EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 2
142-148
Year: 2018

PRESENCE OF SEROTONERGIC 5HT$_4$-TYPE RECEPTORS IN BROILER'S SMALL INTESTINE

[1]Mujezinović Indira, [1]Smajlović Ahmed, [2]*Katica Muhamed, [3]Dukić Behija and [4]Gradaščević Nedžad

[1]Indira Mujezinović, Associate Professor, Department of Pharmacology and Toxicology, Veterinary Faculty University of Sarajevo, Bosnia and Herzegovina.
[1]Smajlović Ahmed, Assistant Professor, Department of Pharmacology and Toxicology, Veterinary Faculty University of Sarajevo, Bosnia and Herzegovina.
[2]*Katica Muhamed, Assistant Professor, Department of Pathophysiology, Veterinary Faculty, University of Sarajevo, Bosnia and Herzegovina.
[3]Dukić Behija, Associate Professor, Department of Microbiology, Immunology and Infectious Diseases, Veterinary Faculty, University of Sarajevo, Bosnia and Herzegovina.
[4]Gradaščević Nedžad, Associate Professor, Department of Radiobiology with radiation Hygiene, Biophysics and Environmental Protection, Veterinary Faculty, University of Sarajevo, Bosnia and Herzegovina.

***Corresponding Author: Katica Muhamed**
Katica Muhamed, Assistant Professor, Department of Pathophysiology, Veterinary Faculty, University of Sarajevo, Bosnia and Herzegovina.

Article Received on 13/12/2017 **Article Revised on 03/01/2018** **Article Accepted on 23/01/2018**

SUMMARY
The aim of the present study was to try to determine the possible distribution of 5-HT$_4$ receptors in small intestine of broilers (duodenum, jejunum and ileum). The disturbances of bowel function are common in broilers and if we know that mentioned dysfunction cause great loss to the poultry industry, due to the high mortality, the results of our research could form the basis for the introduction of new drugs in the pharmacotherapy of this species. Investigation were carried out on isolated smooth muscle of the circular and longitudinal layer of the broilers small intestine (strip dimnesion 3-4 mm x 2 cm). The muscle strips were placed in an isolated organ bath. The mechanical activity of the preparations were recorded via an isotonic force transducer coupled to a pen recorder. This was done following the addition of serotinin (nonselective 5-HT agonist), 2-Me-5HT (5-HT3 agonist) and Y-25130-hydrochloride (selective 5-HT3 antagonist). The sensitivity of the tissues to acetylcholine was tested before starting the experiments. The research established a presence of serotonergic 5HT3 type receptors within the smooth musculature of the small intestines of broilers (COBB 500). The 5HT3 type receptors were present in smooth muscles of duodenum, jejunum and ileum, especially in longitudinal smooth muscles since this layer reacted even to low serotonin concentration (10^{-6}). In the light of these findings, we suggest that investigated substances may have considerable physiological and therapeutic implications in disturbed function of small intestine of broiler's.

KEYWORDS: Broilers, serotonin, serotonergic receptors, isolated smooth muscle, small intestine.

INTRODUCTION

Serotonin is a monoamine neurotransmitter called 5 hydroxytryptamine (5-HT). It was first discovered by Vittorio Erspamer in Rome in 1935. Serotonin was first isolated and named by Maurice M. Rapport, Arda Green and Irvine Page in 1948. The hormone was chemically identified as 5-hydroxytryptamine later by Rapport and became known more broadly as 5HT.[1] Serotonin plays an important role in the regulation of mood, sleep, appetite, vomiting, sexuality, memory and learning, temperature regulation, cardiovascular function and endocrine regulation.[2,3]

