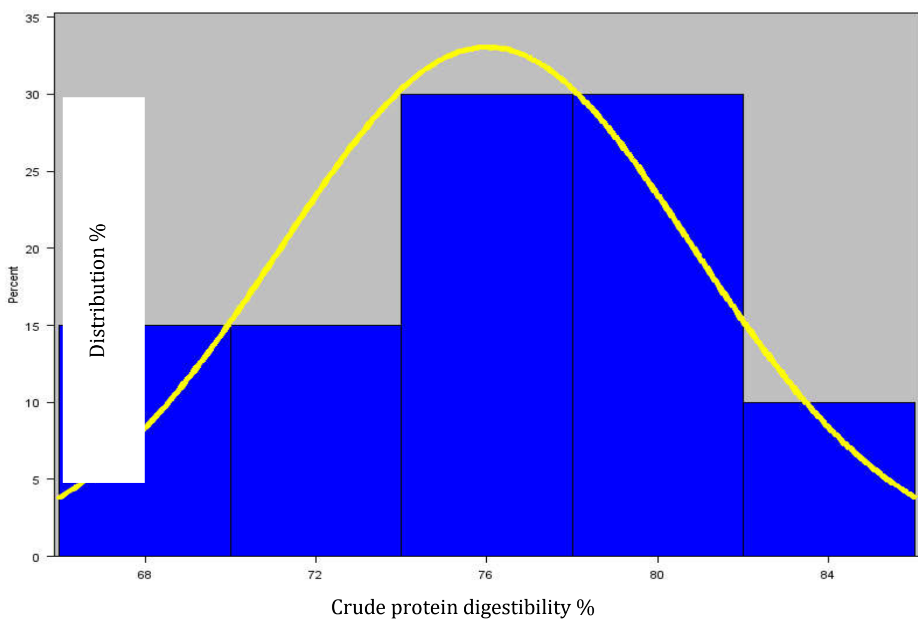

Figure 1: Distribution of apparent protein digestibility in soybean varieties

Table 3
Digestible crude protein yield of different soybean varieties (kg/ha)

Variety	Crop yield	Digestible crude protein yield
ES Mentor	4510	1305
Aires	4020	1247
Prestopro	4100	1242
Seka	3990	1122
Bahia	4260	1107
Pannónia Kincse	4000	1104
ES Gladiator	3780	1088
Sponzor	3940	1032
Borbála	3810	1011
Bólyi 612	3900	920
Bólyi 27	2670	815
Boglár	2800	752

Figure 2 shows the correlation between crude protein content and yield for soybean varieties. Our results indicates, that there is no relationship between the two factors (R^2=0.0013). *Malik et al.* (2006, 2007) and *Jagtap et al.* (1993) also found no correlation between crude protein content and yield. The Prestopro variety had the highest crude protein content of 39.6% and this excellent crude protein content was associated with the second best yield with 4100 kg/ha. The Borbála variety also had a remarkable crude protein content (38.9%) and a yield with 3810 kg/ha but on the other hand the digestible crude protein yield value is quite low (1011 kg/ha). A favourable combination of crude protein content and yield was also observed for the variety ES Mentor, with a yield of 4510 kg/ha and a crude protein content of 38.2%.

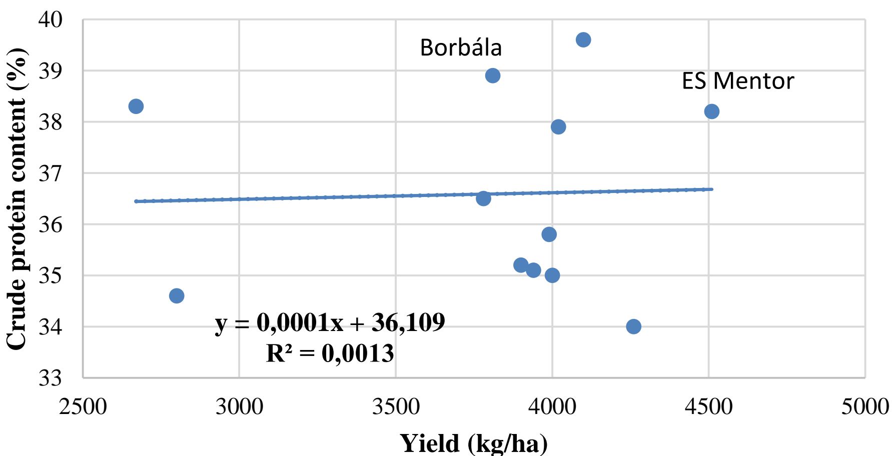

Figure 2: Correlation between the crude protein content and yield for different soybean varieties

Figure 3 shows the test results of the correlation between digestible crude protein content and yield for soybean varieties (R^2=0.0114). No correlation was found. However Aires, Prestopro, and ES Mentor should be highlighted among the varieties with above trend line results, as they have the best yield (4020 kg/ha; 4100 kg/ha, 4510 kg/ha) and digestible crude protein content (31%, 30.3%, and 28.9%). ES Mentor produced the fourth best digestible crude protein content (28.9%) with the best yield (4510 kg/ha).

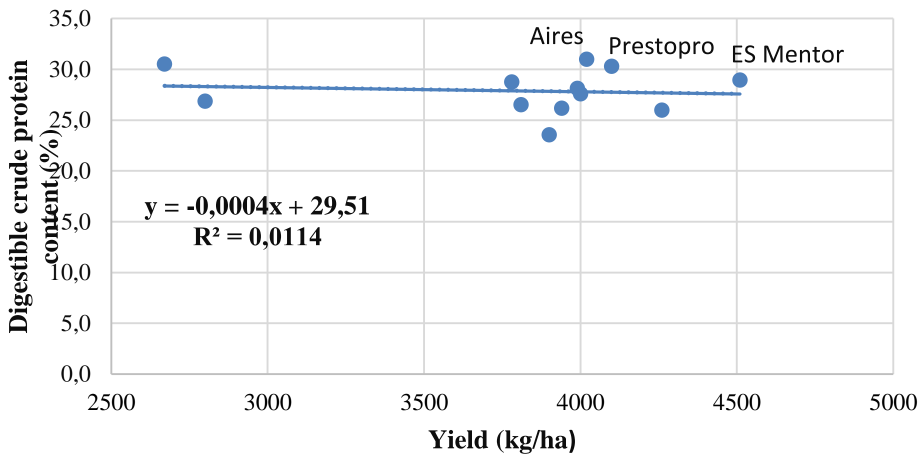

Figure 3: Correlation between the digestible crude protein content and yield for soybean varieties

