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The effects of different plant extracts on bile salt hydrolase activity of *Lactobacillus* strains isolated from the gastrointestinal tract of poultry

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ABSTRACT

The bile salt hydrolysis (BSH) enzyme weakens fat metabolism through bile salt deconjugation and reduces poultry performance, in order to cope with the antibacterial properties of the bile. Therefore, reducing the activity of this enzyme through the use of feed additives is probably a promising alternative to antibiotics for improving poultry performance. Plant extracts have long been used as feed additives for promoting poultry growth. In the current experiment, five *Lactobacillus* strains including *Lactobacillus animalis, Lactobacillus acidophillus, Lactobacillus gallinarum, Lactobacillus lactis,* and *Lactobacillus returi* were obtained from the poultry hindgut and were used as the probiotic application. A plate test and two-step enzymatic reaction method were used for deconjugation activity determination of the *Lactobacillus* strains. Further, four plant extracts (i.e., the aerial parts of Rosemary (*Rosmarinus officinalis*), Roselle calyx (*Hibiscus sabdariffa*), *Berberis vulgaris* root*,* and Green tea) were examined in terms of BSH enzyme inhibitors using the cell-free extracts as the potential antibiotic alternative. Furthermore, the gallbladders of the broilers were freshly collected from the poultry slaughterhouses, and their contents were extracted. The results showed that all *Lactobacillus* strains could hydrolyze the taurocholate acid (TCA) and chicken bile salt mixture (CBSM) to unconjugated bile acid. Moreover, ethanolic extracts of *B. vulgaris* root and Green tea relatively reduced the activity of the BSH enzyme that could potentially be investigated as an appropriate alternative in poultry feed *in vivo*. In conclusion, all five *Lactobacillus* strains were resistant to bile salts (i.e. TCA and CBSM) by BSH activity, and the addition of Green tea and *B. vulgaris* root extracts to the bacterial medium demonstrated inhibitory effects against the BSH enzyme.

Key words: lactobacilli; bile salt hydrolase; plant extracts; antibiotic growth promoters

Introduction

For decades, antibiotic growth promoters (AGP) have been supplemented in poultry feed to improve the growth rate and feed conversion efficiency. Although the exact impacts of these supplementations are not completely recognized, various ideas exist to clarify the principle of antibiotic-mediated growth improvement. The initial concept of AGP is associated with their antibacterial mode which decreases the total number of gut microbiota (FRANCOIS, 1961; VISEK,

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1978), leading to reduced competition for nutrients and microbial substances such as bile catabolism, which improves growth rate (FEIGHNER and DASHKEVICZ, 1987; GASKINS et al., 2002; KNARREBORG et al., 2004).

However, there is concern regarding the development of antibiotic-resistant strains of bacteria that could be considered as a potential hazard to humans and animals (MARON et al., 2013). The World Health Organization strongly supports restraint in AGP use and the European Union banned them entirely in 2006 (MARSHALL and LEVY, 2011). Therefore, researchers are interested in finding alternatives to AGP with a similar impact for poultry production (REID and FRIENDSHIP, 2002; COX and PAVIC, 2010). Although there are several groups of alternatives to AGP in the poultry industry, including probiotics, prebiotics, symbiotics, organic acids, enzymes and phytogenics (medicinal plants), a perfect alternative should have the same beneficial impacts of AGP and ensure the optimization of animal growth and performance (HUYGHEBAERT et al., 2011). Phytogenics, as an interesting group of feed additives, may be a potential alternative to AGP. The biological or therapeutic activity of a phytogenic is completely related to its bioactive compounds and properties, which are variable according their derivatives. On the basis of the plant's biological activity, its antioxidant properties, gut microflora manipulation and immune system improvement are the main modes of action by which phytogenics have a positive impact on the growth performance and health of animals (HASHEMI et al., 2009a, b; GUO et al., 2004).

Probiotic strains, such as *Lactobacillus* spp., are cultures of live microbes that have a beneficial impact on poultry performance by affecting the intestinal microbiota population (FULLER, 1989; SHOKRYAZDAN et al., 2017; HUANG et al., 2004).