Serotonin can be found in the gastrointestinal tract (about 90 percent) and in the bloodstream. In the body, serotonin can be synthesized from the amino acid tryptophan by a short metabolic pathway that consists of two enzymes: tryptophan hydroxylase and amino acid decarboxylase. The first enzyme, tryptophan hydroxylase, has two forms, one that is present in several tissues and the other one in a brain-specific isoform.[4] However, the predominant site of serotonin synthesis, storage and release is the enterochromaffin cells (EC) of the intestinal mucosa with maximal numbers in the duodenum and rectum.[1,4] Within the intestinal mucosa, serotonin released from EC cells activates neural reflexes associated with intestinal secretion, motility and sensation.[1,3,5] Serotonin activates intrinsic and extrinsic primary afferent neurons to, respectively, initiate peristaltic and secretory reflexes and to transmit

information to the central nervous system. Serotonin is also a neurotransmitter utilized by a system of long descending myenteric interneurons.[6]

As mentioned before, serotonin mediates several important physiological functions including neurotransmission, gastrointestinal motility, hemostasis and cardiovascular integrity.[2,3] The function of serotonin is exerted upon its interaction with specific receptors. These receptors are known as the 5-hydroxytryptomine receptors, or as they will be referred to here on out as 5-HT receptors.[7] Several serotonin receptors have been cloned, such as $5HT_1$, $5HT_2$, $5HT_3$, $5HT_4$, $5HT_5$, $5HT_6$ and $5HT_7$.[1,2,3,5,8,9] At least 20 subtypes of 5-HT receptors have been cloned yet.[10,11] These receptors are localized in the brain and in peripheral organs but their distribution is not homogeneous. The majority of 5-HT receptors are postsynaptic, with some exceptions, most notably 5-HT_{1A} and 5-HT_{1B} that are mainly presynaptic and modulate serotonin release.[5,8] Within each group there are subtypes that affect various aspects of body functions. Multiple receptor families explain the broad physiological actions and distribution of this biochemical mediator.[9] Depending on the receptor bound and its localization, serotonin evokes different and, sometimes, opposite responses.[2]

These receptor sites are actually targets for number of medications when treating different gastrointestinal diseases (diarrhea, nausea etc.). Once the serotonin is released, it increase intestinal motility. Research has shown that there is an increase in plasma 5-HT during diarrheal diseases and its decrease when constipation is present.[7,12] $5HT_4$ receptors are located on the enteric nervous system within the GI tract of humans and a number of animal species. Activation of these receptors with agonists evokes the release of excitatory and inhibitory neurotransmitters, with the net result of increasing motility and orthograde peristalsis in the gastrointestinal tract.[13]

The 5-HT_4 receptors are located in the neural circuitry of the intestines. The 5-HT_4 receptor agonists are used as promotility agents to promote gastric emptying and to alleviate constipation. Because of the importance of serotonin in normal gut function and sensation, a number of studies have investigated potential changes in mucosal serotonin signaling in pathologic conditions.[12] The disturbances of bowel function are common in broilers and if we know that mentioned dysfunction cause great loss to the poultry industry, due to the high mortality, the results of our research could form the basis for the introduction of new drugs in the pharmacotherapy of this species. Thus, the aim of the present study was to try to determine the possible distribution of 5-HT_3 receptors in small intestine of broilers (duodenum, jejunum and ileum).

MATERIAL AND METHODS

The research was carried out on 20 broiler chickens (Cobb 500). Broilers had body weight between 2.1 and 2.3 kg and age of up to 42 days. Broilers were obtained and transferred to the local private poultry slaughterhouse, where they were slaughtered in accordance with the regulations.

After the animals were sacrificed, parts of small intestine were taken; i.e. the duodenum, jejunum, ileum strips of 5 cm in size. They were then immersed in a cold, freshly prepared Kreb's bicarbonate solution and transferred to a laboratory for isolated organs. The tissue strips were taken to the procedure 20 minutes after the animal was sacrificed. Small intestines were cleansed of fatty and connective tissues, followed by a preparation of circular and longitudinal strips (2 cm in length and a width of about 3-4 mm) and placed in an isolated organ bath 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 Kreb's bicarbonate solution with a mixture of oxygen and carbon dioxide (95% O_2 and 5% CO_2) at a constant temperature of 41°C. Tissues were suspended under a resting tension of 2 g and were allowed to equilibrate for 45-60 minutes and 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 serotonin was used, the non-selective agonist was added to the bath using an insulin syringe to achieve sufficient concentrations (10^{-7}- 10^{-4} M). The solution of serotonin was then washed after one minute. The period between the individual applications lasted for about 20 minutes. For statistical data processing, application of a single substance was repeated at least six times.