CONCLUSIONS AND RECOMMENDATIONS

The following main conclusions can be drawn from the results of our study. It would be useful to include the digestible crude protein yield per hectare among the plant breeding judging aspects, which could be used as a quality indicator for the use of the crop for feed. In addition, variety selection by the grower should be based on the digestible crude protein yield values if the crop will be used or sold for feed. Our results provide a good basis for selecting varieties that are able to produce the expected yields and have an acceptable digestible crude protein content. It would be an incentive effect for plant growers, if the digestible crude protein content will be among the aspects of the forming of the purchase price so that they would have a greater interest to choose varieties that can produce higher digestible crude protein yields. It would be justifiable to perform this experiment with extracted soybean meal, to determine the extent of difference in digestibility of untreated soybean varies with heat treatment and steeping, and to determine the difference between extracted meals of varieties with different protein digestibility and the extent of the difference.

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ACTA AGRARIA KAPOSVÁRIENSIS *(2021) 25 (2), 41–51; DOI: 10.31914/aak.2626*
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Methodological study

Automatic method for determining the number of *lumbar* and *thoracic vertebrae* in rabbits using Computer Tomography images

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ABSTRACT - There are several studies dealing with the phenotypic variance of the vertebral number in the spinal column of rabbits. According to the literature the number of thoracic and lumbar vertebrae varies between 11-13 and 6-8, respectively. The length of the m. longissimus dorsi (MLD) - a valuable meat part of rabbits - is determined by the length of the vertebral column therefore the number of vertebrae may have economic importance in breeding. The aim of this study was to create an automatic counter using computed tomography (CT) images. In the first step, a skeleton binary mask was created using the radiodensity range between 120 and 3071 HU, then the lumbar and thoracic regions were processed by two different methods. The lumbar part was evaluated based on the frequency of the bone voxels along the axial plane. The number of thoracic vertebrae was determined from the number of ribs. The left and right ribs were processed separately. The developed method was tested on CT examination of 40 Hycole rabbits compared to manual evaluation. The results of the automatic algorithm had few errors: in one case in the lumbar region (2.5%) and in 3 cases in the thoracic region (5%). The automated evaluation process takes a few seconds per individual and then the program visualizes the results on a graph. The incorrectly evaluated rabbits are recognizable on graphs and they can be easily corrected with a minimal time investment.

Keywords: computed tomography, rabbit, vertebra number, automated evaluation

INTRODUCTION

Several studies in several animal species have been shown that the number of vertebrae in different vertebral regions has got a variance (Chilson *et al.,* 2018, *Cunyuan et al.,* 2019, *Donaldson et al.,* 2013, 2014). This has a significant effect on the length of the spine and meat production (*Cunyuan et al.* 2019, *Donaldson et al.,* 2013, *Donaldson et al.,* 2014). The carcass length is a well-inherited trait in pigs and sheeps (*Berge, 1948; Cunyuan et al.,* 2019). Early methods to measure variation in thoracic and lumbar vertebrae number required slaughtering of the animal. As technologies evolved, non-invasive techniques became available for breeding purposes. Computed tomography (CT) besides X-ray gives

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more reliable measuring with 3 dimensional representation in spine traits (*Donaldson et al.,* 2013). Selection research has been going on at the Kaposvár Campus of the Hungarian University of Agriculture and Life Sciences for years *using CT* (*Matics et al.,* 2014*)*. It may be interesting to examine the dorsal skeletal system to provide additional information. The information extracted from this area has never been used in a rabbit selection program. The proposed method is able to automatically count the number of thoracic and lumbar vertebrae using images from CT examinations.

MATERIAL AND METHOD

Animals

All animals were handled according to the principles stated in the *EC Directive 86/609/EEC* regarding the protection of animals used for experimental and other scientific purposes. The study was performed on 40 Hycole rabbits at the age of 11 weeks.

Image acquisition and pre-processing

The image acquisition was carried out with a Siemens SOMATOM Definition AS+ CT scanner in 2021. Animals were immobilized as described by *Matics et al.* (2020). The rabbits were examined with the following parameters: 120 kV tube voltage, 240 mAs X-ray radiation dose, spiral data collection mode with 0.6 pitch, field of view 500 mm according to ISO 9001:2015 quality management system and ISO 14001:2015 environmental management system. Standard DICOM (Digital Imaging and Communications in Medicine) (*The DICOM Standard*) images were reconstructed by Siemens Syngo CT VA48A program with convolution kernel I30f. The resolution of the images was 0.977mm × 0.977mm × 2mm. Each exam series were converted from DICOM to MINC (Medical Image NetCDF) format. This file type allows the series images to be stored in a single file. The images were pre-processed using the OpenIP software package. The individuals were separated, and the plastic container holders were segmented out from the images using an automated pipeline (*Kovács et al.,* 2013). The individual images were converted to NIFTI (*Neuroimaging Informatics Technology Initiative,* 2005) files and the coordinate systems were set with the same starting point located at the bottom left of the first axial slice. The post-processing method was developed in python environment using the following main packages: nibabel, numpy, pandas, scipy, skimage.

Image post-processing

The first image processing approach was the segmentation of the rabbit skeleton by thresholding with 120 HU down threshold value. The skeletal system with associated bones was segmented by connected-component analysis (CCA), where the small bones causing disturbance and first limbs were omitted. The interesting thoracic and lumbar part of the remaining skeleton was cut. The first cutting point was found after the head by signal processing using *slice position* and *1/number of bone voxel counts* data (*Figure 1.1*). The second cutting point was found after the last lumbar vertebra by signal processing of the changes in the *number of bone voxel* by *slice position* (*Figure 1.2*)*.* The results are showed on the *Figure 2.1* and *2.2* where the red lines represent the cutting points.

