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The Effects of Dietary Maoberry (*Antidesma* sp.) Pomace Supplementation on Broiler Growth Performance, Blood Parameters, Carcass Quality and Intestinal Histology

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ABSTRACT

Background: Maoberry (*Antidesma* sp.) pomace (MP) is a waste product and therefore, it is important to exploit this resource as a feedstuff for animals. Hence, the current investigation is aimed to evaluate the effect of dietary supplementation of MP on growth performance, blood parameters, carcass quality and intestinal histology in broilers.

Methods: 288 male (Ross 308), 1-day-old broilers were individually weighed and steel wing tagged before allocation to one of 24 floor pens (12 birds/pen). All birds received the same standard starter diet (7-21 d old) and finisher diet (22-42 d old) to conform with NRC (1994). After week one, six dietary treatments were provided, which contained five different maoberry (*Antidesma* sp.) pomace or MP sources: 0%, 0.1%, 0.2%, 0.3%, 0.4% and 0.5%.

Result: At 42 d, broiler fed 0.1, 0.2, 0.3, 0.4 and 0.5% MP had increased feed efficiency (FE) when compared with broilers on basal diets. Dressing and breast percentages were significantly increased across groups. Broilers fed MP had significantly ($P<0.05$) increased epithelial cell areas and cellular mitosis levels. Supplementing dietary MP exerted positive effects on FE and intestinal histology as they were significantly ($P<0.05$) increased in MP fed birds.

Key words: Broilers, Dressing percentages, Epithelial cell areas, Feed efficiency, Feed intake, Growth, Maoberry (*Antidesma* sp.) pomace, Villus height.

INTRODUCTION

In recent years, researcher have focused on the physiological and biochemical structure and function of natural feed additives, *e.g.* probiotics, organic acids and phytogenic additives (Kaya *et al.,* 2015; Tufarelli *et al.,* 2017; Saleem *et al.,* 2019). However, feed related costs are the main profitability drivers of commercial broiler farms, as feed expenditure accounts for a considerable percentage of livestock production costs, *e.g.* 75%-80% of broiler production (Houndonougbo *et al.,* 2012). Therefore, alternative feed resources have been investigated (Lokaewmanee *et al.,* 2012). The production and processing of fruits into different products, *e.g.* fruit juices, flavours and concentrates, produces large quantities of fruit processing by-products, *i.e.* pomace (Ajila *et al.,* 2015). Various pomace sources from the food industry have been used as alternative feed additives for animal diets (Sirilaophaisan *et al.,* 2015; Vichasilp *et al.,* 2017; Massod *et al.,* 2018) and if correctly treated, these waste materials can be beneficial for both diets and animals. These feedstuffs are relatively cheap, they are waste-products, they create disposal issues for the fruit industry and typically, they are locally available (Mandey *et al.,* 2015).

Antidesma sp. (*i.e.* maoberry in Thailand) is a tropical fruit distributed across northeast Thailand (Hoffmann, 2005). These fruits are believed to be good sources of natural antioxidants (Nuengchamnong and Ingkaninan, 2010) and contain several nutrients including, cathechin, epicatechin, rutin, quercetin, procyanidin B1, procyanidin B2, gallic acid and ferrulic acid (Bulkhup and Samappito, 2008). In Thailand, there are approximately 10 plants processing maoberry products, which produce roughly 70% product from raw material and 30% by-product as waste. Normally, fruit waste have been successfully used for protein enrichment and bioconversion into value-added products, *i.e.* enzymes and other metabolites (Shah and Madamwar 2005). To combat these waste issues, maoberry pomace (MP) can be simply added to the soil as a fertiliser. Equally, several factors also limit the value of maoberry pomace as an animal feed. Maoberry pomace is an abundant source of proanthocyanidins, 97.32 mg-130 mg gallic acid

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equivalent/g of polyphenols (Puangpronpitag *et al.,* 2011) and 11.360 mg/ml anthocyanin (Lokaewmanee, 2016). However, based on these maoberry pomace phytochemical levels, the waste is a good food source for pigs and ducks. Vasupen *et al.* (2011) recommended the addition of 1.5% fresh MP to improve the growth performance and FCR of native (Kadon) pigs. Moreover, Sirilaophaisan *et al.* (2015) suggested that a diet of 0.5% MP could increase the growth performance and decrease cholesterol and triglyceride levels in Cherry Valley ducks.