Substances and solutions used

The following substances were used in the study: Kreb's bicarbonate solution (mM): NaCl 118.4; KCl 4.7; CaCl2 2.5; $MgSO_4$ 1.2; $NaHCO_3$ 25; KH_2PO_4 1.2 and glucose 11.5 (pH 7.3 - 7.4); Serotonin (3-(2-Aminoethyl)-1H-indol-5-ol) (Sigma-Aldrich, Germany); Cisapride (4-amino-5-chloro-n-(1-(3-(4-fluorophenoxy)propyl)-3-methoxy-4-piperidinyl)-2-methoxy-, cis-) (5-HT_4 agonist); SB 203186 (1-Piperidinylethyl-1H-indole-3-carboxylate hydrochloride) (5-HT_4 antagonist) (Tocris Cookson Ltd., Bristol, UK); 2-(Acetyloxy)-N,N,N-tri-methylethanaminum chloride (a solution that the viability of the strips was tested with) (F. Hoffmann-la Roche & Co. Ltd. Basle Switzerland). All the substances used in the experiment were dissolved in distilled water.

Statistical Analysis

Basic statistical data diagnostics was conducted by using Microsoft Excel® (Microsoft Office package, Microsoft, USA).

RESULTS

Results are expressed as percentages of the maximum response (expressed as 100%) produced by acetilcholyne on circular and longitudinal layer of smooth muscle of broiler's duodenum with concentration of 10^{-5} M.

Serotonin (at concentration of 10^{-7} to 10^{-3} M) and its agonist cisapride (at concentracion of 10^{-7} to 10^{-4} M) induced dose-dependent contraction in isolated circular and longitudinal layer of smooth muscle of duodenum, jejunum and ileum of broilers. The circular layer of smooth muscle of small intestine (duodenum, jejunum and ileum) showed lower sensitivity to serotonine and its agonist cisapride in equal concentrations as in longitudinal layer (Fig. 1., 2. and 3).

Results of the effects of serotonin and its antagonist cisapride on smooth muscles of duodenum

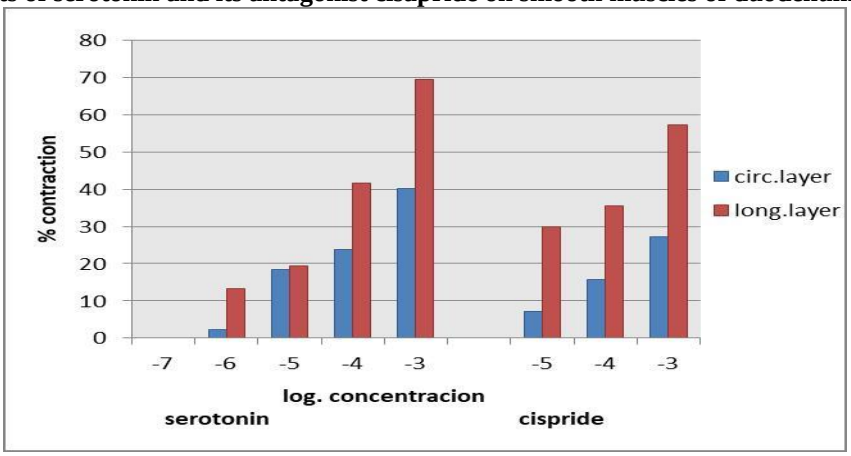

Figure 1: Effect of various concentration s of 5-HT and cisapride, on circular and longitudinal layer of smooth muscles of broiler's duodenum. Contractions were expressed as percentage of muscle strip preparation to acetylcholine (10^{-5} M).