However, a limited number of research experiments have investigated the microbiota products or enzymes that influence growth performance. *Lactobacillus* and *Bifidobacterium* as potent probiotics contain the BSH enzyme which catalyzes primary bile salt to secondary, and changes the host lipid metabolism. It is also known for reducing the level of cholesterol in serum through its direct impact on the host's bile salt metabolism (BEGLEY et al., 2006). For instance, BSH produced by lactobacilli, deconjugates the taurine and glycine of bile acids (LANGHOUT et al., 1999; BEGLEY et al., 2006), which decreases the formation of micelles (MACDONALD et al., 1983) and the performance of broilers subsequently (SHARIFI et al., 2012). According to the literature, the population of *Lactobacillus* strains, the major BSH producers in the chicken intestine, decreased in reaction to AGP (KNARREBORG et al., 2002; DUMONCEAUX et al., 2006; GUBAN et al., 2006; BEGLEY et al., 2006; ENGBERG et al., 2000). Therefore, AGPs may promote chicken performance by reducing BSH activity, as it is an enzyme that exerts a negative effect on host fat digestion and metabolism. Accordingly, the current study sought to evaluate the BSH activity of different *Lactobacillus* strains isolated from the chicken hindgut, as well as the inhibitory impact of different plant extracts as potential alternatives for AGP on the BSH activity of *L. acidophillus*. The decrease in the BSH activity of lactobacilli strains as probiotics is believed to promote their efficiency.

Materials and methods

Bacterial strains and culture conditions. Five *Lactobacillus* strains, including *L. animalis, L. acidophillus, L. gallinarum, L. lactis* and *L. returi*, were obtained from a microbiology laboratory (Urmia University, Iran), having been previously isolated from the gastrointestinal tract of the native chickens. The *Lactobacillus* strains were separately cultured in de Man, Rogosa and Sharpe (MRS) broth (Scharlau, Spain) and stored in 10% glycerol at -20 °C before use.

Preparation of CBSM. The gallbladders of broilers were freshly collected from poultry slaughterhouses, and the contents of the gallbladder were extracted, stored in an oven at 40 °C, dried for 24 hours, and finally, powdered and autoclaved before use in bacterial cultures as CBSM.

Detection of bacterial BSH activity using the plate assay method. The bacteria were cultured in sterile MRS broth and incubated for 20 hours at 37 °C prior to checking the strains' BSH activity. The

deconjugation of bile salt in *Lactobacillus* strains was qualitatively determined by the direct plate assay method with a slight modification (WANG et al., 2012). The MRS agar medium was enriched with bile salts [0.5% w/v, taurocholic acid, Fulka; 0.5% w/v CBSM and $CaCl_2$ (0.37 g/L)] and autoclaved before use. Next, bacterial cells were streaked onto the MRS agar plates and incubated at 37 °C for 5 days anaerobically. MRS agar medium plates were used as the control without supplementing TCA and CBSM. The presence of precipitated unconjugated bile acid around the bacterial colonies (opaque halo) for CBSM, and the formation of opaque granular shiny colonies on the agar indicated BSH activity specific for TCA.

Preparation of cell-free extracts (CFEs). BSH activity in the CFEs of each *Lactobacillus* strain was determined (LIONG and SHAH, 2005; TANAKA et al., 2000; DONG et al., 2012) with some modifications. First, the resting cell suspensions harvested from an overnight culture were centrifuged (10,000×g, 10 min at 4°C) and washed twice with 0.1 M sodium phosphate buffer containing 10 mM dithiothreitol (Merck; pH 7), and then resuspended in the same buffer to obtain a suspension with an optical absorbance ($A_{600\ nm}$) of 3.0. Next, the cell pellet of each *Lactobacillus* strain was submitted to an ultrasonic homogenizer (Hielscher, Germany) for 5 min with a 50% duty circle at level 5 using 75% amplitude and constant cooling. Finally, the mixture was centrifuged for 10 min (20,000 g at 4 °C) and the supernatant was stored as a CFE at -20 °C.

