BF-1406
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RESEARCH ARTICLE Indian Journal of Animal Research, Volume Issue : ()

Growth Performance, Meat Quality, Meat Oxidation and Intestinal Bacterial Contents of Broilers Fed with *Garcinia mangostana* Peel Extract

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10.18805/IJAR.BF-1406

ABSTRACT

Background: Extracts of mangosteen (*Garcinia mangostana*) peels possess various biological activities such as antioxidants and anti-bacteria. The aim of this study was to investigate the effects of mangosteen peel extract (MPE) on growth performance, meat quality, oxidation of meat and bacterial contents in the small intestine of broilers.

Methods: The 192, 7-day-old, chicks were allocated to 4 treatments with 3 replicates per treatment, each replicate containing 16 chicks. Birds were fed diets supplemented with 0, 0.2, 0.4 or 0.8% of MPE for a period of 5 weeks *ad libitum*. Growth performance was evaluated weekly. At 42 days old, the birds were slaughtered and the breast muscles were sampled from 4 birds per replicate for determining meat quality and oxidation. To determine bacterial quantities, the residue of feed in the small intestine (ilium and ceca) was sampled from one bird per replicate.

Result: The results revealed that MPE had no significant effect on growth performance and intestinal bacterial contents. However, it was found that 0.4 to 0.8% of MPE could improve the meat quality by decreasing drip loss and total water loss in muscles. Moreover, 0.8% MPE group tended to be lower in lipid oxidation. This suggests that 0.8% MPE might protect the broiler breast meat against oxidation.

Key words: Broilers, Growth performance, Mangosteen peel extract, Meat quality, Meat oxidation.

INTRODUCTION

In recent years, increasing attention has been given to natural plant compounds that could substitute for antibiotics in farm animals (Cetingul *et al*. 2016; Li *et al.* 2017; Pastsart and Pimpa, 2019). Numerous studies have demonstrated that extracts of the peels of Mangosteen (*Garcinia mangostana*), which are agricultural waste, are rich in phenolic compounds such as xanthones, tannins and anthocyanins (Fu *et al.* 2007; Jung *et al.* 2006; Mahabusarakam *et al.* 2004). These compounds have various biological activities as antioxidants (Jung *et al.* 2006; Kondo *et al.* 2009; Suvarnakuta *et al.* 2011), anti-allergenics (Chae *et al.* 2012), anti-carcinogenics (Akao *et al*. 2008; Mizushina *et al.* 2013), antibacterials, antifungals and antivirals (Pedraza-Chaverri *et al.* 2008).

The phenolic compounds from mangosteen peel have been reported to be antibiotic (Janardhan *et al.* 2017) and enhance the growth performance of broilers (Hidanah *et al.* 2017). These findings indicate that mangosteen peel has potential to serve as an antibiotic growth promoter (AGP) in farm animals. Although a prior study has investigated the effects of mangosteen peel extract (MPE) on growth performance and antibiotic resistance in broilers (Herawati *et al*. 2020), the effects of MPE on meat quality and broilers meat oxidative status remain largely unknown. Therefore, this present study was aimed to examine the effects of dietary supplementation of MPE on growth performance, meat quality and meat oxidation, along with assessing the intestinal bacterial contents of broilers to verify that MPE acts as antibiotic in broilers.

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How to cite this article: Pastsart, U. and Pimpa, O. (2021). Growth Performance, Meat Quality, Meat Oxidation and Intestinal Bacterial Contents of Broilers Fed with *Garcinia mangostana* Peel Extract. Indian Journal of Animal Research. DOI: 10.18805/IJAR.BF-1406.

Submitted: 08-07-2021 **Accepted:** 14-09-2021 **Online:** 08-10-2021

MATERIALS AND METHODS

The experiment was carried out at Scientific Laboratory and Equipment Center, Prince of Songkla University, Surat Thani campus, Thailand during 2016-2019.

