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Article

Maternal cortisol level around conception is associated with offspring sex ratio in captive European wild rabbit (*Oryctolagus cuniculus*)

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ABSTRACT – The sex ratio of the offspring at birth is usually 1: 1 established by natural selection. The sex allocation model predicts that if parents have adequate resources, they would benefit from differentially allocating maternal investment to that sex having higher fitness under the particular environmental conditions. However, little is known about what mechanisms would result in biased sex ratio. One such mechanism could be the interaction of of stress reactivity and progesterone levels in the mothers around conception. In the present investigation the fecal cortisol and progesterone levels at the day of conception were measured in fifteen European wild rabbit does kept in cages. The does were clustered to low and high cortisol response groups and the sex ratio of their progeny was determined. We found a significant correlation between the progesterone levels of mothers and their stress status measured at the mating. This correlation was also reflected in the sex ratio bias in their litters. Even though the litter size was not different, we found higher progesterone levels and more female offspring in the does with higher cortisol levels indicating that maternal status around conception may affect the litter sex ratio.

Keywords: sex allocation, stress, progesterone, glucocorticoids, faecal cortisol

INTRODUCTION

The hypothesis of altering sex distribution (sex allocation) is based on the benefits of adaptive manipulation for parents, i.e., natural selection favors parents by modifying their investment in male and female offspring by maximizing their parental fitness (*Fisher,* 1930; *Charnov,* 1983; *Komdeur,* 2012). According to *Fisher's* (1930) theory of equal distribution, selection does not move in the direction of either sex at the population level, as males and females have, on average, equal genetic contributions to the next generation. *Hamilton (*1967) was the first to point out that within a group interactions have different effects on the fitness of males and females. The balance may be biased to a sex

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that improves relatives' conditions within the group (need for helpers due to resource expansion, such as helping sex in cooperative breeding-nesting birds (*Emlen et al.,* 1986*; Lessells and Avery,* 1987). *Trivers and Willard (*1973*)* hypothesized that if environmental conditions affect the survival of different sexes differently, parents are able to modify the sex ratio of their offspring towards the sex resulting better fitness for them (*Trivers and Willard,* 1973).

Initially, the selective mortality of eggs or embryos remained the only explanation, as in vertebrates the sex is genetically determined (chromosomal), so there is little chance that the sex ratio could be affected, as the average sex ratio is evolving to 0.5 during the random meiosis (*Charnov,* 1983; *Palmer,* 2000). However, *Hardy* (2002) and *West et al.* (2002) suggest some specific factors that correlate with sex ratio even in species with sex chromosomes. These factors include the abundance of food available (*Howe,* 1977; *Desfor et al.,* 2007), the mother's condition, and social status (*Trivers and Willard,* 1973; *Clutton-Brock et al.,* 1982*;* 1984; *Sheldon and West,* 2004).

Studies, performed mainly in birds, have shown that the quality of males can also alter sex ratio *(Weatherhead and Robertson,* 1979). In addition, abnormalities in the production of X- / Y-chromosome sperm have been found in mammals, which may also be an influencing factor (*Chandler et al.,* 2007*; Edwards and Cameron,* 2014). The species-specific social environment can also have a strong impact, modifying the sex ratio in proportion to the benefits and / or costs (*West and Sheldon,* 2002; *Komdeur,* 2004). Similarly, there is competition between siblings, a process that can begin in the uterus, when androgens produced by males during pregnancy can have a detrimental effect on female embryos *(Korsten et al.,* 2009*; Bánszegi et al.,* 2009).

Four optional mechanisms should be highlighted that induce biased sex ratio before and during the pregnancy in the maternal body: (1) the hormone-induced conception bias (*James,* 2004); (2) varying mortality of embryos of different sexes due to excessive glucose metabolism (*Gutiérrez-Adan et al.,* 2006); (3) death of embryos of different sexes due to developmental asynchrony of the embryo and the uterus (*Krakow,* 1995a, 1997) and (4) pregnancy hormone suppression and resource deprivation (*Rivers and Crawford,* 1974*; McMillen,* 1979). Although these processes often have the opposite effect, it should be emphasized that stress has a significant effect on all of them. The association between maternal dominance and testosterone levels and the effect of glucocorticoids on glucose metabolism in rodents have been shown to cause sex allocation (*Pratt and Lisk,* 1989*; Ryan et al.,* 2014).

The aim of the present study was to investigate whether the change in cortisol and progesterone levels are associated with biased sex ratio in European wild rabbits and, if so, in which direction. It has been hypothesized that stress during early pregnancy alters progesterone levels, and higher male embryonic death will be demonstrated similarly to hamsters (*Pratt and Lisk,* 1989).

MATERIAL AND METHODS

Ethical approval

The research was approved by the Committee on the Ethics of Animal Experiments of the Hungarian University of Agriculture and Life Sciences Kaposvári Campus (former Kaposvár University, permit number: MÁB/2-2/2019).

Animals and housing

The study was carried out on 15, one year old European Rabbit does (*Oryctolagus cuniculus*), and their first progeny (n= 76). The rabbits derived from captured wild rabbits, breed in cages for the second generation. The animals were mated by natural breeding. The tested animals had been imprinted during the first week of lactation after birth (*Pongrácz et al.,* 2001) to ensure secure handling (*Bilkó et al*., 2000) by reducing their fear of humans. Does were housed individually in bicellular cages (140*60*45 cm) supplied with outside farrowing boxes (40*25*31 cm) according to the Hungarian and EU legislation in force. The cages were made of welded wire mesh, equipped with a plastic floor, hand-refillable feeders and drinkers, and hay racks on the front wire. In addition, there was a galvanized steel sheet litter tray running on rails under each cage. Water, hay, and commercial rabbit fodder was supplied ad libitum during the study. The sex of the offspring individuals was determined at the age of 60 days.

Hormone assessment

Faecal samples were collected 24 hours after the natural mating. Contamination of the faeces with urine was prevented with a mesh placed in the tray under the cage. Samples were stored at -20 °C until extraction. Cortisol and progesterone levels were measured from faeces based on their breakdown products using an RIA method according to *Benedek et al*, (2021). After freeze-drying, the samples were ground, homogenized, and mixed thoroughly. 200 mg of dry-faeces was then placed in a glass vial and 1.6 ml 80% methanol and 200 µl distilled water were added to extract the hormone metabolites. The vials were capped and vortexed for 30 minutes. Samples were then centrifuged (2450 rpm, 20 min, 4°C) and the supernatant was poured off and stored at -55°C. At the time of use, samples were dried in a chamber (Binder) and reconstituted with ASB buffer at a 1:1 dilution rate. The RIA method used for cortisol and progesterone, was developed for hormone determination in the plasma of food animals using tritium labelled hormones (cortisol and progesterone-1,2,6,7-3H(N)) as well as highly-specific polyclonal antibodies raised against cortisol-21-HS-BSA and 11αOH progesterone11HS:BSA in rabbits.

Statistical analysis

The homogeneity and normal distribution of the samples were tested and confirmed with Levene test and Shapiro Wilk test. The does were classified to two groups using k-means cluster analysis (maximum iterations: 10). The significance of the differences between the two groups in the litter size and the sex ratios was estimated by independent samples t-test. Linear regression was calculated between the faecal cortisol metabolite (FCM) values and the female sex ratio and the progesterone values and the female sex ratio. Pearson correlation was calculated between the progesterone values and the FCM values. All statistical analyses were calculated using SPSS 17.0 software.

RESULTS

The fifteen does included in the study were classified to two groups based on the FCM levels measured on the day of mating; the group with low FCM level (group L: 471.94±69.3) consisted of seven individuals and the remaining eight individuals formed the group with high FCM levels (group H: 797.25±102.7). The average litter size did not differ significantly between the two groups (*Figure 1*, group L: 4.85±1.86, group H: 5.25±1.48, P=0.657). The ratio of the two sexes in the total of 76 offspring individuals was equal (38 male and 38 female). However, the two treatment groups showed significantly different (P=0.024, t=-2,546, df=13) sex ratio (Group L: 0.619, Group H: 1.8). This was mainly due to the significant difference in the number of female individuals in the litters (*Figure 1*, mean number of female offspring per doe: group L:1.85±1.21, group H: 3.37±1.06, P=0.023, t=-2.585, df=13). Although the number of the male individuals in the litters showed opposite tendency, the difference between the two groups was not statistically significant (mean number of male offspring per doe: group L: 3.00±1.15, group H: 1.87±1.24, P=0.094, t=1.804, df=13).

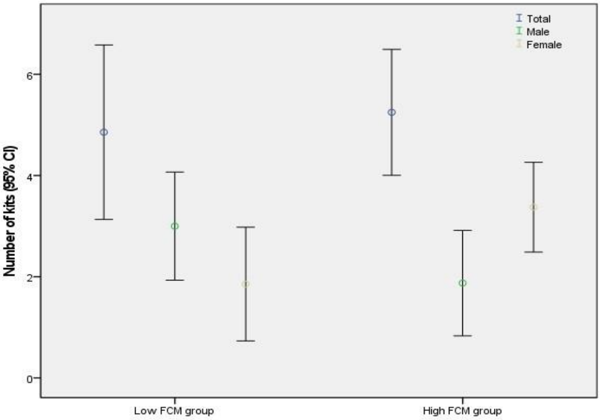

Figure 1. Distribution of the number of the total, male and female kits in the two groups. The differences are significant only in the number of female kits (P=0.023, t-test).

The linear regression between the FCM values measured on the day of conception and the ratio of the female offspring in the litters (female ratio= 0.001*FCM-0,024, Figure 2.) was significant (F=6.997, P=0.020, r^2=0.350). The female individuals have higher ratio in the litters of does with higher cortisol response.

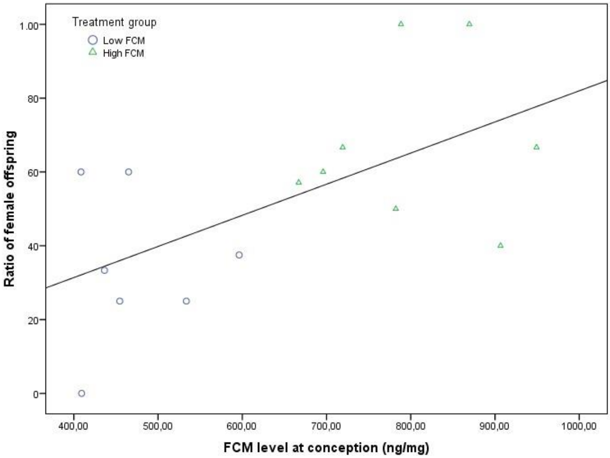

Figure 2. The linear regression between the FCM values measured on the day of conception and the ratio of the female offspring in the litters (F=6.997, P=0.020, r^2=0.350).

The progesterone hormone levels showed similar regression with the ratio of female offspring like FCM level. The linear regression between the two variables (female ratio= 0.001*P+0.086, Figure 3.) was significant (F=7.884, P=0.015, r^2=0.378). The faecal progesterone and cortisol metabolite levels showed a positive, moderate correlation (Pearson correlation, r=0.626, P=0.013, N=15).

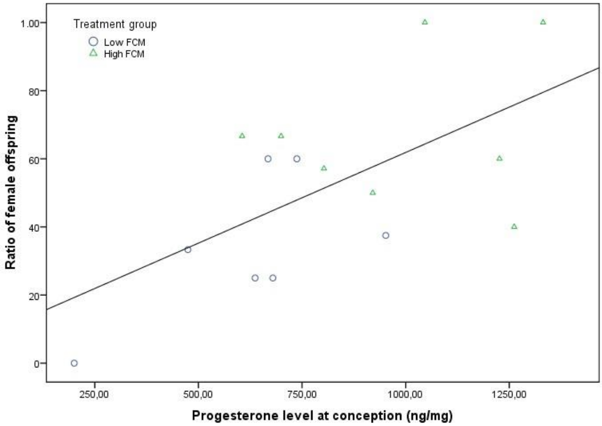

Figure 3. The linear regression between the faecal progesterone values measured on the day of conception and the ratio of the female offspring in the litters (F=7.884, P=0.015, r^2=0.378).

DISCUSSION

The causes of sex ratio bias are complex. Even if focusing only on the two major endocrine axes, the maternal glucose level related to the hypothalamic pituitary adrenal gland (glucocorticoids) and the testosterone and progesterone level related to the gonadal (gonadal) axis in the hypothalamic pituitary gland should be mentioned. Changes in maternal glucose levels in the uterine environment immediately after conception may affect sex ratio proven by experiments (*Larson et al.,* 2001; *Cameron,* 2004). Higher glucose levels favoured male embryos (*Rosenfeld and Roberts,* 2004). Elevated testosterone levels in females due to environmental stress would also be a possible explanation for reinterpreting the atypical sex ratios experienced so far, making the seemingly contradictory results compatible (*Grant* 2007). Several studies have found that a change in non-adaptive sex ratio associated with maternal adrenal hormones

(*James,* 2006) has described the connection between pre-conception testosterone levels in the mammalian female's follicles and the sex of the embryo (*Grant and Irwin*, 2005).

As for wild rabbits, our results show that the amount of FCM during mating, which indicates the stress sensitivity of the individuals, is related to the progesterone metabolite values measured in the faeces. In case of rabbits, it has been shown, that they respond quite differently to stressful situations, which is indicated by remarkable differences in blood serum and glucocorticoid metabolites (*Cabezas et al.,* 2007). Similar to processes in humans, in rabbits the adrenal gland produces significant amounts of progesterone in addition to the ovary, as adrenocorticotropic hormone regulates progesterone production in the adrenal gland (*Fajer et al.*, 1971). In several species (*Bos taurus, Zalophus californianus, Rattus rattus*), progesterone secretion in stressed females has been reported to be as strong in the adrenal gland as in the ovary and a positive correlation has been found between cortisol and progesterone levels. (*Fajer et al.*, 1971). In case of wild rabbits, a positive correlation was also found between cortisol and progesterone values measured on the day of parturition (*Benedek et al.,* 2021), which can be explained with a common metabolic pathway, as some progesterone is released into the bloodstream before it is converted to cortisol (*Beaulieu-McCoy et al.,* 2017).

Furthermore, we found that the number of female offspring among the offspring of high-stress mothers was significantly higher than that of non-stressed mothers.

Furthermore, we found that the number of female offspring among the does exhibit relatively high cortisol values was significantly higher that than of does showing relatively low cortisol values.

The cortico-adrenal stress response can be elicited by external stress (*Teixeira et al.,* 2007) and internal anxiety (*Breazile*, 1988). There may be several outcomes, one of which may be low progesterone production or altered maternal progesterone sensitivity (*Albrecht et al.,* 1978). But the opposite has also been described in pregnant baboons, where progesterone hormone levels were associated with cortisol levels, and with greater stress, progesterone hormone levels became higher (*Albrecht et al.,* 1978). Cortisol has a higher affinity for uterine and placental receptors than progesterone, thereby inhibiting its recognition. Cortisol inhibits uterine blood flow, capillary permeability, thereby inhibiting implantation (*Coubrough*, 1985), thus stress disrupts and impairs the synchronization between the uterus and the embryo. In hamsters, stress around implantation has been shown to result in higher mortality in males than in female embryos (*Pratt and Lisk,* 1989), however, progesterone levels were reduced in this species. In human terms, the mortality of the male embryo was also higher under stress (*Obel et al.,* 2007; *Jongbloet*, 2004), and the growth rate of the male foetus was higher in the early stages of pregnancy, which may explain the differences in differentiation and vulnerability (*Henriksen et al.,* 1995). Stress, or internal anxiety activates the hypothalamic-pituitary-adrenal axis, and cortisol can cause more severe damage to the foetus, so stress may contribute to the recently observed sex ratio bias in humans (*Marcus et al.*, 1998).

The results described herein provide a small step closer to understanding the processes discussed above.