Results of the effects of serotonin and its antagonist cisapride on smooth muscles of jejunum

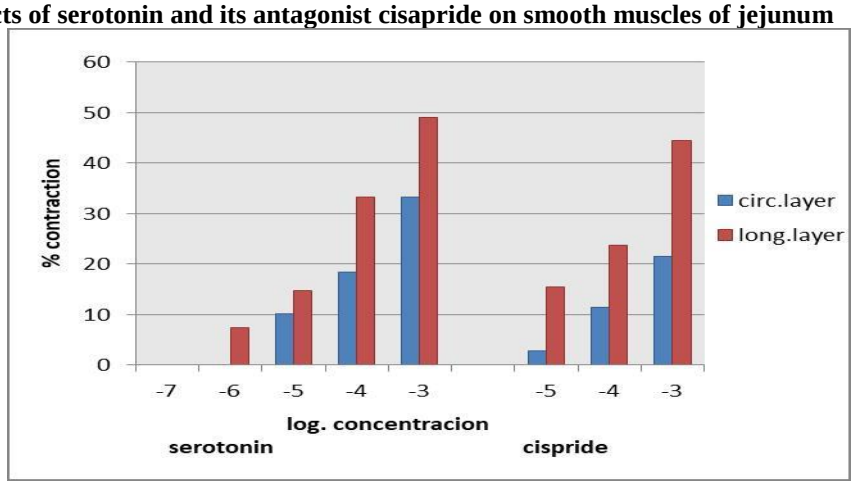

Figure 2: Effect of various concentration s of 5-HT and cisapride, on circular and longitudinal layer of smooth muscles of broiler's jejunum. Contractions were expressed as percentage of muscle strip preparation to acetylcholine (10^{-5} M).

Results of the effects of serotonin and its antagonist cisapride on smooth muscles of ileum

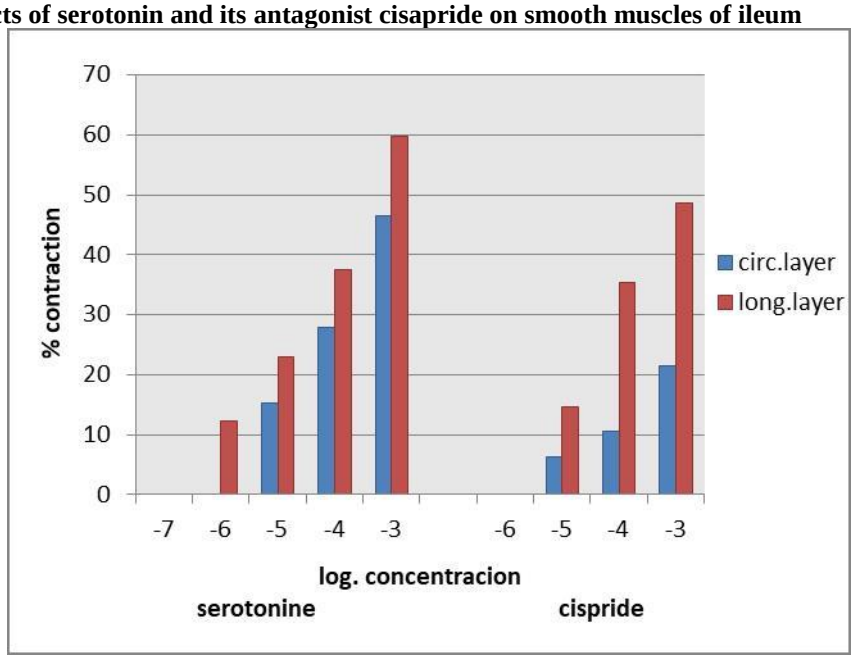

Figure 3: Effect of various concentration s of 5-HT and cisapride, on circular and longitudinal layer of smooth muscles of broiler's ileum. Contractions were expressed as percentage of muscle strip preparation to acetylcholine (10^{-5} M).

