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*Original scientific article*

# PCR-RLFP ON IGFBP-3 GENE AND ITS ASSOCIATION ON GROWTH PERFORMANCE OF LAMBS REARED INTENSIVELY

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IGFBP-3 is responsible for the multiple effects of growth factors in most mammalian species and is considered the major transport factor of growth, used as a marker for different body functions such as growth, metabolism, reproduction, body weight control, immunity, energy balance, and so on. Considered as a candidate gene, used as a marker for the growth and production traits as its essential role in the growth and development of the animals. For the DNA extraction, the blood samples are obtained in the jugular vein using a 10 ml vacutainer containing EDTA as a coagulant in the blood collection of each animal Mis breed of sheep (M), Ile de France (F), and Wurttemberg (W). Isolation of DNA performed using the extraction kit (Quick DNA kit) with primers set the Forward and Reverse. The body weights of lambs from birth to 90 days of age, also been calculated. The results of the agarose gel electrophoresis of PCR amplified IGFBP-3 genes for sheep populations Wurttemberg (W), Mis (M), and Ile de France (F) had 654 bp. In our results showed an absence of polymorphism of the IGFBP-3 gene on the tested sheep populations. The results that there is no polymorphism between the examined sheep breeds, in terms of IGFBP-3 genes, we were interested whether there are differences in the body development of lambs of the mentioned populations because IGFBP-3 is related to the growth of animals. All three breeds have similar weights and growth dynamics, which could link to the growth hormone. Analyzing obtained results, we can suggest that absence of a large difference in the growth of the three breeds of sheep W, M, F does not have to be related to the absence of polymorphism of the IGFBP-3 gene but also other genetic and non-genetic factors can affect this trait. To detect the association between

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genetic polymorphism in IGFBP-3 genes and body development in lambs, DNA sequencing is required, which will be the subject of our future research.

*Key words:* body weight, insulin like growth factor binding protein -3, lambs, polymorphism, sheep breeds

## INTRODUCTION

DNA application analysis can have great practical importance in the management and successful operation of animal farms (ERCEG *et al.,* 1998*;* ZINOVEVA *et al.,* 2015*;* PRIYADI *et al.*,2017*;* PETROVIC *et al.,* 2018*;* CARO PETROVIC *et al.*,2013; 2019; 2021).

A structural gene such as the insulin-like growth factor binding protein-3 (IGFBP-3) gene is associated with the growth and development of the animals (KUMAR *et al.,* 2004*;* ALI *et al.,* 2009) Insulin-like growth factor binding proteins (IGFBPs) belong to a family of at least six homologous proteins that bind insulin-like growth factors (IGFs) and modulate many of their biological actions. IGFBP-3 is responsible for the multiple effects of growth factors in most mammalian species and is considered the major transport factor of growth (ZHAN *et.al.,* 2015). IGFBP-3 have used as a marker for different body functions such as growth, metabolism, reproduction, body weight control, immunity, energy balance, and so on. It is considered as a candidate gene for its use as a marker for growth and production traits considering the essential role in the growth and development of animals (SHARMA *et al.,* 2011*;* OTHMAN *et al.,* 2014*).* The improvement of livestock has generally focused on selective breeding of individuals with superior phenotypes through a simple approach of development of increasingly advanced statistical methods which have maximized selection for genetic gain. However, information now available on the organization and functioning of the genome could be used in breeding programs to improve a range of traits (WILLIAMS, 2005).

Since Wurttemberg breed of sheep used as improver to produce of Mis breed of sheep and Ile de France breed as the terminal sire (PETROVIC, 2007*),* the aim of the study is to find in the PCR RFLP pattern which showed similarities on the three breeds connected with IGFBP-3 gene fragments of each and to find association on their body weight.

## MATERIALS AND METHODS

### *Extraction of DNA*

The Mis breed of sheep (M) kept at the Institute of Animal Husbandry, Ile de France (F) and Wurttemberg (W) maintained by collaborated farmers are used in the study. The blood samples are obtained in the jugular vein using a 10 ml vacutainer containing EDTA as a coagulant in the blood collection of each animal with a total of 45. Isolation of DNA from the blood was perform using the extraction kit (Quick DNA kit) according to the instruction of the manufacturer.