Animals and dietary treatments

One hundred ninety-two Ross 308 one-day-old female broiler chicks were obtained from a local commercial hatchery. Feed and water were supplied for consumption *ad libitum*. Basal starter (weeks 1 to 3) and finisher (weeks 4 to 6) diets were used for broiler chickens according to the nutrient requirement recommendations of NRC (1994). Chicks were weighed at the start of the trial (d7) and randomly assigned to 4 treatment groups, each treatment having three replicate pens (16 birds per pen). The size of the pen was 1x1 m. Chicks of group 1 (Control) were

provided basal feed. The chicks of groups 2, 3 and 4 were provided basal feed supplemented with MPE at 0.2, 0.4 and 0.8% (dry extract by fresh weight of feed), respectively. Daily feed intake, average daily gain (ADG) and feed conversion ratio (FCR) were estimated weekly for both the control groups and the groups with actual dietary treatments. During the trial, all mortalities were removed from the pens and recorded.

Preparation of crude peel extract of mangosteen

The fruits of mangosteen were collected from Southern peninsular Thailand. The fruits were washed thoroughly with distilled water and the white edible pulps were removed. The inedible part, namely peel, was ground and dried in a hot air oven at 50°C for 72 h. A powdered sample of 100 g was soaked in 500 ml of 95% ethanol for 48 h (×3) at room temperature (Tachakittirungrod *et al.* 2007). The filtrates from each extraction cycle were pooled and the solvent was removed under vacuum at 45°C using a rotary evaporator. The obtained crude extracts were stored in vacuum packs at 4°C until use.

Meat samples

At 42 days of age, 4 birds with body weight near the group average were selected from each replicate (making 12 birds per treatment) and transported to a local commercial slaughterhouse. The broilers were fasted for 12 h before transport. Birds were slaughtered and cooled according to common practices, *i.e.* stunning, bleeding, scalding, de-feathering, eviscerating and cooling. Meat quality measurements were performed on the *Pectoralis major* muscle of broiler breast. For the simulated retail display, meat samples were wrapped in oxygen permeable foil and displayed at 4°C under fluorescent light for 24 h per day for 0, 2 and 4 days.

Carcass weight included the whole slaughtered birds, with neck and feet, but without feathers, head, abdominal fat or giblets (Faria *et al.* 2010). Carcass yield (%) corresponded to the ratio between carcass weight after chilling and body weight at slaughter. Breast yield (%) was calculated as the ratio between the breast weight and carcass weight.

Analysis of pH and water-holding capacity

The pH of meat was measured at 1 and 24 h post-mortem in the breast muscle samples. The water-holding capacity of chicken meat was determined in terms of drip loss, thawing loss and cooking loss, according to Uytterhaegen *et al.* (1994) at 24 h post-mortem.

Color measurements

At 24 h post-mortem, chicken meat color was determined. The color was measured in terms of the CIE-Lab coordinates *L**, *a** and *b** with a Hunterlab Miniscan color meter (D65 light source, 10° standard observer, 45°/0° geometry, 1-inch light surface, white standard) following standard procedures (AMSA, 2012). *Hue* and *Chroma* (saturation index) were calculated as Tan^{-1} (b^*/a^*) and $(a^{*2}+b^{*2})^{1/2}$, respectively (Hunter and Harold, 1987).

Oxidation in breast muscle meat

Lipid oxidation was determined in breast muscle samples on days 0, 2 and 4 of display. The lipid oxidation was assessed spectrophotometrically by the thiobarbituric acid reactive substances (TBARS) method (Tarladgis *et al.* 1960) and was expressed as µg malondialdehyde (MDA) per g meat.

Protein oxidation was assessed by determining the carbonyl contents of the samples on days 0, 2 and 4 of display according to the method of Ganhão *et al.* (2010). Protein carbonyls were measured following their covalent reaction with 2,4-dinitrophenylhydrazine (DNPH). This reaction led to the formation of a stable 2,4-dinitrophenylhydrazone product, which was quantified spectrophotometrically at 370 nm, using a molar absorption coefficient of 21.0/(mM.cm). The total carbonyl content was expressed as nmol DNPH incorporated/mg protein by using the formula from Jongberg *et al.* (2011).

The % metmyoglobin (% MetMb) was determined on days 0, 2 and 4 of display using reflectances measured at the wavelengths 520, 530, 570, 580 and 700 nm according to the formulas of Krzywicki (1979) as modified by Lindahl *et al.* (2010).