In experiment designed to investigate effects of antagonists of 5-HT receptors, SB 203186 (5-HT_4 antagonist) was added at concentration 10^{-6} M and 10^{-5} M to antagonized cisapride induced contraction at concentration 10^{-3} M (Fig. 4., 5. and 6.).

Results of the effects of SB 203186 (5-HT_4 antagonist) on smooth muscles of ileum

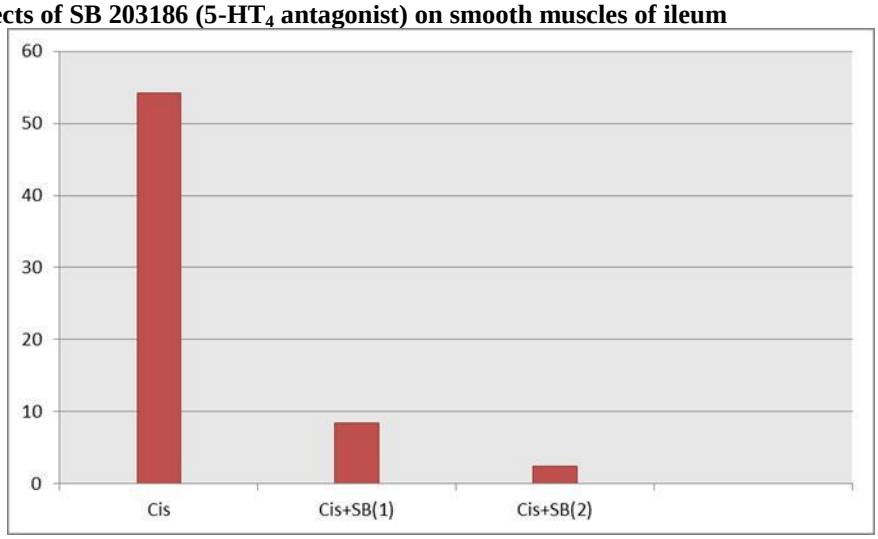

Figure 4: Contractile effect of cisapride (Cis) on the smooth muscles of duodenum alone and in the presence of SB 203186 (5-HT_4 antagonist) (SB) at concentration 10^{-6} M (1) and 10^{-5} M (2).

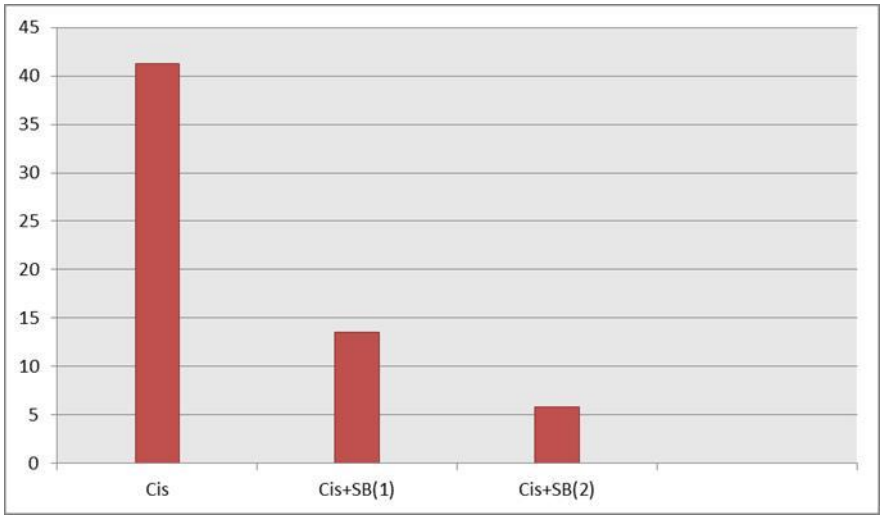

Figure 5: Contractile effect of cisapride (Cis) on the smooth muscles of jejunum alone and in the presence of SB 203186 (5-HT$_4$ antagonist) (SB) at concentration 10^{-6} M (1) and 10^{-5} M (2).