### *The PCR-RFLP of IGFBP3*

Primers likewise are used by other scholars like MACIULLA *et al.* (1997); ALI *et al.* (2009); SHARMA *et al*. (2011) with a set of the Forward: P3: 5`- CCA AGC GTG AGA CAG AAT AC-3 and Reverse: P4: 5`-AGG AGG GAT AGG AGC AAG AT-3 expected to amplify 654 bp of IGFBP3 gene fragment. In preparation for amplification, 20µl of PCR reaction have

prepared by adding 10µl of master mix, 1µl of each primer, 7µl of distilled water, and 1µl of DNA for amplification purposes. The amplification was performed by using the thermal cycler (Qantarus Q Cycler) with the following steps applied; an Initial Denaturation at 94°C for 5 minutes, 35 cycle Denaturation at 94°C, Annealing at 56°C for 1 minute, Elongation at 72°C for 1 minute, and after the end cycle a 5 minutes extension at 72°C.

Expected IGFBP-3 gene fragment checked by PCR products and have analyzed thru agarose gel electrophoresis and have visualized under UV rays. Likewise, the determination of PCR RLFP, the PCR products are having digested with Hae III and incubated for 4 hours at 37°C, 20 minutes at 80°C, 5 minutes at 10°C and hold at 4°C. The digested products have undergone agarose gel electrophoresis for the PCR RFLP fragment of IGFBP3 of every breed.

The statistical processing of the data performed using the software package of the SPSS version 20, where the values of body weight of lambs a total of 300 (100 per genotype) from birth to 90 days of age have calculated, and the effect of genotype on the observed traits have determined by using of the next model:

$$Yij = \mu + Gi + eij$$

Where: Yij = body weight of lambs
µ = the overall mean,
Gi= effect of the ith genotype
eijkl = the residual error.

Significant differences between means within each weight were tested using the significant difference (t 0.05).

## RESULTS AND DISCUSSION

The results of the agarose gel electrophoresis of PCR amplified IGFBP-3 genes for sheep populations Wurttemberg (W), Mis (M), and Ile de France (F) have shown in Figures 1 and 2.

As can be seen, we find that there is no polymorphism between the tested sheep breed in terms of IGFBP-3 genes. Confirmation of our results in the absence of polymorphism of the IGFBP-3 gene, we find in research of other authors. ALI *et al.* (2009) concluded that digestion fragment of IGFBP-3 gene (654 bp) in the four Egyptian sheep breeds with *Hae* III restriction enzyme have yielded single restriction pattern of five fragments of sizes 201, 201, 87, 67, 57 in all the four Egyptian sheep breeds studied that have revealed the absence of polymorphism in those Egyptian sheep breeds. Similar results were reached by SALEH *et al.* (2019). It can be comparable with our results that with RAJASHEKHARA *et al.* (2018) on digestion of 654 bp of IGFBP-3 gene with Hae III restriction enzyme yielded single restriction pattern of four fragments of sizes 201 bp, 201 bp, 87 bp, and 67 bp in all the animals they tested. KUMAR *et al.* (2006) the digestion of 654bp in sheep with Hae III restriction enzyme have yielded single restriction pattern of eight fragments of sizes 201, 201, 87, 67, 56, 19, 16, and 7 bp in all the animals they studied it revealed an absence of polymorphism in sheep. All the fragments obtained by the above scholars have declared the absence of polymorphism which supported the result we have acquired.