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Article

Application possibilities of survival analysis for time-to-event data in animal science

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ABSTRACT – The application efficiency of several statistical methods was tested based on an open field behavior test of mice. The examined trait was the duration time until the animals approached the experimenter’s hand. The available time was fixed in 300 seconds. There were monitored 80 mice belonging to two different species of the Mus genus in equal proportion. Besides, male and female and young and adult animals have also represented the evaluated groups in equal proportions. The data of the examined trait was analyzed with Generalized Linear Models, Kaplan-Meier survival curves, and with Cox Proportional hazard model. The applied statistical procedure provided completely discordant results. According to the GLM results none of the examined factors (species, sex, and age) had significant effects on the examined variable. On the contrary, all factors proved to be significant using a procedure based on the survival analysis. Kaplan-Meier survival curves indicated a higher proportion of individuals successfully approaching the experimenter’s hand in all of the compared groups representing different species sexes and ages, respectively. The estimated Cox regression coefficients were significant indicating the significant effects of the species, sex, and age on the investigated trait. Based on the estimated Hazard ratios the probability that in the next time unit a successful approach of the experimenter’s hand would occur is three times more likely for one species than the other, twice as much for males and the juveniles than for the females and for the adults. Based on the present study it could be concluded that the successful approach of the experimenter’s hand by the mice is clearly “time to event data” thus it is suitable to be analyzed with survival analysis procedures. It could be concluded that applying conventional GLM was not adequate because due to the lack of the successful approach and approach times the censored data should not be used and thus the sample size would largely be reduced.

Keywords: behavior studies, time-to-event data, survival analysis

INTRODUCTION

In any statistical analysis (depending on the research hypothesis) type and distribution of the utilized data will determine the applied statistical method. In animal science, most of the economically important traits are quantitative so that they can be measured and they can be characterized by real numbers providing also the unit of measurement. For these cases, the applied statistical

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methods are generally straightforward and widely known. There are however some other data types that are much less common and consequently, adequate analyzing methods are less frequently used. One of these method is survival analysis which is a statistical method targeting the so called time-to-event data. The meaning of the term "event" could be death (*Moreno-Bentancur et al.,* 2017), developing a disease (*Lánczky et al.,* 2016; *Li et al.,* 2018), or any other well-defined action that can unambiguously be detected on a time scale. Conventionally from the human aspect, the occurrence of the event was usually negative therefore it is often called "failure" but the character of the event can also be positive such as the necessary time for the unemployed people to find a new job (*Brück-Klingberg et al.,* 2011). One of the most peculiar characteristics of the survival analysis is that at the end of the predefined examination period generally not all of the monitored subjects had achieved the event (i.e. they are not yet died from cancer, they may not have found a new job, etc). This phenomenon is called censoring which means that at the end of the study the fate of these subjects is simply unknown. Probably the most invaluable characteristic of survival analysis is that the data of these subjects are not wasted but also utilized in the statistical analysis by including a binomial variable providing the status of every subject (i.e. if the event happened or not). Interestingly for certain diseases like AIDS, the related data is left-censored because of the detection limits of this disease (*Chen et al.,* 2014).

In animal science, the most obvious application possibility is to analyze longevity (*Baginé Hunyadi et al.,* 2016a; *Soltész et al.,* 2016; *Török et al.,* 2021) where longevity is describing productivity and usually covers the period between first parturition and culling. Longevity has high economic importance as culling of the animals is involuntary and related to developing certain diseases or leg problems (*Balogh et al.,* 2015). However, depending on the utilization of the animals' longevity can also describe the length of their successful sport carrier (*Sole et al.,* 2017). Nevertheless, other time-to-event data can also be examined such as farrowing interval (*Baginé Hunyadi et al.,* 2016b). Another area where survival analysis is the adequate method for statistical analysis is the animal behavior studies especially those describing the animals' exploratory behavior (*Hansen et al.,* 2015). In earlier studies, exploratory behavior was investigated based on either novel object test (*Fox et al.,* 2009) or open field test (*Fitzgibbon*, 1994), however according to more recent findings these tests measure several dimensions of the behavior and characterize several areas of personality (*Coleman et al.,* 1998). One of the main benefits of these tests is that results are generally repeatable both in novel and in accustomed environments. Performing these tests provides insight to the area of exploration while in the accustomed environment it characterizes boldness (*Coleman et al.,* 1998). The present study aimed to demonstrate the adequacy of survival analysis using the example of an open field behavior test in mice where its efficiency is compared to an other conventional statistical method. Besides, according to the intention of the authors, the detailed explanation of the survival analysis procedure could also increase its recognition and more frequent use in animal science.

MATERIAL AND METHODS

In the present study, an open field was used to describe mouse behavior. The experiment was performed in the rodent house of the Kaposvár Campus of the Hungarian University of Agriculture and Life Sciences. The rodent house has its mouse breeds, where individuals of known ages, sexes, and backgrounds are kept. The current stock came from several wild populations captured from different parts of the country. The study was performed on 80 laboratory-born offspring of wild animals belonging to two different species of the Mus genus in equal proportion. Besides, male and female and young and adult animals have also represented the evaluated group in equal proportions. Animals were housed in standard laboratory plastic rodent boxes at standard laboratory temperatures of 20-22 ° C. For littering, purified wood chips (LIGNOCELL J. Rettenmaier & Söhne GmbH, Rosenberg, D) were used, complete rodent feed (Ssniff S8106-SO11 Spezialdiäten GmbH, Soest, D) and water were available to the animals ad libitum. For the study, selected individuals were tested individually in a 36.5 x 21 x 18 cm white plastic box. The time of the test is 300 seconds, during which the behavior of the animal was recorded (approach, number of approaches, first approach). Mice were grouped by species, sex and age group. Young mice were selected from 28 to 35 days of age while adult mice from around 500 days.

The statistical analysis of the duration of approaching the experimenter's hand was performed by several methods. After showing with the Shapiro-Wilk normality test that the normal distribution of the data was not given ($p<0.001$) Generalized Linear Model was applied using the SAS 9.4 software (PROC GLM), where species (one and two) sex (male and female) and age (juvenile, adult) were treated as fixed factors. Besides, the duration of approaching the researcher's hand was analyzed separately for the species, sex and age using the Survival function (*Kaplan and Meier*, 1958) where the survival function (S (t)) estimates the probability (P) that the object of the experiment survives longer (T) than some specified time (t): $S(t) = P(T > t)$ in this study, it means that the animal did not yet approach the hand of the experimenter. Significant differences between the various survival function curves were determined by applying the Log-rank test (*Kaplan and Meier*, 1958). Finally, the effects of species, sex and age on the duration of approaching the experimenter's hand was also analyzed applying Cox Proportional Hazard model (*Cox*, 1972) (also called Cox Regression) where the hazard is the probability that after a certain period the event would occur during the next time unit (i.e. the successful approaching will occur in the next second). The Cox regression will provide the regression coefficients (b) for the breed and the age and raising the coefficients to the power of the exponential constant (appr. 2.718) will provide the hazard ratio or the relative hazard:

$$\text{Hazard ratio} = \exp(b)$$

The hazard ratio is constant for the whole examination period (5 minutes in this case). In the present study, the relative hazard shows the ratio of the probabilities that during the next unit of time a successful approach of the experimenter's hand will occur in the examined species, sex, and age groups, respectively (*Woodward*, 2014). These statistical analyses were performed using the SAS 9.4 software package applying the LIFEREG (*SAS Inst. Inc.,* 2013) and PHREG (*SAS Inst. Inc.,* 2014) procedures. To guarantee the repeatability of these procedures all the SAS script with the embedded data is provided as supplementary material (S1.sas).

RESULTS AND DISCUSSION

The descriptive statistics of the analyzed variable are provided in *Table 1*. It has to be mentioned that in 28 cases the animal did not approach the experimenter's hand at all. According to the data pattern of the groups, the animals representing the first species generally approached the experimenter's hand slower than that of the mice in the second group. Besides, the males and juveniles showed shorter approaching times than that of the females and adults, respectively, however, all group means coincided with large standard deviations. Consequently, according to the GLM results none of the examined factors (species, sex, and age) had a significant effect on the examined variable (p>0.05).

Table 1
The descriptive statistics of the duration of approaching the experimenter's hand

Factor	N	Mean	Standard deviation	Min.	Max.
Species1 Sex 1 Age 1	10	140,20	30,982	0	234
Species 1 Sex 1 Age 2	10	121,50	27,237	0	204
Species 1 Sex 2 Age 1	10	61,30	31,232	0	212
Species 1 Sex 2 Age 2	10	82,00	27,395	0	177
Species 2 Sex 1 Age 1	10	89,80	19,994	0	152
Species 2 Sex 1 Age 2	10	52,90	7,288	0	78
Species 2 Sex 2 Age 1	10	102,80	28,082	0	187
Species 2 Sex 2 Age 2	10	55,40	12,387	0	94

The survival function curves for the different breed and age groups were provided in *Figures 1-3*, respectively. The lower curves of a species, sex, or age group indicate a higher proportion of individuals successfully approaching the experimenter's hand compared to the animals of the other group. Log-rank test showed significant effects of all factors ($p<0.05$).

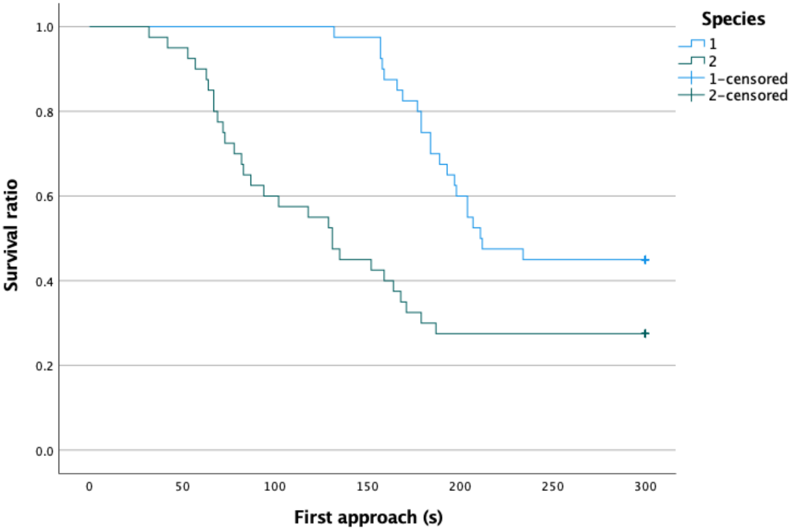

Figure 1. Survival curve of the species

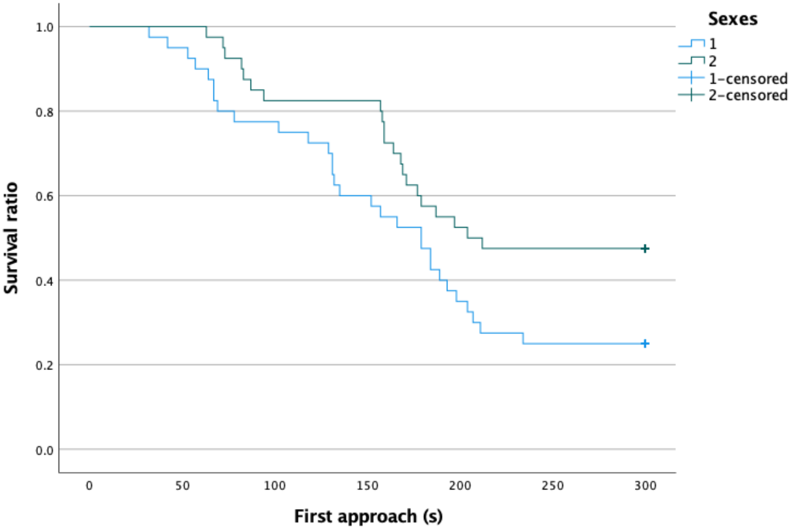

Figure 2. Survival curves for the sexes

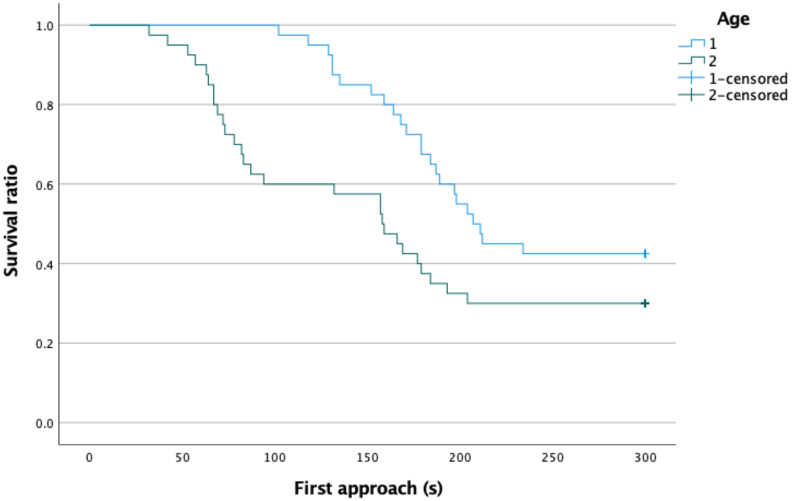

Figure 3. Survival curves for the age groups

In *Figure 1.* it is shown that for the mice of the first group it took appr. 130 seconds until the first animal managed to approach the hand of the experimenter while for the second group this time was 100 seconds faster. The necessary time by which 50% of the mice completed their first hand approach was 211 and 130 seconds, in the first and second groups respectively. The proportion of animals failing to perform any approach was substantially higher in the first species (42,5%) than in the second group (25%). Interpretation of Figures 2-3. are the same and it can be seen that the necessary time by which 50% of the male and female and juvenile and adult mice performed their first approach were 179 and 204 and 158 and 207 seconds, respectively. Besides, the proportion of animals failing to perform any approach was substantially higher in the female (45%) than in the male (22.5%) and in the adult (40%) than in the juvenile (27.5%) group.

The Cox regression parameters estimated by the PHREG procedure are presented in *Table 2.*

The estimated Cox regression coefficients were significant indicating the significant effects of the species, sex, and age on the investigated trait. Based on the estimated Hazard ratios the probability that in the next time unit a successful approach of the experimenter's hand would occur is three times more likely for one species than the other, twice as much for males and the juveniles than for the females and for the adults.

Table 2

Maximum Likelihood estimates of Cox regression coefficients (b) and Hazard ratios examining the effects of species, sex, and age of mice on the duration of their first successful approach to the experimenter's hand.

Factor	b	SE	p	Hazard ratio
Species	1.12	0.30	0.0001	3.08
Sex	-0.77	0.29	0.008	0.46
Age	0.81	0.28	0.005	2.25

Looking at the relevant literature, it is clear that the conventional ANOVA is a frequent evaluation method analyzing behavior tests (*Brantsater et al.,* 2017, *Lee et al.,* 2021). *Brantsater et al.* (2017) even emphasized that ANOVA (rather than Survival Analysis) was used analyzing the approaching time of poultry to a novel object during 120 seconds which trait was a right-censored time to event data. Looking at the observations, of the present study the main problem with ANOVA/GLM was that 28 out of 80 animals did not approach at all the experimenter's hand (S1.sas) which is 35% of the investigated sample. However, a general approach from the researchers that the so-called censored data is often coded as the possible maximal value of the examined trait, and the data is analyzed with ANOVA afterward. This procedure is not appropriate however, as for the censored data the exact value is not known so it should not be included in the analysis at all. The survival function curves are not affected by this problem since the status of the animals (data censoring) has been taken into account. Another advantage of the survival curves is that they are highly informative and provide a clear overview of the number of completed approaches of the experimenter's hand throughout the whole examination period in the examined groups. The only unfavorable characteristic of the Kaplan Meier curves is that they can only describe one factor at a time. Examples for applying Kaplan Meier survival curves can be found in various studies examining for example pig longevity (*Baginé Hunyadi*, 2016a) or mink behavior (*Haage et al.,* 2017). The problem of investigating one factor at a time was not apparent in the Cox regression where the species, sex, and age effects could be examined simultaneously. Using the same methodology as in this study similar results were received in a rabbit behavior study where the running rejection behavior of rabbits on a treadmill was investigated (*Nagy et al.,* 2004). Another example of applying Cox Proportional Hazard was reported in a behavior study of spotted hyenas (*Greenberg et al.,* 2017) where it was observed that the Cox proportional hazards model was significant indicating that subjects in the high-disturbance area were significantly less neophobic than subjects in low-disturbance areas.

CONCLUSIONS

Based on the present study it could be concluded that the successful approach of the experimenter's hand by the mice is clearly "time to event data" thus it is suitable to be analyzed with survival analysis procedures. Applying conventional GLM would not be adequate because due to the lack of successfully approaching the experimenter's hand and approach times the censored data should not be used and thus the sample size would largely be reduced.