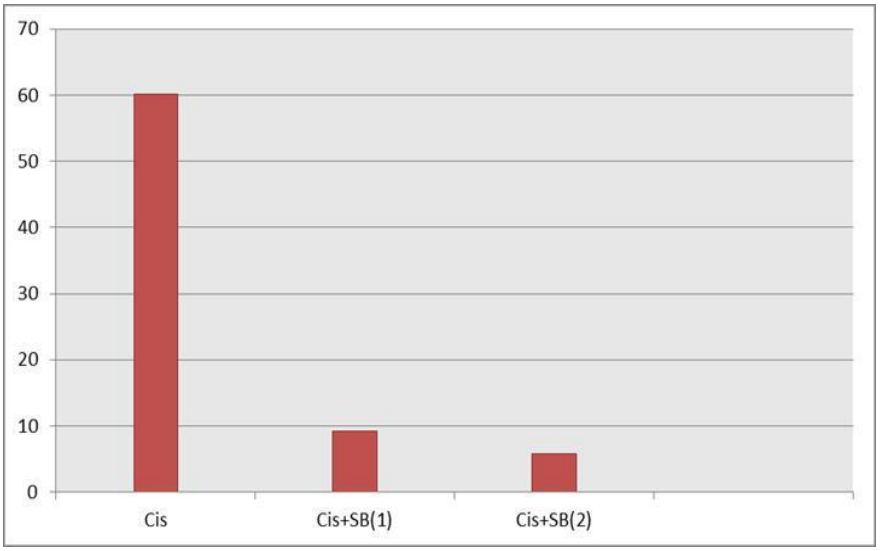

Figure 6: Contractile effect of cisapride (Cis) on the smooth muscles of ileum alone and in the presence of SB 203186 (5-HT$_4$ antagonist) (SB) at concentration 10^{-6} M (1) and 10^{-5} M (2).

DISCUSSION AND CONCLUSION

The 5-HT receptors are abundantly distributed along the gastrointestinal tract, where they may play a role in modulating smooth muscle tone, peristaltic reflex and mucosal secretion.[14,15] The 5-HT$_4$ receptor appears to mediate both inhibition and activation of smooth muscle involving myogenic as well as neural actions.[16] Despite many similarities in its general functionality, the intestinal tract exhibits considerable differences among species. Serotonin receptors for instance are widespread throughout the gastrointestinal tract in small animal models and humans but the proportion and type of receptors at particular regions are different.[16] The function and pharmacological responses of 5-HT receptors in the intestine also varies between small animal models and humans.[16,17]

Many authors tried to identify the 5-HT$_4$ receptors in the guinea pig proximal colon[16,17], human, rat and guinea pig intestine[15], bovine rumen[18], in the canine colon longitudinal[19] and circular muscle[20], in human isolated colon circular muscle[21], in the rat isolated ileum[22] and smooth intestine musculature during the turkey fattening phase.[23]

This study was undertaken to obtain an insight to the distribution of 5-HT$_4$ receptors expressed in the circular and longitudinal layer of the smooth muscle of broiler's small intestine (duodenum, jejunum and ileum). This is the first report to describe the characterization of functional broiler's 5-HT$_4$ receptors *in vitro*.

Serotonin was used at concentrations from 10^{-7} to 10^{-3} M. From the results obtained, it can be concluded that serotonergic receptors are highly present in smooth muscles of longitudinal layer of broiler's small intestine. With the increase in concentration, obtained responses were in the form of stronger (higher) intensity contractions, with lower respond noted in circular layer (Fig. 1, 2 and 3.).

