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EVALUATION OF THE GENOMIC SCORES OF LIMOUSIN CANDIDATE BULLS BY CLUSTER ANALYSIS

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Összefoglalás

A szerzők elemezték a limousin (n = 40) fajtatiszta tenyészbika-jelöltek teljesítményeit (2018-2020). A tenyészbika-jelölteket kis csoportban (2-9) tartották a teszt állomáson, a takarmányozásuk ad libitum gyep szénára vagy lucerna szénára és adagolt abraktakarmányra alapozódott. Az elemzett jellemzők a következők voltak: elősúly a vizsgálat kezdetén és a végén, a napi súlygyarapodás a sajátteljesítmény-vizsgálat során (g/nap), és genomikai pontszámok (GP) 8 tulajdonságra (pl. könnyű ellés, növekedési erély, izomoltság, pontszám). Az adatokat az SPSS 24. programcsomaggal dolgozták fel (klaszter-analízis: K-közép módszer). Az első klaszterbe csoportosított állatok (n=5) nagy növekedési kapacitást és kiváló növekedési intenzitást mutattak jó rámával, ugyanakkor kissé kevésbé kedvező csontfinomsággal. A második és harmadik csoportba tartozó egyedek (n=19, n=16) rámája nem kiemelkedő, csontfinomságuk azonban figyelemre méltó. Klaszter-analízis alkalmazása gyors és hasznos információkkal szolgálhat a tenyésztők számára.
Kulcsszavak: limousin tenyészbika-jelöltek, központi sajátteljesítmény-vizsgáló állomás, genomikai pontszámértékek, klaszter elemzés

Summary

The production of purebred Limousin candidate bulls (n = 40) were analysed between 2018-2020. Candidate bulls were kept in small groups (2-9) at the test station, fed with meadow hay, alfalfa *ad libitum* and dosed fodder. Traits studied were the following: finishing live weight, daily weight gain during the test (g/day) and and genomic scores (GS) for 8 traits (ex: easy calving, vigor of growth, musculatity, score). Data was analysed by SPSS 24.0 software package (K-means cluster analysis). The animals grouped into the first cluster (n=5) showed high growth capacity and excellent growth intensity with good frame size, but at the same time with slightly less favorable bone fineness. The frame size of the individuals belonging to the second and third groups (n=19, n=16) is not outstanding, however, their bone fineness is remarkable. Applying of the K-means cluster analysis can provide the breeders fast and useful information
Keywords: Limousin candidate bulls, central performance test station, genomic scores, K-means cluster analysis

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Introduction

Production control and performance tests are inevitable, important parts of the breeding activity. Various techniques can be applied during these, but it is evident to use accurate, fast, and standardised methods for all traits important according to the breeding aim.

As a result of the professional work, the breeding value of a given animal reflects clearly its real value as a parent in the breeding stock. The real breeding value of the animal should be taken as a base of its economical value in practice.

Organizing the production control in beef cattle, defining its rules was under central, state control in Hungary for a long time (1960-1988). Production control was financed and regulated centrally that time and the staff of experts in the countrywide state organisations (called first OTÁF, afterwards Animal Husbandry Companies) could guarantee the appropriate professional establishment and operation of the test stations (central and farm performance test and progeny performance test stations).

Work of those production control stations (e.g. progeny performance test station in Pély, Hungary and performance test station in Boród, Hungary) was already highly appreciated by international professionals, according to many sources.

Central financing highly decreased after 1988-1995, when social organisations (breeding committees, associations) were established.

Production control and breeding value estimation have to be done according the given breed's breeding programme, taking into consideration the recommendations of the International Committee for Animal Recording – in concordance with the international practice – for the quality professional work.

Production control – according to the Act of Animal Breeding – both in dairy and in beef cattle is done by the breed clubs by themselves or together with other clubs or organisations (such as Animal Breeding Performance Testing Ltd., National Artificial Inseminator Co.). Authenticity of the registered records is proven by the National Food Chain Safety Office (NÉBIH), as registered data is controlled by them, next to the breeders and the breed clubs.

Breeding organisations apply the regulations of the Cattle Performance Test Codex in their breeding programme, with providing some freedom also for themselves.

Performance test in a narrow professional meaning is the pre-selection of candidate bulls born from a contract-mating scheme (bull dam x top breeding bull) for beef production and reproduction traits.

The most important steps in Hungarian literature and practice about performance tests are the following.

