Journal of Medicinal Plants Research Vol. 4(5), pp. 415-420, 4 March, 2010
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875© 2010 Academic Journals

Full Length Research Paper

Hamcho (*Salicornia herbacea*) with probiotics as alternative to antibiotic for broiler production

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Accepted 4 February, 2010

A study was conducted with 140 "Ross" 1-day old broilers having four treatments to know the potentiality of *Salicornia herbacea* (Hamcho) probiotics (SHP) and suitable level in replacing antibiotic. The groups were control (basal diet), antibiotic (basal diet + 0.05% OTC) and SHP 0.5% and 1.0% with basal diet. The birds were arranged in a completely randomized design having 5 replications with 7 chicks per replication in wire cage. Recorded data were analyzed by SAS package program. Addition of feed additives in the diet did not show any adverse effect on growth, feed intake and FCR (feed: gain) in broilers. Addition of SHP has significant effect on increase of crude protein in meat compared to control as well as antibiotic ($p < 0.05$). *S. herbacea* probiotics showed significantly lower fat content in carcass like the antibiotic than control ($p < 0.05$). The thiobarbituric acid (TBA) value of meat in 0.5% SHP group showed significantly lower value compared to control ($p < 0.05$) at fresh and first weeks of preservation although statistically similar with antibiotic for both the levels of SHP up to 3 weeks of preservation. Development of internal organs has no adverse effect among the groups, moreover reducing and positive trend in cecum and large intestine weight after addition of SHP. Considering the findings, 0.5 to 1.0% *S. herbacea* probiotics can be replaceable to antibiotic for broiler production.

Key words: *Salicornia herbacea* probiotics, oxytetracycline (OTC), crude protein, TBA value, fatty acids profile, broiler meat.

INTRODUCTION

Phytogenic feed additives (often called phytobiotics or botanicals) are commonly defined as plant-derived compounds incorporated into diets to improve the productivity of livestock through amelioration of feed properties, promotion of the animals' production performance and improving the quality of food derived from those animals (Windisch et al., 2008). Due to the outbreaks of resistant bacteria and residues of antibiotics in poultry products, using antibiotics is regulated by Korean Government (Yu et al., 2004). Currently there are several kinds of antibiotics alternative developed and used. Among the alternatives, medicinal plants with excellent physiologically activity are getting attention by researchers (Hernandez et al., 2004). Some examples of medicinal plants are green tea, artemisia, acanthopanax and others (Yang et al., 2003; Kwon et al., 2005).

Hamcho (also called glasswort) is one of the most salt tolerant plants growing in the salt marshes and tidal flats along the western coast of Korea (Kim et al., 2009). It has not only been eaten as a food, but also used in folk medicine for treating constipation, obesity and diabetes (Min 1998; Min et al., 2002).

Probiotics are already well established as feed additives in animal nutrition. Probiotics with medicinal plants are being suggested as effective antibiotics alternatives. Probiotics maintains intestinal microbial balance and helps gut mucosa development, improving digestion and absorption rate and thus improving production (Mohan et al., 1996). It has been reported by several studies that single and mixed probiotics increases poultry production (Jin et al., 1998; Mohan et al., 1996). Generally, the digestive organ of healthy animal is dominated by anaerobic organisms and the upper part of it is dominated by *Lactobacillus acidophilus*, the middle part by *Bacillus subtilis* and the lower part by *Streptococcus faecium*. Considering this fact, the effect of probiotics may be improved using mixed probiotics (Yoon et al., 2004). It is

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reported that supplementation of 0.1 and 0.2% of mixed probiotics containing *L. acidophilus, B. subtilis* and *Saccharomyces cerevisiae* improve production of broilers and indirect immunity (Kim et al., 2002).

