УДК 636.082.11

METHOD for ASSESSING and PREDICTING the LEVEL of the SHEEP MEAT PRODUCTIVITY DEVELOPMENT

V. M. Iovenko, Doctor of Agricultural Sciences, Professor
ORCID: 0000-0002-0829-7844
H. O. Yakovchuk
ORCID ID: 0000-0002-2141-8540
I. A. Hladii*
ORCID: 0000-0003-3078-1103
H. I. Rukavnikova
ORCID: 0000-0001-6009-6583

"Ascania Nova" Institute of Animal Breeding in the Steppe Regions named after M. F. Ivanov - National Scientific Selection-Genetics Center for Sheep Breeding
1, Soborna Street, Askania Nova, Chaplynka district, Kherson region, 75230, Ukraine
e-mail: ascitsr_priemnaya@ukr.net

Aim. *Development of a new way to intensify the selection process in sheep breeding.* ***Methods.*** *Molecular genetics, population genetic, biometric.* ***Results.*** *A method has been developed for assessing, selecting and predicting of the sheep meat productivity level development using the growth hormone gene. In the population of the created meat breed, it was found that this gene is characterized by a high level of polymorphism and is controlled by two codominant alleles (A, B), which form three genotypes: AA, AB, and BB. The concentration of the latter is, respectively, 50.6%; 30.0%, 19.4%, which was the basis for the presence of influence varying degrees on the studied animals' trait level. In this context, it was revealed that in terms of growth parameters, lambs with the GH^B allelic gene with a high reliability (p <0.1-0.01) prevail over their peers with an alternative allele (GH^A). In particular, their live weight at birth is 15.0% higher, and the average daily gain is 18.6%. It was also shown that young individuals at the age of 4 months, when they have the best quality lamb meat, had a live weight of 31.6 kg versus 29.0 kg; fresh carcass weight - 12.9 kg versus 11.0 kg; slaughter yield - 43.3% versus 39.4% among peers. In addition, the heart mass in young animals with the GH^B allele is 15.5% higher than in analogs with the alternative allele; and the mass of the liver - by 12.7%; spleen - by 25.8%. Young animals with a homozygous GHBB genotype, which mainly de-*

termines the level of distribution of the GH^B allele, stand out especially positively. These animals have the highest indicators in almost all parameters of meat productivity. ***Conclusions.*** *A method has been developed for assessing and predicting of the sheep meat productivity level development using the growth hormone gene. It has been established that, in terms of growth parameters, young animals with the allelic GH^B gene are highly reliably superior to their peers with the alternative GH^A gene. Young animals with a homozygous GHBB genotype, which mainly determines the level of distribution of the GH^B allele, are seen especially in the positive direction. These animals have high rates in almost all parameters of meat productivity.*

Keywords: sheep, growth hormone, genetic marker, meat productivity.

DOI: : https://doi.org/10.33694/2617-0787-2021-1-14-111-121

СПОСІБ ОЦІНКИ І ПРОГНОЗУВАННЯ РІВНЯ РОЗВИТКУ М'ЯСНОЇ ПРОДУКТИВНОСТІ ОВЕЦЬ

В. М. Іовенко, доктор сільськогосподарських наук, професор

ORCID: 0000-0002-0829-7844

Г. О. Яковчук

ORCID ID : 0000-0002-2141-8540

І. А. Гладій*

ORCID: 0000-0003-3078-1103

Г. І. Рукавнікова

ORCID: 0000-0001-6009-6583

Інститут тваринництва степових районів імені М. Ф. Іванова «Асканія-Нова» - Національний науковий селекційно-генетичний центр з вівчарства

вул. Соборна, 1, смт Асканія-Нова, Чаплинський р-н, Херсонська обл., 75230, Україна

e-mail: ascitsr_priemnaya@ukr.net

Надійшла 02.03.2021

Мета. *Розробка нового способу інтенсифікації селекційного процесу у вівчарстві.* ***Методи.*** *Молекулярно-генетичні, популяційно-генетичні, біометричні.* ***Результати.*** *Розроблено спосіб оцінки, відбору і прогнозування рівня розвитку м'ясної продуктивності овець із застосуванням гену гормону росту. В*

