Atatürk Üniversitesi Vet. Bil. Derg. *Research Article/Araştırma Makalesi*
2018; 13(3): 257-265 *DOI: 10.17094/ataunivbd.349019*

Atatürk Üniversitesi Veteriner Bilimleri Dergisi
http://dergipark.gov.tr/ataunivbd

Effects of Noradrenaline on Smooth Musculature of Small and Large Intestines of Fattening Turkey Under In Vitro Conditions

Muhamed KATICA[1]✉, Indira MUJEZINOVIC[2], Nedzad GRADASCEVIC[3]

1. University of Sarajevo, Veterinary Faculty, Department of Pathophysiology, BOSNİA AND HERZEGOVİNA.
2. University of Sarajevo, Veterinary Faculty, Department of Pharmacology, BOSNİA AND HERZEGOVİNA.
3. University of Sarajevo, Veterinary Faculty, Department of Radiobiology with Radiation Hygiene, Biophysics & Environmental Protection, BOSNİA AND HERZEGOVİNA.

Geliş Tarihi/Received	Kabul Tarihi/Accepted	Yayın Tarihi/Published
03.11.2017	30.03.2018	25.12.2018

Bu makaleye atıfta bulunmak için/To cite this article:
Katica M, Mujezinovic İ, Gradascevic N: Effects of Noradrenaline on Smooth Musculature of Small and Large Intestines of Fattening Turkey Under In Vitro Conditions. *Atatürk Üniversitesi Vet. Bil. Derg.*, 13 (3): 257-265, 2018. DOI: 10.17094/ataunivbd.349019

Absract: The aim of the study was to determine the possible presence of adrenergic receptors in the smooth musculature of small and large intestines of heavy hybrid turkey, using different concentrations of non-selective adrenergic receptor agonists, noradrenaline, under in vitro conditions. The research was carried out on 24 fattening turkeys. Tests of circular and longitudinal layers of muscular duodenum, jejunum, ileum, cecum and colon in the organ isolation apparatus, using different concentrations of noradrenaline hydrogentartarate solution, were tested. The research has established the presence of adrenergic receptors within the smooth musculature of the small and large intestines of fattening turkeys. The effect of the noradrenaline on the longitudinal layer was stronger (P<0.001) than the effect on the circular layer of *tunica muscularis* in small and large intestines of fattening turkeys. There were also statistically significant differences in the results obtained with regard to the use of different concentrations of noradrenaline. Significant differences in the effects of noradrenaline in different parts of the small and large intestines have also been established. Further research into smooth musculature of the small and large intestines of fattening turkeys is needed, with the use of selective agonists and α and β receptor antagonists.

Keywords: Adrenergic receptors, Fattening turkeys, Noradrenaline, Small and large intestines, *Tunica muscularis.*

Noradrenalinin İn Vitro Koşullar Altında Besi Hindilerinde İnce ve Kalın Bağırsak Düz Kasları Üzerine Etkisi

Öz: Bu çalışmanın amacı heavy hybrid hindilerin ince ve kalın bağırsaklarındaki düz kaslarda bulunan adrenerjik reseptörlerin olası varlığını in vitro koşullar altında farklı konsantrasyonlarda non-selektif adrenerjik reseptör agonisti, noradrenalin, kullanarak belirlemektir. Çalışma 24 besi hindisinde gerçekleştirildi. Organ izolasyon cihazında duodenum, jejunum, ileum, çekum ve kolonun müsküler longitudinal ve sirküler tabakalarında testler farklı konsantrasyonlarda noradrenalin hydrogentartarate solüsyonu kullanılarak test edildi. Çalışma besi hindilerinin ince ve kalın bağırsaklarındaki düz kaslarda bulunan adrenerjik reseptörlerin olası varlığını ortaya koymdu. Noradrenalin'in longitudinal tabaka üzerindeki etkisi, besi hindilerinin ince ve kalın bağırsaklarında ki *tunica muscularis* sirküler tabaka üzerinde ki etkisinden daha güçlüydü (P<0.001). Farklı konsantrasyonlarda noradrenalin kullanımına ilişkin olarak elde edilen sonuçlarda da istatistiksel olarak anlamlı farklılıklar vardı. Noradrenalinin ince ve kalın bağırsakların farklı bölgelerindeki etkilerinde de önemli farklılıklar bulundu. Selektif agonistler ve α ve β reseptör antagonistleri kullanılarak, besi hindilerinin ince ve kalın bağırsak düz kaslarında daha fazla araştırma yapılması gerekmektedir.