In practice, meat is stored and cooked for consumption (Cortinas et al., 2005). Lipid oxidation is one of the primary mechanisms of quality deterioration in meat products through adverse changes in flavor, color, texture and nutritive value (Ura et al., 2008). Chicken meat enriched with polyunsatuarated fatty acids contains longer fatty acids (FA) with a high number of double bonds, which increases the susceptibility of meat to oxidation (Maraschiello et al., 1999; Ruiz et al., 1999; Grau et al., 2001a and b). One of such product is malondialdehyde (MDA), which has long been considered as an index of oxidative rancidity. Among many methods proposed for assessing MDA, the 2-thiobarbituric acid (TBA) has been widely adopted as a sensitive assay method for lipid oxidation in animal tissues. Lee at el. (2007) concluded that *Salicornia herbacia* may have anti-oxidative and anti-inflamatory activities by modulating redical induced toxicity and various pro-inflamatory responses.

The purpose of this study was to assess possibility of *S. herbacia* with probiotics to be used as broiler feed additives for substituting antibiotic and their effect on productivity, meat quality and organ development.

MATERIALS AND METHODS

Animals and experimental design

One hundred and forty 1-day old "Ross" broiler chicks were considered for this study. The chicks were housed in a close, ventilated caged-broiler house in which they were raised for 24 h of daily light. From 1 to 14 days of age, supplemental heat was provided by electric heater which was placed inside the chicken house; thereafter the room temperature was kept at 22 ± 2℃ through a supplemental heating system. The birds were assigned to 4 treatments with 5 replications following completely randomized design (CRD). There were four dietary treatments, control, antibiotic (basal + 0.05% oxytetracycline), and hamcho (basal + 0.5% and basal + 1.0%). The feed and drinking water were provided *ad libittum.*

Experimental diets and feeding

Experimental diets were divided into two phases: starter 0 - 3 weeks and finisher 4 - 5 weeks of age. Diets were formulated following NRC (1998). The chemical compositions of experimental diets are given analyzed by AOAC (1990) method and shown in Table 1. The probiotics used in the formulation of *S. herbacia* probiotics (SHP) are given in Table 2.

Parameters studied

Body weight gain, feed intake, feed conversion ratio and mortality

Body weights were measured on weekly basis from initial day to the final day of the experiment. Feed intake had been determined by measuring feed residue on weekly basis since the beginning of the experiment. Feed conversion ratio was obtained by dividing the feed intake to body weight gain. Dead birds were recorded and accordingly adjusted the feed intake.

Carcass composition and organ development

At the end of the experiment, 16 chickens were slaughtered and samples were collected from breast and thigh muscle. The chemical composition of the carcass was determined according to common method of AOAC (1995). The organs development was measured by taking weight and length of the broilers after slaughtering and calculated in respect of body weight.

Lipid oxidation determination

For the determination of lipid oxidation (carcass rancidity) of broiler meat, the method described by Witte et al. (1970) was used with little modifications. For this analysis, 10 g of thigh and breast meat mixture (equal amount of each) was blended at full speed for 1.5 min in chilled stainless watering blender cup with 25 ml of extracting solution containing 20% trichloroacetic acid (TCA) in 2 M phosphoric acid. The resulting sediment was transferred quantitatively to 50 ml volumetric flask with 20 ml distilled water and diluted by shaking and homogenized. A 25 ml portion was filtrated through Whatman No.1 filter paper. Then 5 ml filtrate was transferred to a test tube and 5 ml of 2-thiobarbituric acid (0.005 M in DW) was added. The solution was shaken in a water bath at 80℃ for 30 min. After cooling, the color development was measured at 530 nm in a spectrophotometer, biochrom, Libra S22 (Biochrom Ltd., Cambridge, England). Thiobarbituric acid (TBA) reactive substance values were expressed as micromole of malondialdehyde (MDA) per hundred gram of meat.

Statistical analysis

The data obtained from this study were analyzed by general linear models (GLM) of SAS Package Program (1990) to estimate variance components for a completely randomized design. Duncan's multiple comparison tests (1955) were used to examine significant differences between treatment means. Differences were statistically assessed at $P < 0.05$.