середовищі створюваної м'ясної породи встановлено, що цей ген характеризується високим рівнем поліморфізму і детермінується двома кодомінантними алелями (А, В), котрі утворюють три генотипи: АА, АВ, ВВ. Концентрація останніх складає відповідно 50,6%; 30,0%; 19,4%, що стало підставою для наявності різного ступеню їх впливу на рівень досліджуваної ознаки тварин. В цьому контексті виявлено, що за параметрами росту ягнята з алельним геном GH^B з високою вірогідністю (p<0,1-0,01) переважають своїх ровесників з альтернативним алелем GH^A. Зокрема, їх жива маса при народженні на 15,0% вища, а середньодобові прирости – на 18,6%. Також показано, що молоді особини у віці 4-х місяців, коли у них найкращі якості баранини, мали живу масу 31,6 кг проти 29,0 кг; масу парної туші – 12,9 кг проти 11,0 кг ; забійний вихід – 43,3% проти 39,4% у їх ровесників. Крім цього, маса серця у молодих тварин з алелем GH^B на 15,5% більша, ніж у аналогів з альтернативним алелем, а маса печінки – на 12,7%; селезінки – на 25,8%. Особливо у позитивний бік виділяється молодняк з гомозиготним генотипом GHBB, котрий в основному визначає рівень розповсюдження алеля GH^B. Ці тварини мають найвищі показники практично за всіма параметрами м'ясної продуктивності. ***Висновки.*** *Розроблено спосіб оцінки і прогнозування рівня розвитку м'ясної продуктивності овець із застосуванням гену гормону росту. Встановлено, що за параметрами росту молоді тварини з алельним геном GH^B з високою вірогідністю переважають своїх ровесників з альтернативним алелем GH^A. Особливо у позитивний бік виділяється молодняк з гомозиготним генотипом GHBB, котрий в основному визначає рівень розповсюдження алеля GH^B. Ці тварини мають найвищі показники практично за всіма параметрами м'ясної продуктивності.*

Ключові слова: вівці, гормон росту, генетичний маркер, м'ясна продуктивність.
DOI: : https://doi.org/10.33694/2617-0787-2021-1-14-111-121

СПОСОБ ОЦЕНКИ И ПРОГНОЗИРОВАНИЯ УРОВНЯ РАЗВИТИЯ МЯСНОЙ ПРОДУКТИВНОСТИ ОВЕЦ

В. Н. Иовенко, доктор сельскохозяйственных наук, профессор
ORCID: 0000-0002-0829-7844

А. А. Яковчук
ORCID ID: 0000-0002-2141-8540
И. А. Гладий*
ORCID: 0000-0003-3078-1103
Г. И. Рукавникова
ORCID: 0000-0001-6009-6583

Институт животноводства степных районов имени М. Ф. Иванова «Аскания-Нова» - Национальный научный селекционно-генетический центр по овцеводству
ул. Соборная, 1, пгт. Аскания-Нова, Чаплинский р-н, Херсонская обл., 75230, Украина
e-mail: ascitsr_priemnaya@ukr.net