Anahtar Kelimeler: Adrenerjik reseptör, Besi hindisi, İnce ve kalın bağırsaklar, Noradrenalin, *Tunika muskularis.*

✉Muhamed KATİCA
University of Sarajevo, Veterinary Faculty, Department of Pathophysiology, BOSNİA AND HERZEGOVİNA.
e-mail: muhamed.katica@vfs.unsa.ba

INTRODUCTION

The production of noradrenaline originates in part from the core of the adrenal gland, and is partially released as a neurotransmitter from postganglial adrenergic threads (1-4). Unlike adrenaline, the principal representative of catecholamines, noradrenaline has 5 to 10 times less metabolic action (4,5).

It is a non-selective agonist and acts on the target cells through specific adrenergic receptors located on the CNS cell surface and in almost all peripheral tissues, and thus leads to blood vessel contraction in the body, increased cardiac muscle activity, relaxation of the smooth musculature of the stomach and bowels, dilation of pupils etc. (4,6,7).

In the smooth muscles of rumen of small and large ruminants in terms of distribution of adrenergic receptors, there were contrasting results depending on whether studies were conducted under *in vivo* or *in vitro* conditions. In small ruminants, specifically in sheep, the presence of adrenergic receptors in the smooth muscles of rumen was significantly higher than in comparison to cattle, where studies found receptors only in traces. Especially low levels of adrenergic receptor representation were found in the smooth muscles of dorsal and ventral bag of rumen (7,8).

Many studies have confirmed the presence, role and influence of various physiologically and pharmacologically active substances such as acetylcholine, catecholamines, histamine, serotonin, enkephalin, some prostaglandins and so on, which have the role of neurotransmitters or autacoids in digestive systems of humans and most animals. They greatly affect the motility and secretion in this part of the organism, and therefore participate in the normal maintenance of its function. In some animals this role is more known than it is in others (4,8,9).

Both adrenoreceptor types (α and β) are represented in different proportions in the gastrointestinal system of domestic animals, where they participate in regulation of motorics and secretion (10,11).

Inadequate knowledge of representation and role of these substances in the functioning of digestive tract in domesticated poultry, especially turkeys, has led us to try to illuminate their role in this type of animal. In accordance with the aforementioned, the aim of the study was to determine the possible presence of adrenergic receptors in the smooth musculature of small and large intestines of heavy hybrid turkey, using different concentrations of non-selective adrenergic receptor agonist, noradrenaline, under *in vitro* conditions.

MATERIALS and METHODS

Experimental Animals

The research was carried out on 24 fattening turkeys, heavy hybrid BIG BUT 600 (British United Turkey). All the individuals used in this research came from the same parental flock.

Fattening turkeys were of a body weight between 7 and 10 kg, and of the age of 91 to 125 days. Breeding turkeys were transferred to the local poultry slaughterhouse, where they were druged and had their neck veins transected in accordance with the regulations in force.

After the animals were sacrificed, parts of small and large intestines were taken; i.e. the duodenum, jejunum, ileum, cecum and column strips of 5 cm in size. They were then immersed in a freshly prepared *Krebs solution* and transferred to a laboratory for isolated organs. The tissue strips were taken to the procedure at farthest 20 minutes after the animal was sacrificed. Small and large intestines were cleansed of fatty and connective tissue, followed by a preparation of circular and longitudinal strip (2 cm in length and a width of about 3-4 mm) and placed in an isolated organ bathroom with a volume of 10 ml.

In order for conditions to be as close as possible, 4 strips were used simultaneously. The strips were placed in two 2-chamber baths for isolated organs of a volume of 10 ml, made by „Ugo Basile“, Italy, where

freshly prepared *Krebs solution* was found. Such suspended strips were aerated in *Krebs' solution* with a mixture of oxygen and carbon dioxide (95 % O_2 and 5% CO_2) at a constant temperature of 41 ° C. In 1 hour adaptation time and 2 -gram loading period, the comics were rinsed every fifteen minutes. Movement registration was performed on a single-channel printers of isometric transducers, made by „Ugo Basile“, Italy. Vitality of the strips was verified by adding acetylcholine at a concentration of 10^{-5} M at the beginning and/or the end of the experiment.