Colorimetric BSH assay. A two-step standard BSH assay (TANAKA et al., 2000) was performed to determine the BSH activity quantitatively with some modifications by the amount of amino acid released from the conjugated bile salts (TCA and CBSM) using *Lactobacillus* strains of CFEs. Briefly, 10 µL of CFEs, 10 µL of conjugated bile salts (100 mM TCA and 3% w/v CBSM) were added to 180 µL of reaction buffer (0.1 M sodium-phosphate, with a pH of 6.0). Then, the reaction mixture was conducted at 37 °C for 30 min. Next, 200 µL of 15% (w/v) trichloroacetic acid was immediately added to stop the reaction, and the sample was centrifuged to remove the precipitates. For the second reaction, the supernatant (100 µL) was completely mixed with 1.9 mL of ninhydrin reagent (0.5 mL of 1% (w/v) ninhydrin in 0.5 M sodium-citrate buffer with a pH of 5.5, 0.2 mL of 0.5 M sodium-citrate buffer with a pH of 5.5, and 1.2 mL of glycerol), and the mixture was vortexed and boiled for 14 min. After subsequent cooling for 3 min in tap water, the absorbance at 570 nm was determined using glycine or taurine as the standard. One unit of BSH activity (U/mL) was defined as the amount of enzyme that liberated 1 mmol of amino acid from the substrate per min.

Microplate precipitation-based BSH activity assay. The fast evaluation of BSH determination is a precipitation-based check, through which the hydrolysis of the conjugated bile acid substance leads to deconjugated bile acids that are insoluble at the reaction pH, and can easily be observed as a white precipitate (TANAKA et al., 2000; SMITH et al., 2014). Briefly, 10 µL of CFEs was added to the wells of a 96-well microplate with a flat bottom. Then, 190 µL of reaction mixture containing 178 µL of reaction buffer (0.1 M sodium-phosphate with a pH of 6.0), 10 µL of TCA (100 mM), and 2 µL of 1 M dithiothreitol were added for a total reaction volume of 200 µL, to find out whether BSH positive activity in each strain in the wells became turbid immediately. In addition, the plates were incubated at 37 °C for up to 6 hours and the precipitation of insoluble unconjugated bile salts was monitored every 30 min by optical monitoring, concomitant to absorbance measurement at 600 nm (A_{600nm}) using a microplate reader (ELISA Reader Model DENA 3200). In order to assay BSH activity, different plant extracts with a serial dilution of stock concentrations were used as BSH inhibitor (1%-0.015% v/v) to find the best inhibitor. Before adding the reaction mixture, 10 µL of the plant extracts was added to the 10 µL CFEs of *L. acidophillus* in the bottom of each well and gently mixed by pipetting and incubated for 30 min. Then, the reaction mixture was added and the precipitation of insoluble unconjugated bile salts was monitored every 30 min by optical monitoring, concomitant to absorbance measurement at 600 nm (A_{600nm}) using a microplate reader (ELISA Reader Model DENA 3200). Subsequently, the potential BSH inhibitor

candidates at various concentrations were checked through the above-mentioned two-step standard BSH assay based on precipitation using the 96-well microplate assay. Finally, the percentage inhibition was measured by dividing the inhibited activity (i.e., the mean activity of the control minus the mean residual activity in the presence of a plant extract) relative to the mean activity of the control and then multiplied by 100.

Preparation of extracts. The plants (the aerial parts of Rosemary (*Rosmarinus officinalis*), Roselle calyx (*Hibiscus sabdariffa)*, *B. vulgaris* root, and Green tea) were purchased, air-dried at room temperature, and ground to a mesh size of 1 mm. Then, 100 g of each sample of the fine powder was dissolved in 1000 mL of 70% ethanol for 96 h, followed by filtering and concentrating to a small volume in order to remove the entire ethanol using a rotary evaporator. The plant extracts were kept at 4 °C for further studies.