Цель. *Разработка нового способа интенсификации селекционного процесса в овцеводстве.* ***Методы****. Молекулярно-генетические, популяционно-генетические, биометрические.* ***Результаты.*** *Разработан способ оценки, отбора и прогнозирования уровня развития мясной продуктивности овец с использованием гена гормона роста. В популяции создаваемой мясной породы установлено, что этот ген характеризуется высоким уровнем полиморфизма и контролируется двумя кодоминантными аллелями (А, В), которые образуют три генотипа: АА, АВ, ВВ. Концентрация последних составляет соответственно 50,6%; 30,0%, 19,4%, что явилось основанием для наличия различной степени влияния на уровень исследованного признака животных. В этом контексте выявлено, что по параметрам роста ягнята с аллельным геном GH^B с высокой достоверностью (p<0,1-0,01) преобладают над своими ровесниками с альтернативным аллелем (GH^A). В частности, их живая масса при рождении на 15,0% выше, а среднесуточные приросты – на 18,6%. Также показано, что молодые особи в возрасте 4-х месяцев, когда у них наилучшее качество баранины, имели живую массу 31,6 кг против 29,0 кг; массу парной туши – 12,9 кг против 11,0 кг; убойный выход – 43,3% против 39,4% у ровесников. Кроме этого, масса сердца у молодых животных с аллелем GH^B на 15,5% больше, нежели у аналогов с альтернативным аллелем; а масса печени – на 12,7%; селезенки – на 25,8%. Особенно положительно выделяется молодняк с гомозиготным генотипом GHBB, который в основном определяет уровень распространения аллеля GH^B. Эти животные имеют наивысшие показатели практически по всем параметрам мясной продуктивности.* ***Выводы.*** *Разработан*

способ оценки и прогнозирования уровня развития мясной продуктивности овец с применением гена гормона роста. Установлено, что по параметрам роста молодые животные с аллельным геном GH^B с высокой вероятностью преобладают над своими ровесниками с альтернативным аллелем GH^A. Особенно в положительную сторону виделяется молодняк с гомозиготным генотипом GHBB, который в основном определяет уровень распространения аллеля GH^B. Эти животные имеют высокие показатели практически по всем параметрам мясной продуктивности.

Ключевые слова: овцы, гормон роста, генетический маркер, мясная продуктивность.
DOI: : https://doi.org/10.33694/2617-0787-2021-1-14-111-121

The progress of sheep breeding, along with other measures, requires the earliest possible assessment of the animals' productive qualities. This issue is gaining special attention in the current difficult economic conditions, when the industry is in a very difficult situation.

Today, in the developed countries of the world, scientific and practical research on the genomic selection of animals is becoming more and more widespread, since the use of only selection methods based on the use of means for assessing individuals by phenotype, in many cases, inhibits the effectiveness of breeding work. This is especially true for the sheep breeding industry, where the breeding and improvement of animals is carried out using traditional methods.

To speed up the breeding process, in recent years, scientists have focused their efforts on finding links between genetic and molecular markers and commercial traits of farm animals. Many works are known in this direction. In individual breeds, types and herds, a number of multidirectional relationships were found between the development level of the farm animals main selected traits and individual and complex genotypes for alleles of blood groups and polymorphic protein loci of blood. However, the proposed developments are not always effective. Their main disadvantage is that the genes of proteins or blood groups, expressed by a separate genotype, are far from polygenic quantitative traits in nature. More promising is the involvement of genes of quantitative traits (QTL-genes) in the development of selection and genetic methods for assessing and predicting the level of sheep productivity, the nature of which is known in the context of controlling the development the certain productive traits of animals.

The growth hormone gene (somatotropin) is one of the most demanded objects in the genetics of farm animals and poultry. Its various

allelic variants are associated with a very wide range of economically useful traits, ranging from performance indicators to disease resistance factors [3, 4]. This gene plays an especially important role in the development of the animals' meat qualities. At the same time, it can accelerate the metabolism in the body and stimulate the growth of many organs and tissues, especially bones, muscles and internal organs, has a direct effect on the synthesis and secretion of the hormone and, as a result, promotes the intensive growth of individuals [1, 2].

In this context, the goal of our work was to develop a highly effective method for assessing, selecting and predicting the sheep meat productivity level using the growth hormone polymorphic gene types as markers, the polymorphism level of which is determined by polymerase chain reaction (PCR).

Material and research methods. The research was carried out on a crossbred young sheep of the "Ascania Nova" breeding plant of the Kherson region, obtained from crossing the Ascanian Fine-Fleeced and Texel breeds when creating a new meat breed for the conditions of the Ukraine south (n = 50). Alleles and genotypes of the polymorphic locus of the growth hormone (GH) gene are used as molecular genetic markers.