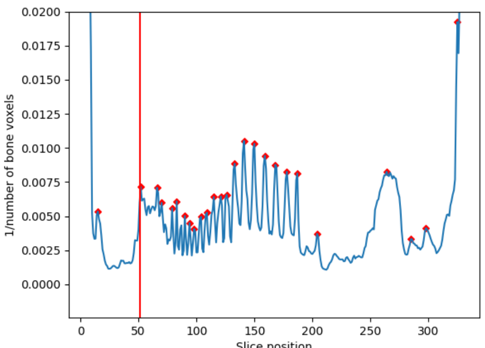

Figure 1.1: Determining of the cutting point after the head

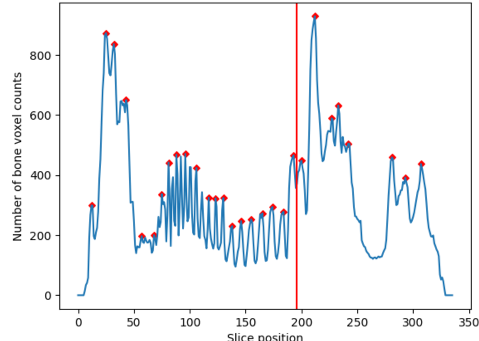

Figure 1.2: Determining of the cutting point after the last lumbar vertebra

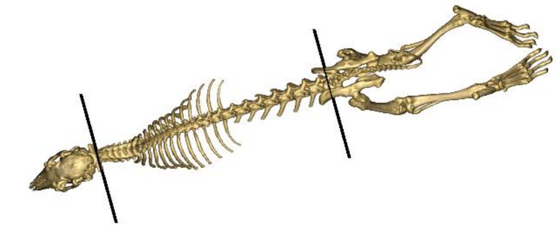

Figure 2.1: Two cutting plane on the truncated skeletal system

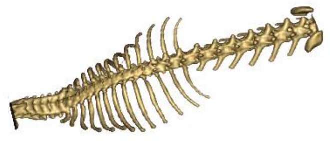

Figure 2.2: 3D rendered image of the cut skeletal system

The curved ribs complicate the image evaluation, therefore we cut it close to the vertebrae by elastic cylinder (*Figure 3.1 and 3.2*). The cylinder was placed on a curve defined by two functions with 10 degrees of freedom (*Figure 4.1 and 4.2*). These functions represented the axial centerline of the skeleton.

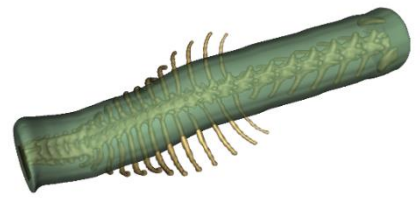

Figure 3.1: Fitted elastic cylinder on the vertebrae

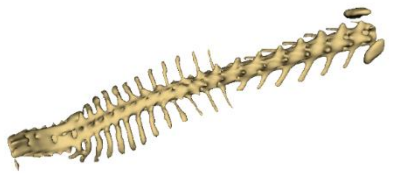

Figure 3.2: 3D rendered image of the cut skeletal system

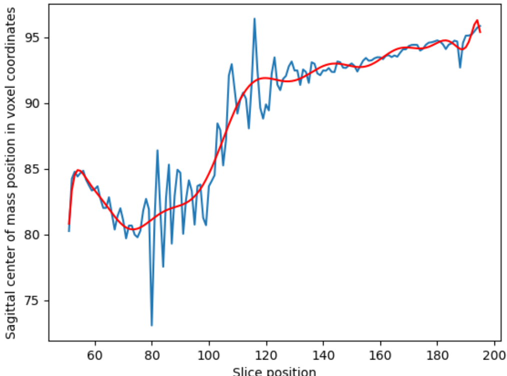

Figure 4.1: Fitting ten degree of freedom equation on the sagittal mass centres along the axial axis

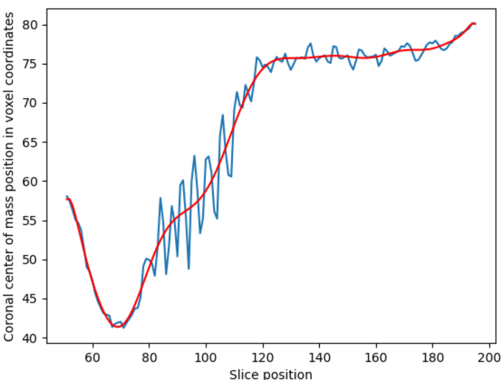

Figure 4.2: Fitting ten degree of freedom equation on the coronal mass centres along the axial axis

After the removal of the disturbing parts, we could easily separate the lumbar and thoracic regions by signal processing of *distance from middle* and *slice position* (*Figure 5.1, 5.2*). At the thoracic part, the number of vertebrae was examined by rib detection. The right and left ribs were examined separately, because the two-sides disturbed each other (*Figure 6.1, 6.2*). The number of vertebrae at the lumbar part was determined by signal processing of the skeletal voxel frequency with the slice position (*Figure 7.1, 7.2*). The above-described image post-processing method is fully automated.

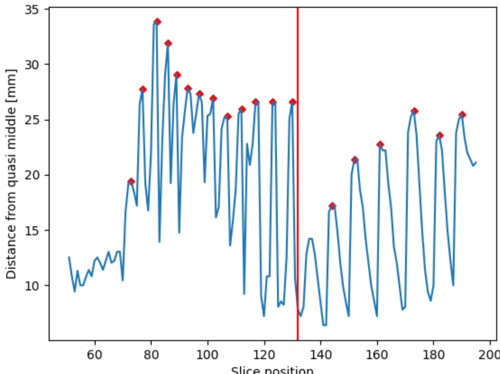

Figure 5.1: Detection of separating point between lumbar and thoracic part

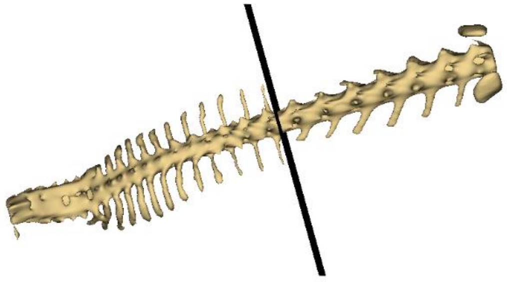

Figure 5.2: Separating plane between lumbar and thoracic part

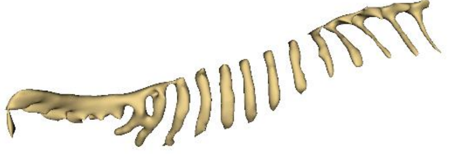

Figure 6.1: 3D rendered image of the left cut vertebrae.