RESULTS AND DISCUSSION

Growth performance

The results in Table 3 stated that body weight gain, FCR (feed conversion ratio) and feed intake of broiler chicks were not significant among the treatment groups. Kim et al. (2002) reported that weight gain and feed conversion ratio improved when 0.1 to 0.5% probiotics with *Lactobacillus* sp., *Bacillus* sp. and Yeast were fed to broilers. According to Ryu et al. (2003), supplementation of 0.1 to 0.3% mixed probiotics containing *L. acidophilus*, *B. subtilis*, *S. cerevisiae* to broiler chicks fed diets improved weight gain and feed conversion ratio (FCR) as well as feed intake. In Table 3, weight gain and FCR showed statistically similar among the groups although the same probiotics were used, the reason might be

Table 1. Chemical composition of the experimental broiler starter and finisher diets (%).

Treatments	Feed type	Control	Antibiotics	SHP	
				0.5%	1.0%
Moisture	Starter	10.00	10.16	10.24	10.27
	Finisher	10.32	9.91	9.63	9.87
Crude ash	Starter	5.12	5.14	5.52	5.41
	Finisher	5.65	5.92	5.44	5.46
Crude fat	Starter	7.10	7.19	6.18	7.12
	Finisher	5.82	7.74	6.90	7.59
Crude fiber	Starter	25.65	25.11	23.95	25.66
	Finisher	25.35	25.11	23.95	25.66
Crude protein	Starter	18.41	18.80	18.84	18.89
	Finisher	18.06	18.86	18.25	18.66
NFE (nitrogen free extract)	Starter	34.02	33.59	35.27	32.65
	Finisher	34.45	32.52	35.24	32.52

SHP: *S. herbacia* probiotics.

Table 2. The species and strain of microflora used in *Salicornia herbacia* probiotics with chemical composition.

Species of microflora	Strain	Composition of SHP (%)	
L. acidophilus	KCTC 3111	Moisture	39.31
L. plantarum	KCTC 3104	Crude protein	11.83
B. subtilis	KCTC 3239	Crude fat	1.76
S. cerevisiae	KCTC 7915	Crude fiber	9.22
		Crude ash	13.62
		NFE	24.26

SHP, *S. herbacia* probiotics; NFE, Nitrogen free extract; KCTC, Korean collection of type culture.

Table 3. Effects of *S. herbacea* probiotics on growth performance of broiler.

Items	Control	Antibiotics	SHP*	
			0.5%	1.0%
Initial weight (g)	45.00	45.00	45.00	45.00
Final weight (g)	1909 ± 67	1855 ± 29	1924 ± 57	1905 ± 56
Weight gain (g)	1864 ± 66	1810 ± 28	1879 ± 56	1860 ± 56
Feed intake (g)	2922 ± 116	2887 ± 78	2961 ± 92	2946 ± 59
FCR(Feed/Gain)	1.57 ± 0.02	1.58 ± 0.01	1.58 ± 0.01	1.59 ± 0.02
Mortality (%)	5.71	0	0	0

Values are mean ± Standard error (n = 4). Means in the same row are not significantly different (P > 0.05). *SHP=*S. herbacia* probiotics.

addition of *S. herbacia* with the probiotics. Uuganbayar (2004) reported that 0.5 to 1.5% green tea supplement in broiler diet had effect to reduce body weight gain and feed intake of the chicks. Sarker et al. (2009) found that the growth performance between 0.5 and 1.0% addition of both green tea and fermented green tea probiotics groups did not show any statistical difference. No dead birds were observed in antibiotic and SHP supplemented treatments except 2 birds (5.71%) were dead in control treatment (Table 3).

Carcass composition

The meat composition of broilers is shown in Table 4. The crude protein content was increased significantly

Table 4. Effects of *S. herbacea* probiotics on the carcass composition of broiler (%).

Treatments	Control	Antibiotics	SHP*	
			0.5	1.0%
Moisture	74.00 ± 0.61	74.10 ± 0.59	73.36 ± 1.31	73.84 ± 0.41
Crude protein	21.94^{b} ± 0.04	22.20^{b} ± 0.63	23.89^{a} ± 0.04	23.89^{a} ± 0.27
Crude fat	1.04^{a} ± 0.11	0.68^{b} ± 0.07	0.82^{ab} ± 0.10	0.73^{b} ± 0.10
Crude ash	1.25 ± 0.02	1.25 ± 0.11	1.31 ± 0.08	1.26 ± 0.01

Values are mean ± Standard error (n = 4). Means with different letters within the row are not significantly difficult (P < 0.05). *SHP = *S. herbacia* probiotics.