- The first study in Hungary according breeding value estimation and improving traits of economical importance in beef cattle is by *Nagy* (1974). This paper deals in details with the traits and their measures: fertility, calf rearing ability, feed conversion, early maturing and conformation. This study was gap filling in case of breeding value estimation that time, and made the bases of the system applied nowadays in Hungary with the detailed description of the parts of the performance control system already used in practice abroad. Performance test for purebred Limousine breeding bulls started in 1972, at the experimental farm of the Department of Animal Breeding, Gödöllő, Hungary. The foundation of that work were the studies and their results made in the stock breeding farm of the State Farm Hajdúszoboszló, Hungary, which enlights the importance of the connectedness of theory and practice.
- *Nagy et al.* (1985) analysed the results of the Hungarian central performance tests per breeds, in comparison to the breed standards set in Great Britain (Meat and Livestock Commission,

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MLC). The paper analysed the performance of Hereford, Limousin, Charolais and Hungarian Fleckvieh feeders/yearlings in the Performance Control Station Borópuszta, belonging to the Animal Breeding Company Szekszárd between 1980-1984. They concluded that all four breeds are below the MLC standard in case of the corrected live weight at 200 days. Worst performance was measured in Charolais, which was 50 kg (83.3%) under the standard value. Limousin approached closest the MLC value, was only 21 kg (91.8%) bellow it. Hungarian Fleckvieh and Hereford showed 85.5% and 87.5% performance, respectively. Difference among the four breeds was lower in case of live weight at 300 days of age, and all were closer to the standard, with performance over 90%. Limousin breed was closer to the comparative value (97.3%, 10 kg). At 400 days of age all four breeds already exceeded the MLC values, with the following extents: Limousin, 103.4%, Hereford 103.2%, Hungarian Fleckvieh 102.7% and Charolais 101.9%. Authors concluded, that the performance of these breeds in Hungary is under the MLC breed standard at younger age, but they can compensate it during rearing, and even exceed the standard.

- It was proved by analysing the results of farm and central performance tests (*Nagy et al.*, 1989) that there's a significant difference between the same Charolais sire lines (central performance test, 8 breeding line, n = 74, farm performance test, 6 breeding line, n = 122) in growth capacity and growth intensity, with individuals tested in central station being better.
- Studying Charolais (n = 95), Hereford (n = 55) and Limousin (n = 120) candidate bulls in a central station in Boród, Hungary showed that – under same keeping and feeding circumstances – French breeds had higher weight gain and better feed conversion ratio than the Hereford. The measured values during the performance test were in weigh gain Ch: 1809 g/day, He: 1648 g/day, Li: 1676 g/day, while in feed conversion ratio Ch: 13.0 NEg Mj/kg, He: 15.3 NEg Mj/kg, Li: 13.3 NEg Mj/kg (*Tőzsér et al.*, 1987).
- Performance in measured in farm environment not always show the productive ability of the breeds, especially in case of growth intensity, due to different feeding. Therefore central performance tests have a great importance. In Hungary the breed clubs for Hereford, Angus, Hungarian Fleckvieh, Charolais and Limousin frequently send the young candidate bulls to central stations for performance tests.
- The heritability values (h^2) estimated based on 548 candidate bulls from two breeding stations between 1992-1999 were the following: live weight corrected to 365 days 0.28, conformation traits in connection with life productivity (0.13), length measures 0.23, width measures 0.17, muscularity 0.13 (*Tőzsér*, 2006).
- It's advised to measure the progeny of the bulls who performed well in farm tests in central stations in case if there's a genotype-environment interaction in a trait with great importance in the given breed and in the breeding aim. That kind of studies can be accompanied with testing the siblings born from embryo splitting in farm and in central station in parallel.
- Measuring the circumfence of *m. longissimus dorsi* by ultrasound scanner *in vivo* is already applied in the practice of beef cattle husbandry in Hungary, in case of several breeds, in concordance with international experiments.
- Ultrasound equipment with different wavelength (3.5-7.5 MHz) sensors are suitable for studying reproduction status in cows (ovaries, fallopian tube, uterus, etc.) and in bulls (testicle measures, tissue structure, etc.) *in vivo*, without causing a tissue damage, according to the review *Griffin and Ginther*, 1992.

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- Based on international experiences, selection for beef production in candidate bulls can be highly more effective by measuring feed consumption and feed conversion performances, using so called electric gates (*INRA*, 1995).
- *Gáspárdy et al.*, (1998) used two types of animal models for estimating the weaning weight corrected to 205 days of bull calves. That study showed the importance of utilizing animal model in performance tests. As applying animal model is getting widespread, the role and importance of performance tests is increasing, because breeding value of the individual can be accurately estimated based on its own performance and the performance of its relatives.
- Furthermore, it's important to continue estimating the genomic breeding values of the candidate bulls based on central performance tests for the more efficient selection.

The aim of our study was the investigating the possibility of grouping breeding bulls based on type according their central performance test results by cluster analysis.