In the next part of our experiment, the presence of $5HT_4$ type receptors in intestinal smooth muscle of broilers was investigate. In the clinical practice, 5-HT_4 receptor agonists like cisapride are used to relieve patients suffering from gastro-oesophageal reflux diseases, dyspepsia or gastroparesis[24] 5-HT_4 receptors are involved in the regulation of cisapride stimulated orocaecal transit in human.[25] We used cisapride at concentrations from 10^{-7} to 10^{-3} M. Cisapride has affinity for a number of 5-HT receptors, such as 5-HT_{2A} and 5-HT_3 receptors[26] but is an agonist only in 5-HT_4 receptors.[24] In this *in vitro* study cisapride evoked dose-dependent contractions on circular and longitudinal layer of smooth muscles of broiler's duodenum, jejunum and ileum (Fig. 1., 2. and 3.). From the results obtained, it can be concluded that 5-HT_4 receptors are highly present in smooth muscles of longitudinal layer of broiler's small intestine than in circular layer.

Some of 5-HT_4 receptor antagonists might be useful in the treatment of irritable bowel syndrome in human medical practice[27] and 5-HT_4 receptor antagonists inhibited normal colonic motor activity in dogs.[28]

The cisapride effects (at concentration 10^{-3} M) on longitudinal layer of duodenum, jejunum and ileum was blocked by SB 203186 (5-HT_4 antagonist) at concentrations of 10^{-6} and 10^{-5} M. The inhibitory effects of 5-HT_4 antagonist was dose dependent (Fig. 4., 5. and 6).

Our data clearly indicate that under the applied conditions, 5-HT_4 receptors mediate the contractions of the broiler's small intestine (duodenum, jejunum and ileum), especially in longitudinal smooth muscles since this layer reacted even to the low serotonin concentration (10^{-6}). In accordance with our findings, we suggest that investigated substances may have therapeutic utility in the treatment of peripheral disorders such as disturbed function (motility, secretion etc.) of broiler's small intestine.

REFERENCES

1. Riviere, J.E., Papich, M.G. Veterinary Pharmacology and Therapeutics. 9th edition. Wiley-Blackwell: 2009.
2. Cirillo C., Berghe, V.P., Tack J. Role of serotonin in gastrointestinal physiology and pathology. Minerva Endocrinologica, 2011; 36(4): 311-24.
3. Kato S. Role of serotonin 5-HT4 receptors in intestinal inflammation. Biol Pharm Bull, 2013; 36(9): 1406-9.
4. Spiller R. Serotonin and GI clinical disorders. Neuropharmacology, 2008; 55(6): 1072-1080.
5. Rang H.P., Dale M.M., Ritter J.M. Pharmacology. 7th edition. Churchhill Livingstone: 2011.
6. Gershon M.D., Tack J. The Serotonin Signaling System: From Basic Understanding To Drug Development for Functional GI Disorders. Gastroenterology, 2007; 132(1): 397-414.
7. Camilleri M. Serotonin in the Gastrointestinal Tract. Curr Opin Endocrinol Diabetes Obes, 2009; 16(1): 53–59.
8. Darmon M., Awabdh S., Emerit M.B., Masson J. Insights into Serotonin Receptor Trafficking: Cell Membrane Targeting and Internalization. Progress in Molecular Biology and Translational Science, 2015; 132: 97-126.
9. Kaufman D.M., Milstein M.J. Neurotransmitters and Drug Abuse. In Kaufman's Clinical Neurology for Psychiatrists. 7th edition, Elsevier Saunders: 2013.
10. Hannon J., Hoyer D. Serotonin receptors and systems: endless diversity. Acta Biolog Szeged, 2002; 46: 1-12.
11. Berger M., Gray J.A., Roth B.L. The expanded biology of serotonin. Annu Rev Med, 2009; 60: 355-366.
12. Costedio M.M., Hyman N., Mawe G.M. Serotonin and its role in colonic function and in gastrointestinal disorders. Dis Colon Rectum, 2007; 50(3): 376-88.
13. Gale J.D., Mori I. Comprehensive Medicinal Chemistry II. Elsevier Ltd., 2007; 671–69.
14. Hegde S.S., Eglen R.M. Peripheral 5-HT4 receptors. FASEB., 1996; 10: 1398–1407.
15. Grider J.R., Foxx-Orenstein A.E., Jin J.G. 5-Hydroxytryptamine 4 receptor agonists initiate the peristaltic reflex in human, rat and guinea-pig intestine. Gastroenterology, 1998; 115: 370–380.
16. Wouters M.M., Farrugia G., Schemann M. 5-HT receptors on interstitial cells of Cajal, smooth muscle and enteric nerves. Neurogastroenterol Motil, 2007; 2 suppl.: 5-12.
17. Coupar I.M., Irving H.R., Manallack D.T., Tan Y. Y., Ayad F., Di Iulio J. L., Tochon-Danguy N., Iskander M.N. Assessment of the pharmacological properties of 5-methoxyindole derivatives at 5-HT4 receptors. J Pharm Pharmacol, 2012; 64: 1099–1106.
18. Muminović M., Hadžović S., Abdagić I., Smajlović A. The Effect of Serotonin on the isolated smooth muscles of the bovine rumen. Veterinaria, 2000; 49: 65-77.
19. Prins N.H., Briejer M.R., Schuurkes J.A.J. Characterization of the contraction to 5-HT in the canine colon longitudinal muscle. Br. J. Pharmacol, 1997; 120: 714–720.
20. Prins N.H., Briejer M.R., Schuurkes J.A.J., Akkermans L.M.A. Contractile effects of carbachol and 5-HT in the canine colon circular muscle. Naunyn-Schmiedebergs' Arch. Pharmacol, 1998a; 358 suppl.: 40.21.
21. Prins N.H., Briejer M.R., Schuurkes J.A.J., Akkermans L.M.A. Optimisation of a 5-HT4 receptor bioassay in human isolated colon circular muscle. Neurogastroenterol. Motility, 1998b; 10: 478.
22. Tuladhar B.R., Costall B., Naylor R.J. Pharmacological characterization of the 5-hydroxytryptamine receptor mediating relaxation in