Gas chromatography-mass spectrometry (GC-MS) analysis of plant extracts. The extract contained both the polar and nonpolar components of the plant material, and 2 µL of the sample of the solutions was used in GC-MS to analyze different compounds. The GC-MS analysis of the ethanol extract of the plant extract was performed using an Agilent 7890. A gas chromatograph coupled to a 5975A mass spectrometer was used with a HP-5 MS capillary column (5% Phenyl Methyl polysiloxane, 30 m length, 0.25 mm i.d., 0.25 µm film thickness).

The initial temperature of the oven was kept at 80 °C for three minutes and then increased at 8 °C/min to 180 °C and remained at the same temperature for 3 minutes. Further, helium was used as the carrier gas at a flow rate of 1 mL/min, the electron impact was 70 eV, the injector was set in a split mode (split ratio of 1:500), and mass range acquisition was from 40 to 500 m/z. Furthermore, the plant extract constituents were identified using the calculated linear retention indices (MONDELLO, 2008; NIST, 2005) and mass spectra were determined on the basis of those reported in NIST 05. The relative percentage amount of each component was calculated by comparing its average peak area to the total area. Table 1 presents the phytochemicals present in plant extracts, qualitatively detected using standard screening tests.

Results

Qualitative BSH activity. In this experiment, different *Lactobacillus* strains (i.e., *L. animalis, L. acidophillus, L. gallinarum, L. lactis,* and *L. returi*) were qualitatively checked for BSH activity by direct plate assay. In general, as shown in Fig. 1, all *Lactobacillus* strains can hydrolyze TCA and CBSM to unconjugated bile acid which precipitates from the solid MRS medium. All five *Lactobacillus* strains produced the formation of characteristic precipitate halos around the colonies, and opaque granular shiny colonies on the agar.

Quantitative BSH activity. Table 2 shows the BSH activity of *Lactobacillus* strains on TCA and CBSM. In the quantitative assay, cell-free extracts obtained from *Lactobacillus* strains were tested for BSH activity in a two-step procedure, as previously described by TANAKA et al. (2000). Moreover, all the *Lactobacillus* strains demonstrated BSH activity towards TCA ranging from 4.06 ± 0.057 to 4.21 ± 0.017 U/mL for *L. acidophillus* with the highest total of BSH activity units (4.21 ± 0.017), compared to *L. animalis* with the lowest total BSH activity (U/mL).

Moreover, *Lactobacillus* strains isolated from chicken indicate the ability to deconjugate CBSM. The BSH activity of these strains with respect to CBSM ranges from 3.78 ± 0.015 to 4.01 ± 0.035 U/mL. The lowest CBSM deconjugation activity (3.78 ± 0.015 U/mL) is related to *L. returi* whereas the highest activity belongs to *L. acidophillus* and sodium taurocholate.

Microplate precipitation-based BSH activity assay. The BSH activity of each *Lactobacillus* strain was tested by microplate assay in different concentrations and types of the bile salts. However, the reaction mix in the wells (containing the bile salts and cell-free extracts) became turbid due to the precipitation of unconjugated bile salts by BSH activity. This was visualized by the naked eye as a white and yellow precipitate, and measured by a microplate reader. Then, different plant extracts, which are usually used as dietary supplements in

poultry feeds, were added to identify their ability to decrease BSH activity. Various plant extracts, their inhibition activity, and the percentage inhibition effects of each extract are illustrated in Fig. 2. Green tea and *B. vulgaris* root ethanol extracted showed a higher inhibitory effect on BSH activity than the others.