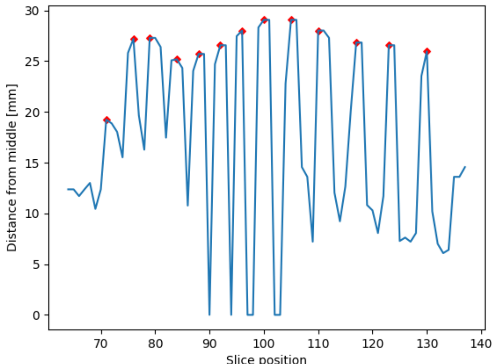

Figure 6.2: Counting of left thoracic ribs

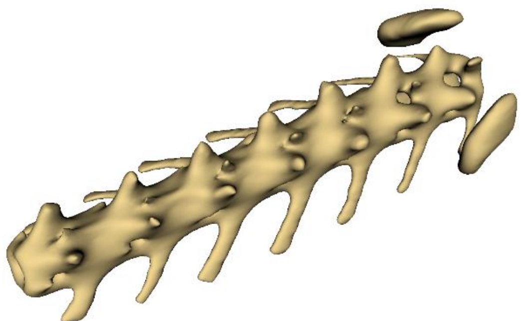

Figure 7.1: 3D rendered image of the lumbar vertebrae

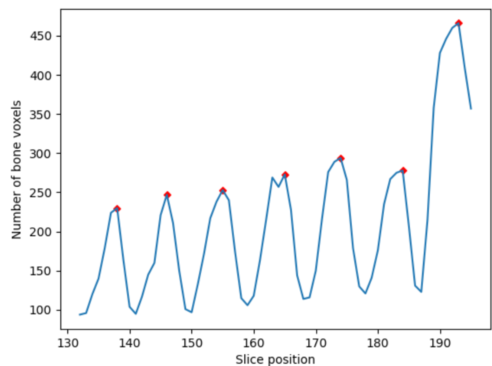

Figure 7.2: Counting of the lumbar vertebrae

Difficulties:

1. High-density substances in the intestines originated from feed. These materials adhere to the intestinal wall and may fuse to the ribs on the CT images. The rib becomes inseparable using our proposed method.
2. A non-typical vertebra was found with characteristic of the lumbar and thoracic vertebra (*Figure 8*). This case cannot be handled by the program. The vertebra was classified as a part of lumbar region.

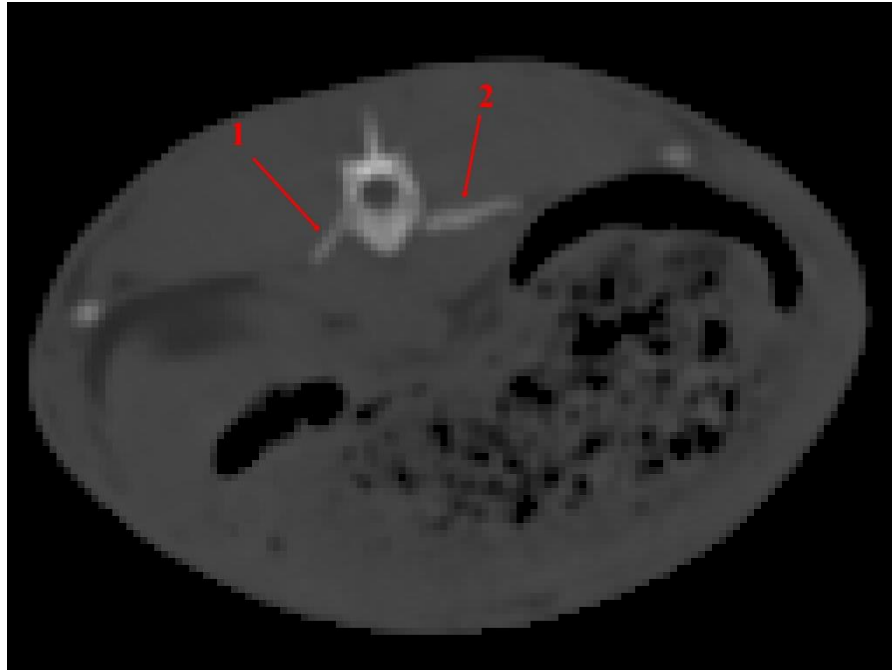

Figure 8: Typical transverse process of lumbar vertebra (left side of image, red mark 1). Rib like structure beside of the same lumbar vertebra (right side of image, red mark 2).

In addition, the length of each vertebral region (thoracic and lumbar) was calculated using the differences between the appropriate anatomical points.

Statistical analysis

The normality (Kolmogorov-Smirnov) and variance equality (two side F-test, conf. level 0.95) of lengths by groups were tested. All of the grouped lengths

are normally distributed and there are no significant differences in variances between examined groups, therefore analysis of variance was performed. R statistical software were used for all statistical processing.

RESULTS

After manual and automated examination of all the rabbits, the results were compared to each other. At the lumbar region one error occurred while at the thoracic region we found three errors.

Frequencies of the dorsal vertebrae system in rabbits are described in *Table 1*. It is encouraging that the number of cases where either the number of lumbar or thoracic vertebrae increases is more frequent than when only one increases and the other decreases.

Table 1
Variability of the vertebral skeletal system

Number of lumbar vertebrae	Number of thoracic vertebrae	Frequency [animals]	Frequency [%]
7	12	23	57.5
8	12	8	20
7	13	8	20
6	13	1	2.5

All of the collected and comparable lengths were summarized in *Table 2.*

Table 2
Lengths of spine regions according to the number of vertebrae (mm)

Region	Number of vertebrae	Mean	Standard Deviation	Minimum	Maximum
Thoracic	12	108.3 [a]	3.1	100	116
	13	122.9 [b]	5.0	114	130
Lumbar	7	110.4 [a]	3.8	100	118
	8	130.0 [b]	3.2	126	136
Thoracic-lumbar	12-7	229.8 [a]	5.8	220	240
	12-8	251.0 [b]	5.2	246	262
	13-7	249.7 [b]	5.3	240	256

a, b: means with different superscripts within the same region differ significantly ($P<0.001$)

Based on analysis of variance, length of thoracic region and length of lumbar region grouped by number of vertebrae have got significant differences. Process the two regions' lengths (thoracic and lumbar) together with analysis of variance by multiple comparisons of means (Tukey Contrast) grouped by thoracic-lumbar vertebrae there is not significant differences between 13-7

and 12-8 and significant differences are between 12-8 and 12-7, 13-7 and 12-7. Generally, the larger number of vertebrae resulted longer regions.