After the solution of noradrenaline hydrogentartarate was used, the non-selective agonist was added to the bath of a volume of 10 ml using an insulin syringe to achieve sufficient concentrations (10^{-7}-10^{-4} M). The solution of noradrenaline hydrogentartarate was then washed after one minute. The period between the individual applications lasted for about 20 minutes. For statistical data processing, researching a single substance was repeated at least six times.

Substances and Solutions Used

The following substances were used in the study: *Krebs solution* (mM): NaCl 118.4; KCl 4.7; $CaCl_2$ 2.5; $MgSO_4$ 1.2; $NaHCO_3$ 25; KH_2PO_4 1.2 i glukoza 11.5 (pH 7.3 - 7.4), acetylcholine chloride (a solution that the viability of the strips was tested with), *2-(Acetyloxy)-N,N,N-tri-methylethanaminum chloride* (F. Hoffmann-la Roche & Co. Ltd. Basle Switzerland) and noradrenaline hydrogentartarate (non-selective agonist), (R)-4-(2-Amino-1-hydroxyethyl)-1.2-benzenediol (Sigma-Aldrich, Germany). All the substances used in the experiment were dissolved in distilled water.

Statistical Analysis

The results were processed in the Minitab 14® (12). Two-factor variance analysis (ANOVA Two-way) was used with a post hoc Tukey test to detect the significance of differences between group pairs.

RESULTS

Results of the effects of noradrenaline on smooth musculature of duodenum

By applying the concentration of noradrenaline (10^{-7} -10^{-6} M) to the longitudinal and circular muscular layers of duodenum, no reaction was found, but at higher concentrations of 10^{-5} - 10^{-4} M, both muscular layers were less responsive in terms of relaxation, with longitudinal layer being slightly more sensitive, as can be seen in Figure 1. (A) and (B), as well as in Table 1.

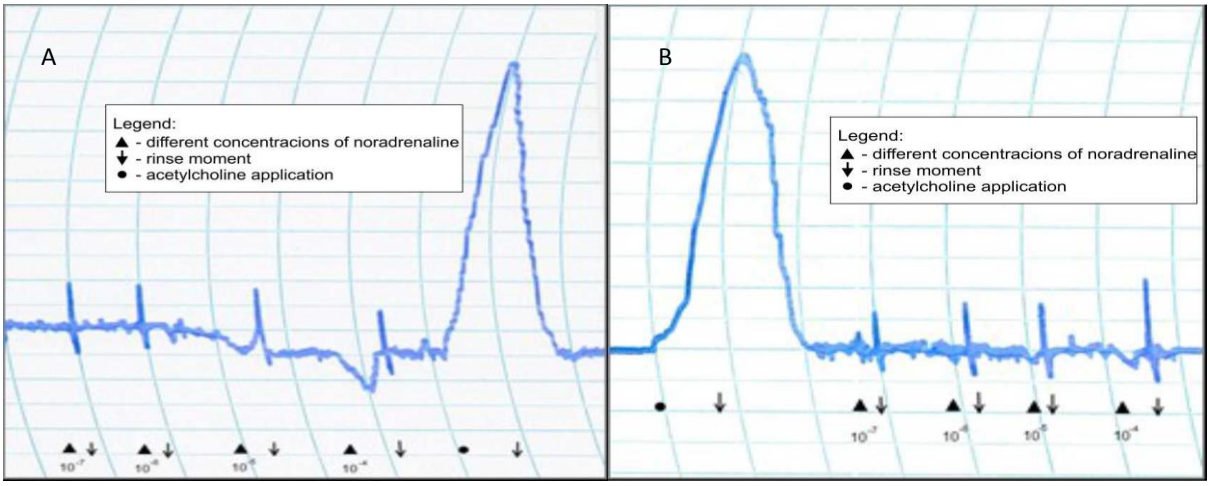

Figure 1. The application of noadrenaline at different concentrations (▲ 10^{-7}- 10^{-4}) to the longitudinal (A) and circular (B) muscular layer of duodenum of a fattening turkey.
Şekil 1. Besi hindi duedonum longitudinal (A) ve sirküler (B) kas tabakasına farklı konsantrasyonlarda noradrenalin (▲ 10-7 - 10-4) uygulaması.