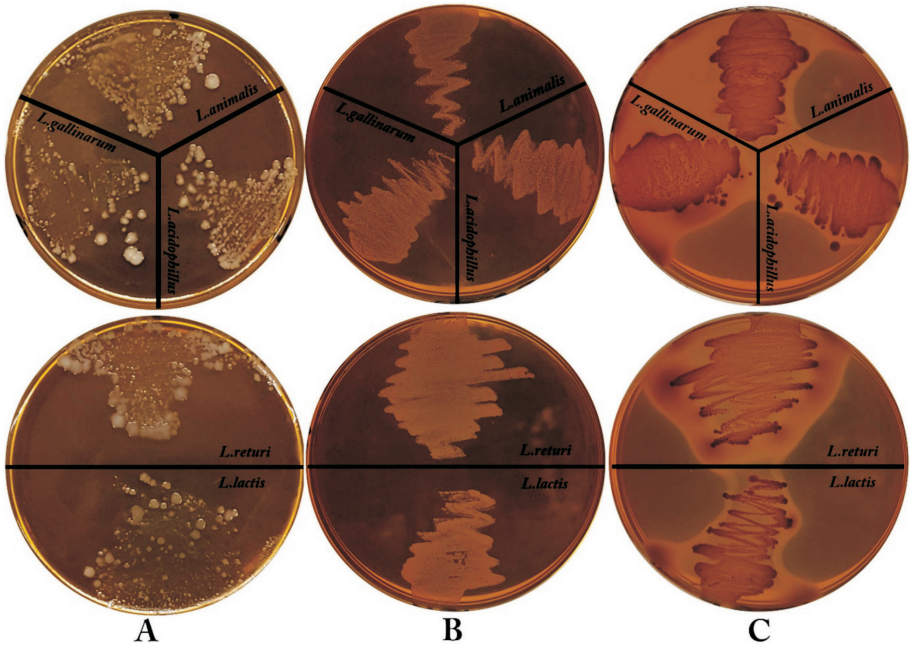

Fig. 1. The manifestation of BSH activity by lactobacilli on MRS agar medium. A, B, and C-labelled panels are control plates, assay plates containing 0.5% TCA, and assay plates containing 0.5% CBSM, respectively.

Table 1. Phytocomponents identified in ethanolic plant extracts by GC-MS

Rosemary					
Peak	Retention time	Area (%)	Molecular weight	Compound name	Type/Nature
4	5.251	0.82	$C_{10}H_{16}$	(1R)- (+)-Alpha-Pinene	Terpene
5	7.282	15.51	$C_{10}H_{18}O$	Eucalyptol	Monoterpenoid
8	9.909	3.99	$C_{10}H_{16}O$	Camphor	Terpenoid
10	10.389	5.03	$C_{10}H_{18}O$	Borneol	Monoterpenoid
12	10.864	0.49	$C_9H_{14}O$	4-isopropyl-2-cyclohexenone	Cryptone
15	11.711	3.92	$C_6H_6O_3$	furfural	Hydroxymethyl
19	16.472	33.49	$C_9H_6O_2$	2H-1-Benzopyran-2-one	Coumarin
25	22.148	9.82	$C_{10}H_8O_3$	7-Methoxy-2H-1benzopyran-2 one	Herniarin
27	26.434	0.89	$C_{16}H_{32}O_2$	n-Hexadecoic acid	Palmitinic acid
28	28.431	3.08	$C_{20}H_{40}O$	Phytol	Diterpene

Table 1. Phytocomponents identified in ethanolic plant extracts by GC-MS (continued)