Natural products, which improve gut health are potential feed alternatives (Rattanawut, 2014). Gut morphology is an important indicator of intestinal health (Zang *et al.,* 2009). Moreover, intestinal villi plays significant roles in the final phase of nutrient digestion and assimilation (Incharoen *et al.,* 2016). For broilers increased physiological performance at the intestinal level could enhance broiler performance (Abdullah *et al.* 2010). Moreover, in tropical and subtropical areas, stress caused by high environmental temperatures is widely recognised as a primary issue for broiler production (Aberra *et al.,* 2005). This has stimulated research to fine new feed supplements can be effectively reduce stress and increase antioxidant capacity in broilers.

Maoberries contain rich sources of natural antioxidants and are a potential source of bioactive phenolic compounds such as polyphenols, flavonoids and anthocyanins (Butkhup and Samappito 2011). Based on this scientific evidence, it is proposed that maoberry pomace could function as a natural feed additive in broiler diets. In a previous study, maoberry pomace supplementation improved egg-laying rates, without negatively affecting egg quality (Lokaewmanee, 2016). However, no reports have yet investigated histological intestinal pathology in broilers fed basal diet supplemented with MP. Therefore, this study is designed to demonstrate the effects of dietary MP on the growth performance, blood parameters, carcass quality and intestinal histology of broilers.

MATERIALS AND METHODS

Animal ethics

Experimental procedures were in accordance with Kasetsart University and Kasetsart University Animal Experimental Committee guidelines and code of practice. Ethical approval was granted before the study commenced. Ethics reference number was ACKU60-ETC-022.

Bird management

This study was conducted at the Department of Agricultural and Resources Faculty of Natural Resources and Agro-Industry, Kasetsart University Chalermprakiat Sakon Nakhon Province Campus, 47000, Thailand. The study was conducted between December, 2019 and August, 2020.

In total, 288 male (Ross 308), 1-day-old broilers were purchased from a commercial hatchery (Charoen Pokaphan Group, Thailand). Upon arrival, broilers were individually weighed and steel wing tagged before allocation to one of 24 floor pens (12 birds/pen), equally distributed open-sided housing, so that each pen had a similar initial total BW. Pens were separated by solid walls to prevent contact between treatment groups. Feed and water were available *ad libitum* throughout the study. Rice husks were used as litter material. The lighting schedule was maintained at 23 light hours: 1 hour dark throughout the study period.

Experimental design and treatments

A randomised study design (4 replicate pens per treatment, with 12 birds per pen) was adopted. All birds received the same standard starter diet (7-21 d old); crude protein: 230 g/kg; metabolizable energy 13.40 MJ/kg and finisher diet (22-42 d old) 200 g/kg; metabolizable energy 13.40 MJ/kg as shown in Table 2. The diets were formulated to conform with National Research Council (1994). After week one, six dietary treatments were provided, which contained five different maoberry (*Antidesma* sp.) pomace or MP sources: 0%, 0.1%, 0.2%, 0.3%, 0.4% and 0.5%.

Table 1: Chemical composition of MP.

Chemical analysis	(g/kg)
Dry matter	965.5
Crude protein	98.9
Crude fibre	29
Crude fat	154.1
Crude ash	35.09
Gross energy (MJ/kg)	18.23

Table 2: Ingredients and nutrient composition (g/kg) of the starter (7-21 days old) and finisher diet (22-42 days old).