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Instituional Review Board statement: This research was approved by the Committee on the Ethics of Animal Experiments of Kaposvár Campus (permit number: MATE KC MÁB/21-2/2022). The authors declare that all experiments were performed in accordance with approved guidelines and regulations.

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Article

In vivo classification of two closely related species of mice, mound-building mouse (*Mus spicilegus*) and house mouse (*Mus musculus*)

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ABSTRACT – Correct identification of similar, closely related species with overlapping distribution is a crucial point in field biology. In small mammal studies, species identification is particularly problematic in population studies using trapping where live animals need to be identified. The aim of our research was to develop a method making the classification of the two Hungarian mouse species, mound-building mouse (*Mus spicilegus*) and house mouse (*Mus musculus*) possible based on morphometric characters. The basis to obtain reference data was the captive populations of caged animals housed in our laboratory where the true species classification was known for every animal. Body weight, body length, tail length, and tail diameter were measured for 56-56 individuals from both species. From these measurements the ratio of the body length/tail length was also calculated. Besides, the sex and age of these animals were also recorded. Data analysis consisted of stepwise discriminant procedure and discriminant analysis, respectively. The stepwise discriminant procedure restricted the morphometric characters to the ratio of the body length/tail length and tail diameter. Performing the discriminant analysis to these body measures a perfect classification was obtained even using cross-validation. Thus, applying the obtained discriminant function to the classification of any live trapped mice is feasible.

Keywords: small mammal studies**,** live-trapping, classification

INTRODUCTION

Rodents (*Rodentia*) is one of the most populous order in the Mammalian class, and due to their excellent adaptability, its representatives can be found on all continents (*Bihari*, 2007). They play an essential role in the ecosystem as the primary food for many of our protected and highly protected species of carnivorous birds (*Bihari,* 2007).

One of the most common methods of surveying live rodents is by trapping them with various devices. Trapping small mammals is a direct method that besides faunistic and point mapping tasks allows us to estimate total population size as well.

The genus *Mus* belongs to the family of Mice (*Muridae*) and consists mainly of nocturnal terrestrial species (*Musser and Carleton*, 1993). In Hungary, the

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Mus genus is represented by two species: the eastern subspecies of the house mouse (*Mus musculus musculus* Linneaus 1758) and the mound-building mouse (*Mus spicilegus spicilegus* Petényi 1882). Based on external morphological features the two mice species are difficult to distinguish (*Demeter et al.,* 1996). In Hungary, both the mound mouse and the house mouse are 5-8 cm long, the color of the back coat is gray or greyish brown. Characters such as coat color, body, tail, or leg length are poorly distinguished from relatives of the genus Mus (*Auffray and Britton-Davidian,* 2012). The first scientific description of the mound-building mouse was published by a paleontologist-zoologist János Petényi Salamon (*Petényi,* 1882). The species can play an essential role in nature conservation as an indicator of traditional farming, as in Hungary the individuals of this species can typically be encountered in abandoned agricultural areas (*Bihari,* 2004; *Sokolov et al.,* 1998), where they build their mount during the winter (*Simenovska-Nikolova and Gerasimov,* 2000). On the contrary, the house mouse invades human settlements as the temperature drops. However, both species of mice spend the growing season on the agricultural landscape. Identifying the two species in non-laboratory conditions (with genetic testing) has been limited so far to owl sputum analysis, where the remnant of the animal (e.g. skull bones) is examined in its original habitat. In addition to owl sputum analysis, it would be important to find morphometric differences in live-trapped animals to help species' classification. Hence, an accurate picture of the distribution of mound-building mice and house mice in Hungary could be obtained. The aim of our research was to develop a method making the classification of the two Hungarian mouse species, mound-building mouse (*Mus spicilegus*) and house mouse (*Mus musculus*) possible based on morphometric characters.

MATERIAL AND METHODS

The present study was carried out at the rodent laboratory of the Kaposvár Campus (of the Hungarian University of Agriculture and Life Sciences). The animals were kept in accordance with current legislation, the study is not an animal experiment. The number of animals used in the study was reduced as little as possible (1998. XXVIII. Hungarian law on the protection and welfare of animals).

The founders of our captive mouse populations were wild-caught mound-building mice and house mice from several populations throughout Hungary. The descendants of the founder animals were bred for several generations forming our two captive laboratory populations where the age and species of all animals were known.

Animals were housed in standard T4 laboratory polycarbonate rodent boxes on a 12-12 hour reverse day-night cycle. Red neon fluorescent lamps provided the night illumination. The holding temperature is standard 20-22 ° C and humidity is 30-60%. For bedding, wood shavings (Lignocell J. Rettenmaier and Söhne GmbH, Rosenberg, Germany) and hay were used. Complete rodent feed (Ssniff S8106-SO11 Spezialdiäten GmbH, Soest, Germany) and water *ad libitum* were available.

From the two available captive mouse populations, 56 adult mound-building mice and 56 adult house mice were randomly selected within the species with a sex ratio of 1:1. As no sexual dimorphism was found in a preliminary analysis the sexes were not used in the later statistical analysis.

During the data collection, the species, sex, and age of the individuals were recorded, and their body weights were measured on a lab analytical digital balance scale. The body length of the individuals was measured in millimeters from the tip of the nose to the anus. This measurement was performed with a 3 cm diameter wide and 10 cm long transparent cylinder open at each end and scaled at every 1 mm. The tail length of the animals was measured with a digital caliper from the anus to the tip of the tail to the nearest 0,01 mm (*Figure* 1).

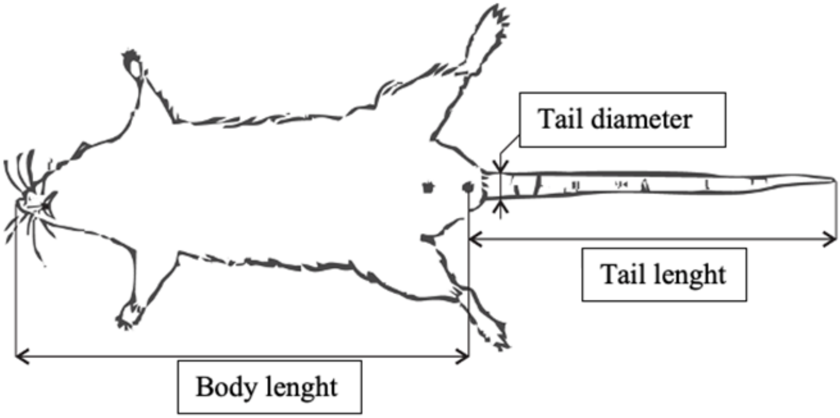

Figure 1. Body variables measured on a live animal. Body length: from nose to anus. Tail length: from the anus to the end of the tail. Tail diameter: the diameter measured at the base of the tail.

Using a digital caliper measuring with two decimal places, we measured the tail diameter to place in the tail base. The body length/tail length ratio and the body length-tail length difference were derived from the measured recordings. The group means of all measured traits were depicted using Excel figures.

Analysis of the above mentioned morphometric characters consisted of stepwise discriminant procedure and discriminant analysis, respectively applying the STEPDISC (*SAS Institute Inc.,* 2013) and DISCRIM (*SAS Institute Inc.,* 2013) procedures of the SAS 9.4 statistical software where the known species of the animals served as the class variable. The equality of variance-covariance matrices of the different species and the equality of their vector means were tested with the POOL and MANOVA options of the DISCRIM procedure. To ensure the reliability of the calculated discrimination function classification of the analyzed animals were tested using cross-validation.

RESULTS AND DISCUSSION

The means of the measured morphometric characters were depicted by the two examined species (*Figures 2-5*). It can be seen that several group means were similar and there was an obvious overlapping between the species for all traits.

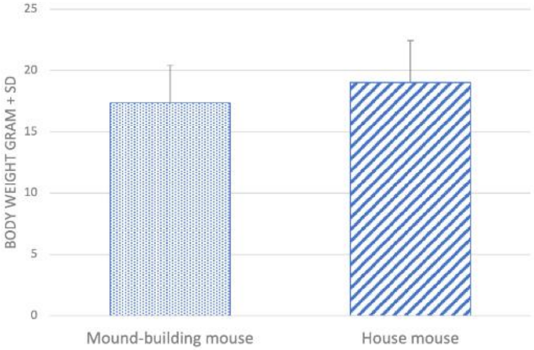

Figure 2. Bodyweight of the two species

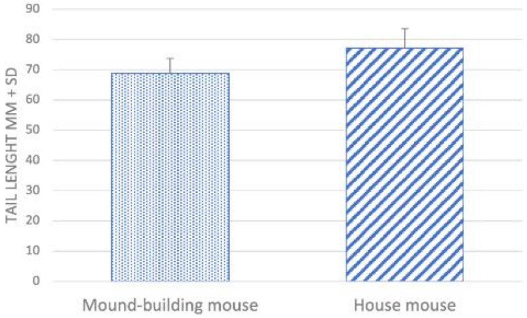

Figure 3. Tail length of the two species

Using the STEPDISC procedure a stepwise discriminant analysis was performed to select a subset of the measured morphometric characters for use in discriminating between the species. In this study, the FORWARD option was used and at each step, PROC STEPDISC entered the variable that contributed most to the discriminatory power of the model. The results of the procedure being provided in *Table 1.* Based on these results the DISCRIM procedure was performed using only the tail diameter and the body length/tail length ratio, respectively.

Table 1

Stepwise selection summary

Step	Morphometric Character	Partial R square	F value	Pr > F
1	Tail diameter	0.84	587	0.0001
2	Body length/tail length ratio	0.12	16	0.0001

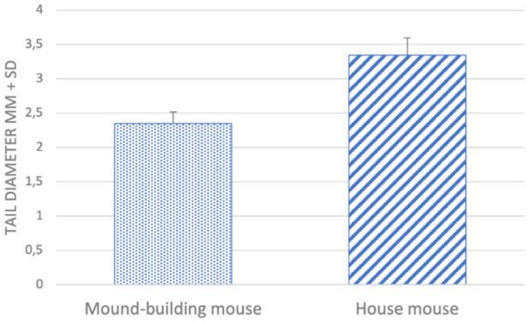

Figure 4. Tail diameter of the two species

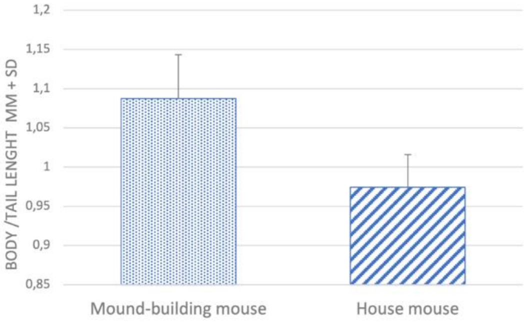

Figure 5. Body length/tail length ratio of the two species

Based on the discriminant analyis both the variance-covariance matrices ($Chi^2 = 0.0019$) and the vector of means (Wilks lambda = 0.0001) were different thus quadratic discriminant analysis was used. Based on the calculated discriminant function classification of the reference data without and with cross-validation is presented in *Tables 1-2*, respectively. It can be seen that the applied quadratic discriminant function resulted that 100% of the measured individuals could be properly assigned to their species even with cross-validation.

Table 2.
Classification summary for calibration data

Number of observations classified into species			
From: Species	1	2	Total
1	56	0	56
2	0	56	56
Total	56	56	112

Table 3.
Cross-validation summary

Number of observations classified into species			
From: Species	1	2	Total
1	56	0	56
2	0	56	56
Total	56	56	112

Regarding the body sizes of living individuals of the two socially different mouse species, we tested several variables which could help the differentiation of the two species. The first trait was body weight, but we do not consider this a good determinant, as body weight varies with age, sex, and even seasonality. Based on preliminary analysis body length did not differ between the two species, but the tail length did, although the difference in tail length alone does not allow the identification of the two species on the field. If we examine the length of the tail in relation to the length of the body, we see a difference between the two species. The tail of the mound-building mouse is significantly shorter than that of the house mouse, where the body length and tail length are nearly the same or slightly longer than the body. *Lindquist* (2003) found a similar difference in relative tail length in the two American mouse species he studied, the deer mouse (*Peromyscus maniculatus*) and the very similar white-footed mouse (*Peromyscus leucopus*). The morphology of the deer mouse and the white-footed mouse is very similar, challenging to distinguish from each other, just as in the case of the mound-building and the house mouse. In field studies, the two American species can be distinguished from each other based on relative tail length, the tail of the deer mouse is longer than that of the white-footed mouse (*Horner,* 1954). Our other derived variable i.e., the difference between body length and tail length, is also a well-measurable marker on the field; the tail of a mound-building mouse is shorter than its body, and the tail of a house mouse is longer than the body.

A significant difference was obtained for the tail diameter. The tails of house mice are much thicker than those of mound-building mice. *Sokolov* (1998) also mentions that the tail of the mound-building mouse is the thinnest within the genus Mus, but we have not had quantified data on it so far. The tail diameter of the house mice is over 3 mm at the base of the tail, while that of the mound-building mice is around 2 mm. The question arises whether different tail lengths and thicknesses may be related to the different habitat use of the two species. The tail may be an important factor in overcoming certain field obstacles. Thus, we would like to continue investigating this question, as we know that house mice move to buildings for the winter (*Carlsen*, 1993), where they need the ability to climb, while mound-building mice spend the whole year on abandoned fields (*Bihari,* 2004, *Sokolov et al.,* 1998). In previous open-field tests comparing the two species, *Sokolov et al.* (1990) found that house mice climb on landmarks while exploring the terrain while the mound-building mice remain on the ground.

Concerning species classification based on mouse morphometric analysis, only few studies were found. Based on mouse skulls recovered from owl-pellets *Cserkész et al.* (2008) managed to distinguish *Mus spicilegus* from Mus musculus with almost 100% success based on discriminant function analysis of cranial measurements. Analyzing different body measures (various length, width, and height) of live animals in several *M. m. domesticus* populations *Slabova and Frynta* (2007) also received very high (93-97%) reclassification success for the Czech and Syrian mouse populations applying discriminant function analysis.

It is important to note that the data of our study only characterize the Hungarian population because our data are from this range; there are local differences within the species in the geographic range of the species complex, so our findings can only be applied to the Hungarian population. Based on this knowledge, we have the opportunity to examine the mound-building mice during the field trapping of small mammals; thus, we could get an overall picture of the condition of the Hungarian populations. With the growth of intensive agriculture, the habitat of mound-building mice decreases, and in the absence of the set-aside of arable land, the success of their overwintering may also decrease significantly.

CONCLUSIONS

It can be concluded that the detected morphological differences between the mound-building and the house mouse, may have great importance in the reliable classification of the two species in field studies. The two species could be distinguished based on the tail diameter and the body length/tail length ratio. These variables are well-measurable in the field and from preliminary studies, it is known that these traits are not sex-dependent. Further studies are needed on the possible age effect on these variables because, in field studies, adult and juvenile individuals can also be encountered, where body proportions may be different.

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Review

Water balance calculation capability of hydrological models

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ABSTRACT - Currently, in the world, there are many different hydrological models built and developed to solve problems related to the hydrological cycle. Each model has its specific mathematical foundations to describe physical processes in nature. Therefore, each model has its various characteristics: setting up the model, input data requirements, model calibration and verification, and output results. Water balance is still playing an important role in the effective management and use of water resources for agriculture. Based on the results of the hydrological parameter's calculation, the water balance of the study basin can be calculated by the user or by the separated module of each model. Each hydrological models have its advantages and disadvantages. However, it is impossible to simulate hydrological processes and water balance completely accurately in nature. Still, simulation results can give us a view of the changing trend of hydrological components and the water balance. Model developers are gradually completing the shortcomings and improving the efficiency and accuracy so that the model can simulate reality with the highest accuracy. This paper sets out to review the fifteen hydrological models currently widely used in the world. Within the frame of the present study, some models are only briefly introduced; the rest are considered in more detail, from more aspects, from specific examples so that readers can decide for themselves which model is suitable for their study area and simulation needs, especially in the identity of the complex and unpredictable impacts of climate change on the agricultural sector.