the rat isolated ileum. Br. J. Pharmacol, 1996; 119: 303–310.
23. Katica M. Testing the effects of physiologically and pharmacologically active substances and their antagonists in smooth intestine musculature during the turkey fattening phase. University of Sarajevo, Veterinary Faculty, Bosnia and Herzegovina: 2015.
24. Briejer M.R., Akkermans L.M.A., Meulemans A.L., Lefebvre R.A., Schuurkes J.A.J. Cisapride and a structural analogue, R 76,186, are 5-hydroxytryptamine4 (5-HT4) receptor agonists on the guinea-pig colon ascendens. Naunyn-Schmiedebergs' Arch. Pharmacol, 1993; 347: 464–470.
25. Bharucha A.M., Camilleri S., Haydock I., Ferber D., Burton S., Cooper D., Tompson K., Fitzpatrick R., Zinsmeister A. Effects of a serotonin 5-HT4 receptor antagonist SB-207266 on gastrointestinal motor and sensory function in humans. Gut, 2000; 47(5): 667–674.
26. Gommeren W., Renders J., van Gompel., Lesage A., Leysen J., Jurzak M. Extensive pharmacological study of the G protein-coupled fraction of human 5-HT receptors using agonist radioligand binding. Naunyn-Schmiedebergs' Arch. Pharmacol, 1998; 358: P8.42.
27. Sanger G.J. 5-Hydroxytryptamine and functional bowel disorders. Neurogastroenterol. Motility, 1996; 8: 319–331.
28. Morita H., Mochiki E., Takahashi N., Kawamura K., Watanabe A., Sutou T., Ogawa A., Yanai M., Ogata K., Fujii T., Ohno T., Tsutsumi S., Asao T., Kuwano H. Effects of 5-HT2B, 5-HT3 and 5-HT4 receptor antagonists on gastrointestinal motor activity in dogs. World J Gastroenterol, 2013; 19(39): 6604–6612.