Results of the effect of noradrenaline on the smooth musculature of jejunum.

From Figure 2. (A) and (B), as well as from Table 1., it can be seen that the circular layer of jejunum was less responsive to different concentrations of noradrenaline in comparison to the longitudinal layer. By applying higher concentrations of noradrenaline 10^{-5} - 10^{-4} M, greater relaxation occurred in the longitudinal layer.

.

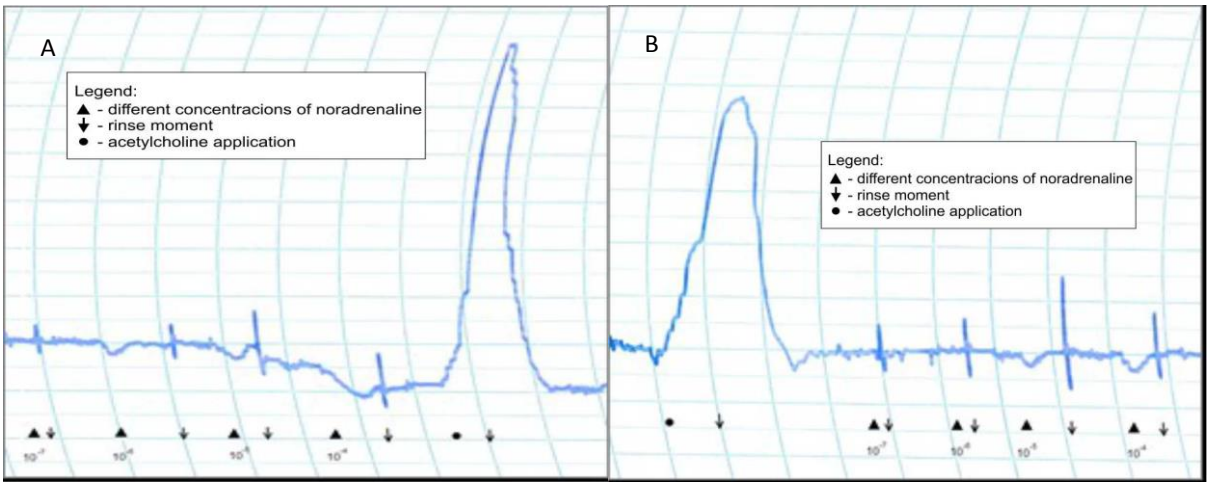

Figure 2. The application of noradrenaline at different concentrations (▲ 10^{-7} - 10^{-4}) to the longitudinal (A) and circular (B) muscular layer of jejunum of a fattening turkey.
Şekil 2. Besi hindi jejenum longitudinal (A) ve sirküler (B) kas tabakasına farklı konsantrasyonlarda noradrenalin (▲ 10-7 - 10-4) uygulaması.

Results of the effects of noradrenaline on the smooth musculature of ileum.

Both muscular layers of ileum react similarly in terms of relaxation at different concentrations of noradrenaline, as well as in other small intestine parts. The circular layer reacted with weaker reactions, while the longitudinal layer at the highest concentration of 10^{-4} M reached the maximum response, as shown in Figure 3. (A), as well as in Table 1.