B. vulgaris					
1	2.156	1.31	C_7H_{14}	cis-1,3-Dimethyl cyclopentane	Cyclopentane
2	2.190	1.80	C_7H_{14}	1,2-dimethyl-Cyclopentane	Cyclopentane
11	9.789	3.67	$C_6H_8O_4$	4H-Pyran-4-one, 2,3 dihydro-3,5-dihydroxy-6-methyl	
12	13.737	16.83	$C_9H_{10}O_2$	2-Methoxy-4-vinylphenol	Guaiacol
23	24.506	10.95	$C_{12}H_{16}O_4$	2-(3,4-Dimethoxyphenyl) tetrahydropyran	Elemicin
24	26.434	1.55	$C_{16}H_{32}O_2$	n-Hexadecanoic acid	Palmitinic acid
Green tea					
10	13.594	0.53	$C_{11}H_{18}O_2$	2,6-Octadien-1-ol, 3,7-dimethyl- acetate	Geranyl acetate
13	15.024	3.87	$C_6H_6O_3$	1,2,3-Benzenetriol	Pyrogallol
21	24.786	76.96	$C_8H_{10}N_4O_2$	Caffeine	Alkaloid
22	25.038	1	$C_7H_8N_4O_2$	Theobromine	Alkaloid
23	26.434	0.85	$C_{16}H_{32}O_2$	n-Hexadecanoic acid	Palmitinic acid
24	28.431	3.04	$C_{20}H_{40}O$	Phytol	Diterpene
Roselle calyx					
1	2.155	0.41	C_7H_{14}	1,3-dimethyl-cyclopentane	Cyclopentane
3	3.597	2.94	$C_5H_4O_2$	2-Furaldehyde	Furfural
5	5.818	0.69	$C_7H_6O_4$	Methyl-5-formylfuran	
10	9.789	0.43	$C_6H_8O_4$	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	
15	11.774	5.01	$C_6H_6O_3$	Hydroxymethylfurfurole	Levulinic acid
26	26.451	1.13	$C_{16}H_{32}O_2$	n-Hexadecoic acid	Palmitinic acid
29	28.980	1.68	$C_{18}H_{32}O_2$	9,12-Octadecadienoic acid	Linoleic acid ethyl ester
30	29.054	1.16	$C_{20}H_{36}$	Ethyl linoleolate	Linoleic acid

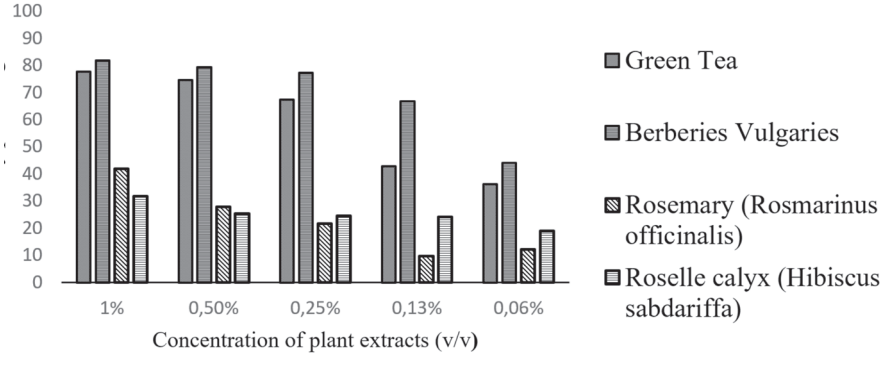

Fig. 2. Percentage inhibition of different plant extracts on BSH enzyme

Table 2. BSH Activity of *Lactobacillus* Strains on TCA and CBSM [2]

Strains	BSH Activity[1]	
	Total activity (U/mL) of CBSM[3]	Total activity (U/mL) of TCA[3]
L. animalis	3.94 ± 0.032	4.13 ± 0.026
L. lactis	4.01 ± 0.058	4.19 ± 0.005
L. gallinarum	3.96 ± 0.020	4.17 ± 0.021
L. acidophillus	4.01 ± 0.35	4.21 ± 0.017
L. returi	3.78 ± 0.015	4.06 ± 0.057

[1] The results are provided as means ± standard deviation; the values are in triplicate; [2] BSH activity from cell-free extracts of *Lactobacillus* strains grown on MRS broth supplemented with 3% w/v CBSM and 100 mM TCA; [3] CBSM: Chicken bile salt mixture; TCA: Taurocholic acid.

Discussion

The most important trait of probiotics is to survive while passing through the gastrointestinal tract in order to preserve their beneficial impacts (ELLI et al., 2006). Many parameters such as gastrointestinal pH, bile salts, and others may influence the survival capability of the probiotic. The exact beneficial effects of the BSH enzyme in probiotic bacteria such as lactobacilli and bifidobacteria (BEGLEY et al., 2006) are still not well recognized. But there are some hypothesizes about the roles of BSH in bacterial physiology based on some evidence related to certain commensal bacteria and conjugated bile salts (GENG and LIN, 2016). It has been demonstrated that hydrolysis of conjugated bile acids via BSH could supply cellular carbon, nitrogen, sulfur and energy source for some bacteria species (VLAHCEVIC et al., 1996; TANAKA et al., 2000; RIDLON et al., 2006).