When carrying out genetic studies, DNA isolation from experimental blood samples was carried out according to the standard method using a set of reagents, DNA sorb-B (Ampli Sens). The following primers were used to amplify the selected fragment of the GH gene - GHF-5-CTCTGCCTGCCCTGGACT-3' and GHF-5-GGACAAGCAGA-AGGCAAC-3'.

Amplification was carried out using a Libe Line thermal cycler according to an appropriately selected program.

Determination of this gene fragment was carried out using the PCR-RFLP method. PCR was carried out with the following temperature regime: one cycle — denaturation at 94 ° C for 5 min; 35 cycles - denaturation at 95 ° C for 30 s; annealing - 30 s at 65 ° C and elongation - 72 ° C for 45 s; one cycle - final relaxation 72 ° C 7 min. The length of the amplified fragment was 422 bp. When processing the amplified fragment, the restriction enzyme Nae III (GG / CC) was used, obtained in accordance with the standard procedure of the manufacturer "Thermo Scientific", USA. The restriction product was separated by horizontal electrophoresis in 2.5% agarose gel at a voltage of 80 V for 30 min. Genotypes were visualized using ethidium bromide in the ultraviolet spectrum. The size of the restriction fragment was determined by a molecular weight marker (Gene Ruler TMSO) bp DNA Ladder "Fermentas" pUC19 / MSpi "Sib Enzym". Genotyping of animals was carried out by analyzing the obtained foregrams.

Animal testing for this gene was carried out at the age of 3 months. Growth parameters of lambs and their slaughter characteristics were used as performance indicators.

Research results. As a prototype of the development, we took a method for assessing the sheep productivity level using immunogenetic and genetic-biochemical markers combined with individual economically useful traits [5]. The author found that in Caucasian sheep with the AD type of the transferin locus (Tf), live weight is 16% higher compared to the average for the herd, and with sheep carriers of homozygotes Tf AA and Tf DD, respectively by 13.7 and 44, 6%. In animals with the type of hemoglobin AB, an increase in wool density by 31.7% compared with the homozygote Hb BB was noted.

The analysis of the prototype revealed the following disadvantages:

- genes of the given polymorphic systems, expressed by separate genotypes not associated with quantitative traits of sheep productivity;
- another negative property of the established relationships - their chaotic nature when comparing different populations of animals;
- a low recurrence level of such relationships in a number of adjacent generations.

The first domestic specialized meat breed of sheep for the conditions of the southern region of Ukraine is being created at the "Ascania Nova" Institute of Animal Breeding in the Steppe Regions. For a successful and in a short time to carry out the relevant work, it is necessary to search and introduce methods that would ensure an increase in the efficiency of selection and breeding work.

In this context, a method has been developed for assessing, selecting and predicting the development of sheep meat productivity level using a polymorphic gene of growth hormone, since somatotropin has a powerful anabiotic and anti-catabolic effect, enhances protein synthesis and inhibits its breakdown, and also helps to reduce the deposition of subcutaneous fat, enhance fat burning and an increase in the ratio of muscle mass to fat. That is, in general, this gene accelerates the metabolism in the body and stimulates the growth of many organs and tissues, especially bones, muscles and internal organs, and in general controls the process of growth and development of meat productivity of individuals.

The stated problem is solved by the fact that on the basis of genetic certification of young sheep according to the growth hormone types of polymorphic locus, it is determined by the genotype of the experimental individual. Then, the analysis of meat productivity in groups of young animals with different genotypes for the specified genome is carried out. If a significant advantage in the development of the productive traits lev-

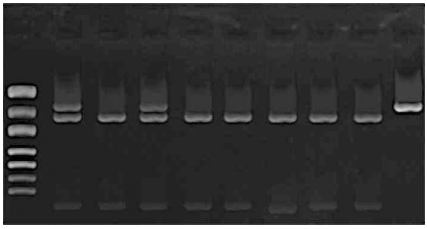
el a certain genotype is found, then such animals are selected for further breeding use, which is an important element of genomic selection.

Below is a sequence of relevant studies to establish the correlation between the markers of the applied gene and the meat productivity development level of hybrid young sheep.