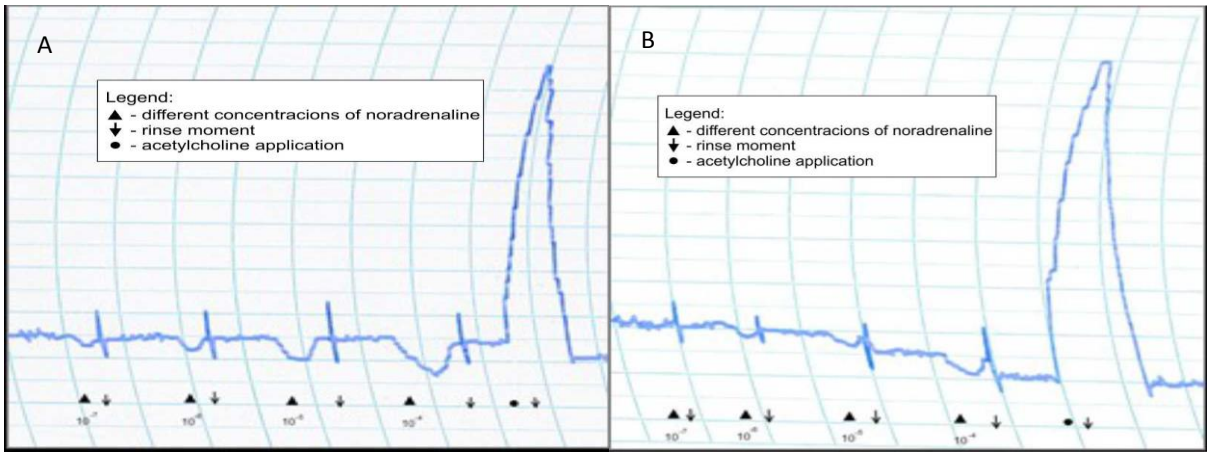

Figure 3. The application of noradrenaline at different concentrations (▲ 10^{-7} - 10^{-4}) to the longitudinal (A) and circular (B) muscular layer of ileum of a fattening turkey.
Şekil 3. Besi hindi ileum longitudinal (A) ve sirküler (B) kas tabakasına farklı konsantrasyonlarda noradrenalin (▲ 10-7 - 10-4) uygulaması.

Results of the effects of noradrenaline on smooth musculature of cecum

By applying different concentrations of noradrenaline to the muscular layer of cecum, Figure 4. (A) and (B), as well as Table 1. show uniform reactions of both muscular layers in terms of relaxation. In the muscular part of cecum, longitudinal layer had more intense responses at higher concentrations of 10^{-5} - 10^{-4} M, unlike the circular layer which did not give a response to the noradrenaline of 10^{-7} - 10^{-6} M.

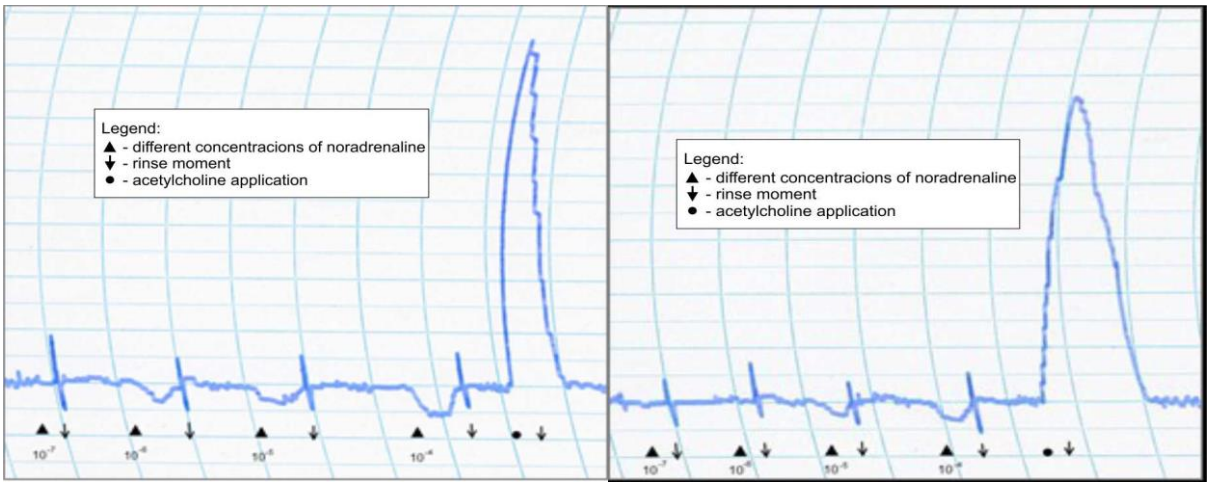

Figure 4. The application of noradrenaline at different concentrations (▲ 10^{-7} - 10^{-4}) to the longitudinal (A) and circular (B) muscular layer of cecum of a fattening turkey.
Şekil 4. Besi hindi çekum longitudinal (A) ve sirküler (B) kas tabakasına farklı konsantrasyonlarda noradrenalin (▲ 10-7 - 10-4) uygulaması.