Keywords: integrated watershed modelling, water balance

INTRODUCTION

Hydrologic problems are usually calculated based on statistical methods; hydrologic time series are analysed based on autoregressive components methods, and components of the hydrological cycle are calculated based on differential equations. *Freeze and Harlan (1969)* questioned the application of mathematical descriptions to the modelling of hydrological systems, which included equations to simulate individual hydrological processes and the interactions between hydrological components. The two authors laid the main foundations for the model structure, model parameter values and model execution *(Clark*

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et al., 2017). As a result of the developments in information technology, applying the geographical information system (GIS) and various mathematical- and physical-based hydrological modelling software has also become feasible for related research. Since then, several hydrological models have been built and developed according to the development of information technology in general and the technologies of hydrology, geographical information system and remote sensing. During the hydrology and water balance research in different study areas, scientists have also found that only integrated modelling methods can understand the ongoing hydrological processes. In the following sections of the study, some specific examples related to the necessity of hydrological models in the simulation, analysis of the results of water balance, and hydrological parameters calculation. The question is that among many hydrological models widely used, which model is an effective tool and suitable to achieve the research purpose and what criteria to choose the model or the combination with other models? In this study, we try to give insight into hydrological models. The models were analysed in detail, including technical background, data requirements, calibration, and some application fields. Based on this information, readers can make the right decision in choosing the suitable model to apply to their research.

APPLICATIONS FOR PROCESS-BASED HYDROLOGICAL MODELLING

Fatichi et al. (2016) defined the process-based hydrological model as*: "a mathematical formulation that explicitly represents and/or incorporates through assimilation approaches, the hydrologic state variables and fluxes that are theoretically observable and can be used in the closure of assumed forms of the laws of conservation of mass, energy, and momentum at temporal scales characterizing the underlying physical processes".* Process-based hydrological models based on the observation and scalability of hydrological processes have a history of development since the 1960s. At first, this approach was popularised, but later, scientists realised that it is difficult to provide accurate simulation results for climate conditions, hydrological regimes, and different catchments with mathematical modelling based on physical laws. Besides hydrological process nonlinearity, spatial and temporal scale variation, and the ability to observe, heterogeneity and parameter equifinality has made it necessary for the user to consider the simulation objectives and purposes, as well as the data availability conditions, to be able to consider the usefulness of the approach or to proposals of other alternatives *(Fatichi et al., 2016).* However, this method is applied to make hydrological forecasts in non-stationary climate conditions and the effects of land-use change. Below are the process-based hydrological models that are widely used today *(Table 1.).*

Table 1.
Descriptions of process-based hydrological models

Model	**Category**	**Type**	**Description**
HEC-RAS	Hydraulic modelling	1D	HEC-RAS is a 1D hydraulic model used for steady and unsteady open channel flow computations. HEC-RAS includes tools for running sediment transport and water quality analyses. HEC-RAS is capable of modelling subcritical, supercritical, and mixed flow regime profiles and can model inline channel structures such as culverts, weirs, and bridges.
HEC-HMS	Hydrologic modelling	1D/2D	HEC-HMS is a lumped parameter/quasi-distributed hydrologic model that includes support for MODClark - a quasi-distributed hydrologic model. HEC-HMS has most of the functionality in HEC-1 and is used for modelling single or multiple storm events. It supports several different options for modelling rainfall, losses, unit hydrographs and stream routing.
SWAT	Soil and Water process-based model	2D	This model can simulate the quality and quantity of surface water, as well as groundwater. Besides, it is also possible to determine the impacts of land use, as well as climate change on the environment.[1]
PAWS	Parallel hydrologic, process-based watershed	3D	The PAWS model can simulate hydrological processes based on the laws of conservation of energy such as evapotranspiration, vegetation growth, surface and groundwater, flow in the unsaturated zone, and interactions between the components.[2]
AQUAVEO SMS	Surface-water Modeling System	3D	SMS include full range of coastal, riverine solution modules for simulating riverine and flood modeling processes.[3]
AQUAVEO WMS	Watershed Modeling System	3D	WMS include complete watershed solution such as GIS, web-based data, Terrain data. The model has features such as automated watershed delineation & hydrologic modeling, hydraulic modeling & floodplain mapping, storm drain modeling and export to Google Earth.[4]

[1] https://swat.tamu.edu/

[2] http://water.engr.psu.edu/shen/PAWS/PAWS_Documentation.pdf

[3] https://www.aquaveo.com/

[4] https://www.aquaveo.com/software/wms-watershed-modeling-system-introduction

INTEGRATED HYDROLOGICAL MODELLING ENVIRONMENTS

Integrated hydrological models can simulate hydrological processes occurring on the surface and subsurface at a wide range of spatial and temporal scales (*Paudel and Benjankar, 2022*). Especially, the approach integrates hydrodynamic models to simulate river hydraulics, water quality, sediment transport, floods and forecasting changes in surface flows. Thus, integrated hydrological models enable the experts to look into interactions across the entire hydrologic cycle. The research group of *Kollet et al. (2017)* reviewed and compared integrated hydrological models, including Cast3M, ATS, CATHY, HydroGeoSphere (HGS), GEOtop, MIKE-SHE, and ParFlow (PF). Due to the limitation of the paper, below we only review the technical background of the Cast3M, ATS, CATHY, HydroGeoSphere (HGS), GEOtop models based on the research paper of *Kollet et al. (2017)* and go into a more detailed analysis with MIKE SHE and ParFlow models. In addition, LISEM, PAWS, WATERISK, and InHM models were also considered in more detail. Thus, a total of 12 integrated hydrological models are considered in this paper.

Cast3M

The Cast3M model is developed based on the French Alternative Energies and Atomic Energy Commission (CEA) in France with the primary purpose of solving problems related to solid and fluid mechanics. The model can also solve hydrology and hydrogeology problems in finite elements or finite volumes. Cast3M uses the Darcy multidomain approach to integrate surface flows and subsurface to apply to 2-D and 3-D configurations (*Weill et al., 2009)*. Surface flow is calculated based on a 3D porous layer. The equations are discretised with a finite volume scheme to combine the Darcy and Richards equations in the subsurface with the diffuse wave approximation of the Saint Venant equations to the surface flows into a single generalised Richards equation (*Kollet et al., 2017*). According to the researchers, the Richards equation can also be integrated into the advection, diffusion, and dispersion of transport equation.

Advanced Terrestrial Simulator

According to *Kollet et al. (2017)*, the Advanced Terrestrial Simulator (ATS) model is a specialized model that simulates ecosystem hydrology processes based on the Amanzi model and the Arcos multiphysics management strategy. The utilities of the ATS model include freeze processes, a surface energy balance, snow processes and thermal hydrology in the surface and subsurface. To determine dynamic vegetation and deformation capacity, carbon cycle, and transport response, the ATS model used both large-leaf models for combination and execution. To determine the subsurface and the diffuse wave model for the surface Richards equation was applied to the ATS model. Surface-to-surface coupling via modelled continuous pressure formula. The finite difference method has been used by ATS model to accurately compute unstructured meshes and layering structures for hydrology applications.

CATchment HYdrology

The CATchment Hydrology (CATHY) model applies Richards 3D equation with the finite element approach, based on 1-D kinematic approximation of the Saint Venant equation. Based on a time decomposition process that solved subsurface-surface coupling, this work requires iterative updating of boundary conditions to be able to automatically split potential fluxes into actual flows on the land surface *(Kollet et al., 2017)*. CATHY uses the mass balance equation to determine variations in subsurface and surface storage. The CATHY model can also be combined with the Noah-MP land surface model, in addition, the vegetation models can also be integrated with boundary layer dynamics and hydrogeophysical inversion.

HydroGeoSphere

3D control volume and finite element simulator used in the HydroGeoSphere (HGS) model to simulate the entire terrestrial part of the hydrological cycle *(Aquanty, 2015).* To solve the problem of the differential wave equation for surface water and Richards' equation for subsurface flow is solved by global implicit method. Synthesized review and analysis of important components of the hydrologic cycle such as soil evaporation, evaporation from water bodies, evapotranspiration based on LAI values, root depth, root density, snow processes. The HGS model can also use the integration of subsurface-surface interactions to calculate contaminant and energy transport processes at the surface and in the subsurface. To simulate atmosphere, surface, and subsurface interactions, the HGS Model can be combined with the Weather Research and Forecasting (WRF) model *(Davison et al., 2015).* To solve the non-linear points in the governed flow equations, the model used Newton's method in combination with an iterative sparse matrix solver *(Kollet et al., 2017).*

GEOtop

GEOtop is a distributed, grid-based hydrological model developed to describe in 3D water flow in the surface of topsoil. The model can also account for the exchange of water and energy with the atmosphere and considers vegetation processes and the influence of complex topography on radiant flux *(Kollet et al., 2017)*. The equations of heat and water flow are solved by the Newton-Raphson method and expressed as a 3D finite volume. Water content in the saturated zone is calculated based on the retention curve according to the van Genuchten formula, while in the saturated zone the concept of specific storability is applied *(Kollet et al., 2017).*

WEAP

WEAP is an integrated hydrological model for planning complex systems of the distribution of water resources. The purpose is to use an integrated approach to the calculation of water systems and the direction of policy for water resources management.

The WEAP model features a detailed analysis of water demand for rural, urban, and agricultural use. WEAP can establish water use management methods and water users and set up a model to calculate the amount of water loss and to manage water reuse. In addition, WEAP can also do financial analysis, thus enabling the effective assessment of profit and cost models of water use. This is one of the strengths of WEAP to help decision-makers, stakeholders and farmers have a better view of water as a commodity and calculate more efficient use of water. This is very important in the context of the ongoing climate change, which is more unpredictable and extreme than before. In addition, water gradually becomes the most affected resource. This model can give the necessary results for rational planning and integrated use of water resources.

The WEAP model does not have a separate module to calculate the basin's water balance. *Kandera and Výleta (2020)* used the WEAP model to estimate the quantitative water balance of surface water in the Hron River basin from 2010 to 2015. They stated that the model does not need too much input data, however, it provides an integrated approach to solving problems in water resource management. *Kirilov and Bournaski (2019)* published the steps to perform water balance modelling based on describing relevant computational aspects of water movement through the water cycle. *Avilés et al. (2020)* used the WEAP model to evaluate the system's water balance by considering the allocation of available water to satisfy the demands of the different sectors. *Bozorgy et al. (2012)* used the WEAP model to estimate the amount of water lost by an existing irrigation infrastructure and the amount of water that could be saved by modernising the scheme. They proved the possibility of saving significant water resources, thereby improving profits. The WEAP model can also be used to assess the current water balance and change the water balance in the future, to build a scientific basis for the integrated management of water resources in Benin's largest catchment Ouémé *(Höllermann et al., 2010)*.

MIKE SHE

The MIKE SHE integrated hydrological model was developed based on the blueprint of *Freeze and Harlan (1969).* The model can simulate important hydrological cycle processes such as evapotranspiration, overland flow, unsaturated flow, groundwater flow, channel flow and their interactions. *Tran et al. (2019)* applied the MIKE SHE model combined with the IFS rain forecasting products to forecast discharge to the reservoir in the Tra Khuc-Song Ve basin. Some studies use integrated modelling of surface water and groundwater *(Hughes and Liu, 2008)*. Other studies apply MIKE SHE and GIS to simulate hydrological processes for several basins *(Paparrizos and Maris, 2015; Právetz et al., 2015)* and assess the effects of land change and climate change on groundwater and the ecosystem by *Keilholz et al. (2015)*. The process of modelling inland excess water with MIKE SHE and using satellite imagery for validation by *Van Leeuwen et al. (2016)*. Combining models of MIKE SHE, MIKE URBAN, and MIKE 11 to create probabilistic inundation maps (*Oliver et al., 2018*). *Nagy et al. (2019)* built a MIKE SHE model to simulate the accumulation processes of excess water, water storage, and excess water maps.

One of the most advanced and flexible features of MIKE SHE is its separate water balance calculator. The water balance calculation module is an effective water balance calculation tool as it can simulate the water balance at the entire catchment and local scale in any phase of the simulation *(DHI, 2017).* The results are presented in many different formats, such as text format, times series file, and map formation. Below is the MIKE SHE water balance flow chart created by authors for the Hungarian part of the Berettyó river catchment *(Figure.1).*

Asadusjjaman and Farnaz (2014) use MIKE SHE to calculate water balance and investigate the effect of land-use changes on water balance components in the subsurface. *Loliyana and Patel (2020)* the model's effectiveness in simulating hydrological processes in land-use change, groundwater variability and irrigation practices.

MIKE SHE provides several different approaches to calculating water movements, including overland flow, river and lakes, unsaturated flow, evapotranspiration, and saturated flow. The essential input data required to perform the simulation of hydrologic processes are topography, precipitation, reference evapotranspiration, air temperature, solar radiation, sub-catchment delineation (for runoff distribution), river morphology (geometries, cross-

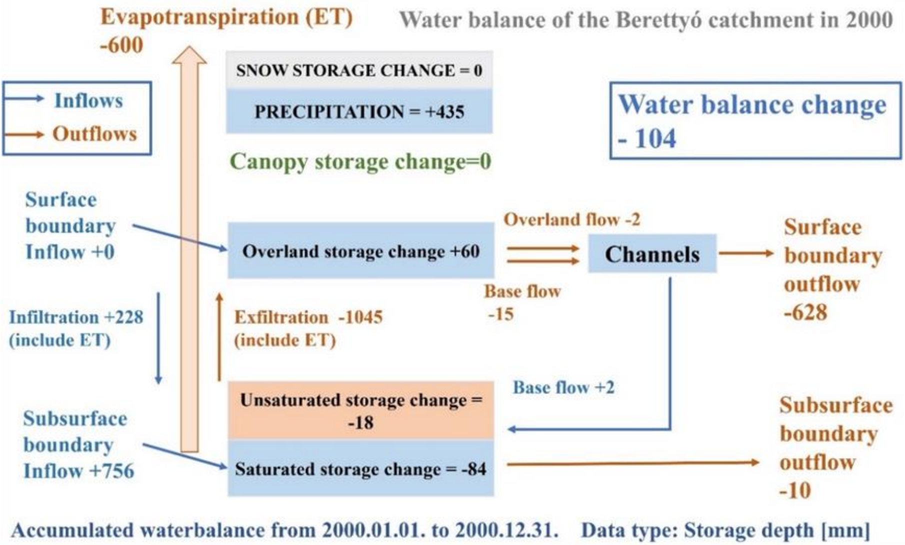

Figure 1. Water balance of the Hungarian part of the Berettyó river catchment in the dry year 2000 using the MIKE SHE model

sections for river flow and water level calculations) and land use distribution (for vegetation and paved runoff calculations), soil distribution (for infiltration and runoff), and subsurface geology (for calculating groundwater flow).

In the case of a lack of input data for simulation and the complexity of the processes taking place in the unsaturated zone, MIKE SHE has the solution to apply the linear reservoir module for simulation. *Sun et al. (2006)* identified several advantages of the MIKE SHE model including 1) it is a distributed model, and most of the algorithms in describing the complete water cycles are physically based 2) it simulates explicitly groundwater-surface water interactions, so it is ideal for wetland dominated systems as well as storage-based systems commonly found in humid regions, 3) it has been commercialised, and a GIS user interface was built in the system that can directly use spatial GIS databases for model inputs. Also, the model has an advanced visualisation facility that makes the interpretation of modelling outputs much easier.

PARFLOW

According to *Maxwell et al. (2019),* ParFlow model (PARallel FLOW) is an integrated hydrological model that can simulate surface and underground flows.

The main function of the ParFlow model is to compute 3-dimensional saturated groundwater flows in heterogeneous porous layers *(Maxwell, 2019)*. In this complex environment, ParFlow is implemented in three different modes: 1) variably saturated; 2). steady-state saturated; and 3). integrated–watershed flows. Capable of handling large scale problems on single and multi-processor computing platforms. ParFlow uses a modular architecture and contains a flexible communication layer to encapsulate parallel interaction across a wide range of platforms. The ParFlow model contains a flexible communication layer that encapsulates parallel interaction across a range of platforms based on a modular architecture. PARFLOW has also combined surface water and groundwater to simulate synthetically the process of slope surface runoff and channel runoff, besides it has also integrated a surface land-use model. *Kuffour et al., (2019)* provide an overview, applications, and ongoing development of ParFlow, for a small watershed threatened by flash floods in Germany.

According to *Maxwell et al., (2019),* ParFlow uses the to calculate the water balance for the 3D Richards' equation, overland flow, and land surface model (Community Land Model) capabilities. For shallow overland flow, the 2D kinematic wave equation can be chosen. To calculate the flow depth-discharge relationship, Manning's equation was used. Community Land Model is fully integrated into the ParFlow model as a module and can be run in parallel with other simulations. ParFlow is built to be easily connected to applications (e.g. climate models, land surface models).