Table 5. Effect of hamcho probiotics in the broiler meat (μmol MDA[1]/100 g).

Storage time	Control	Antibiotic	SHP[2]	
			0.5%	1.0%
Fresh	1.74 ± 0.12^{a}	1.44 ± 0.06^{ab}	1.36 ± 0.03^{b}	1.63 ± 0.14^{ab}
1st	6.07 ± 0.82^{a}	4.06 ± 0.31^{ab}	3.12 ± 0.14^{b}	5.57 ± 0.89^{a}
2nd	15.19 ± 2.13	10.40 ± 2.02	10.85 ± 1.72	9.81 ± 2.11
3rd	20.09 ± 4.24	10.38 ± 2.25	15.21 ± 2.37	15.46 ± 2.59

Data: means±standard error. ^{a,b}Means with different superscripts within same raw are significantly different (P < 0.05). [1]MDA = Melondialdehyde, [2]SHP = *S. herbacia* probiotics.

after addition of *S. herbacia* probiotics compared to the other treatment (P < 0.05).

In Table 4, the crude fat contents were reduced in antibiotic and probiotics groups compared to control (P < 0.05). The crude ash increased in broilers fed diets containing 0.5 and 1.0% of SHP groups compared to the control and antibiotic groups (P > 0.05). Davis et al. (1975) reported that the crude protein content and the crude fat of meat are negatively correlated to each other. In other words, if the crude protein content is higher, the crude fat content tended to be lower, which is in agreement with our findings (Table 4).

Lipid oxidation of meat

The thiobarbituric acid (TBA) reactive substance test is the most widely used method for quantifying lipid oxidation development in meat and meat products. The TBA test determines the amount of malondialdehyde (MDA), a major secondary byproduct of lipid oxidation, in an oxidized lipid. In Table 5, TBA value of fresh broiler meat was significantly lower in 0.5% SHP group (1.36 umol/100 g), while control was significantly high (1.74 umol/100 g) (P < 0.05). In case of one week of storage, TBA value of broiler meat was significantly higher in control group compared to the other treatments (P < 0.05). The average TBA value (0 to 3 weeks) of broiler meat kept at 4℃ in a cool chamber is shown in Figure 1. Addition of SHP (0.5 and 1.0%) significantly reduced the lipid oxidation like antibiotic fed broilers compared to control. Lipid oxidation causes loss of nutritional and sensory values as well as the formation of potentially toxic compounds that compromise meat quality and reduce its shelf life (Cortinas et al., 2005). The storage time also had an effect on these values, and the highest TBA occurred at the end of the storage period. This result agrees with the findings of Muhammet et al., 2005.

Sarker et al. (2009) showed a similar trend in reducing lipid oxidation when broilers are fed with medicinal plant (green tea) with the same probiotics (0.5 and 1.0%) levels (Figure 1). Uuganbayar (2004) reported that 0.5 to 1.5% green tea supplement in broiler diet had effects to reduce the TBA value on broiler meat compared to the control and Yang et al. (2003) also reported that the TBA value of broiler meat was decreased significantly when broilers were fed with diets with 0.5 to 2.0% green tea by-products supplement diet. Mountney (1976) reported that the rancidity of broiler meat arises faster than the red meat of pork and beef because of more unsaturated fatty acids content. It can be assumed that the hamcho supplementation with probiotics in broiler diet may reduce the rancidity of the broiler meat. Our result revealed with the Kim et al. (2009) and Lee et al. (2007) stated the antioxidative potentiality of *S. herbacia*, our result agreed with their findings.