CONCLUSIONS

The described technique provides a quick automated image processing method (2-3 seconds per individual) for counting the lumbar and thoracic vertebrae. The software visualizes the results on graphs. The incorrectly evaluated rabbits are recognizable on the graphs. It is possible to correct the data with minimal time investment and the perfect result can be achieved. Examination of the dorsal skeletal system can serve extra information for the rabbit selection program. The robustness of the proposed method may increase with better image resolution. Based on the virtual measurements, the larger number of vertebrae resulted longer regions.

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Mini-review

Determination of *Fusarium* mycotoxin exposure in humans based on urine samples, using One Health approach

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ABSTRACT - The role of mycotoxins has been recognized in the etiology of a number of human diseases. Therefore, biomonitoring of human mycotoxin exposure is very important. One of the possible ways to do this is the urinary biomarker-based exposure determination. Over the past few decades, such studies have been conducted in many countries around the world on volunteers of different ages, genders, and eating habits, although these studies do not always use the same measurement, and calculation methods. This review focuses on the most important *fusarium* mycotoxins (deoxynivalenol (DON), zearalenone (ZEA), fumonisins (FUM), T-2, and HT-2 toxins). Because of the presence of mycotoxins in the environment-feed-food chain, One Health strategies should be adopted for the prevention of their exposure.

Keywords: *Fusarium* mycotoxins, human exposure, urinary biomarker

INTRODUCTION

Mycotoxins are secondary metabolites of molds. They cause great economic losses through their frequent occurrence in the food chain and pose a serious health risk to both animals and humans. These toxins are aggressive cytotoxins, resistant to gastric juice, insensitive to high temperatures (100-200 °C), may accumulate in various organs, directly and/or indirectly inhibit the body's specific defence mechanism (*Jávor* and *Szigeti*, 2011 a and b).

Different mycotoxins develop different diseases in different species (liver carcinoma, esophageal cancer, kidney damage, etc.). When different mycotoxins are occurring at the same time, synergistic, additive, or antagonistic interactions can occur (multi-mycotoxic effect) (*Kovács et al.,* 2016).

Fusarium mycotoxins occur worldwide in cereal grains. Mammalian cell cultures were used to show the cytotoxicity of the most common *Fusarium* mycotoxins; deoxynivalenol (DON), zearalenone (ZEA), fumonisin B1 (FB1) and moniliformin (MON). For each *Fusarium* mycotoxin the most sensitive cell line was determined for further toxicological experiments as an alternative of living animal testing. For DON and FB1 Chinese hamster ovary cells (CHO-K1) were found to be the most sensitive, the IC50 values were 0.27 and 85.5 µg/ml,

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respectively, after 48-h exposure. For MON the hepatocellular carcinoma cells (HepG2) showed the highest sensitivity, the IC50 values were 39.5 μg/ml for 48 h and 26.8 μg/ml for 72-h exposure. For ZEA Balb/c mice keratinocyte cell line (C5-O) was found to be the most sensitive, the IC50 value was 24.1 μg/ml after 72-h exposure. In this study DON was found to be the most cytotoxic of all the mycotoxins they tested, MON was the second most cytotoxic, followed by ZEA, and FB1. The results suggests that CHO-K1, C5-O, and HepG2 cells are the appropriately sensitive cell lines for biomonitoring of DON, ZEA and MON contaminated feed and food extracts (*Cetin & Bullerman*, 2005).

Methods for testing mycotoxins and their residues have evolved a lot, and become widely known and available.

Formation of mycotoxins can vary between fungal species as well as within a given species. Numerous physical, chemical, and biological methods have been developed to address the mycotoxin problem, but large-scale, practical, and cost-effective methods for treating mycotoxin-containing feeds are not currently available. For the contaminated foods and feeds detoxification strategies should be used to reduce or eliminate the adverse effects of mycotoxins, improving food and feed safety and prevent economic losses (*Manal et al.,* 2012). Depending on their mode of action, feed additives may act either by binding mycotoxins to their surface (adsorption), or by degrading or transforming them into less toxic metabolites (biotransformation) (*Kolosova & Stroka*, 2011). From the several mycotoxin reduction methods, it should not be stated that a single method is unconditionally effective to eliminate mycotoxin contamination of plants or to prevent the resulting health effects. Prevention strategies, cleaning and sorting methods are widely used as they serve as a first-line barrier to rid the material of various contaminants, including mycotoxins. However, other feed production technologies that also have a mycotoxin reducing effects, such as milling, dehulling and thermal methods can be controversial and limited by different practical conditions. Some physical removal methods results high weight loss, which can be a dilemma in practical manufacturing. Feed additives specifically against mycotoxins are promising but they are still in their infancy, as in the in vitro performance of some products is inconsistent and their in vivo performance requires more evidence (*Peng et al.,* 2018).

Applying the One Health approach helps to protect the population from the direct (on health) and indirect (economic, on trade and livelihood) effects of mycotoxins. The practical application of this approach is useful for the development of a functioning risk management system. Development initiatives for management systems for the early prevention of toxic exposure are important

(*Ladeira et al.,* 2017). A basic and effective measure to reduce fungal contamination in facilities for the storage of susceptible plants is to regulate the environment by manipulating ecological factors. This minimizes the entry of mycotoxins from fungi into the feed and food chain and ultimately reduces their adverse effects on animal and human health (*Imran et al.,* 2020).

It is a worldwide problem, that mycotoxins contaminate food and feedstuffs. Acute mycotoxicosis caused by high doses is currently rare. But ingestion of low and medium doses of *Fusarium* mycotoxins is quite common. These low amounts may weaken immune function and intestinal health, has effect on pathogen fitness and host-pathogen interactions, thus it can cause a different outcome of the exposure. The exposure of DON and other *Fusarium* mycotoxins generally makes worse the infection with viruses, bacteria and parasites in wide range of host species. For example: coccidiosis in poultry (*Girgis et al.,* 2008; *Girgis et al.,* 2010), enteric septicaemia of catfish (*Manning et al.,* 2005; *Manning et al.,* 2013), salmonellosis in pigs (*Vandenbroucke et al.,* 2011; *Verbrugghe et al.,* 2012) and mice (*Tai & Pestka*, 1988) and necrotic enteritis in poultry (*Antonissen et al.,* 2014 b). On the other hand, the T-2 toxin decreases the colonization capacity of Salmonella in the pig intestine. Although the effect of *Fusarium* mycotoxin exposure on infectious disease in human is less known, the animal model based extrapolations suggest possible aggravation of e.g. salmonellosis and colibacillosis in human, as well (*Antonissen*, 2014 a).