ParFlow's water balance conceptual models are incomplete because, in fact, the groundwater division is difficult to determine; in many cases, the watershed of surface water and groundwater do not coincide, even in some basins, for example, in Dong-er catchment, surface water flow depends largely on underground flow from outside *(Tran, 2021)*.

In the ParFLOW model, there is no mode to visualise the results. The special advantage of ParFlow is that with only the TCL script, users can set up and can fully automate hundreds of simulations. PARFLOW has the advantage of parallel simulation platforms of saturated flows in equilibrium layers, variable saturated heterogeneous porous, and the use of complex octree-space partitioning algorithms to simulate aggregate flow in the catchment.

WateRisk

The WateRisk integrated hydrological model was developed by *Koncsos et al. (2011),* the model is developed based on 1D and 2D models integrations. The processes described in 1D are a hydrodynamic model of water movements.

The 2D model describes hydraulic models representing precipitation, snow accumulation, evaporation, and surface spreading, as well as infiltration and groundwater movement. The model is developed based on the distribution of cells with homogeneous physical properties. By writing the hydrological balance equations on the cells, a three-dimensional description of the flow can be obtained *(Kardos and Koncsos, 2018)*. The conceptional algorithm and components of the WateRisk model are built on top of the ARES (Flood protection decision support system) model system *(Koncsos et al., 2011)*. The structure of algorithms is modular, which means that simulations at cells don't only work individually but can be combined to simulate more complex processes in space and time. The WateRisk model aims to solve problems such as describing hydraulic processes in the channel, reducing the number of cells, reducing iteration steps, and improving the model's calibration capabilities. Besides, the WateRisk model also applies the method of analysis of change scenarios to predict changes in hydrological processes and includes economic analysis methods. The model can simulate large basins (~50-5 000 km^2) with a long simulation time (1-30 years) with detailed spatial and temporal resolution and fast computation speed. However, the model does not account for processes in the atmosphere and in groundwater movements.

Koncsos et al. (2011) compared the WateRisk model with the HEC-RAS model. Accordingly, the authors made a comparison based on the results of the water surface profile, the water level in the upper and middle sections under time series, water flow, and downstream water level under boundary conditions. Based on the results, the WateRisk 1D hydrodynamic model gives the same results as simulated by the HEC-RAS model. *Kozma (2013)* applied the WateRisk model to simulate hydrologic and hydrodynamic processes for the integrating surface and subsurface in both the two pilot areas, Szamos-Kraszna Interfluve (with hydrologic characteristics like wetlands) and Danube-Tisza Interfluve (characterized by drought). As a result, an inland water hazard map was created, integrating climate change scenarios and cost-benefit analysis into the water resources assessment. In a research paper by *Kozma (2013)*, water balance analysis was performed including surface storages water balance, surface - subsurface water system water balance and comparison of natural factors and factors affecting water balance at Szamos-Kraszna Interfluve inland water area. Also, in this study area, *Kozma (2013)* used the WateRisk model to conduct risk-related studies and build flood maps. The results show that the water balance calculations of the model can determine which hydrological factors can predict inland excess water occurrence.

Integrated Hydrologic Model

The Integrated Hydrologic Model (InHM) model was developed in partnership between Tampa Bay Water and the Southwest Florida Water Management District in the late 1980s. This model belongs to the advanced model of integrated hydrology modelling (IHM), which helps users to simulate and better understand the complex hydrological processes taking place at the surface and subsurface. This model is a combination of two models that have been widely applied in research, namely Hydrologic Simulation Program–FORTRAN (HSPF) and MODFLOW. The HSPF model is responsible for simulating relationships and processes taking place in the surface and vadose zone. MODFLOW is aimed to simulate the flows taking place in saturated layers.

Table 2.
Comparison the features of the models

Capability	HEC-RAS	HEC-HMS	SWAT	AQUAVEO ecosystem	HGS	WEAP	MIKE SHE	ParFLOW	PAWS	WateRisk	InHM
GIS support	X	X	X	X	X	X	X	X	X	X	X
One-Dimensional (1D) & Open channel flow	X	X	X	X	X	X	X	X	X	X	X
Two-Dimensional (2D) modelling	X	X	X	X	X	X	X	X	X	X	X
Three-Dimensional (3D) modelling				X	X		X	X	X	X	X
Sediment transport	X	X	X	X	X		X			X	X
Water quality modelling		X	X	X		X	X			X	
Evapotranspiration modelling		X	X	X	X	X	X	X	X	X	X
Hydraulic analysis	X	X		X	X	X	X	X	X	X	X
Financial analysis						X					
Separated water balance module							X				
Source code modifiability			X			X		X			
River analysis component	X	X		X	X	X	X	X	X	X	X
Interaction between surface and subsurface water				X	X	X	X	X	X	X	X
Detailed user manual	X	X	X		X	X	X				
Free of charge	X	X	X			X			X	X	X
Calibration tools			X				X				
Integrated approach				X	X	X	X	X	X	X	X

The InHM model provides simulation tools for space (from small to 25 000 km^2) and for long time interval (from 1 day to tens of years). Some studies using InHM model, such as *Loague et al. (2004)* have shown that the uncertainty in initial soil-water content is a major limitation in event-based simulations.

The InHM model with a quasi-physically based rainfall-runoff model performed well in the peak stormflow simulations but poorly for the storm flow depth estimations. *Heppner et al. (2006)* used erosion experiments to determine the performance of the sediment transport component. They demonstrated the interaction between surface water hydrology and sediment transport caused by rain and flood. *Tang et al. (2019)* used the InHM model to simulate the effects of the check dam system on hydrologic responses and landforms at Hilly-Gully Catchment, Loess Plateau. The results also show that the InHM model has worked effectively, helping to understand better the impacts related to hydrology and predict changes in sediment transport.

CONCLUSIONS

Among the process-based models, the SWAT and HEC-HMS can simulate the hydrological processes taking place on the surface and in the channel, evapotranspiration, and precipitation. However, since the catchment is usually assessed as separate sub-basins and HRUs, it is limited or impossible to calculate the hydrological processes that take place periodically, and the spatial changes in the groundwater table cannot be calculated *(Kozma, 2013).* The database of the SWAT model has not yet met the practical application needs of each region, especially Hungary, and needs revision.

The WEAP model has features suitable for simulating hydrological processes and is widely used in hydrology. However, the WEAP does not have a separate water balance calculation capability. This can make it difficult and time-consuming to calculate the water balance. AQUAVEO company has built an ecosystem of models that can support each other. To simulate surface water and groundwater, AQUAVEO company provides two separate models for implementation. Furthermore, the Watershed Modelling System (WMS) includes 20 auxiliary models for watershed simulation, including several popular models such as HEC-1, HEC-HMS, HEC-RAS. This may cause difficulties for users when they have to approach too many different models at once and then connect them into a unified whole. In the current AQUAVEO, there are no models or modules with dedicated water balance calculation capability.

Hydrological models such as ATS specialise in simulating ecosystem hydrology processes. Cast3M model specialises in solving problems related to solid and fluid mechanics. The CATHY model is widely used to simulate the variation of soil moisture, groundwater flow and surface runoff in space and time. However, there is no separate water balance calculation module. GEOtop is a distributed hydrological model for the description of soil and surface water flows based on the developed grid system. HydroGeoSphere includes features like surface and groundwater modelling, thermal and solute transport modelling, and advanced numerical methods.

The MIKE SHE model is an integrated model, so many outputs are computed. MIKE SHE has its own module to get the water balance simulation results, including total water balance, error of each component, snowmelt component, canopy interception component, overland flow, unsaturated zone, saturated zone, irrigation component. MIKE SHE is very focused on this module, so the results are presented in many different formats such as text format, times series file, and map formation. It can be said that MIKE SHE provides a full range of *"perspectives"* to help users analyse, evaluate, and determine water balance accurately, from detailed to general. ParFlow can calculate water balance, but the manual for the water balance section is very limited. To use ParFlow effectively and easily, users need to have the skills to understand the Linux/UNIX system and the ability to compose and execute commands in different programming languages. PAWS model can simulate large watersheds. Thus, the basic components of the water cycle can be simulated, and it is also possible to simulate the interaction between the water cycle and other natural cycles such as carbon/nitrogen and the ecosystem. Another disadvantage of the model is that there is no separate water balance calculation module. Besides, the data presented through different software makes the balance calculation more complicated. The main task of the WateRisk model is to solve problems such as describing the hydraulic processes in the channel, reducing the number of cells, reducing repeated steps, and improving the model's calibration. However, it has not been widely used, especially among international researchers. This model does not include a separate water balance calculation module. The InHM model has strengths in calculating and analysing sediment transport processes. The InHM model is an advanced model specialised in simulating complex processes, including auxiliary models such as HSPF and MODFLOW to realise the connection between surface water and groundwater.

Each model has its characteristics, strengths, and weaknesses *(Table 2.).* The best model is the one that can simulate most closely the processes occurring in nature but requires the least and simplest data. The choice of a reasonable model must be based on many factors. What is the purpose of simulation? What are the characteristics of the study area (soil heterogeneity, soil permeability, extreme hydroclimatic conditions, the role of groundwater, etc.)? What does the result want to achieve? Is the scope of the study large or small? What is the research object or process? Avoid using complex models that require many input parameters but only simulate a simple hydrological process. Many factors govern the choice of one model or the other or a combination of models to support each other to achieve simulation purposes. Criteria for selecting a model or combining it with a specific model are based on 1) spatial and temporal interval, which defines simulation objectives and features of the study basin; 2) Demand for input data, costs to have data; 3) uncertainties of the model (uncertainties of parameters, aggregated parameters, empirical equations) 4) previous studies 5) knowledge of model theory. It is, therefore, necessary to have an overview of the models, especially their application to basins with a high percentage of agricultural lands, like Hungary. This is a difficult task because most studies have specific reasons for applying a particular model. Very few studies compare hydrological models and comment on the capabilities, advantages, and disadvantages. The result of our study is to give an overview of the hydrological models currently widely used in the world. On this basis, we hope that users can choose a suitable model to perform the proposed task.

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Article

Examination of the evapotranspiration dynamics of maize in Thornthwaite-Mather type compensation evapotranspirometer

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ABSTRACT - According to the data provision of the National Meteorological Service, since the early 1980's an intense warming has begun and it is also reflected in domestic observations. In Hungary, just as in other Central European countries, the extremes of weather events are becoming more common. As a main crop, maize (sweet corn) has an outstanding national and global significance. Certainly, global warming and changes in water supply will harmfully affect the cultivability of maize too. Water stress reduces the leaf surface, therefore because of the less captured photosyntetically active radiation, biomass production and yields will be reduced. Weeds with a wider tolerance range than crops may also become increasingly dangerous competitors in field crop production because of their wide tolerance range, fertility and strong adaptability to changing climate- and precipitation conditions. In this research the effect of climate change on the evapotranspiration of maize was investigated at the Agrometeorological Research Station of MATE Georgikon Campus in Keszthely, between 21 May 2021 and 1 September 2021 in Thornthwaite-Mather type compensation evapotranspirometer. The aim of the study was to assess the main characteristics (like leaf area index, daily evapotranspiration, and yield) of sweetcorn under optimal water supply conditions. Furthermore it was also an aim to determine how weeding affects plant characteristics so half of the treatment (1 vessel of the evapotranspirometer) was kept weed-free, while the other half was exposed to natural weeding. In terms of results, positive relation between temperature and evapotranspiration was found and it has been established, that maximum temperature has a greater effect on evapotranspiration, than daily mean temperature. In case of yield indicators, the negative effect of weeding was statistically detectable and it was also pointed out, that the presence of weeds can negatively affect the quantity of crops. The results of the study was compared to a number of other researches on the subject, and it was concluded that the negative consequences of climate change, especially the increasing frequency of drought-hot periods could pose a major threat to successful maize production in the future.

Keywords: sweetcorn, evapotranspiration, weeding, leaf area index, climate change

INTRODUCTION

According to the data of the Central Statistical Office, in 2019 the sown area of corn was 1.03 million hectares in Hungary, which means a notable area compared to the complete 9.3 million hectares of the country. Corn is a widely used

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crop. On the one hand it has an important role in human consumption (sweetcorn, popcorn, corn poridge, flour, beer base) and on the other hand, in livestock feeding (fodder, silage etc.), furthermore it is also suitable for industrial purposes (furfural, isosugar and starch production, corn oil), and it's stalks and cobs are also used for heating or recycling back into the soil. In addition an important way to use corn is the production of ethyl-alcohol, which is mainly used as gasoline biofuel additive (*Ranum et al*., 2014).

Production needs

Corn is a heat-demanding plant, which is suitable to be cultivated in warmer areas. The minimum required soil temperature for germination is 10 °C but on 16-18 °C germination will be faster. 32 °C is the critical temperature, that can adversely affect yields at any stage of developement. Frost also threatens corn at any stage, especially the leaves of ripe plants (*Plessis*, 2003). The amount of heat during the growing season significantly affects the yields of corn. It is favorable if it is between 1250-1750 °C (*Dénes*, 2005).

Under domestic conditions, a minimum amount of water is available for producing corn. The water demand of corn, grown under optimal conditions is 550-670 mm in Hungary, depending on climate conditions and agronomic factors. In seed stage and initial growth phase it does not require much water, so soil moisture is sufficient. Irrigation is not necessary in the vegetative phase either, but the water use of corn peaks in the early reproductative phase, so it is justified to avoid water stress during tasseling, flowering and pollination. Drought during flowering is the most dangerous as dehydration of pistils and pollens can result in poor fertilization (*Kranz et al*., 2008). The depth of rooting is greatly influenced by the amount of water stored in the soil from the previous year and the amount of rainfall, during the growing season. The main water use period of corn is from mid-June to mid-August. Drought during ripening speeds up the ripening process, preventing the grains from reaching their potential size and weight (*Lamm et al*., 2009).

Taking the agro-ecological properties of Hungary and the water demand of corn into account, it would be justified to irrigate crops. Experiences also show that irrigation in appropirate time, according to the needs of plants increases the yield (*Smith et al*., 1992). In Hungary only about 2% of corn fields are irrigated due to small farm sizes and the lack of integrated water management systems (*Gaál et al*., 2014). Expanding the irrigation network and optimizing irrigation systems would be one of the most effective adaptation measures to mitigate the effects of drought in maize production (*Hanquing et al*., 2019). In addition, drought tolerance of maize hybrids would also be an important component of successful cultivation in the future, especially in areas prone to drought (*Cooper et al*., 2014).

Soils with extreme conditions or poor water management such as sandy or saline soils are not suitable for growing corn (*Udvardy*, 2010). It is desirable if soil has a deep fertile layer, favorable morphological characteristics, optimal moisture content, suitable chemical properties (pH: 6.5-7.5) and sufficient amount of accessible plant nutrients (*Plessis*, 2003). The most favorable soils for corn are humus- and nutrient-rich, easily warming chernozem, brown forest, chernozem brown, meadow chernozem and meadow soils.

The expected effects of climate change in Hungary

In 2021, the Sixth Assessment Report of the United Nations Intergovernmental Panel on Climate Change drew attention to the fact that, if current emission trends continue, global warming will be exceeded by 1.5 ^{o}C, thereby warm seasons will be prolonged, while cold seasons will be shortened, and the frequency of extreme warm weather events will be increased and they will increasingly exceed the upper tolerance limit of agriculture (*IPCC*, 2021).

In their study, *Ciscar et al*., (2011) predicted a global temperature rise of 2.3-3.1 ^{o}C, which is worse than the number, specified by the IPCC. The majority of studies on the subject agree that Europe (including Hungary) will be more affected by the negative effects of climate change than the world average. As a result of the intensification of the greenhouse effect, domestic climate may become warmer, dryer and richer in sunlight (*Mika*, 2002). The IPCC report also points out, that as a result of climate change, the water cycle may become more intense, so that while rainfall would increase sharply in some areas, droughts would worsen elsewhere. Agriculture is the most directly affected economic sector by global warming. Farming needs to adapt to changed environmental and biological conditions so some desertified and eroded areas should even be completely excluded from agricultural production (*Farsang et al*., 2015).