Determination of total bacteria and selected bacterial populations in the intestinal contents

For a determination of total bacteria and some selected micro-organisms in intestinal digesta from 12 birds (3 birds per treatment), the contents of ileum and caecum were separately collected, cooled and subjected to microbial assays. The populations of total bacteria, *Escherichia coli* and *Salmonella* spp. were expressed as Log10 CFU/g. A 1 g sample of fresh intestinal contents was added to 9 ml sterilized 0.9 % NaCl (1:10) and then subsequent dilutions were prepared. The total bacterial count was assessed using Nutrient agar. The *Escherichia coli* was cultured on eosin methylene blue agar (EMB) and *Salmonella* spp. were quantified using xylose lysine deoxycholate (XLD) agar and incubation under aerobic conditions for 48 h at 37°C.

Statistical analysis

All data were analyzed for fixed effects of the treatment. Post-hoc tests were performed at a significance level of $P<0.05$ using Duncan's new multiple range test. The analyses were done using SPSS.

RESULTS AND DISCUSSION

Growth performance

As shown in Table 1, daily feed intake was the lowest compared for the control group among the treatment groups ($P<0.05$). On the other hand, final body weight, ADG, FCR and mortality (%) of birds were not significantly different between the treatments ($P>0.05$) during 7 to 42 days of rearing.

The present study analyzed the effects of adding MPE to broiler feed on body weight gain, feed intake, FCR and mortality rate. We found that there were no effects from supplementing MPE on productive performance of broiler chickens. This was supported by the work of Herawati *et al*. (2020) reported that MPE did not have any significantly different effects on body weight gain and FCR of broilers compared with Colistin AGP and it can be considered a useful natural AGP promoting growth and production performance of broilers without contributing to antibiotic resistance of bacterial strains. However, the results of Hidanah *et al.* (2017) demonstrated that the performance of broiler chickens can be enhanced by the addition of mangosteen peel powder (5%) or a combination of mangosteen peel powder (2.5%) and ginger rhizome powder (2.5%) to feed formulations. The lack of significant influence of the MPE on growth performance in this study could be attributed to the composition of the basal diet and/or the environmental conditions as stated by Toghyani *et al.* (2010) that AGP may have more impact when the diet used is less digestible and it is known that well-nourished, healthy chicks that were housed under clean and disinfected conditions do not respond to antibiotic supplements provided.

Carcass yield and meat quality

The results of carcass yield are presented in Table 2. The data show that the carcass yield (%) from the group of birds receiving 0.2% of MPE was higher than the other treatments ($P<0.05$), but there were no significant differences in % breast in carcass ($P>0.05$).

Along with growth performance, the present study also aimed to assess the influences of MPE on the carcass and on breast yields. The data indicate that the carcass yield was higher for the group fed the 0.2% MPE supplemented diet than for the other dietary treatment groups and the mechanism underlying this observation is not fully understood.

Regarding meat quality, it was observed that pH_1 for the group of birds that received 0.2% of MPE was significantly higher than for the others. The drip loss and total water loss of meat significantly decreased with supplemental 0.4 and 0.8% MPE. Moreover, meat yellowness (*b**) with 0 and 0.4% MPE was greater than with 0.2 and 0.8% MPE groups ($P<0.05$). The color saturation index (*Chroma*) of 0.2% MPE was lower than those of 0 and 0.4% MPE groups ($P<0.05$). However, the differences in pH_{24}, cooking loss, *L**, *a** and *Hue* of breast muscles were not significantly different between the groups ($P>0.05$; Table 2).

Table 1: Effects of mangosteen (*Garcinia mangostana*) peel extract in broiler feed on productive performance indicators from 7 to 42 days of age.

Item	Concentration of mangosteen peel extract (%)				SEM	P-value
	0	0.2	0.4	0.8		
Initial body weight (g)	137.50	137.50	137.50	137.50	0.77	1.00
Final body weight (g)	2000.00	2053.33	2000.00	2000.00	19.12	0.75
Average daily gain (g/day)	53.21	54.74	53.21	53.21	0.56	0.77
Daily feed intake (g/day)	93.79^{b}	103.81^{a}	105.47^{a}	101.57^{a}	1.58	0.01
Feed conversion ratio	1.76	1.90	1.98	1.91	0.03	0.09
Mortality rate (%)	2.08	2.08	4.17	4.17	0.67	0.90

^{a,b} Within a row, mean values with different superscripts differ significantly at $P<0.05$

Table 2: Effects of mangosteen (*Garcinia mangostana*) peel extract in broiler feed on carcass yield and meat quality.