Mycotoxin-producing fungi mainly infect cereals, partly already in the production area (field molds) and partly during storage (storage molds). These toxins can be found in larger quantities mainly in whole-grain bakery products, bread, pasta, cereals, muesli. These compounds are resistant to various food production operations and can accumulate in the body when consumed. Many mycotoxins are renal and/or hepatotoxic and carcinogenic compounds, neurotoxics or endocrine disruptors. At-risk groups include those aged 0-5, those over 70, expectant mothers, and those with chronic diseases that weaken the various immune responses. Consuming cereals from controlled sources is particularly important in these groups. Furthermore, in the case of developing schoolchildren and young people, care should be taken not to overdo the consumption of whole grain-cereals (*NNK,* 2020).

In different type of foods, Regulation (EC) No 1126/2007 also regulates the maximum levels for DON and ZEA (*Table 1*). Commission Recommendation 2013/2007 / EU sets maximum recommended concentrations of T-2 and HT-2 toxins in different type of foods (*Table 2*).

Table 1
Maximum levels for DON and ZEA in foods

Mycotoxin	Product	Maximum levels (ppm)
DON	Cereals intended for direct human consumption, cereal flour, bran and germ as end-product marketed for direct human consumption	0,75
	Pasta (dry)	0,75
	Bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals	0,5
	Processed cereal-based foods and baby foods for infants and young children	0,2
ZEA	Cereals intended for direct human consumption, cereal flour, bran and germ as end-product marketed for direct human consumption	0,075
	Refined maize oil	0,4
	Bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals, excluding maize-snacks and maize-based breakfast cereals	0,05
	Maize intended for direct human consumption, maize-based snacks and maize-based breakfast cereals	0,1
	Processed cereal-based foods (excluding processed maize-based foods) and baby foods for infants and young children	0,02

(Commission of the European Communities Regulation (EC) No 1126/2007)

Table 2
Maximum recommended levels for T-2 and HT-2 toxins in food

Mycotoxin	Product		Maximum recom-mended levels (ppm)
T-2+HT-2	Cereal products for human consump-tion	Oat bran and flaked oats	0,2
		Breakfast cereals including formed cereal flakes	0,075
		Bread (including small bakery wares), pastries, biscuits, cereal snacks, pasta	0,025
		Cereal-based foods for infants and young children	0,015

(European Commission Recommendation 2013/165 / EU)

Commission Regulation (EC) No 1126/2007 regulates, inter alia, maximum levels for fumonisins (FUM) in different type of foods. The concentrations specified in the Regulation are given for FB1 + fumonisin B2 (FB2) (*Table 3*).

Table 3
Maximum levels for fumonisins in food

Mycotoxin	Product	Maximum levels (ppm)
FB1+FB2	Unprocessed maize, excepted for unprocessed maize intended to be processed by wet milling	4
	Maize intended for direct human consumption, maize-based foods for direct human consumption, excepted for foodstuffs listed in * and **	1
	* Maize-based breakfast cereals and maize-based snacks	0,8
	** Processed maize-based foods and baby foods for infants and young children	0,2

(Commission of the European Communities Regulation (EC) No 1126/2007.)

Exposure can be determined by two different approaches, one indirect by combining food consumption and contamination data, and another, a direct approach based on biomarkers. In both approaches, exposure is expressed as probable daily intake (PDI).

In risk assessment of mycotoxins, food consumption data and occurrence data from the corresponding foods are normally used to estimate population exposure. However, this method cannot estimate the individual intake, usually does not consider all kinds of sources of contamination, so biomarker-based methods are hence more and more used to assess dietary exposure from blood or urine concentrations (*Turner et al.,* 2012).

The determination of maximum tolerable contamination levels for mycotoxins is commonly based on estimations of tolerable daily intakes (TDIs) regarding comprehensive food consumption databases in single countries or regions. The European Food Safety Authority (EFSA) defined TDIs (*Table 4*).

Table 4
Tolerable Daily Intake values (TDIs) defined by European Food Safety Authority (EFSA)

Mycotoxin	TDI (µg/kg body weight/ day)	Source of information
DON	1	EFSA, 2017
ZEA	0,25	EFSA, 2011 a)
T-2+HT-2	0,1	EFSA, 2011 b)
FB1	1	EFSA, 2018

(EFSA, 2017; EFSA, 2011 **a**; EFSA, 2011 **b**; EFSA, 2018)

BIOMARKERS USED FOR EACH *FUSARIUM* TOXIN

FB1 levels in human urine show huge variability even under controlled conditions, which suggests that regulating the urinary excretion of fumonisin is a complex process. Nevertheless, the results confirm that urinary FB1 content is

a useful biomarker to assess exposure in ongoing population-based studies. If the level of exposure is relatively constant, there shouldn't be significant difference between morning and afternoon urine samples. However, it is complicated to link urinary FB1 content to dietary fumonisins because there are significant differences between individuals and the rate of excretion may also vary. Nevertheless, monitoring urinary FB1 levels - combined with the use of multiple-mechanism biomarkers - is an important tool in the investigation of fumonisins (as contributing factor of the development of human diseases such as esophageal cancer), especially in areas where the population consumes large amounts of maize and thus high exposure is probable. Eight volunteers from Guatemala consumed foods contaminated with FB1 (mean 2.94 ± 0.55 μg/kg). The urinary recovery of FB1 in these cases averaged 0.5 ± 0.24% of the dose (*Riley et al.,* 2012).

FB1 is a structural analogue of sphinganine, that is why FB1 is a specific inhibitor of the ceramide synthetase enzyme, thus interfering with the formation of complex sphingolipids (*Wang et al.,* 1992). In animal experiments blood and urine sphinganine/sphingosine (SA/SO) concentrations predicted fumonisin toxicity early, specifically and sensitively. However, it is not a sensitive indicator of the extent of FUM uptake in humans and is not a good biomarker for estimating human exposure (*Van der Westhuizen et al.,* 2008). In the case of fumonisins, urinary FB1 level is probably the most appropriate exposure biomarker in humans. Furthermore, DON, which was detected in urine, was also found to be strongly correlated with the amount of DON consumed (*Turner et al.,* 2011 a).

Shephard et al. (2013) found that urinary biomarker-based mycotoxin measurement is a valuable and efficient method for the detection of various mycotoxins (including DON, ZEA, α-zearalenol (α-ZOL), β-zearalenol (β-ZOL) and FB1). This is especially true in areas where it is difficult to collect food samples and it is hard to study food consumption data. This was the first publication on urinary DON, ZEA, α- and β-ZOL.