In their research *Pásztor et al*., (2010) tried to assess the agroecological potential of Hungary and its future during climate change, using a 4M simulation model. In terms of their results, the negative effects of climate change will prevail in Hungary, so according to the forecast, the average yield of maize will also decrease. The reason of maize yield loss is the rising temperature and extremes in the precipitation phenomena, especially during the generative period (*Széles et al*., 2018).

In their study *Meza et al*., (2018) concluded, that the yield loss of maize, due to climate change will be 10-30%, depending on the climate change scenario and the applied hybrid.

Aware of these informations, irrigation of maize may have a great importance in the future.

Evapotranspiration of maize under drought and warm conditions

Evaporation of the water content of soil and transpiration of plants take place in parallel, forming the evapotranspiration complex. The extent of current evapotranspiration is influenced by a number of factors, such as soil cover, soil structure, moisture content, crop type and growth phase, and weather factors, like radiation, temperature, relative drought and wind speed (*Lich et al*., 2017). Water requirements of maize grown with adequate water supply is equal to the sum of the daily evapotranspirations measured during the growing season (*Basso et al*., 2018).

Plants release about 95-98% of their water intake in form of water vapor (*Loch et al*., 2004). A corn plant evaporates about 200 litres of water during the growing season, which if cannot be replaced from the soil, needs to be returned by irrigation.

The rate of evapotranspiration can also be reduced with proper nutrient supply. The value of transpiration is the lowest with satisfying nutrient supply (*Loch et al*., 2004). An optimal dose of phosphorus and potassium reduces water consumption by about 25-30%.

The effects of weeding in maize fields

Maize, as a large-scale crop (70-75 cm row spacing) cannot compete with weeds without systematic human weed control (*Bozsik et al*., 1997). In their study, *Varanasi et al*., (2016) stated that weeds are likely to show greater resistance and adaptability to changes in CO_2 concentration and rising temperature than cultivated plants, due to their diverse gene pool and plasticity. Next to the variety of environmental conditions, weeding is one of the main reasons of the decrease in maize yields (*Rajcan et al*., 2001).

Former domestic studies have attempted to detect a correlation between weediness and crop depression in maize and have concluded that the average yield of maize grown without weed control was only 23.5% compared to the weed-free control treatment (*Bozsik et al*., 1997). Nevertheless, this value cannot be considered constant because the weed composition of maize may vary considerably depending on the ecological and agrotechnical characteristics of the specific area.

The multiplication of weeds may lead to a large loss of nutrients, which slows down the developement of maize. In addition, next to the loss of nutrients, competition for space, water and light between industrial plants and weeds are also cannot be neglected (*Zimdahl,* 2004).

The purpose of the research

For the better understanding of the effects of climate change on maize production, the aim of the study was to assess the evapotranspiration dynamics of maize under optimal water supply conditions, using Thornthwaite-Mather type compensation evapotranspirometer in the quite arid and warm growing season of 2021 which was suitable to model the expected future conditions related to global warming. An other aim was to determine, how weeding affect yields and other performances of maize.

MATERIAL AND METHODS

The study took place at the Agrometeorological Research Station of the Hungarian University of Agricultural and Life Sciences, Georgikon Campus, Keszthely (N: 46°, 44’, 7.93”, E: 17°, 14’, 16.65”, altitude: 114.2 m).

The research was set up in a Thornthwaite-Mather type compensation evapotranspirometer (*Figure 1.*), which consists of a variable number of 4 m^2 surface, 100 cm deep culture vessels and a measuring cellar, which are connected to each other via a pipeline. Soil is placed in culture vessels according to natural stratification (*Simon et al.*, 2020). The Thornthwaite-Mather type compensation evapotranspirometer is commonly used to measure the potential evapotranspiration of plant populations. The culture vessel is connected to a compensation tank via a pipeline, so the water level below the crop can be kept at a constant depth, which is equal to the water level of the compensation tanks. It is controlled by a float and an overflow pipe, so if the water level drops due to the co-evaporation of the plants and the soil, the float will open a tap and the water from the dosing tank (tank 1.) will be replenished. If the water movement is in the opposite direction (due to precipitation), the water flows into a water collection tank (tank 2.), through an overflow. Potential evapotranspiration can be determined by knowing the daily values of depletion from tank 1. and the values of backflow to tank 2. (*Gombos*, 2011).

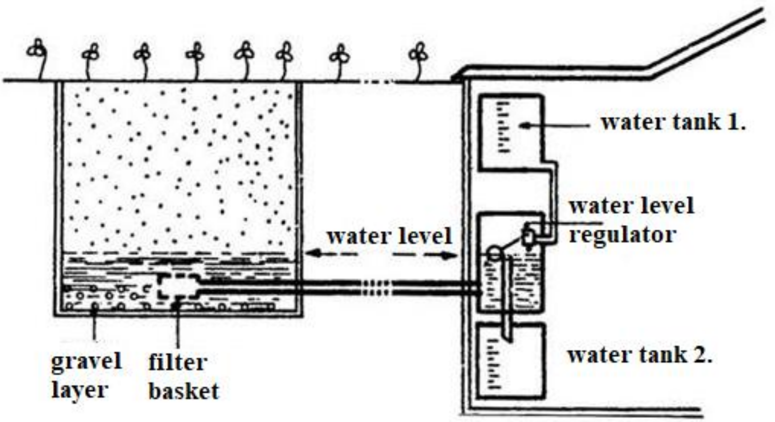

Figure 1: The structure of the Thornthwaite-Mather type compensation evapotranspirometer (*Gombos,* 2011)

Ramann brown forest soil was available in the culture vessels of the evapotranspirometer, on which the necessary soil preparation works were carried out, in the autumn of 2020. The soil preparation tasks, such as soil rotation, soil dusting, leveling and keeping the area weed free were done with manual tools. In addition to the establishment of appropirate soil condition, satisfying supply of nutrients was also provided. Nitrogen (225 kg/ha) was applied in 3 portions: 50% on the 14. day after sowing, 25% immediately before reaching the five-leaf stage and a further 25% immediately before flowering. 150 kg/ha of superphosphate and 100 kg/ha of 60% potassium salt granules were also applied on the experimental plots. Sowing took place on May 21, 2021, during which GSS 8529 supersweet maize hybrid was sown in 2 growing vessels, 3-3 rows per vessel with 75 cm row spacing, 12-13 cm plant distance and about 4-5 cm deep. To ensure the stock effect, the culture pots were sorrounded on all sides with maize to prevent border effect. Border effect means that certain parameters (like wind speed, temperature, or the mass of certain (weed) species) at the edge of the population change, compared to the internal section (*Margóczy*, 1998). The purpose of avoiding border effect was to get an uniform population without lower plants at the edges (which may could have modified our results) and to protect plants at the edges of the vessels from greater physical pressure to transpirate.

In this study 2 different treatments were set up. The 2 vessels were connected to the evapotranspirometer, which provided them a continuous optimal water supply. One of the vessels was exposed to weeding, while the other one was kept weed-free throughout the experiment. Weeds were not planted into the culture vessels. They were settled in a natural way therefore the area specific species were able to settle.

Measurement of leaf area

To determine leaf area, LI-3000C portable leaf area meter was used which has the advantage of working in fine resolution (1 mm^2), additionally it is suitable for non-destructive field measurements and ideal for long-term samplings due to its battery capacity.

The first leaf area measurement was performed on June 29, 2021 and from then on, repeated leaf area measurements were performed weekly until tasseling, in order to determine the leaf area of 5-5 representative sample plants. During the measurements always newly completely developed leaves were measured, then their surface was added to the results of previous measurements. By averaging the area of each leaf of 5 sample plants per vessel, the leaf area of the average plant per vessel was obtained.

Determination of leaf area index

The following formula was used to estimate leaf area index: $LAI_0 = (LA_0 \text{ x } LN \text{ x } PN) / T$, where LAI_0 is the leaf area index of the given species, LA_0 is the area of the average leaf size of the given species, LN is the average number of leaves per plant, PN is the number of plants in the study area and T is the size of the study area (*Richter*, 2009).

The highest productivity of maize can be achieved at LAI values ranging from 4 to 5 (*Lykhovyd et al*., 2019).

Monitoring of weather elements

Evapotranspiration data were compared with air temperature and precipitation data from the QLC-50 device located at the Agrometeorological Research Station. The QLC-50 measuring device, which is available for ten-minute sampling has been operating in the area of the Research Station since 1996 (*Kocsis*, 2008). The meteorological elements measured by the QLC-50 measuring machine are: wind speed, wind direction, air temperature, humidity, precipitation, soil surface temperature and soil temperature (at a depth of 5, 10, 20, 50 cm).

Determination of reference evapotranspiration

The concept of reference evapotranspiration provides comparability according to internationally accepted standards for the determination of evapotranspiration (*Irmak et al.*, 2011). The equation can be described as follows:

$$ET_0 = \frac{0.408\Delta(R_n - G) + \gamma \frac{900}{T_a + 273} u(e_s - e_a)}{\Delta + \gamma(1 + 0.34u)}$$

In the equation ET_0 is the reference evapotranspiration [mm day^{-1}], Rn is the radiation balance [MJ m^{-2} day^{-1}], G is the thermal flux of the soil [MJ m^{-2} day^{-1}], T_a is the daily mean temperature [^{o}C], u is wind speed at 2 m altitude [m s^{-1}], e_s is the saturation water vapor pressure [kPa], e_a is the actual water vapor pressure [kPa] and e_s-e_a is the saturation deficit [kPa], Δ is the slope of the temperature-saturation vapor pressure function [kPa ^{o}C^{-1}] and γ is the psychrometric constant [kPa ^{o}C^{-1}] (*Allen et al.*, 1998).

To determine the evapotranspiration of a plant under favorable conditions, the plant coefficient Kc (also known as the plant constant) need to be obtained by comparing the measured evapotranspiration (ETc) of the plant to the reference evapotranspiration (ET_0) (Kc = ETc / ET_0). In knowledge of reference evapotranspiration, the evapotranspiration of a given plant can be calculated (*Allen et al.*, 1998). (ETc = Kc x ET_0) The plant coefficient of maize varies widely, usually between 0.15 and 1.2 depending on the phenophase, but its value is influenced by several factors (*Rácz*, 2014).

Extraction of crop data

Representative sampling of the experimental maize crop took place on 27 August 2021. For each treatment, the length and weight of the collected cobs (unfolded from their husks) were measured, then averaged, in order to compare the results of the treatments.

The experimental maize was let to dry until constant weight (11-12 % moisture content). The seeds were crushed from the tubes, and thousand-seed weight [g] was calculated by working with 5-5 repetitions per treatment. 50-50 randomly selected seeds per treatment were counted, measured by digital analytical scales, multiplied by 20, then averaged to get the most accurate result regarding to thousand-seed weight.

Statistical methods used

Two-way analysis of variance (ANOVA) tests were performed on the averages of the samples in IBM SPSS program, furthermore T-tests were also performed in Excel to confirm any significant differences.

RESULTS AND DISCUSSION

Weather conditions of the growing season

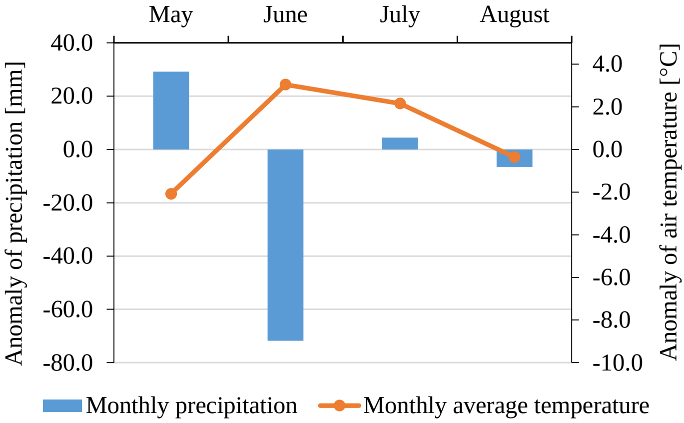

Figure 2. Deviation of the monthly precipitation amounts and the average monthly temperature of the 2021 growing season from the climate standard (1981-2010)

Due to the cold and rainy May, our experimental maize could only be sowed late, on the 21st of May (*Figure 2.*). June was quite arid and warm – a total of 3 mm precipitation fell on 2 rainy days. July and August did not show a significant difference from the 30 year average. In July, 69.2 mm of rain fell on 8 rainy days and in August 66.8 mm on 13 days. These months included days with larger amounts of precipitation (e.g.: July 17: 18.4 mm, July 30: 27.9 mm, August 1: 18.5 mm, August 26: 19.6 mm). Due to the drought year, it was not necessary to install a rain protection system.

The average temperature in May was 14.0 °C, which showed a notable deviation from the climate standard in a negative direction. Compared to that, June with an average temperature of 22.1 °C also showed a remarkable difference, but in a positive direction. In July, the average monthly temperature was

23.2 °C, which also deviates positively from the climate standard. With an average temperature of 20.1 °C, August did not show a significant deviation from the 30-year average.

In terms of temperature, it is important to examine not only the average but the maximum temperatures as well.

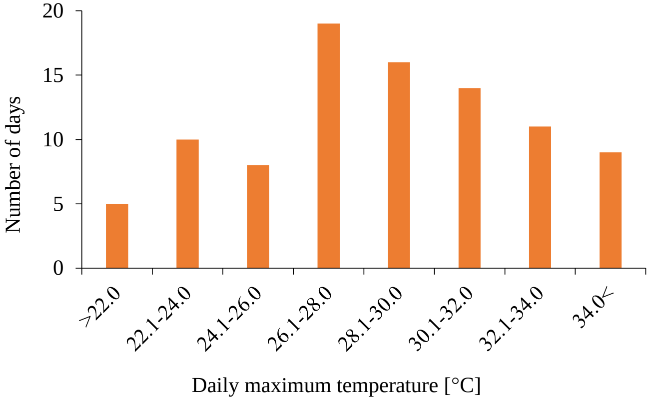

Figure 3. Distribution of daily maximum temperatures during the growing season

There were a total of 20 days in the growing season when the maximum temperature exceeded 32 °C, which is considered critical for maize in every growing phase. There were 9 days where it exceeded 34 °C too (*Figure 3.*).

Developement of leaf area during the growing season

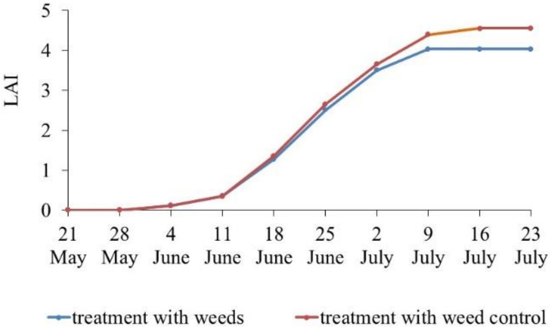

Figure 4. Developement of LAI (Leaf Area Index) of different treatments

In the treatment with weeds, the restraining effect of weeds on the leaf area began to become more pronounced from July 2 (*Figure 4.*). The presence of weeds reduced LAI by 11.5%. Based on the results of the two-way ANOVA, the presence or absence of weeds had a significant effect ($p = 0.003$) on the leaf area of maize. In connection with weeding, *Ngome et al.* (2012) described a significantly negative correlation ($p < 0.01$, $R^2 = 0.31$-0.51) between the LAI of maize and weed biomass.