Moreover, the presence of conjugated bile salts in the environment of the small intestine, is the most unfavorable and toxic parameter to the survival of probiotics (BEZKOROVAINY, 2001). Thus, the ability of probiotic Lactobacilli to detoxify bile salts through the production of the BSH enzyme is often included among the criteria for probiotic strain selection (NORIEGA et al., 2006). Furthermore, *Lactobacillus* strains isolated from the gastrointestinal tract are more likely to be BSH positive than those without exposure to bile salts (TANAKA et al., 1999). In the present study, all the *Lactobacillus* strains isolated from the gastrointestinal tract of chickens clearly revealed BSH activity for TCA and CBSM through the direct plate and quantitative assays in the current experiment.

BSH is an enzyme that changes the state of conjugated bile salt to unconjugated and free primary bile acids (GILLILAND and SPECK, 1977). Therefore, BSH enzyme in probiotic bacteria could have reciprocal impacts on the host physiology by disturbing conjugated bile acid-mediated fat metabolism and endocrine functions (JONES et al., 2014; JOYCE et al., 2014). Hence, these abilities of the BSH enzyme are associated with reduced serum cholesterol levels, and are gaining much more attention in human patients. To date, limited research has been conducted about the BSH activities of intestinal bacterial on animal performance (FEIGHNER and DASHKEVICZ, 1987; GUBAN et al., 2006; LIN, 2014).

However, some investigations have indicated that probiotic supplementation to broiler diets weakens performance by reducing fat digestibility that can have an undesirable effect on animal production (MOUNTZOURIS et al., 2010; SHARIFI et al., 2012). Additionally, several researchers have reported a correlation between BSH activity in the intestine and AGP, and concluded that using AGP leads to a reduction in BSH activity, and improves animal growth performance by higher fat digestibility (GUBAN et al., 2006; FEIGHNER and DASHKEVICZ, 1987). In addition, others indicate

that conjugated bile acids play a crucial role in lipid digestion and micelle formation (DIBNER and RICHARDS, 2005; BEGLEY et al., 2006).

Similarly, AGP have been used in animal nutrition for more than 60 years, to improve the growth rate and feed conversion ratio while preventing infections (CASTANON, 2007). Despite the important positive effects of AGP on growth rate, mortality and higher disease resistance, these types of additives have a number of disadvantages. These concerns include the development of antimicrobial resistance, which is a potential threat to human health (WHO, 2012). According to LIN et al. (2014) there is an inverse relationship between BSH activity as a potent characteristic of bacteria in the intestine and body weight gain.

Therefore, reducing BSH activity in bacteria by BSH inhibitors could be one of the main objectives for developing novel alternatives to AGP. WANG et al. (2012) recommended that research on BSH enzyme activity can be the main mechanistic microbiome target for new alternatives to AGPs, and hence scientists have focused on a potent BSH enzyme from a chicken *L. salivarius* probiotic strain, to find BSH inhibitors as alternatives to AGPs (GENG and LIN, 2016). They have suggested some chemical compounds as BSH inhibitors, such as copper and zinc in high dosages which were known growth promoters in poultry nutrition in the past (LIU et al., 2011; WANG et al., 2012). However, there are some undesirable effects of these kinds of metals being used long-term in animal feed as growth promoters, such as toxicosis due to extended exposure and soil pollution via increased amounts excreted in the feces.