In the case of DON, urinary metabolites and in vitro results indicate that the major detoxification pathway is glucuronidation (*Maul et al.,* 2012). The epoxidation pathway is likely to be less significant in humans (*Piekkola et al.,* 2012). T-2 and HT-2 toxins and their metabolites are rare in human urine (*Fan et al.,* 2019).

ANALYTICAL DETERMINATION OF URINE BIOMARKERS

Numerous studies have been published worldwide on risk assessment of urine-based biomarkers. The urine sample on which the test is based is collected as follows: after the volunteer has accurately recorded the food which they consumed for 3 days the volunteer collects urine for 24 hours on the fourth day. Participants complete a questionnaire about their health status before conducting the studies. Samples from individuals with liver and /or kidney disease are generally not considered due to potential disturbances in mycotoxin and creatinine metabolism. Samples are stored frozen before transport to the site of analysis. Frozen samples are assayed for multi-mycotoxins (for example DON, DOM-1, FB1, FB2, ZEA, α-ZOL, β-ZOL, ochratoxin A (OTA), aflatoxin M1 (AFM1), T-2 toxin, HT-2 toxin, nivalenol (NIV), etc.). Urine samples are thawed and centrifuged. The samples are then treated with β-glucuronidase / sulfatase enzyme. The hydrolyzed urine is then diluted with water and usually purified using an immuno-affinity column. (The column is specific for the measured mycotoxins. For example when DON is measured, they use an immuno-affinity column specific for DON. In case of a multi mycotoxin measurement, they can use multi-mycotoxin immuno-affinity columns, which are specific for several mycotoxins.) The analyses are carried out by high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS). Toxin content is usually expressed in "µg/L urine" (*Solfrizzo et al.,* 2014; *Gerding et al.,* 2014; *Heyndrickx et al.,* 2015; *Gambacorta et al.,* 2018; *Mitropoulou et al.,* 2018; *Franco et al.,* 2019; *Lemming et al.,* 2020).

EVALUATION OF RESULTS (CALCULATION OF PDI)

Using the urine biomarker concentrations measured in different urine tests, the PDI of each mycotoxin can be calculated according to the following formula (*Solfrizzo et al.,* 2014; *Gerding et al.,* 2014):

$$PDI = (C \times V \times 100) / (W \times E)$$

where: PDI =probable daily intake of mycotoxin (µg/kg body weight/day)
C - human urinary biomarker concentration normalized for creatinine (µg/L)*
V - 24 h human urine volume measured for each volunteer (L)
W- human body weight measured for each volunteer (kg)
E - mean urinary excretion rate of mycotoxin*

*Urinary creatinine levels provide important data on excretion rate and renal function. Urine creatinine content is usually determined by a kinetic colorimetric assay based on the Jaffe method (Toora & Rajagopal, 2002). The measured toxin concentrations are normalized to the urinary creatinine level (mycotoxin concentration/creatinine concentration).

The results obtained are finally usually compared with TDIs.

*A wide variety of excretion rates are used, which greatly influences the PDI value and whether it will exceed the TDI value. For example, *Gambacorta et al.* (2013) found strong correlations between the amount of relevant biomarkers excreted in 24 h post-dose urine and the amount of mycotoxins ingested in piglets. Many studies used these excretion rates in the past years. They found that the mean percentages of dietary mycotoxins excreted as biomarkers in 24 h post-dose urine was 36.8% for ZEA, 28.5% for DON and 2.6% FB1. On the other hand *Shephard et al.* (2013) found, that the excretion rate for FB1 varies only 0,5-0,8%. *Riley et al.* (2012) also found a 0.5% excretion rate in the case of FB1 and they calculated with a 50% excretion rate for DON in human. In contrast, *Warth et al.* (2013) found, that the excretion rate for DON is 68%, but for ZEA is only 9,4% in humans.

ESTIMATES OF EXPOSURE IN TROPICAL AND SUBTROPICAL AREAS

In sub-Saharan Africa (Cameroon) measurements from human urine samples have shown that some Cameroonians are highly exposed to various mycotoxins. For example, for fumonisins, DON, and NIV the PDI has been found to exceed the TDI in some cases. Concentrations measured for FB1 are of concern, as both the average probable daily intake (APDI) and the maximum probable daily intake (MPDI) exceed the TDI (*Abia et al.,* 2013).

In a survey from Egypt: only 2% (n = 69) of urine samples from pregnant women were positive for DOM-1 with values between 0.1 and 0.12 ng/ml. It was concluded that deepoxy-deoxynivalenol (DOM-1) is not the main route of detoxification (*Piekkola et al.,* 2012).

During experiments in Brazil, mycotoxins were detected in 53% of food samples and 93% of urine samples. Based on the results, high exposure of the studied population to DON and fumonisins was established. Although the incidence of aflatoxins was low, the measured concentrations reached potentially hazardous values for health. Incidence and exposure levels showed an inverse pattern in food and urine samples: measurement based on food samples showed smaller, while measurement based on urine samples showed higher exposure (*Franco et al.,* 2019).

Investigations in South Africa showed that urinary FB1 levels are adequate to indicate FB1 exposure. The use of this biomarker improves the evaluability of exposure data, thereby contributing to the mapping of FUM contamination, the development of contamination reduction strategies, and the mapping of

health effects (*Van der Westhuizen et al.,* 2011). In South Africa, scientists detected FB1 in nearly 96% of urine samples in a group of home-grown corn-consuming people (*Shephard et al.,* 2013). Urine studies in Mexico have found the LC / MS-MS method to be sufficiently sensitive for the detection of FB1 (*Gong et al.,* 2008). The One Health approach is particularly justified in rural areas of Africa and can prevent population from direct (on health) and indirect (e.g. economies) effects of mycotoxins, which represent a serious health problem as well considerable economic losses. In these poor regions usually no regulations are available, the infrastructure for prevention and controlling food contamination is less developed and they do not allow the rejection of contaminated food. A complex risk management system is needed (*Ladeira et al.,* 2017).

In urine samples from Haiti, and Bangladesh (n = 42 and 95, respectively), FB1 was not detectable in samples from Bangladesh and only 1% of Haitian samples contained FB1 (*Gerding et al.,* 2015).