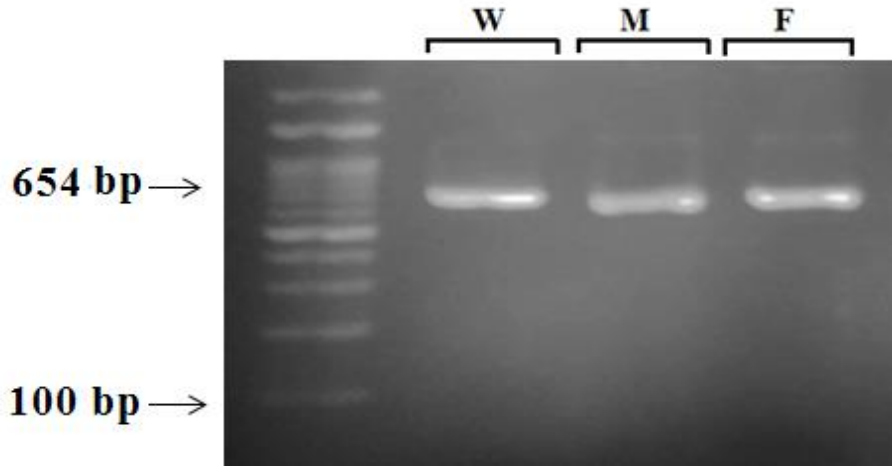


Figure 1. PCR product of IGFBP-3 gene of the three breeds of sheep Wurttemberg, Mis and Ile de France using a 100-bp ladder marker

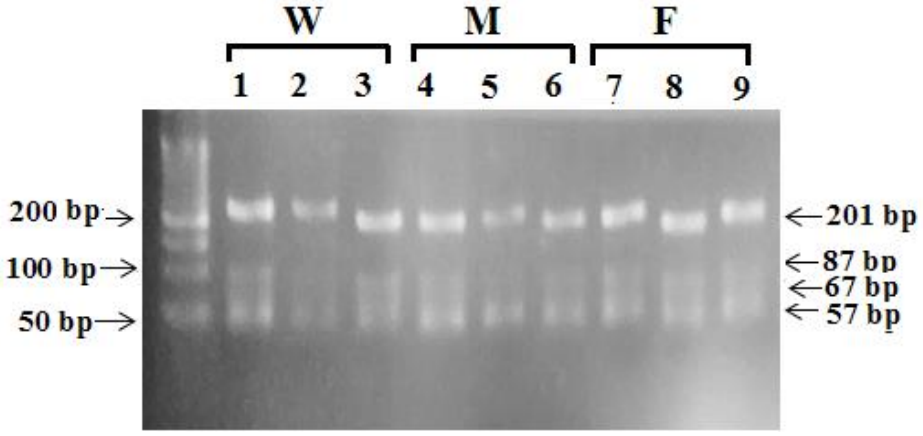


Figure 2. Restricted PCR product using 50-bp ladder marker (Wurttemberg, Mis, Ile de France)

Starting from the results that there is no polymorphism between the examined sheep breeds in terms of IGFBP-3 genes, we were interested in whether there are differences in the

body development of lambs of the mentioned populations because IGFBP-3 is related to the growth of animals. The research results are shown in the following tables.

*Table 1. Body weight of lambs at birth and at 30 days of age*

| Descriptive Statistics | | | | |
|---|---|---|---|---|
| Genotype | Minimum | Maximum | Mean | |
| | Statistic | Statistic | Statistic | Std. Error |
| M1 | 3.00 | 6.00 | 4.1700 | .09750 |
| W1 | 3.00 | 5.50 | 4.0440 | .05509 |
| F1 | 3.10 | 5.60 | 4.2240 | .05053 |
| M30 | 10.00 | 17.00 | 13.0400 | .18198 |
| W30 | 10.00 | 16.00 | 12.5900 | .14432 |
| F30 | 10.00 | 16.00 | 13.4200 | .14155 |

*Table 2. Paired samples significance test of difference in body weight of lambs at birth and 30 days of age*

| Genotype | | Mean | Std. Error Mean | 95% Confidence Interval of the Difference | | t | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|
| | | | | Lower | Upper | | |
| Pair 1 | M1 – W1 | .12600 | .10586 | -.08406 | .33606 | 1.190 | .237 |
| Pair 2 | M1 – F1 | -.05400 | .10936 | -.27100 | .16300 | -.494 | .623 |
| Pair 3 | W1 – F1 | -.18000 | .06931 | -.31753 | -.04247 | -2.597 | .011 |
| Pair 4 | M30 -W30 | .45000 | .09987 | .25183 | .64817 | 4.506 | .000** |
| Pair 5 | M30 - F30 | -.38000 | .22820 | -.83280 | .07280 | -1.665 | .099 |
| Pair 6 | W30 - F30 | -.83000 | .18589 | -1.19885 | -.46115 | -4.465 | .000** |