Work begins with a polymerase chain reaction to establish the level of polymorphism of the studied blood locus in experimental animals.

It was found that in this sheep population, the polymorphism of the used gene is controlled by two codominant alleles (GH^A, GH^B), which form three genotypes, two homozygotes (A / A, B / B) and heterozygote A / B.

It was revealed that the A / A genotype is represented by two fragments, 366 and 56 bp in length, the A / B genotype is represented by three fragments, 422, 366, and 56 bp in length. (Fig. 1). The B / B homozygote is characterized by the presence of a 422 bp nonrestriction product.



bp 1 2 3 4 5 6 7 8 9 10

1 – DNA marker MspI (501, 404, 331, 242, 190, 147, 111); 2, 4 – genotype AB (422, 366, 56 bp); 3, 5, 6, 7, 8, 9 – genotype AA (366, 56 bp); 10 – PCR product (422 bp)

Figure 1. Electrophoregram of the restriction products distribution GH gene (Hae III restriction enzyme)

In the herd studied by us, the prevalence of distribution was gained by the homozygote AA (50.6%), the second place is occupied by the heterozygote AB (30.0%), the third is the homozygote BP (19.4%).

At the next stage, the analysis of the level of animals' meat productivity in three specific groups was carried out. The analysis results are shown in Table 1.

It was shown that in terms of growth parameters, young animals with the GHB allelic gene (genotypes AB, BB) with a high probability ($p < 0.1$-0.01) prevail over their peers with the alternative GHA allele. In particular, their live weight at birth is 15.0% higher than that of their peers, and their average daily weight gain is 18.6%.

In addition, the table shows that young individuals at the age of 4 months, when they have the best meat qualities of young lamb, had a

live weight of 31.6 kg versus 29.0 kg of weight; fresh carcasses - 12.9 kg versus 11.0 kg; slaughter yield - 43.3% versus 39.4%.

An interesting picture is observed in the weight of the young animals' main internal organs. Thus, the heart mass of young animals with the GH^B gene is 15.5% more than that of their peers, and the liver mass is 12.7%; spleen - by 25.8%.

Table 1. Growth parameters of lambs and their slaughter qualities

Index	Genotype			Allele		The average in the herd
	AA	AB	BB	A	B	
Live weight at birth, kg	5,28	6,25	7,20	5,70	6,70	5,70
Live weight at 4-month age, kg	29,6	28,7	34,5	29,0	31,6	29,8
Average daily gains, g	125,1	160,0	205,0	142,5	175,0	163,5
Slaughter qualities:						
Pre-slaughter mass, kg	27,2	28,5	31,0	27,8	29,8	28,9
Fresh carcasses weight, kg	10,0	11,0	12,9	11,0	12,0	11,6
Slaughter yield, %	36,8	38,6	41,6	39,2	40,2	39,0
Internal fat, g	361,3	317,5	318,0	339,4	317,7	352,0
Heart, g	123,5	130,0	170,0	126,7	150,0	141,2
Lungs, g	334,2	322,5	370,0	328,3	346,2	335,2
Liver, g	468,0	520,1	610,8	494,0	565,5	498,0
Kidneys, g	100,3	108,0	108,0	104,2	108,0	127,8
Spleen, g	44,5	44,0	60,0	44,2	52,0	46,3

Young animals with a homozygous GHBB genotype, which mainly determines the level of distribution of the GH^B allele, stand out especially in the positive direction. These animals have high rates in almost all parameters of meat productivity.

A corresponding advantage has been established in comparison with the herd average.

Based on the analysis of the data obtained, it was concluded that the increase in the sheep meat productivity is due to the genetic influence of the growth hormone allelic gene B, which, due to more developed internal organs, provides an intensive metabolism in the body, and hence an increased sheep live weight level. Based on the foregoing, it is advisa-

ble to use the established dependence in breeding work in the selection of high-value animals, especially for obtaining young lambs, which will be used as ram sires.