Ingredients	Starter diet (g/kg)	Finisher diet (g/kg)
Maize	513	620
Soybean meal	328	250
Fish meal	61	34
Rice bran oil	64	63
Oyster shell	11	11
Di-calcium phosphate	9	8
Salt	4	4
DL-methionine	2	2
Concentrate mixture[1]	8	8
Nutrient composition (g/kg)		
Crude protein	230	200
Crude fiber	40	40
Crude fat	40	60
Calcium	10	8
Available phosphorus	5	4
Metabolizable energy (MJ/kg)	13.40	13.40

[1]Concentrate mixture included (per kg of diet): Trans-retinyl acetate 12,000 IU, cholecalciferol 2,000 IU, DL-α-tocopheryl acetate 12 IU, menadione 1.50 mg, thiamine 1.50 mg, riboflavin 4 mg, pyridoxine 2 mg, cyanocobalamine 15 µg, biotin 0.30 mg, pantothenic acid 10 mg, folic acid 0.5 mg, nicotinic acid 60 mg, copper 6 mg, manganese 60 mg, zinc 60 mg, iron 20 mg, preservative 6.25 mg and feed supplement 25 mg.

Measured traits

MP was analysed for proximate constituents (AOAC, 2006) and gross energy using Bomb calorimetry (Table 1). FI, body weight gain (BWG) and FE in each replicate were recorded at days 7, 14, 21, 28, 35 and 42. The weight of dead broilers was determined and their BW and FI data included in FE calculations per pen.

At the end of the study (day 42), blood samples were collected from the basilica vein of eight broilers per treatment (two birds/replicate). Blood was collected in blood tubes and centrifuged at 2000 g for 15 min. After this, the serum was decanted into sterile vials and stored at -20°C until chemical analysis. The triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), haemoglobin and haematocrit levels were analysed using chemistry analysis kits (Human Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany) while blood counts and white blood cell types (*i.e.* heterophils, lymphocytes, monocytes, eosinophils and basophils) were analyzed using the Hemavet Multi-Species Haematology System (Drew Scientific, Inc., Oxford, CT, USA). Heterophil: lymphocyte (H:L) ratios were also calculated using a previously described method (Cotter, 2015).

Carcass quality

At the end of the feeding experiment, 12 birds from each group were weighed individually and killed by decapitation under light anaesthesia with diethyl ether. The digestive organs were carefully removed. Their feathers removed to eliminate feather weight and the head, viscera and shanks were also removed. The carcass was left for 1 h to drain excess water and then weighed. Thighs, drumsticks, wings and breasts were removed and weighed individually. Total visceral organs and abdominal fat were carefully excised and weighed individually.

Tissue collection and histological analyses

A gut segment (approximately 2 cm) from the middle of the duodenum was excised, rinsed in cold physiological saline (0.9%) and immediately placed in Bouin's fluid. Thereafter, samples were transferred into 70% ethanol within 24 h. Samples were then embedded in paraffin and sliced into 5 µm sections for intestinal histological examination. Six cross-sections per broiler were processed using standard hematoxylin and eosin staining, as described by Owusu-Asiedu *et al.* (2002). Villus height was measured from the tip of the villus to the crypt-villus junction. In total, 16 villi were examined from different sections in each broiler. The area of the villus was calculated using villus height, basal width and apical width; villus area = (basal width + apical width) × villus height. This approach was based on Iji *et al.* (2001). Epithelial cell area was measured at the middle part of the villus and divided by the number of cell nuclei. In total, eight sections were counted per broiler. The number of mitotic cells having homogeneous, strongly-stained basophilic nuclei was also determined (Tarachai and Yamauchi, 2000). Total mitotic cell numbers were counted from four different sections. The mean value from four broilers in each group was expressed as mean cell mitosis. Villus height, villus area, epithelial cell area and cell mitosis numbers were processed using an image analyser (Nikon Cosmozone IzS, Nikon Co., Ltd., Tokyo, Japan).

Statistical analysis

Data were analysed using PROC MIXED in SAS (version 9.2; SAS Inst. Inc., Cary, NC, USA) using the following statistical model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where,

Y_{ij} = observation in the j^{th}, μ = general mean, T_i = fixed MP level effects and e_{ij} = random error component in the $(i \cdot j)^{th}$ unit. Data differences were considered statistically significant at a probability level of 5% ($P<0.05$).