In Table 1, we can see that lambs of all three breeds have a similar body weight at birth, in the interval of 3.0-6.0 kg, with an average of 4.0-4.2 kg, which means that they theoretically have the same starting position for body development. Small differences in weight were not statistically significant (P> 0). According to recent research (RASOULI *et al*., 2017; SALEH *et al.*, 2019) insulin-like growth factor binding protein-3 genes (IGFBP-3) are involved in regulating body growth from birth to weaning. Let us now look at our results in Tables 2, 3 and 4.

It showed that there were significant differences (Table 2) in body weight of lambs in 30 days between Mis and Wurttemberg as well as between Wurttemberg and Ile de France (P <0.01). At the age of 60 days there is a significant difference in body weight only between lambs of Wurttemberg and Il de France breed (P <0.01), while at the age of 90 days too between Mis and Wurttemberg, as well as between Wurttemberg and Ile de France (P <0.01). Regardless of the existing differences in the body weight shown by statistical analysis, all three breeds have similar weights and similar growth dynamics, which may be related to the growth hormone. We

must be kept in mind that all three breeds are of the fleshy type sheep with intense growth potential. Analyzing obtained results, we can suggest that absence of a large difference in the growth of the three breeds of sheep W, M, F does not have to be related to the absence of polymorphism of the IGFBP-3 gene but also other genetic and non-genetic factors can affect this trait.

*Table 3. Body weight of lambs at 60 and 90 days of age*

| Descriptive Statistics | | | | | | |
|---|---|---|---|---|---|---|
| Genotype | N | Min | Max | Mean | | Std. Deviation |
| | Statistic | Statistic | Statistic | Statistic | Std. Error | Statistic |
| M60 | 100 | 20.00 | 26.00 | 22.4500 | .19300 | 1.92996 |
| W60 | 100 | 18.00 | 26.00 | 22.0200 | .21414 | 2.14137 |
| F60 | 100 | 19.00 | 26.00 | 22.6100 | .21691 | 2.16909 |
| M90 | 100 | 28.00 | 37.00 | 32.2360 | .20033 | 2.00331 |
| W90 | 100 | 28.00 | 36.00 | 31.7360 | .20348 | 2.03482 |
| F90 | 100 | 28.00 | 36.00 | 32.3380 | .21682 | 2.16822 |
| Valid N (listwise) | 100 | | | | | |

*Table 4. Paired samples significance test of difference in body weight of lambs at 60 and 90 days of age*

| Genotype | | Mean | Std. Error Mean | 95% Confidence Interval of the Difference | | t | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|
| | | | | Lower | Upper | | |
| Pair 7 | M60 -W60 | .43000 | .26978 | -.10529 | .96529 | 1.594 | .114 |
| Pair 8 | M60 - F60 | -.16000 | .27879 | -.71317 | .39317 | -.574 | .567 |
| Pair 9 | W60 - F60 | -.59000 | .17815 | -.94348 | -.23652 | -3.312 | .001** |
| Pair10 | M90 - W90 | .50000 | .14320 | .21587 | .78413 | 3.492 | .001** |
| Pair 11 | M90 - F90 | -.10200 | .13729 | -.37441 | .17041 | -.743 | .459 |
| Pair 12 | W90 - F90 | -.60200 | .15808 | -.91567 | -.28833 | -3.808 | .000** |

PETROVIC *et al.* (2011) examined the influence of external factors on body weight variability of some domestic pramenka populations. The authors state that the body weight of lambs depends on the effect of the mother's age. The body of lambs at a later age also depends on the body weight at birth, as well as the type of birth. Lambs born in the spring-summer season have a higher body weight than offspring born in the autumn-winter season. UNAL *et al.* (2006) studied some growth characteristics of lambs and found the genotype did not show a significant effect on the subject properties. However, the said authors state that the sex of the lambs and the birth type showed significant effects in lambs from birth to six months of age.