Results of the effects of noradrenaline on the smooth musculature of colon

In Figure 5. (A) and (B), as well as in Table 1., it can be seen that both muscular layers of colon did not respond to the concentration of noradrenaline 10^{-7} - 10^{-6} M. At higher concentrations of 10^{-5} - 10^{-4} M both the circular and longitudinal layers reacted equally in terms of relaxation.

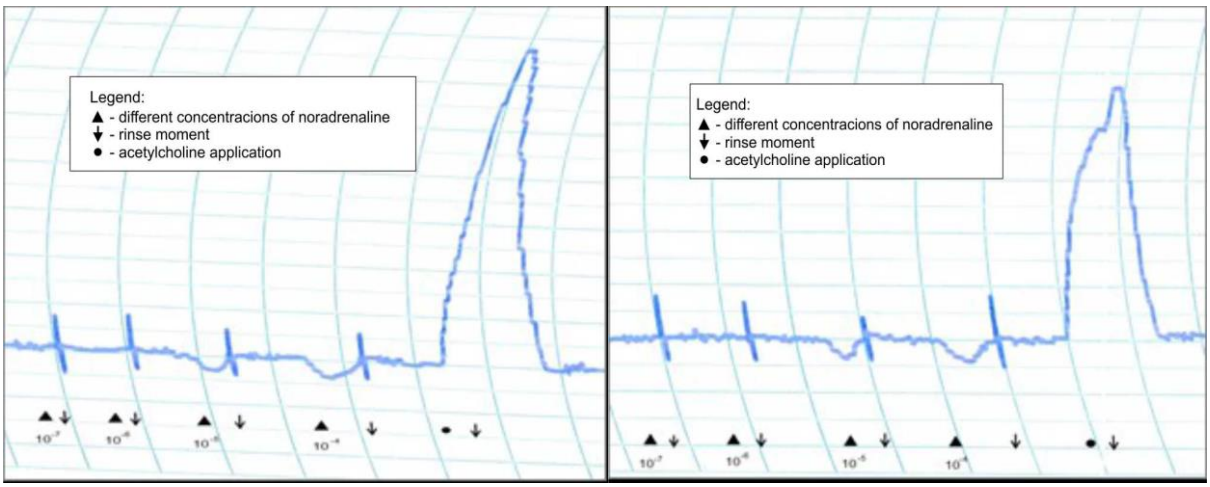

Figure 5. The application of noradrenaline at different concentrations (▲ 10^{-7} - 10^{-4}) to the longitudinal (A) and circular (B) muscular layer of colon of a fattening turkey.
Şekil 5. Besi hindi kolon longitudinal (A) ve sirküler (B) kas tabakasına farklı konsantrasyonlarda noradrenalin (▲ 10-7 - 10-4) uygulaması.

Table 1. Mean values (%) of different concentrations of noradrenaline compared to the maximum response (100%), as well as P≤ of small intestines of fattening turkeys.
Tablo 1. Farklı noradrenalin konsantrasyonlarının ortalama değerlerine (%) göre maksimum yanıtın (%100) ve aynı zamanda besi hindilerinin ince bağırsaklarının P≤'lerinin karşılaştırılması

Conc.	Duodenum				Jejunum				Ileum				Ceacum				Colon				p≤		
	Circ layer	SD	Long layer	SD	Circ layer	SD	Long layer	SD	Circ layer	SD	Long layer	SD	Circ layer	SD	Long layer	SD	Circ layer	SD	Long layer	SD	Int.	musc	Int. × musc
10-7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	22.00	0.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.001	0.001	0.001
10-6	0.00	0.00	0.00	0.00	0.00	0.00	28.00	1.58	20.00	2.37	40.00	0.70	0.00	0.00	33.33	2.58	0.00	0.00	0.00	0.00	0.001	0.001	0.001
10-5	6.66	1.22	66.66	2.09	33.33	1.67	40.00	1.58	43.33	2.75	66.66	3.72	30.00	0.79	40.00	0.70	46.66	2.53	40.00	0.03	0.001	0.001	0.001
10-4	26.66	1.91	76.66	2.54	41.33	1.48	70.00	0.50	60.00	1.76	100.0	0,00	53.33	0.80	66.66	2.34	56.66	1.04	60.00	0.91	0.001	0.001	0.001