Weight of relative internal organs

In Table 6, the results showed that the caecum weight was significantly reduced in broilers fed diets containing 0.5% SHP compared to the other treatment groups (P < 0.05). The reduction of caecum weight in addition of SHP

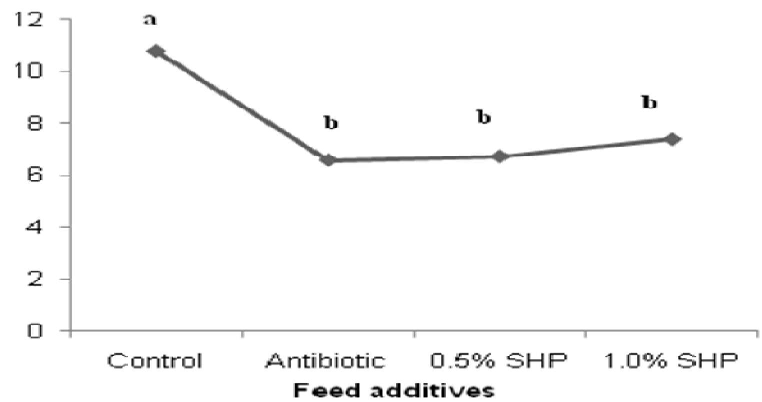

Figure 1. Effect of feed additives on thiobarbituric acid (TBA) value in broiler meat. Data with different superscript are significantly different (P < 0.05). MDA, Malondialdehyde.

Table 6. Effects of *S. herbacea* probiotics on relative organ weight in broiler.

Internal organs (%)	Control	Antibiotics	SHP*	
			0.5	1.0%
Crop wt.	0.28 ± 0.5	0.48 ± 0.12	0.37 ± 0.09	0.29 ± 0.1
Heart wt.	0.50 ± 0.03	0.51 ± 0.01	0.49 ± 0.05	0.48 ± 0.02
Liver wt.	1.60 ± 0.06	1.73 ± 0.02	1.76 ± 0.09	1.67 ± 0.1
Gizzard wt.	0.89 ± 0.09	1.04 ± 0.18	0.9 ± 0.08	0.98 ± 0.13
Pancreas wt.	0.17 ± 0.01	0.17 ± 0.01	0.15 ± 0.01	0.19 ± 0.1
Cecum wt.	0.60^{ab} ± 0.07	0.74^{a} ± 0.1	0.44^{b} ± 0.03	0.47^{ab} ± 0.11
Kidney wt.	0.63 ± 0.03	0.64 ± 0.04	0.63 ± 0.02	0.61 ± 0.05
Small intestine wt.	2.57 ± 0.24	2.70 ± 0.56	2.40 ± 0.2	2.41 ± 0.07
Large intestine wt.	0.17^{a} ± 0.02	0.11^{b} ± 0.01	0.13^{ab} ± 0.01	0.18^{a} ± 0.02
Abdominal fat wt.	2.12 ± 0.28	1.84 ± 0.4	2.38 ± 0.23	2.39 ± 0.32
Proventriculus wt.	0.54 ± 0.12	0.67 ± 0.17	0.52 ± 0.18	0.45 ± 0.07

Values are mean ± Standard error (n = 4). Means with different letters within the row are not significantly difficult (*P* < 0.05). *SHP = *S. herbacia* probiotics.

was probably due to better microbial activity. Caecum has a muscular wall that can massage the contents to speed up absorption. The function of caecum is to absorb water and salts from undigested feed.

The abdominal fat weights were not changed statistically for broilers fed diets. Broilers fed 0.5% SHP showed reduced large intestine weight like antibiotic fed birds compared to control (P < 0.05). Some other researcher fed medicinal plant to broilers and got similar result. Uuganbayar (2004) reported diets containing 0.5% green tea showed a significant weight loss of the small intestine compared to the control diet, which is similar to our study. Kim et al. (2006) also expressed same opinion. Other internal organs showed in the Table 6, did not show any adverse effect after addition of medicinal plants mixed with probiotics.

The findings of the present study indicate that diets containing 0.5 to 0.1% *S. herbacia* (hamcho) with probiotics can be added for substituting oxytetracycline for broiler production. More research is needed to clarify our under standing on the optimal levels of hamcho with probiotics in different species of poultry.

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