Dependence of evapotranspiration on meteorological elements

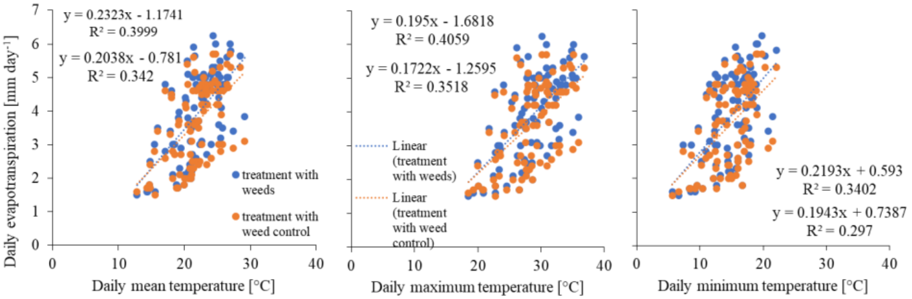

Figure 5. Relations between daily mean, maximum and minimum air temperature [°C] and daily evapotranspiration of maize [mm day^{-1}]

On *Figure 5.*, a dot band, sharply separated from the linear line can be observed, where relatively low evapotranspiration values are associated with high temperature values. A reason for this is due to the fact that after germination, in late May – early June, the experimental plants did not have a large leaf area, so their transpiration was much lower, than in their fully developed state. Furthermore, during this initial period, the temperature was unusually high compared to the average. The reason for the upper jumps may be other effects that were not examined, e.g. the wind. Based on the results, a positive relationship was found between temperature and evapotranspiration: the higher the temperature, the higher the evapotranspiration is. The relationship is positive, but based on R^2 the maximum temperature (treatment with weeds: $R^2 = 0.4059$, treatment with weed control: $R^2 = 0.3518$) had a greater effect on evapotranspiration than the daily mean temperature (treatment with weeds: $R^2 = 0.3999$, treatment with weed control: $R^2 = 0.342$). The least close connection was detected in case of minimum temperature (treatment with weeds: $R^2 = 0.3402$, treatment with weed control: $R^2 = 0.297$).

Comparison of daily evapotranspiration and daily reference evapotranspiration

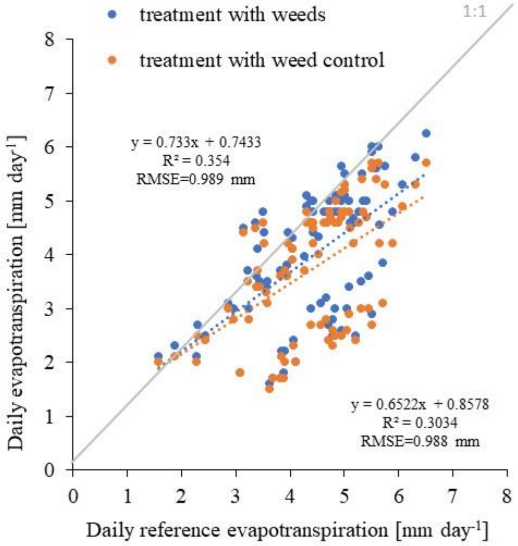

Figure 6. Relationship between daily reference evapotranspiration and daily evapotranspiration of maize

The small leaf area at the beginning of the growing season can also be observed on *Figure 6*. During this period, the reference evapotranspiration was much higher than the measured evapotranspiration values. Until the development of the first leaves, almost only the evaporation of the soil surface was observed. Based on the data measured during the growing season, the Penman-Monteith equation slightly underestimates our data. The reasons for the variability in daily evapotranspiration may have been due to both variabilities in meteorological elements and biological characteristics. During the growing season, the treatment with weeds transpirated a total of 350.2 mm of water, while the treatment with weed control transpirated a total of 328.9 mm. Comparing the evapotranspiration of the two treatments by T-tests, no significant difference ($p = 0.1744$) was observed. The cumulative Penman value was 388.5 mm. The daily mean evapotranspiration was 3.9 ± 1.2 mm for the treatment with weeds and 3.6 ± 1.2 for the treatment with weed control. The daily mean value of the reference evapotranspiration calculated by the Penman Monteith equation was 4.4 ± 1.0 mm. Comparing the individual treatments and reference evapotranspiration by T-tests, a significant difference was found for both treatments (treatment with weeds – reference evapotranspiration: $p = 0.0137$, treatment with weed control – reference evapotranspiration: $p < 0.001$).

In their research, *Tyagi et al*. (2003) examinated the evapotranspiration of maize under optimal water supply, in India. In their results they reported a cumulative evapotranspiration of 354 mm in the growing season.

Examination of the effect of weeds on crop

Based on the results of the two-way analysis of variance, the presence and absence of weeds had a significant effect ($p < 0.001$) on the development of the cob weights.

According to the data of the measured cob length, the average length was 10.86 ± 4.01 cm in the treatment with weeds and 16.97 ± 5.84 cm in the treatment with weed control. The expected value of cob length of GSS 8529 maize hybrid (20-22 cm), specified by the distributor was approached only with regular weed control. In connection with weeding, *Iderawumi et al*. (2018) pointed out, that in treatments where weed control does not happen within 4 weeks after sowing, corn yield drastically fall back, especially where there is no weed control at all.

Based on the results of the ANOVA and T-tests, weeds had also a significant effect ($p < 0.001$) on thousand-seed weights.

In agricultural industry, the decrease of cob weight, cob length and thousand-seed weight can also cause large losses of income.

CONCLUSIONS

The weather during the 2021 growing season was suitable for modelling the expected adverse effects of climate change. The research shows that the competition with weeds may cause significant changes in indicators, such as LAI, cob weight and cob length, which may lead to remarkable economical losses. The accelerated evapotranspiration which lead to insufficient water supply may also cause losses in yield, especially if weed control is unsatisfactory. The results of the study were compared with a number of other studies on the subject. Based on the results, the study also prove that the negative consequences of climate change, especially the acceleration of evapotranspiration, caused by the increase of drought, hot periods and the accelerated spread of weeds may pose a major threat to successful maize cultivation in the future. Therefore, it would be expedient to urge the preparation of agriculture for changing circumstances and to carry out further studies to assess the expected impacts of climate change on crop production.

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ACTA AGRARIA KAPOSVÁRIENSIS *(2022) 26 (1), 71–81; DOI: 10.31914/aak.2700*
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Hungarian University of Agriculture and Life Sciences (MATE), Kaposvár Campus, Kaposvár

Tudományos közlemény

Előzetes eredmények egyes szarvasmarha húsrészek zsírtartalmának és zsírsavösszetételének összefüggéséről

SZOKOL Balázs [1], VARGÁNÉ VISI Éva [1*]

[1] Magyar Agrár- és Élettudományi Egyetem, Élettani és Takarmányozástani Intézet, Élettani és Állat-egészségügyi Tanszék, 7400 Kaposvár, Guba S. u. 40.

ABSTRACT - **Preliminary results about the relationship between fat content and fatty acid composition of some meat parts of beef**

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This work aimed to investigate whether the lipid profile differs among beef cuts purchased from retail stores. Fat content, fatty acid composition, and conjugated linoleic acid content of different cuts were determined. Moreover, the relationship between the fat content and fatty acid composition was evaluated to see whether the change in the fat content of beef affects the fatty acid composition of beef lipids. During five weeks, the thick flank, fore rib, thick rib, and neck samples were obtained at a local store. In addition, conjugated linoleic acid content and fatty acid composition were measured with a gas chromatograph equipped with a flame ionization detector (GC-FID) after transesterification of acyl lipids in the form of fatty acid methyl esters (FAME). There were only a few significant differences among beef cuts. Regarding the measured nutrients, the ratio of arachidonic acid and monounsaturated fatty acids (MUFA) within lipids differed among cuts. In contrast, the ratio of the other fatty acids and the conjugated linoleic acid content were not affected by the type of cut. Nevertheless, when the pulled data were analysed, irrespectively of the cuts, it was found that the more fat in the beef was, the less healthy the lipid profile became. The fat content had a significant positive correlation ($r=0.663$, $P=0.001$) with the ratio of saturated fatty acids (SFA) and a significant negative relationship ($r=-0.621$, $P=0.004$) with the ratio of polyunsaturated fatty acids (PUFA) within beef lipids. Consequently, enhanced fat deposition can impair the quality of meat not only with the higher fat and less lean meat content of carcasses but also can compromise the fatty acid profile, causing a significantly more saturated character. This tendency could be seen clearly, although this preliminary research used only a limited number of samples.

Keywords: conjugated linoleic acid, PUFA, MUFA, SFA, FAME

BEVEZETÉS

Globális szinten nézve a marhahús az egyik legértékesebb állati eredetű élelmiszer alapanyag, ugyanis fehérjéinek aminosav-összetétele kiváló, emellett fontos vitamin- és ásványianyag-forrás (*Oh és mtsai, 2016).* Táplálkozásélettani szempontból viszont a marhahús hátrányos tulajdonsága, hogy lipidjei jelentős mennyiségben tartalmaznak telített zsírsavakat (*Garcia és mtsai, 2008).*

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Mindemellett, a kérődzők zsírja, így a marhahús is jelentős mennyiségben tartalmaz konjugált linolsavakat (conjugated linoleic acid, CLA) amelyek fogyasztása előnyös az egészségre, mivel csökkentik a zsírok szintézisét, valamint hozzájárulhatnak egyes daganatos megbetegedések (pl. mellrák) és a 2-es típusú diabetes mellitus megelőzéséhez is (*Poulson és mtsai, 2004*).

A marhahús kémiai összetételét, köztük zsírtartalmát és zsírsavösszetételét számos tényező befolyásolja, amelyek közül a legfontosabbak a genetikai tulajdonságok, a takarmányozás és az életkor (*Oh és mtsai, 2016; Schönfeldt és mtsai, 2010; Warren és mtsai, 2008*), valamint az egyes húsrészek zsírtartalma és zsírsav összetétele is különbözhet egymástól *(Pavan és Duckett, 2013)*. Az idézett vizsgálatok fő célja általában egy egészségre előnyösebb zsírsavprofil kialakítási lehetőségének vizsgálata volt. Az idézett tanulmányokban a zsírtartalomra és a zsírsavösszetételre ható faktorok hatását egyenként vizsgálták olyan módon, hogy a többi tényező szintjét standardizálták.

A tudományos vizsgálatokban alkalmazott protokollal szemben a fogyasztó (a kiskereskedelmi forgalomba hozott húsrészeket vásárló átlagember) különböző genetikai hátterű, takarmányozású és eltérő korú állat húsát szerezheti be, ugyanazon húsrész neve alatt. Emiatt célul tűztük ki annak vizsgálatát, hogy a kiskereskedelmi forgalomban beszerzett szarvasmarha húsrészek zsírtartalmában és zsírsavösszetételében kimutatható-e szignifikáns különbség, illetve azonos néven vásárolt húsrész esetében a fenti összetevők értékei milyen mértékű szóródást mutatnak. Ezen kívül a vizsgálat tárgyát képezte az is, hogy van-e eltérés a jótékony élettani hatásairól ismert CLA mennyiségében az egyes húsrészek között. Mindezen kérdések precíz megválaszolására széleskörű, az ország egész területére kiterjedő vizsgálat lenne szükséges. Jelen vizsgálatban, előkísérletként, egy kisebb mintaszámú, helyi gyűjtést végeztünk, és az így kapott mérési adatok elemzését végeztük el. A húsrésztől függetlenül célul tűztük ki továbbá annak vizsgálatát is, hogy a marhahús zsírtartalma befolyásolja-e annak zsírsavösszetételét; azaz gyakorol-e bármilyen pozitív vagy negatív hatást a zsírsavösszetételre a hús zsírtartalmának csökkenése vagy növekedése.

ANYAG ÉS MÓDSZER

A vizsgálatokhoz felhasznált húsmintákat egy helyi húsüzem boltjából szereztük be. Négy különböző húsrészből (felsál, rostélyos, lapocka és nyakhús) húsrészenként egy kilogrammnyi mennyiségét vásároltunk meg egy-egy alkalommal. A vásárlást öt héten keresztül, heti egy alkalommal ismételtük meg.

A húsminták nedvességtartalmát az MSZ ISO 1442, zsírtartalmát az MSZ ISO 1443 szabvány alapján határoztuk meg.

A zsírsavösszetétel-meghatározásához lipid-extrakciót követően zsírsav-metil-észtereket képeztünk, amelyek analízisét lángionizációs detektorral ellátott gázkromatográffal (GC-FID) végeztük (*Salamon és mtsai, 2009*). Az egyes zsírsavak azonosítása tesztelegy (Supelco 37 Component FAME Mix) segítségével történt az egyes zsírsav-metil-észterek retenciós ideje alapján. A zsírsavösszetételt a csúcsok alatti terület alapján határoztuk meg, területszázalékos módszerrel.

A konjugált linolsavak mennyiségi meghatározása során a mintából kivont acil-lipidekből származékképzéssel zsírsav-metil-észtereket képeztünk, amelyek elválasztása és detektálása GC-FID-del, a mennyiségi meghatározás pedig külső standard kalibrációs módszerrel, a visszanyerés figyelembevételével történt (*Varga-Visi és mtsai, 2012).*

Az egyes húsrészek kémiai összetételének összehasonlításához egytényezős varianciaanalízist alkalmaztunk. A középértékek azonosságára vonatkozó nullhipotézist akkor utasítottuk el, ha a megfigyelt szignifikanciaszint (P-érték) kisebb volt, mint 0,05. Amennyiben szignifikáns különbség volt a csoportok között, az átlagok páronkénti összehasonlítására a Tukey *post hoc* tesztet alkalmaztuk. A valószínűségi változók közötti összefüggés meglétéről vagy hiányáról a lineáris regresszióanalízisek F-próbája alapján döntöttünk, az összefüggések szorosságát korrelációanalízissel vizsgáltuk. A statisztikai kiértékelést az IBM SPSS Statistics 20. programcsomag segítségével végeztük el.

EREDMÉNYEK ÉS ÉRTÉKELÉSÜK

Az egyes húsrészek zsírtartalmának és zsírsavösszetételének elemezése

Az egyes húsrészek szárazanyag-tartalma, nyerszsír-tartalma, valamint konjugált linolsav-tartalma az *1. táblázatban* látható. A vizsgált kémiai összetevők jelentősen nem különböztek egymástól (P≥ 0,05).

1. táblázat

Kiskereskedelmi forgalomban vásárolt szarvasmarha húsrészek egyes kémiai összetevői (átlag ± szórás, n= 5)

Vizsgálat	Húsrész				Varianciaanalízis F-próbája	
	felsál	rostélyos	lapocka	nyakhús	F	P
SZA (%)	74,7±1,43	73,5±1,68	74,9±1,32	76,1±0,54	3,221	0,051
NYZS (%)	1,5±1,05	3,5±1,74	3,1±1,68	2,2±0,81	2,044	0,148
CLA (mg/g minta)	0,059±0,05	0,116±0,06	0,063±0,02	0,086±0,02	1,931	0,165
CLA (mg/g zsír)	3,6±0,94	3,3±0,69	2,4±1,15	4,1±0,9	2,698	0,081

SZA = szárazanyag-tartalom; NYZS = nyerszsír-tartalom, CLA = konjugált linolsav

A szignifikáns különbség hiánya arra vezethető vissza, hogy a hibavariancia nagy volt a húsrész okozta varianciához képest (az F-érték kicsi), így a húsrésznek a kémiai összetételre gyakorolt hatása nem bizonyult szignifikánsnak. Az egyes húsrészek szárazanyag- és zsírtartalma, valamint konjugált linolsav-tartalma nem különbözött szignifikánsan, amely – részben – azzal indokolható, hogy az egyes csoportokon belül nagy volt a szórás. A szárazanyag-tartalom esetében a relatív szórás (a szórás és az átlag hányadosa, szorozva százzal) az egyes mintacsoportoknál mindössze 2 % körüli érték volt. Ezzel szemben, a nyerszsírtartalom esetében volt olyan csoport, a felsál mintáké, ahol a relatív szórás megközelítette a 70 százalékot. Az elemi minták zsírtartamában mért nagy különbség miatt nem nyílt lehetőség az egyes húsrészek zsírtartalma közötti különbség kimutatására. Hasonlóképpen, az egyes elemi minták konjugált linolsav-tartalma is nagyon különböző volt, ami azzal indokolható, hogy ezek a zsírsavak a lipid-frakcióban találhatók, így annak változásával a konjugált linolsavak mennyisége is megváltozik. Összehasonlítva az általunk mért átlagokat a mások által mért értékekkel (*Pavan és Duckett*, 2013), a rostélyos húsrész szárazanyag tartalmában és zsírtartalmában is hasonló értéket kaptunk (73,5 % v.ö. 72,6 % valamint 3,5% v.ö. 3,0%).

A *2., 3.* és *4. táblázatban* a zsírsavösszetétel vizsgálatok eredményei láthatóak. Az egyes húsrészek között nem volt szignifikáns különbség sem a telített, sem az egyszeresen telítetlen zsírsavak összetételében (*2. és 3. táblázat,* P≥ 0,05). A többszörösen telítetlen zsírsavak közül egyedül az arachidonsavnál (C20:4 n-6) tapasztaltunk jelentős különbséget, amelynek átlaga a felsál mintákban szignifikánsan nagyobb volt, mint a rostélyos mintákban *(3. táblázat)*.