Materials and methods

The database for our study was made up from results of the bull candidates monitored in the central performance test station of our associations (Bos-Genetic Co., Martonvásár, Hungary), between 2018-2020 (six starts, n=40 animals).
The central performance test was performed according to the rules of the Breeding Programme of the Limousin and Blonde d' Aquitaine Breeder's Association (*LBTE*, 2018), the main characteristics are followings:

1.) Starting and transport:
- Time of weaning is 180-210 days of age.
- Candidate bulls have to be delivered to the station not older than 240-250 days.
- Between the delivery and the tests start candidate bulls spend 30 days in a quarantine stable.

2.) Housing:
- Aspects of grouped housing (2-9 animals): identical origin, same size and not more than 30 days age difference.
- Paddocks, compartments and boxes need to be equipped with feeder and drinking-trough and technology suitable for handing the animals, e.g. individual neck extenders. Floor: concrete and deep litter.

3.) Nutrition:
- Ad libitum, based on grass or alfalfa hay, and fodder mixture (15% protein content), which provides the necessary energy, mineral salts, vitamins, fats and carbohydrates for the body. Fodder dose is adjusted to 1500 g/day daily weight gain, which is defined based on the last measured live weight.

4.) Measures, judging:
- Weighing: every 30-day.
- Conformation judgement: according to the official judging system (16 traits, 1-9 scores)
- Body measures: height at withers, hip height, withers width, rump width I and II.

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- Genomic scores (GS): muscularity, weight gain, frame, bone fineness, calving ease, milk production, diameter of the inner pelvis (1-12 scores). Genomic scores (EvaLim® test, 54K Illumina chip for SNP determination, 12,800 breeding bull SNP data and associated performance and offspring test results form the reference base; *Ingenomix*, 2020). The French reference population is divided into 10 parts based on their characteristics. The bulls tested are classified in the class in which the individuals with the most similar SNP patterns are located, based on their SNP data. Point 1 includes the sample animals that achieved the worst 10% phenotypic result for a given trait, while point 10 includes those with the best 10%. If the breeding bull is most similar to the best 5% and 1% best individuals of the reference population for the given trait in terms of DNA-SNP data, then 11 or you get 12 points (*Szűcs*, 2018).

5.) Closing and qualification:

- Length of the self-performance test 150 days.
- Age at qualification: 465-460 days.
- Animals starting in the same group and qualified at the same time.
- Qualification: based on the law by the competent authority and the directives in the breeding programme and the constitution of the association.
- Animals that didn't qualify cannot be sold for breeding purposes.

Statistical analysis

K-mean cluster analysis was used for analysing the relations among the measured and calculated data from the central performance test. Different groups were estimated based on Euclidean distance (*Sváb*, 1979). SPSS 24.0. statistical package was used for the analysis.

Results and discussion

There's a worldwide tendency among cattle breeders to focus on type instead of breed for the sake of marketable products. Farmers are looking for types, which are able to produce economically under the given ecological, economical, etc. circumstances. This tendency is clearly visible in number of animals, as the ratio of culture breeds (Holstein-Frisian, Hereford, Angus, Charolais, etc.) is increasing compared to local breeds, both in dairy and in beef cattle (*Szabó et al.*, 2002).

Types, which are able to produce more effectively compared to traditional types due to their higher adaptability, are also developed within beef breeds (e.g. the English or the USA-Canadian type of Hereford). Separating and evaluating animals belonging to breeding, butchery and mixed types (Hereford, Angus, Charolais, Limousin, Blonde d' Aquitaine breeds) is the aspiration of breeders in the Hungarian beef cattle branch. There's a new idea about expressing the value of the animals in money as well, based on its productive parameters. Hungarian Charolais Lightweight Breeding Index and Hungarian Charolais Heavyweight Breeding Index would play an important role in nucleus breeding stations. Hungarian Charolais Terminal Product Index, applied according to the profile of the market breeding farm could help significantly in choosing the breeding bulls (*Török*, 2020).

Based on the data of the animals in our study 5 individuals grouped to the first cluster, 19 to the second, while 16 to the third by K-means cluster analysis. Analysis started from the initial cluster

centres (*Table 1.*). It is shown, that based on parameters the data of two clusters out of three (the first and second) are close to each other. Number of iterations was 4 during the study (*Table 2.*).