SD - Standard deviation; Circ layer- Circular layer; Long layer - Longitudinal layer; Int. - Intestine; musc- T. muscularis.

DISCUSSION and CONCLUSSION

The effects of catecholamines (natural, artificial) or adrenergic drugs in the body can occur due to direct action on adrenergic receptors or indirectly by stimulating the release of catecholamines from adrenergic neurons. Catecholamines and other medicinal substances their effects on digestive tract achieve by acting on adrenergic α and β receptors (13-15).

The primary task of this study was to investigate direct action on adrenoreceptors while the indirect mechanisms of catecholamine action were not considered.

In the original, scanned readouts, clearly established are reactions of both layers of *tunica muscularis*, in terms of relaxation, indicating the apparent presence of adrenergic receptors in small and large intestines of the heavy hybrid turkeys of the BIG BUT 600 origin. The obtained results showed that tested tissue did not react at the noradrenaline concentration level of 10^{-7} M, except in the longitudinal layer of *tunica muscularis* ileum. With increasing noradreanaline concentrations, the effects of relaxation were more pronounced. The concentration of noradrenaline significantly influenced its effect on smooth musculature irrespective of the intestinal part and the muscular layer.

In addition to the above, the analysis of the effect of noradrenaline significantly differs depending on the intestinal part and that its effect is significantly different in different layers of *tunica muscularis*. The recorded interaction between the intestinal part and the muscular layer was also significant.

The effect of noradrenaline on the longitudinal layer was stronger than the effect on the circular layer of *tunica muscularis*, irrespective of the concentration and part of the intestine. The same effect was also present when the parts of the bowels were observed individually, except for the colon where the effect on circular musculature was slightly more pronounced. The effect of noradrenaline significantly differed in different parts of the small intestine, irrespective of the concentration and the muscular layer, and was significantly more pronounced in the musculature of the ileum than in the other parts of the intestine.

The effect of noradrenaline on circular musculature was different depending on the part of the intestine, regardless of the concentration. The weakest effect was found in duodenum, and the strongest in ileum.

The results of the research correspond to similar research, Katica (9) where measured were the effects of other types of synthetic catecholamines (adrenaline) on the smooth musculature of small and large intestines of fattening turkeys. Maximum relaxation occurred in the longitudinal layer of ileum at 10^{-3} M adrenaline concentration, which is fully compatible with the maximum relaxation of this study with the concentration of noradrenaline at 10^{-4} M. Since noradrenaline has several times weaker metabolic activity than adrenaline (4,5), there is a clear difference in the efficacy of adrenaline and noradrenaline concentrations.

The differences between these two studies are manifested in different effects on smooth musculature when using different concentrations of noradrenaline and adrenaline. The effects of adrenaline in some segments of the small intestinal tract (concentration of 10^{-6} M) (9) had the same or greater effect in comparison to the noradrenaline (concentration of 10^{-4} M) in this study.

Likewise, comparing to other similar research, such as Mujezinović (4), a partial correlation was found between the presence of adrenergic receptors in circular and longitudinal layers of *tunica muscularis* in small intestine of heavy hybrid turkeys and broilers. According to Mujezinović (4), different concentrations of noradrenaline (10^{-7} - 10^{-4} M) did not produce any effects in the circular layer of *tunica muscularis* of duodenum, jejunum and ileum of the broilers, indicating the absence of adrenergic receptors in that part of the smooth musculature. In this study, it was established that the concentrations

of noradrenaline (10^{-5} - 10^{-4} M) in the same tissues of fattening turkeys produced effects of less relaxation indicating dose and response immutability (the so-called dose-response link), or presence of adrenergic receptors. By analyzing the results in the longitudinal layer of the small intestine of fattening turkeys and broilers, it was established that the results obtained were fully compatible, just like the maximum response (relaxation), indicating the most sensitive part; longitudinal layer of smooth musculature of ileum, or the place of the highest representation of the examined receptors in both species of tested animals.