In this study, *B. vulgaris* root and Green tea extracts had a high inhibitory effect on BSH activity in *L. acidophilus*. LIN et al. (2014) discovered some potent BSH inhibitors for a phylogenetically distant BSH from *L. acidophilus*. SMITH et al. (2014) considered more than 2000 compounds as BSH inhibitors by high-throughput screening technology, and suggested some natural and chemical substances as potential alternatives to AGP. These studies recommended that Carnosic acid (the most bioactive compound of Rosemary), epichatechin monogallate (the most bioactive compound of Green tea), and gossypetin (the most bioactive compound of Roselle calyx) were potential natural BSH inhibitors. Therefore, more investigations will be needed into modified designs of probiotics based on BSH and BSH-producing bacteria, to decrease the detrimental effects of BSH on fat digestibility and energy harvesting, and hence conserve the other valuable impacts of probiotics on animal health and the immune system. Furthermore, this will help to develop safe BSH inhibitor-based, non-antibiotic feed additives for improving feed efficiency and growth rate.

Conclusion

In general, this study found that all five *Lactobacillus* strains isolated from the native poultry hindgut were resistant to bile salts (i.e., TCA and CBSM) throughout BSH activity. Among the plant extracts of the present study, Green tea and *B. vulgaris* root showed relative inhibitory effects against the BSH enzyme. Therefore, these plant extracts could be regarded as potential alternatives to AGP, along with the *lactobacilli* as probiotics that may result in improving poultry production by harvesting the energy of increased fat digestibility, and also providing advantages for health status.

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DIBAMEHR, A., M. DANESHYAR, A. TUKMECHI, S. M. ABTAHI FROUSHANI: Učinak različitih biljnih ekstrakata na aktivnost hidrolaze žučnih soli sojeva bakterije *Lactobacillus* izolirane iz probavnog sustava peradi. Vet. arhiv 91, 89-99, 2021.

SAŽETAK

Enzimi hidrolaze žučnih soli (BSH) oslabljuju metabolizam masti dekonjugacijom žučnih soli što dovodi do smanjenja proizvodnosti u peradi. Smanjenje aktivnosti ovog enzima, upotrebom dodataka prehrani, mogla bi biti obećavajuća alternativa za primjenu određenih antibiotika u peradarstvu. Biljni ekstrakti dugo se upotrebljavaju kao dodaci prehrani za poticanje rasta. U ovom je istraživanju pet sojeva bakterije *Lactobacillus*, uključujući *Lactobacillus animalis, Lactobacillus acidophillus, Lactobacillus gallinarum, Lactobacillus lactis* i *Lactobacillus returi*, dobiveno iz stražnjeg dijela crijeva peradi te upotrijebljeno kao probiotik. Test na ploči i enzimska reakcija u dva koraka primijenjene su za utvrđivanje aktivnosti dekonjugacije u sojeva *Lactobacillus*. Nadalje, četiri biljna ekstrakta - nadzemni dijelovi ružmarina (*Rosmarinus officinalis*), hibiskusa (*Hibiscus sabdariffa*), korijen obične žutike (*Berberis vulgaris*) i zeleni čaj - istraživana su s obzirom na inhibitore enzima BSH upotrebom izvanstaničnih ekstrakata kao moguća zamjena antibiotiku. Osim toga, nakon usmrćivanja, prikupljeni su svježi žučni mjehuri brojlera te je izvađen njihov sadržaj. Rezultati su pokazali da svi sojevi bakterije *Lactobacillus* mogu hidrolizirali tauroholatnu kiselinu i žučne soli pilića (CBSM) u nekonjugiranu žučnu kiselinu. Štoviše, ekstrakti etanola korijena *B. vulgaris* i zelenog čaja relativno su smanjili aktivnost BSH enzima što bi se moglo istražiti u hranidbi peradi *in vivo*. Zaključno, svih pet sojeva bakterije *Lactobacillus* bilo je otporno na žučne soli npr. tauroholičnu kiselinu (TCA) i (CBSM) putem BSH aktivnosti, a dodatak zelenog čaja i ekstrakta korijena *B. vulgaris* mediju s bakterijama pokazali su inhibitorne učinke protiv BSH enzima.

Ključne riječi: laktobacili; hidrolaza žučnih soli; biljni ekstrakti; antibiotici kao promotori rasta