RESULTS AND DISCUSSION

Bird performance

Overall, no differences were observed for FI and BWG. Broilers fed with 0.5% dietary MP had a statistically higher FE ($P<0.01$) when compared with those fed 0%, 0.1%, 0.2%, 0.3% and 0.4% dietary MP (Table 3).

Blood parameters

No differences ($P>0.05$) were observed for triglyceride, HDL, LDL, haemoglobin, haematocrit, heterophils, lymphocytes, monocytes, eosinophils, basophils and H:L ratios (Table 4).

Carcass quality

Dressing and breast percentages were significantly increased in the 0.5% dietary MP group ($P<0.05$). Total visceral organ weight, thighs, drumsticks, wings, breast and abdominal fat were not significantly different ($P>0.05$) between groups (Table 5).

Table 3: Feed intake (FI), body weight gain (BWG) and feed efficiency (FE) in broilers given 0%, 0.1%, 0.2%, 0.3%, 0.4% and 0.5% dietary MP during the study (7-42 days old; mean ± SEM, N = 4).

	MP percentages							
	0%	0.1%	0.2%	0.3%	0.4%	0.5%	SEM	P-value
FI (g)	4758	4301	4747	4165	4158	4070	62.58	0.57
BWG (g)	2853	2853	2842	2826	2880	2837	22.17	0.21
FE	0.60^{b}	0.60^{b}	0.65^{b}	0.68^{b}	0.69^{b}	0.71^{a}	0.04	0.01

^{a,b}Data in the same row with different superscripts are significantly different ($P<0.05$).

Light microscopy observations

The villus height and villus area of all intestinal segments did not change in all groups (P>0.05). Duodenal and jejunal cell areas and cell mitosis in the ileum were significantly increased in 0.3%, 0.4% and 0.5% dietary MP groups (P<0.05). Cell mitosis in the duodenum was significantly increased in the 0.4% and 0.5% dietary MP groups (P<0.05), whereas cell mitosis in the jejunum was significantly increased in the 0.1%, 0.2%, 0.3%, 0.4% and 0.5% dietary MP groups (P<0.05; Table 6).

The present study investigated the effects of feeding MP on the growth performance, blood parameters, carcass quality and intestinal histology in broilers. Results indicated no significant differences (P>0.05) in growth performance parameters, except FE. For poultry farmers, it is very important to produce chicken meat economically, without using antibiotics (Khonyoung *et al.,* 2011). The FE of MP groups was improved when compared with the 0% MP (control group) and was optimal in the 0.5% MP group which might be attributed to total polyphenolics in MP. The total polyphenolics identified in raw mao seeds and marc materials were 120.68-161.22 and 40.55-52.68 mg gallic acid eqivalents (GAE)/g, respectively (Puagpronpitag *et al*., 2008). Additionally, mao seeds and mao marcs showed similar antioxidant levels as grape seed proanthocyanidin extracts. Antioxidant activity mechanisms in mao seed and marc extracts are likely to be activated from free radical scavenging by 2,2-diphenyl-1-picrylhydrazyl (DPPH) or 2,2-azobis (3-ethylbenzothialzoline-6-sulfonic acid) (ABTS) radicals and may be directly mediated by polyphenolic compounds in mao seed and marc (Puangpronpitag *et al.,* 2011).

The present study indicated that feeding 0.5% dietary MP had resulted in increased body weight gain and was related to dressing and breast percentages. This observation may be related to several nutrient components present in MP (Table 1), including bioactive constituents such as carotenoids, phytosterols, vitamin C, total phenolics, antioxidant molecules (Judprasong *et al.,* 2013), anthocyanins and flavonoids (Chaikham *et al.,* 2016). Puangpronpitag *et al.* (2011) reported that maoberry seed and skin-pulp residue extracts exerted anti-apoptotic and anti-inflammatory effects in human breast epithelial cells and also displayed inhibitory effects against some pathogenic

Table 4: Triglyceride concentrations (mg/dl), high density lipoprotein; HDL (mg/dl), low density lipoprotein; LDL (mg/dl), haemoglobin concentration (g/100 ml), haematocrit concentration (%), counts and type of white blood cells (WBC; × 10^3/µL) and heterophils: lymphocytes ratios in broilers given 0% 0.1%, 0.2%, 0.3%, 0.4% and 0.5% dietary MP during the study (7-42 days old; mean ± SEM, N=4).