Results of the present study on the effect of genotype and environmental factors on the birth weight and weaning weights in lambs were similar to other studies. MOMANI *et al.* (2010) stated that genotype of lambs significantly affected average daily gain, birth weight and body weight of lambs at 15, 30, 45 and 60 days.

PETROVIC *et al.* (2015) stated that the genotype and the environmental factors have an important effect on lambs' growth from birth to weaning. Effect of dam age shows that young and old mothers gave birth to lighter lambs, while mature sheep have heavier lambs at birth. It also observed that lambs in both genotypes were heavier at birth if born from heavier ewes. Maternal weight also influenced the weight of lambs at weaning. The birth type and sex had an effect on the body weight of lamb. The effect of year on the birth and weaning weights was statistically significant. Lambing season shows that lambs born in spring-summer had a higher body weight at birth and at weaning.

Research results of CARO PETROVIC *et al.* (2015) showed a highly significant effect of genotype on lambs' body weights at birth, at ages 30, 60, and 90 days. Male lambs showed better growth efficiency compared with females. There were highly significant effects of sex on all ages. Likewise in their result showed that the interaction between genotype x sex showed a very significant effect on birth weight.

## CONCLUSION

Our researches show that there is no polymorphism between the tested sheep breeds in terms of IGFBP-3 genes. There are certain differences in the weight and growth of lambs, which can be due to various genetic and environmental factors. As a conclusion based on our research results, we can point out that polymorphism in IGFBP-3 gene cannot be a sufficient indicator of the connection with the body development of lambs. To more reliably detect the association between genetic polymorphism in IGFBP-3 genes and body development in lambs, DNA sequencing is required, which will be the subject of our future research.

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# PCR-RLFP NA IGFBP-3 GEN I NJEGOVA ASOCIJACIJA NA PERFORMANSE RASTA JAGNJADI KOJA SE INTENZIVNO GAJE

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Izvod

IGFBP-3 je odgovoran za višestruke efekte faktora rasta kod većine vrsta sisara i smatra se glavnim transportnim faktorom rasta. Koristi se kao marker za različite telesne funkcije kao što su rast, metabolizam, reprodukcija, kontrola telesne mase, imunitet, energetski balans i tako dalje. Smatra se genom kandidatom i koristi se kao marker za osobine porasta i proizvodnje kao njegove suštinske uloge u rastu i razvoju životinja. Za ekstrakciju DNK, uzorci krvi se uzimaju u jugularnoj veni korišćenjem vakutajnera od 10 ml koji sadrži EDTA kao koagulans u krvi svake životinje Mis rase ovaca (M), Il de France (F) i Virtemberg (V ). Izolacija DNK je izvedena korišćenjem kompleta za ekstrakciju (Quick DNK kit) sa prajmerima postavljenim napred i nazad. Izračunate su i telesne mase jagnjadi od rođenja do 90 dana uzrastai. Rezultati elektroforeze u agaroznom gelu PCR amplifikovanih gena IGFBP-3 za populacije ovaca Vurttemberg (V), Mis (M) i Ile de France (F) su imali 654 bp. Naši rezultati su pokazali odsustvo polimorfizma gena IGFBP-3 na ispitivanim populacijama ovaca. Obzirom da ne postoji polimorfizam između ispitivanih rasa ovaca, u pogledu gena IGFBP-3, zanimalo nas je da li postoje razlike u razvoju tela jagnjadi navedenih populacija jer je IGFBP-3 povezan sa rastom životinja. Sve tri rase imaju sličnu telesnu masu i dinamiku rasta, što bi moglo biti povezano sa hormonom rasta. Analizirajući dobijene rezultate, možemo sugerisati da odsustvo velike razlike u rastu tri rase ovaca V, M, F ne mora biti povezano sa odsustvom polimorfizma IGFBP-3 gena već i drugih genetski i ne-genetski faktori mogu uticati na ovu osobinu. Da bi se otkrila povezanost između genetskog polimorfizma u genima IGFBP-3 i razvoja tela kod jagnjadi, potrebno je sekvenciranje DNK, što će biti predmet našeg budućeg istraživanja.

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