2. táblázat

A kereskedelmi forgalomban vett húsminták zsírsavösszetétele, a telített zsírsavak aránya (%)(átlag ± szórás, n= 5)

Vizsgálat	Húsrész				Varianciaanalízis F-próbája	
	felsál	rostélyos	lapocka	nyakhús	F	P
C14:0	1,83±0,45	2,28±0,23	2,12±0,65	2,19±0,39	0,896	0,465
C15:0	0,35±0,1	0,48±0,25	0,43±0,07	0,49±0,03	1,142	0,362
C16:0	23,75±2,32	26,83±1,38	25,01±2,42	25,22±2,5	1,633	0,221
C17:0	1±0,26	1,29±0,52	1,17±0,17	1,27±0,12	0,968	0,432
C18:0	17,09±3,35	20±3,83	15,36±3,27	16,82±2,13	1,748	0,198

Az adatok g/100g zsírsavra vonatkoznak.

3. táblázat

A kereskedelmi forgalomban vett húsminták zsírsavösszetétele, a telítetlen zsírsavak aránya (%) (átlag ± szórás, n= 5)

Vizsgálat	Húsrész				Varianciaanalízis F-próbája	
	felsál	rostélyos	lapocka	nyakhús	F	P
C14:1	0,33±0,11	0,38±0,22	0,56±0,22	0,47±0,2	1,123	0,369
C16:1	2,72±0,36	2,9±1,17	4,11±1,22	3,65±0,85	2,294	0,117
C18:1 n-9c	37,11±3,18	38,68±1,77	41,00±3,01	40,14±2,4	2,102	0,140
C18:2 n-6c	9,27±4,94	4,62±1,6	6,35±2,26	6,18±0,77	2,293	0,117
C18:3 n-3	0,66±0,45	0,48±0,19	0,53±0,25	0,52±0,13	0,375	0,772
CLA c-9, t-11	0,39±0,15	0,36±0,12	0,32±0,03	0,44±0,02	1,278	0,316
C20:2 n-6	0,13±0,08	0,06±0,02	0,09±0,03	0,09±0,02	2,223	0,125
C20:3 n-6	0,81±0,52	0,3±0,17	0,47±0,21	0,38±0,08	2,987	0,062
C20:4 n-6	4,17±2,73^{b}	1,24±0,68^{a}	2,19±0,95^{ab}	1,99±0,38^{ab}	3,558	0,038
C20:5 n-3	0,38±0,38	0,11±0,08	0,2±0,2	0,12±0,04	1,642	0,219

$^{a, b}$ *Az azonos sorban lévő és egymástól eltérő betűvel jelölt átlagok között szignifikáns különbség van (P<0,05). Az adatok g/100g zsírsavra vonatkoznak.*

A zsírsavösszetételt a zsírsavak telítettsége szerint csoportosítva is elemeztük *(4. táblázat)*. A lapocka több MUFA-t tartalmazott, mint a felsál. A *3. táblázat* adatai alapján ugyanakkor nem volt szignifikáns különbség a lapocka és a felsál minták lipidjeinek palmitoleinsav (C16:1) és olajsav (C18:1 n-9c) tartalmában (4,11 v.ö. 2,72 P=0,117 palmitoleinsavra, illetve 41,00 v.ö. 37,11 P=0,117 az olajsavra, *3. táblázat*). Az adatok alapján viszont az is látható, hogy a tendencia mindkét zsírsavnál azonos volt, azaz a lapocka telítetlenebb jellegű volt a MUFA szempontjából. Mivel az olajsav és a palmitoleinsav dominál a MUFA frakcióban, ezért együttes arányváltozásuk már szignifikáns eltérést eredményezett.

A PUFA jelentős hányadát adó arachidonsav aránya ugyan nagyobb volt a felsálban, mint a rostélyosban *(3. táblázat)*, azonban e két húsrész zsírján belül a PUFA aránya (15,83 a felsál, 7,17 a rostélyos esetében) nem különbözött jelentős mértékben (P=0,054, *4. táblázat*).

Összességében tehát elmondható, hogy az egyes húsrészek zsírsavösszetétele között csak néhány esetben lehetett szignifikáns különbséget kimutatni.

4. táblázat
A kereskedelmi forgalomban vett húsminták zsírsav-csoportok szerinti zsírsavösszetétele (%) (átlag ± szórás, n= 5)

Vizsgálat	Húsrész				Varianciaanalízis F-próbája	
	felsál	rostélyos	lapocka	nyakhús	F	P
SFA	44,01±6,17	50,87±4,44	44,16±3,05	45,99±2,89	2,723	0,079
MUFA	40,15±2,99^{a}	41,96±2,64^{ab}	45,68±2,58^{b}	44,3±2,37^{ab}	4,256	0,022
PUFA	15,83±8,2	7,17±2,51	10,16±3,28	9,71±0,92	3,141	0,054

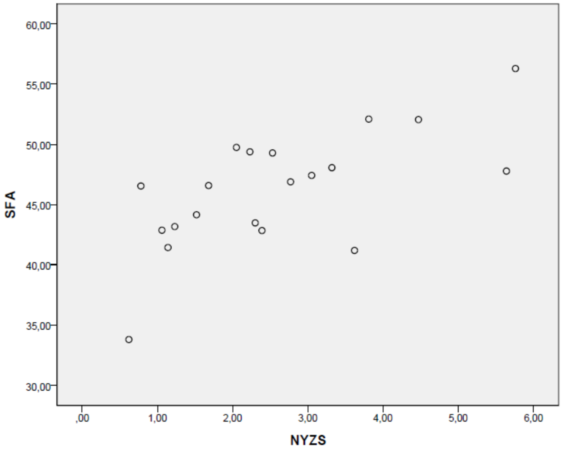
$^{a, b}$ Az azonos sorban lévő és egymástól eltérő betűvel jelölt átlagok között szignifikáns különbség van (P<0,05). Az adatok g/100g zsírsavra vonatkoznak.

Pavan és Duckett (2013) kísérletében rostélyos minták átlagosan 46,10% SFA-t, 36,61% MUFA-t és 5,07% PUFA-t tartalmaztak, míg saját vizsgálatunkban a zsírsavösszetételen belül az SFA átlagosan 50,87 %, a MUFA 41,96 % és a PUFA 7,17 %-ot tett ki. Az eltérésben a zsírsavösszetételt befolyásoló számos egyéb tényezőn kívül az is szerepet játszott, hogy a kisebb mennyiségben előforduló zsírsavak közül voltak olyanok, amelyeket csak *Pavan és Duckett* (2013) (C22:5 n-3 és C22:6 n-3), míg másokat csak mi azonosítottunk (C15:0; C17:0; C20:2 n-6 és C20:3 n-6), így eltérő módon történt a SFA és a PUFA tartalom kiszámítása. A CLA tartalomban volt tapasztalható a legjelentősebb különbség (saját vizsgálat: 0,33 g/100 g zsírsav, illetve *Pavan és Duckett* (2013): 0,68 g/100 g zsírsav). *Pavan és Duckett* (2013) vizsgálatában a takarmányozás teljes egészében legelőre alapozott volt. A legeltetett, és/vagy több tömegtakarmányt fogyasztó szarvasmarhák húsában az összes zsírsav tartalmon belül általában nagyobb a CLA aránya, mint a koncentrált takarmányt fogyasztó állatok zsírjában (*Poulson és mtsai*, 2004).

Az eredmények alapján úgy tűnik, hogy a húsrészen kívüli egyéb (nem vizsgált és nem standardizált) tényezők a zsírtartalom, a CLA-tartalom és a zsírsavösszetétel olyan jelentős szóródását eredményezték, hogy az egyes húsrészek között meglévő esetleges különbségek statisztikailag nem voltak kimutathatók.

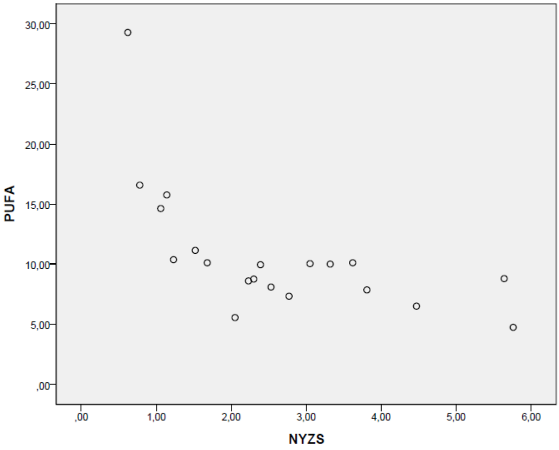
A zsírsavösszetétel és a nyerszsír tartalom közötti összefüggés

Annak érdekében, hogy a marhahús zsírtartalma és zsírsavösszetétele közötti kapcsolatot feltérképezzük, először grafikusan ábrázoltuk a változókat, hogy esetleges összefüggésük, vagy annak hiánya láthatóvá váljon. A független változó minden esetben a nyerszsír-tartalom volt, míg a függő változó az SFA *(1. ábra)*, a PUFA *(2. ábra),* illetve a MUFA *(3. ábra)* volt.



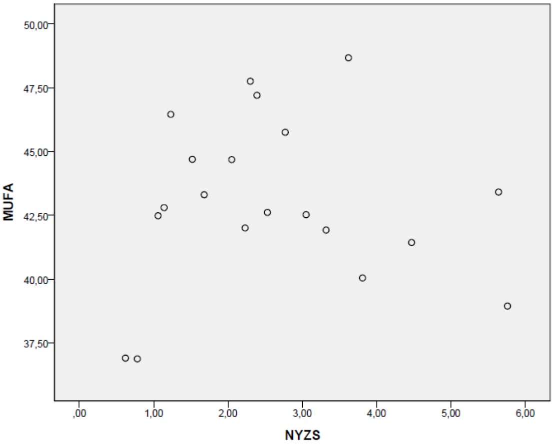
1. ábra. A telített zsírsavak százalékos arányának (SFA) ábrázolása a nyerszsír-tartalom (NYZS, %) függvényében

A *1. ábra* alapján egyenes arányosság látszott a telített zsírsav tartalom és a nyerszsír tartalom között, tehát minél nagyobb volt a marhahús zsírtartalma annál több telített zsírsavat tartalmazott.



2. ábra. A többszörösen telítetlen zsírsavak százalékos arányának (PUFA) ábrázolása a nyerszsír-tartalom (NYZS, %) függvényében

A többszörösen telítetlen zsírsavak esetében viszont fordított arányosság látszott a nyerszsír-tartalom és a PUFA között *(2. ábra),* tehát minél nagyobb volt a zsírtartalom annál kisebb volt a többszörösen telítetlen zsírsavak aránya. Az egyszeresen telítetlen zsírsavak aránya és a zsírtartalom között ugyanakkor nem volt szemmel látható összefüggés *(3. ábra).*



3. ábra. Az egyszeresen telítetlen zsírsavak százalékos arányának (MUFA) ábrázolása a nyerszsír-tartalom (NYZS, %) függvényében

A grafikus ábrázolásokban fellelhető tendencia megfigyelhető volt a Pearson féle korrelációs együtthatókat (r) bemutató táblázatban is *(5. táblázat).* Az r-értékek és a regresszióanalízis F-próbájának megfigyelt szignifikancia-szintjei (P) alapján elmondható, hogy a zsírtartalom és az SFA között szignifikáns egyenes arányosság, míg a zsírtartalom és PUFA között jelentős fordított arányosság van, ezzel szemben a zsírtartalom és a MUFA között nincs szignifikáns összefüggés (P=0,467). Adataink részben összhangban vannak *Pavan és Duckett* (2013) megfigyeléseivel, akik szignifikáns pozitív korrelációt (r=0,55 P<0,0001) mutattak ki az SFA és az összes zsírsavtartalom között (ami arányos a zsírtartalommal), valamint jelentős negatív korrelációt (r=-0,66 P<0,0001) találtak a PUFA és az összes zsírsavtartalom között. Hivatkozott szerzők a MUFA és a zsírtartalom közti összefüggést is jelentősnek detektálták, azonban szemben az előbbi P-értékekkel, csak sokkal kisebb mértékben (r=-0,22 P<0,05). Jelen vizsgálatban a MUFA és a nyerszsírtartalom között szignifikáns kapcsolatot nem tudtunk kimutatni, továbbá a másik két változópár esetében is gyengébb volt az összefüggés (P=0,001, illetve 0,004), mint *Pavan és Duckett* (2013) vizsgálataiban. Ennek feltehetőleg az az oka, hogy hivatkozott szerzők azonos körülmények között tartott és takarmányozott, azonos fajtájú hízóbikák húsát vizsgálták, amelyek kora és testtömege is hasonló volt, míg jelen vizsgálatban a beszerzett minták fenti paraméterei nem voltak azonosak. A kémiai összetételt mindezen tényezők együttesen befolyásolják, emiatt az egyes kémiai paraméterek közötti összefüggés csak kevésbé volt jelentős. Jelen vizsgálat nem fajta- vagy takarmányspecifikus volt, csupán kis mintaszámú „fogyasztói előkísérletnek” tekinthető. Ennek ellenére mégis szignifikáns összefüggés volt kimutatható a zsírtartalom és az egyes zsírsav csoportok (SFA, PUFA) lipideken belüli aránya között.

A regressziós modellek korrelációs együtthatóiból (r) számított determinációs együtthatók (r^2) értékei alapján elmondható, hogy az SFA varianciájának 44,0 százaléka, míg a PUFA varianciájának 38,6 százaléka magyarázható a nyerszsír tartalom lineáris változásával. A szignifikánsnak talált kapcsolatok lineáris regressziós modellje alapján az is látható *(5. táblázat),* hogy egy százalékos nyerszsír tartalom növekedés hatására az SFA százalékos aránya 2,18-dal nőtt, míg a PUFA aránya közel ugyanennyivel, 2,22-dal csökkent. A zsírtartalom növekedésének hatására bekövetkező „zsírsav telítődés” a PUFA rovására valósult meg, a MUFA-t azonban kevésbé, vagy egyáltalán nem érintette. Ezt az összefüggést támasztják alá *Pavan és Duckett* (2013) eredményei is, ugyanis vizsgálatukban a nyolc húsféleség közül a legtöbb zsírt (19,93 g/100g nyers hús) tartalmazó darált marhahús tartalmazta a legtöbb SFA-t (48,95%) és ezzel egyidejűleg a legkevesebb PUFA-t (1,99 %).

5. táblázat

A nyerszsír-tartalom (NYZS), valamint az egyes zsírsav csoportok (SFA, PUFA, MUFA) közötti összefüggés marhahúsban (n=20), valamint a nyerszsír-tartalom és az egyes zsírsav csoportok közötti lineáris regresszió paraméterei és szórásuk.

Független változó	Függő változók		
NYZS	SFA	MUFA	PUFA
r	0,663	0,020	-0,621
P	0,001	0,467	0,004
B_0	40,6±1,73	-	16,5±1,98
B_1	2,18±0,58	-	-2,22±0,66

NYZS = nyerszsír-tartalom (%). A becsült regressziós egyenlet az alábbi volt: $Y = B_0 + B_1 \cdot X$ *ahol* B_0*=regressziós állandó,* B_1*=regressziós együttható, X=nyerszsír tartalom, Y=SFA vagy PUFA.*

Felmerül a kérdés, hogy mi állhat a zsírtartalom változással összefüggő zsírsavprofil-módosulás hátterében. Ennek feltételezhető oka, hogy eltérő zsírtartalomnál a neutrális lipidek és a biológiai membránokban található amfipatikus lipidek aránya különbözik egymástól, valamint ez a két lipid frakció eltérő zsírsavösszetétellel rendelkezik. A foszfolipidekben több PUFA, például arachidonsav található, mint a triacilgliceridekben (*Käkelä és Hyvärinen*, 1995). A sovány hús lipidjeiben arányaiban több foszfolipid található, mint a zsíros húsban (*Domínguez és mtsai*, 2019), amely viszont több telítetlen zsírsav jelenlétét is jelentheti. A hús zsírtartalmának növekedésével a zsírsejtek (adipociták) mérete, és ezzel párhuzamosan a triglicerid frakció mennyisége is nő; a membránlipidek (foszfolipidek) mennyisége azonban ehhez képest kevésbé változik.

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