Table 1: **Initial cluster centers**

Traits (1)	Clusters (2)		
	1	2	3
Body weight at the and of test, kg (3)	593	592	501
Daily weight gain during self performance test, g/ day (4)	1934	1589	1212
GS for easy calving: easy birth of calves (5)	4	10	9
GS for vigor of growth (6)	7	3	3
GS for musculatity (7)	6	5	10
GS for frame size (8)	7	5	1
GS for size of bone (9)	2	4	4
GS for easy calving: offspring (10)	4	6	6
GS for milk production (11)	4	3	11
GS for inside diameter of the pelvice (12)	7	5	3

1. tablázat: A vizsgálat kezdeti klaszter közepek

Tulajdonságok (1), Klaszterek (2), Vizsgálat végi élősúly, kg (3), Vizsgálat alatti súlygyarapdás, g/nap (4), Genomikai pontszám könnyű ellésre: borjak könnyű születése (5), Genomikai pontszám növekedési erélyre (6), Genomikai pontszám izmoltságra (7), Genomikai pontszám rámára (8), Genomikai pontszám csontváz finomságra (9), Genomikai pontszám könnyű ellésre: utódok könnyű ellése (10), Genomikai pontszám tejtermelésre (11), Genomikai pontszám medence belső átmérőjére (12)

***Table 2*: Iteration history**

Change in Cluster Centers (1)		
1	2	3
55,26	40,49	134,69
37,53	4,60	5,65
27,71	8,52	0,00
0,00	0,00	0,00

2. táblázat: Iterációk

Klaszterközepek változása (1)

Data of the final cluster centres is summarized in *Table 3*. In live weight of the breeding bulls at the end of the test, the mean of the animals grouped in the first and second cluster was significantly higher (+65 kg, +59 kg, $P \leq 0.001$), than those belonging to the third cluster (524 kg). Similarly, in case of weight gain during the test results of animals in the first and second cluster were higher, compared to the third one (1.8 kg/day, 1.5 kg/day, 1.3 kg/day, $P \leq 0.001$).

It can be seen that the first and second groups are heavier than the third group and their growth vigor is also more significant.

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Table 3: **Final cluster centers**

Traits (1)	Clusters (2)		
	1	2	3
Body weight at the and of test, kg (3)	589a	583b	524ab
Daily weight gain during self performance test, g/ day (4)	1819c	1539d	1350cd
GS for easy calving: easy birth of calves (5)	6	7	8
GS for vigor of growth (6)	6	4	4
GS for musculatity (7)	6	7	7
GS for frame size (8)	6e	3	2e
GS for size of bone (9)	5 fg	7f	7g
GS for easy calving: offspring (10)	5	4	4
GS for milk production (11)	5	5	6
GS for inside diameter of the pelvice (12)	6	5	6

Mean values with same letters (a, b, c, d: P≤0.001, e: P≤0.01, f,g: P≤0.10) withine the row are significantly different

3. tablázat: A vizsgálat végi klaszter közepek

Tulajdonságok (1), Klaszterek (2), Vizsgálat végi élősúly, kg (3), Vizsgálat alatti súlygyarapdás, g/nap (4), Genomikai pontszám könnyű ellésre: borjak könnyű születése (5), Genomikai pontszám növekedési erélyre (6), Genomikai pontszám izmoltságra (7), Genomikai pontszám rámára (8), Genomikai pontszám csontváz finomságra (9), Genomikai pontszám könnyű ellésre: utódok könnyű ellése (10), Genomikai pontszám tejtermelésre (11), Genomikai pontszám medence belső átmérőjére (12)

Seeing the data of the second and third groups in genomic scores for vigor of growth (4, 4 score) were consistent with the trend of weight gain between the groups.

The scores obtained for muscularity (6,7,7 score) in the three groups suggest that muscularity my be similary in groups of individuals with different mean weights.

Regarding frame size, the result of the third group (2 score) was significantly lower than the result of the first group (6 score) so a higher body weight can mean a bigger frame size.

Size of bone appeared to be more favorable in the second (7 score) and third (7 score) groups compared to the first one (5 points).

In the last three parameters (easy calving: offspring, milk produktion, inside diameter of the pelvice), the results of the three groups were identical.

We have to highlight the following results based on the cluster analysis:

- Based on the distances of the cluster centres (*Table 4*) first cluster is closer to the second, than to the third one. The lowest distance is (197.7) between the second and the third groups, while the distance is the highest between the first and third clusters (473.1).
- The animals grouped into the first cluster showed high growth capacity and excellent growth intensity with good frame size, but at the same time with slightly less favorable bone fineness.
- The frame size of the individuals belonging to the second and third groups is not outstanding, however, their bone fineness is remarkable.

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There were no previous studies in the Hungarian literature about using K-mean cluster analysis for the GS of bulls. *Tőzsér et al.* (2000) have used this method previously for analysing the conformation of Charolais cows. They've clearly separated four cow groups, which means assistance in defining the types.

***Table 4*: Distances between final cluster centers**

Cluster	1	2
1		
2	279,9	
3	473,1	197,7

4. Táblázat: A végső klaszter centrumok közötti távolság

Conclusions

Identifying and separating homogenous groups of animals is an important step in the practice of animal breeding. Based on our results, K-mean cluster analysis proved to be suitable for that task. Applying that method can provide the breeders fast and useful information. These kinds of studies can help to understand the relationships among given components of the type.

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