Item	Concentration of mangosteen peel extract (%)				SEM	P-value
	0	0.2	0.4	0.8		
Carcass (%)	81.29^{b}	83.33^{a}	81.41^{b}	80.67^{b}	0.32	0.02
Breast (%)	23.61	23.73	22.64	24.13	0.26	0.20
pH_1	5.99^{b}	6.29^{a}	5.98^{b}	5.81^{c}	0.04	0.00
pH_{24}	5.64	5.69	5.71	5.67	0.01	0.32
Drip loss (%)	7.90^{a}	8.17^{a}	6.57^{b}	6.17^{b}	0.22	0.00
Thawing loss (%)	9.50^{a}	8.20^{ab}	7.36^{b}	6.58^{b}	0.33	0.01
Cooking loss (%)	28.73	30.96	28.73	28.34	0.41	0.12
Total water loss (%)	46.12^{a}	47.43^{a}	42.37^{b}	41.80^{b}	0.68	0.00
*L**	58.65	59.43	59.62	59.81	0.33	0.64
*a**	6.84	5.85	6.78	6.71	0.16	0.08
*b**	18.75^{a}	16.99^{b}	17.98^{a}	17.02^{b}	0.19	0.00
Hue	1.22	1.24	1.21	1.20	0.01	0.26
Chroma	19.98^{a}	17.99^{c}	19.24^{ab}	18.32^{bc}	0.20	0.00

^{a,b,c} Within a row, mean values with different superscripts differ significantly at $P<0.05$.

In our study, the pH_1 for the group of 0.8% MPE was lower than others. This could be explained by the differences in the balance of muscle energy metabolism. According to Warriss (2010), which stated that drip loss of meat is related with ultimate pH_{24} and meat with higher ultimate pH will have a lower drip loss and higher water holding capacity. However, in this study, no relationship between ultimate pH_{24} and drip loss was found for the meat samples. In the present study, the drip loss and total water loss were found to be decreased in the treatment groups with 0.4 and 0.8% MPE. This result is in accordance with those reported previously by Kołodziej-Skalska *et al*. (2011), who showed that pigs receiving a plant extracts mixture, including oregano, cinnamon and Mexican pepper which were natural sources of antioxidants had a lower drip loss and cooking loss in M. *longissimus dorsi* muscle.

Oxidation in breast meat

The lipid oxidation in meat allows determining the effects of crude plant extracts in feed on the oxidative stability of meat during cold storage (Table 3). There was no effect of MPE in feed on breast meat lipid oxidation (P>0.05) during 4 days of cold storage. However, the 0.8% MPE dietary treatment tended to reduce lipid oxidation on day 4 of display (P=0.07). The oxidation of lipid, pigment and protein is a major cause of food quality deterioration of muscle meat. Lipid, pigment and protein oxidative processes in meat appear to be linked. The oxidation of one of these leads to the formation of chemical species that can exacerbate oxidation of the others (Chaijan, 2008; Faustman *et al*. 2010; Estévez, 2011). In the present study, meat from 0.8% MPE tended to reduce lipid oxidation by day 4 of display. This might suggest that the supplementation of 0.8% MPE has probably potential to protect against lipid oxidation in broiler filet. This result supports the findings of Lopez-Bote *et al*. (1998), who also reported that the meat from broilers fed on a diet containing spice extracts had lower concentrations of lipid oxidation than meat from the control group. The TBARS method in this study was used to assess the secondary products (MDA) from lipid oxidation. However, the peroxide value that indicates primary products from lipid oxidation should also be assessed to investigate the effects of a diet on oxidative stability of meat during storage. The key factors affecting lipid oxidation in meat are total fat content, fatty acid composition and iron (Fe) (Min *et al*. 2008). Fe is the transitional metal in myoglobin pigment and has been suggested to play important roles as a catalyst and an initiator of lipid oxidation *in vivo* and *in vitro via* the Fenton reaction. This produces hydroxyl radicals (•OH) and ferrylmyoglobin from interactions of H_2O_2 and MetMb. The free radicals and ferrylmyoglobin can remove a hydrogen from a polyunsaturated fatty acid, which initiates lipid oxidation. It is well known that chicken breast has lower myoglobin and fat contents than pork or beef, so chicken meat has the least lipid oxidation among these animals. This may result in no significant difference in lipid oxidation in our study was observed.