T-2 and HT-2 toxins were also not detected from urine samples from India (n=60) (*Warth et al.,* 2014). Neither T-2, HT-2 and nor HT-2-4 glucuronic acid (HT-2-4-GlcA) were detected in samples from Bangladesh and Haiti (n = 42 and 95, respectively) (*Gerding et al.,* 2015). Furthermore, HT-2 was not detected in the urine samples collected in Nanjing (China) (n=260) and only 2.0% of the samples contained T-2 (mean concentration, 2.45 μg/L; range, 0.742–3.61 μg/L) (*Fan et al.,* 2019).

ESTIMATES OF EXPOSURE IN EUROPE

Based on the first approach interpretation of urinary ZEA concentrations, PDIs do not exceed TDI (0.25 μg/kg body weight) for European samples (however, for urine samples from Haiti and African countries, PDIs exceed TDI). There is no difference between men and women in the urinary α-ZoL/ZEA ratio. This ratio ranges from 0.83 to 10. Furthermore, the data support that estimation based on urine biomarkers is a suitable method to biomonitoring the ZEA exposure (*Mally et al.,* 2016).

In the UK adult urine survey for DON and DON glucuronides (n = 34), only two samples from the same volunteer were positive at very low DOM-1 concentrations (0.5-0.8 ng/ml), that was about 1% of the detected DON + DON glucuronides (57.9 and 61.8 ng/ml). It was concluded that deepoxy-deoxynivalenol (DOM-1) is not the main route of detoxification. In contrast, deoxynivalenol conjugated with glucuronic acid (DON-GlcA) by uridine diphosphate

glucuronyltransferase (UDP-GT) appears to be the main metabolite. In addition, unconjugated DON also persisted in the body and was excreted in the urine in 68% of the studied group (*Turner et al.,* 2011 b).

In urine and blood serum studies of Swedish adolescents, and it was found that the concentration of DON in the urine is generally low, however, in 2% of cases, the PDI exceeds the TDI. In the case of DON, a significant correlation was found between cereal consumption and exposure (*Lemming et al.,* 2020).

Extended urine multi-biomarker analysis of Swedish adults and children found that biomonitoring of mycotoxins is a useful tool to confirm mycotoxin exposure and in trend analyses. Furthermore, this test method is also an important tool to support the association of exposure with the consumption of certain food groups, at least when there is a major source of mycotoxin intake. In addition, the method has a role in exploring the influence of other factors (such as the socio-economic situation). The development of these studies is highly dependent on the validation of sampling procedures and analytical methods, as well as the development of reference materials and toxicokinetic studies in humans (*Mitropoulou et al.,* 2018).

In two Italian volunteers, DOM-1 was not detected in the urine even after treatment with β-glucuronidase enzyme. In parallel, a 1.7-fold increase in DON concentration was observed using β-glucuronidase enzyme (*Lattanzio et al.,* 2011). In contrast, when analyzing the urine samples of 32 Belgian volunteers, 25% of the samples were positive for deepoxy-deoxynivalenol glucuronic acid (DOM-1-GlcA) (*Huybrechts et al.,* 2015).

In southern Italy, mycotoxins (including DON, FB1, ZEA, α-ZOL and, β-ZOL) were determined by urinary biomarkers. Several mycotoxins were found in all urine samples of volunteers in the study. The PDI estimated by the urinary biomarker approach for DON, FB1, and ZEA was found to fit well with the intake calculated from the dietary approach reported in the literature (*Solfrizzo et al.,* 2014).

In urine samples from Germany (n=50), FB1 was not detectable in the samples (*Gerding et al.,* 2015). In general, low FB1 concentrations can be found in human urine samples (*Vidal Corominas et al.,* 2018). That is because of low oral bioavailability of FB1, which is 5% or less (*Schelstraete et al.,* 2020). Neither T-2, HT-2 and nor HT-2-4 glucuronic acid (HT-2-4-GlcA) were detected in samples from Germany (*Gerding et al.,* 2015).

In Central Europe, studies based on urinary biomarkers have found that mycotoxin exposure in the German population is low, except DON and DON-GlcA, which had a higher incidence and, PDIs calculated from the measured concentrations were close to the bid values and exceeded the bid values in

12% of cases. No significant correlation could be found between the dietary habits of the participants and the mycotoxin exposure. This is presumably due to the relatively low number of samples and low exposure values (*Gerding et al.,* 2014). However, a strong quantitative correlation was found between dietary DON and urinary DON content. Furthermore, urinary monitoring of DON was found to be essential for experiments investigating DON exposure and health effects (*Turner et al.,* 2010).

In a study of 27 Austrian volunteers, the mean measured DON+DON-GlcA concentration was found to be 20.4 ng/ml (the LOD was 4 ng/ml for DON and 6 ng/ml for DON-GlcA). 96% of the samples were positive for DON-GlcA, and in 22% was free of DON. However, DOM-1 was not detectable. On average, 86% (79–95%) of total DON (DON and DON metabolites) was DON-GlcA (*Warth et al.,* 2012). Conjugation is probably the main route of detoxification and de-epoxidation is less important (*Schelstraete et al.,* 2020).

In Hungary, fumonisin exposure was estimated based on population consumption data of the Hungarian National Food Chain Safety Office (NÉBIH) and Hungarian Central Statistical Office (KSH), and FB1 & FB2 contamination of edible maize-based foods. The results showed that the average toxin intake of the population was well below the reference values set by *JECFA* (2016): 2 µg/kg bw/day FB1+FB2+fumonisin B3 (FB3) and EFSA (2018): 1 µg/kg bw/day FB1. However, in 1% of the subjects (n = 60), the PDI (in one case it was 1,81 µg/kg body weight (bw)/day) exceeded TDI (1 µg/kg bw/day). Children's involvement was 2.5 times bigger than the mean (*Zentai et al.,* 2019). On the other hand, when exposure assessment was carried out based on urine multi-mycotoxin analysis, the ratio of volunteers with PDI exceeding TDI was approx. 12% (calculated by the excretion rate in pigs, according to *Gambacorta et al.,* 2013) (unpublished data).

CONCLUSIONS

Urine biomarker-based research are widespread worldwide and are widely used. The method has a significant scientific background. In different countries, volunteers of different ages, genders, and diets were studied using this method. Urine biomarker-based research is considered a very good method to determine *Fusarium* mycotoxin exposure and assess the human health risk they pose. However, it is important to mention that in some cases huge differences can be found between the excretion rates, and in addition, the data and calculation methods found in the literature are not always uniform. For this reason, the different results can only be compared by taking these into account.

Furthermore, the holistic approach reported by One Health is typically not taken into account when evaluating results.

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