Now the assessment of rams by the offspring quality requires significant financial and labor costs, since you need to grow them to the state of ram sire, then get offspring from them, evaluate this offspring by their own productivity (by phenotype), and only then can the level of the breeding value of the ram sire be established. Today, the cost of keeping one sheep is more than UAH 3000 thousand per year. With the proposed method, there is no need for a long (more than two years) process of its keeping. After birth, at the age of 2-3 months, replacement young lambs are certified and their genotype is established by the growth hormone gene, and young animals with genotypes containing the allelic GH^B gene are selected for further breeding use. All the rest will be ranged for quality lamb.

Conclusions. A method has been developed for assessing and predicting the level of the sheep meat productivity development using the growth hormone gene. It was found that, in terms of growth parameters, young animals with the GH^B allelic gene with a high probability prevail over their peers with the alternative GH^A allele. In particular, their live weight at birth is 15.0% higher, and the average daily gain is 18.6%. It was also shown that young individuals at the age of 4 months, when they have the best qualities of mutton, had a live weight of 31.6 kg versus 29.0 kg; fresh carcass weight - 12.9 kg versus 11.0 kg; slaughter yield - 43.3% versus 39.4% among their peers. In addition, the heart weight in young animals with the GH^B gene is 15.5% more than in other sheep, and the liver weight is 12.7%; spleen - by 25.8%. Young animals with a homozygous GHBB genotype, which mainly determines the level of distribution of the GH^B allele, stand out especially in the positive direction. These animals have high rates in almost all parameters of meat productivity.

The use of the proposed method increases the meat productivity level of sheep by 12-15%.

References

1. Akers R.M., 2006. Major advances associated with hormone and growth factor regulation of mammary growth and lactation in dairy cows. *Journal of Dairy Science.* 89(4): 1222-1234. https://doi.org/10.3168/jds.s0022-0302(06)72.

2. Depison N. et al, 2017. Associations of growth hormone polymorphism characteristics of thin-tailed sheep using PCR-RFLP in jamli province. *African J. Biotechnol.* 16. 20. pp. 1159-1167. https://doi.org/10.5897/AJB2016.15783.

3. Ibrahim M., African J., 2016. Polymorphism of growth hormone gene and its association with wool traits in Egyptian sheep breeds. *Biotechnol.* 15. 14. pp. 549-556. DOI: 10.5897/AJB2015.14928.

4. Kumari R., Kumar R., Meena A. et al, 2014. Genetic polymorphism of growth hormone gene in native sheep breeds of India. *Indian Journal of Small Ruminants.* 20(2): 15 – 18.

5. Chizhova, L. M. (2004). Biokhimicheskie test-sistemy, geneticheskie markery pro-duktivnosti, ikh ispol'zovanie v selektsii ovets [Biochemical test systems, genetic markers of productivity, their use in sheep breeding]. *Extended abstract of Doctor's thesis.* Stavropol [in Russian].

Список використаної літератури

1. Akers R.M., 2006. Major advances associated with hormone and growth factor regulation of mammary growth and lactation in dairy cows. *Journal of Dairy Science.* 89(4): 1222-1234. https://doi.org/10.3168/jds.s0022-0302(06)72.

2. Depison N. et al, 2017. Associations of growth hormone polymorphism characteristics of thin-tailed sheep using PCR-RFLP in jamli province. *African J. Biotechnol.* 16. 20. pp. 1159-1167. https://doi.org/10.5897/AJB2016.15783.

3. Ibrahim M., African J., 2016. Polymorphism of growth hormone gene and its association with wool traits in Egyptian sheep breeds. *Biotechnol.* 15. 14. pp. 549-556. DOI: 10.5897/AJB2015.14928.

4. Kumari R., Kumar R., Meena A. et al, 2014. Genetic polymorphism of growth hormone gene in native sheep breeds of India. *Indian Journal of Small Ruminants.* 20(2): 15 – 18.

5. Чижова Л. М. Биохимические тест-системы, генетические маркеры продуктивности, их использование в селекции овец : автреф. дис. … д-ра с.-х. наук : 06.02.01. Ставрополь, 2004. 40 с.