The results of the research on the distribution of adrenergic receptors in large intestine (*ceacum*) of fattening turkeys are consistent with similar research, Katica (10).

In conclusion, it could be stated that research established a presence of adrenergic receptors within the smooth musculature of the small and large intestines of fattening turkeys, the provenance (BIG BUT 600). Further, more precise research with the use of selective agonists and α and β receptor antagonists is necessary. More precise information on the presence of α and β receptors in this part of *tubus alimentarius* could ultimately be the basis for potential introduction of new, more effective drugs in the treatment of impaired bowel function in turkeys.

REFERENCES

1. Cupic V., Muminovic M., Kobal S., Velev R., 2014. Textbook of Veterinary Pharmacology. Belgrade, Sarajevo, Ljubljana, Skopje, 2nd edn., p. 291-294, 304-305, National Library of Serbia, Belgrade.
2. Gvozdic D., 2012. Pathophysiology of Endocrinology System. In.: Bozic T., Textbook of Pathophysiology of domestic animals. 2nd edn., p. 394-396, Mladost Biro d.o.o., Belgrade, Serbia.
3. Hodzic A., Hamamdzic M., 2012. Endocrinology of domestic animals. p. 80-97, University textbook, Sarajevo, Bosnia and Herzegovina.
4. Mujezinovic I., 2007. The impact of physiological and pharmacological active substances and their antagonists in the small intestine smooth muscle of broilers. University of Sarajevo, Faculty of Veterinary Medicine, Bosnia and Herzegovina.
5. Guyton AC., Hall JE., 2003. Textbook of Medical Physiology. 10th edn., p. 408-418, Medicinska naklada, Zagreb, Croatia.
6. Bylund DB., Eikenberg DC., Hieble JP., Langer SZ., Lefkowitz RJ., Minneman KP., Molinoff PB., Ruffolo RR., Trendelenburg AU., 1994. IV. International union of pharmacology nomenclature of adrenoceptors. Pharmacol Rev, 46, 121-136.
7. Katica M., Katica A., Mlaco N., 2016. The role of catecholamines in maintenance of homeostasis in digestive tract of domestic animals. Atatürk University Journal of Veterinary Sciences., 11, 106-111.
8. Muminovic M., 1991. The effects of physiological and pharmacological active substances and some of their antagonists in the rumen of cattle in order to improve pharmacotherapy. University of Sarajevo, Faculty of Veterinary Medicine, Bosnia and Herzegovina.
9. Katica M., 2015. Testing the effects of physiologically and pharmacologically active substances and their antagonists in smooth intestine musculature during the turkey fattening phase. University of Sarajevo, Faculty of Veterinary Medicine, Bosnia and Herzegovina.
10. Katica M., 2015. Presence of adrenergic recptors in the wall of caecum during Turkey Fattening Phase. International Congress, One World-One Health-One Vision, Book of Abstracts, p. 113- 115, Sarajevo, Bosnia and Herzegovina.
11. Bylund DB., 2005. Alpha-2 adrenoceptor subtypes: are more better? British J Pharmacol, 144, 159-160.
12. Minitab Inc., 2003. Minitab Statistical Software. Release 14 for Windows, State College, Pennsylvania.
13. MA PM., 1997. Catecholaminergic systems in the zebrafish. III Organization and projection pattern

of medullary dopaminergic and noradrenergic neurons. J Comp Neurol, 381, 411-427.

14. Manara L., Croci T., Aureggi G., Guagnini F., MaVrand JP., Le Fur G., Mukenge S., Ferla G., 2000. Functional assessment of adrenoceptor subtypes in huma colonic circular and longitudinal (taenia coli) smooth muscle. Gut, 47, 337-342.
15. De Ponti F., Giaroni C., Cosentino M., Lesshini S., Frigo G., 1996. Adrenergic mechanism in the control of gastrointestinal motility: From basic science to clinical application. Pharmacol Therapeutics, 69, 1, 59-78.