Determination of the protein carbonyl content by the DNPH method is the most common procedure to assess protein oxidation in meat and meat products (Estévez, 2011). Protein oxidation is initiated by several reactive oxygen species and is affected by the same factors that also influence lipid oxidation. In the present study, the protein carbonyl contents did not significantly differ between the treatment groups (P>0.05) (Table 3). In contrast, our previous study found that dietary wild betel leaf powder (2%) reduced protein oxidation in chicken meat (Pastsart and Pimapa, 2019).

The oxymyoglobin turns to MetMb, producing an undesirable brown color during storage. The %MetMb formation in meat is shown in Table 3. The results indicate that there was no significant difference in %MetMb on days 2 and 4 of display among the groups (P>0.05). On day 0 of display, % MetMb in 0.8% MPE group was lower than 0 and 0.4% MPE groups (P<0.05).

Total bacteria and selected bacterial populations in the intestinal contents

The colony forming units of total bacteria, *Escherichia coli* and *Salmonella* spp. in the ilium and ceca were not significantly different (P>0.05). However, supplementation of the crude extract at 0.4% in feed slightly tended to reduce the amount of *Salmonella* spp. in the ceca (P=0.15) (Table 4).

Table 3: Effects of mangosteen (*Garcinia mangostana*) peel extract in broiler feed on breast meat oxidation.

Item	Concentration of mangosteen peel extract (%)				SEM	P-value
	0	0.2	0.4	0.8		
TBARS day 0 (µg MDA/g meat)	1.15	1.16	1.06	1.17	0.04	0.73
TBARS day 2 (ìg MDA/g meat)	1.86	1.79	1.73	1.77	0.06	0.89
TBARS day 4 (ìg MDA/g meat)	2.14	2.01	1.90	1.88	0.04	0.07
Protein carbonyls day 0 (nmol/mg protein)	3.16	3.16	3.18	3.07	0.11	0.99
Protein carbonyls day 2 (nmol/mg protein)	6.19	5.55	5.31	4.75	0.24	0.21
Protein carbonyls day 4 (nmol/mg protein)	7.64	8.04	6.70	7.54	0.53	0.93
MetMb day 0 (%)	38.51^{a}	35.83^{ab}	37.11^{a}	32.72^{b}	0.69	0.02
MetMb day 2 (%)	53.70	50.83	49.10	49.91	1.03	0.43
MetMb day 4 (%)	59.88	58.81	62.59	59.74	1.00	0.59

^{a,b,c} Within a row, mean values with different superscripts differ significantly at P<0.05.

Table 4: Effects of mangosteen (*Garcinia mangostana*) peel extract in broiler feed on bacterial quantities in Ileum and Caecum of broilers (Log10 CFU/g).

Item	Concentration of mangosteen peel extract (%)				SEM	P-value
	0	0.2	0.4	0.8		
Ileum						
Total bacteria	6.43	6.70	6.44	6.47	0.27	0.99
Escherichia coli	5.61	6.96	4.20	4.67	0.96	0.81
Salmonella spp.	3.16	6.13	5.26	3.54	0.65	0.35
Caecum						
Total bacteria	6.61	7.11	6.27	6.35	0.18	0.38
Escherichia coli	4.81	6.95	4.25	6.77	0.78	0.57
Salmonella spp.	3.98	7.20	1.97	5.82	0.87	0.15

Salmonella spp. and *Escherichia coli* are the two most important food-borne pathogens of public health in poultry production. This study aimed to determine the effect of MPE on their levels, including total bacteria counts in the intestinal contents. Although it is important to consider that some feed additives originating from plant products have a profound impact on gut microflora either directly or indirectly (Cowan, 1999), this study showed that chickens fed the diets containing plant crude extracts had similar colony counts of total bacteria, *Escherichia coli* and *Salmonella* spp. as the control group on conventional diet.

CONCLUSION

The findings of our study suggest that dietary supplementation with 0.4 or 0.8% MPE can be beneficial to broiler breast meat quality, increasing its water holding capacity and tending to reduce lipid oxidation by day 4 of display. However, dietary supplementation with MPE had no effects on growth performance, mortality, meat protein oxidation, or microbial colony counts in the ileo-cecal digesta.

ACKNOWLEDGEMENT

The authors acknowledge the financial support for this study from the Research and Development Office, Prince of Songkla University, Thailand.

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