	MP percentages							
	0%	0.1%	0.2%	0.3%	0.4%	0.5%	SEM	P-value
Triglyceride	26.10	23.51	23.75	23.02	24.09	24.16	7.46	0.45
HDL	75.25	77.50	76.00	81.00	86.00	81.00	4.19	0.48
LDL	42.69	48.64	82.38	41.30	51.68	47.60	8.58	0.61
Haemoglobin	7.16	6.50	7.41	6.83	6.86	8.33	1.60	0.25
Haematocrit	21.50	20.50	20.25	20.50	20.50	20.00	0.53	0.25
White blood cells								
Heterophils	9.00	8.75	8.50	8.75	9.75	8.25	0.65	0.95
Lymphocytes	13.50	16.00	15.00	15.25	15.50	15.50	0.04	0.65
Monocytes	1.25	1.25	1.25	1.00	1.00	1.25	0.23	0.99
Eosinophils	1.75	1.50	1.00	1.50	1.75	1.50	0.08	0.83
Basophils	2.50	2.50	2.25	2.50	2.00	2.50	0.10	0.65
H:L ratio	0.67	0.55	0.57	0.56	0.63	0.53	0.02	0.21

Table 5: Dressing percentages; weight of visceral organs, thighs, drumsticks, wings, breasts and abdominal fat weight in broilers given 0%, 0.1%, 0.2%, 0.3%, 0.4% and 0.5% dietary MP during the study (7-42 days old; mean±SEM, N=4).

	MP percentages							
	0%	0.1%	0.2%	0.3%	0.4%	0.5%	SEM	P-value
Dressing (%)	78.81^{b}	78.93^{ab}	79.00^{ab}	79.15^{ab}	78.93^{ab}	79.13^{a}	0.22	0.03
Total visceral organ weight (% BW)	8.66	8.49	8.35	8.73	8.34	8.67	0.24	0.44
Thighs (% BW)	13.39	13.32	13.30	13.47	12.73	12.38	0.35	0.16
Drumsticks (% BW)	11.97	11.85	11.47	11.61	11.46	11.71	0.19	0.39
Wings (% BW)	9.23	8.92	9.10	9.10	9.14	9.06	0.19	0.91
Breasts (% BW)	18.42^{b}	20.78^{ab}	20.67^{ab}	20.83^{ab}	20.97^{ab}	22.11^{a}	0.57	0.02
Abdominal fat (% BW)	1.18	1.07	1.06	1.26	1.27	1.25	0.09	0.36

[a,b]Data in the same row with different superscripts are significantly different (P<0.05).

Table 6: Villus height and area, cell area and cell mitosis of duodenum, jejunum and ileum sections in broilers given 0%, 0.1%, 0.2%, 0.3%, 0.4% and 0.5% dietary MP during the study (7-42 days old; mean±SEM, N=4).

	MP percentages							
	0%	0.1%	0.2%	0.3%	0.4%	0.5%	SEM	P-value
Villus height (mm)								
Duodenum	1.41	1.47	1.50	1.51	1.51	1.54	0.14	0.98
Jejunum	0.98	1.09	1.10	1.13	1.15	1.14	0.07	0.57
Ileum	0.56	0.57	0.57	0.57	0.61	0.61	0.02	0.58
Villus area (mm^2)								
Duodenum	0.20	0.18	0.24	0.21	0.27	0.25	0.03	0.26
Jejunum	0.10	0.15	0.15	0.13	0.15	0.16	0.01	0.14
Ileum	0.06	0.07	0.07	0.06	0.08	0.08	0.01	0.15
Cell area (μm^2)								
Duodenum	248.53^{c}	269.67^{b}	268.92^{b}	288.62^{a}	283.24^{a}	285.64^{a}	28.08	0.01
Jejunum	162.97^{c}	164.23^{c}	173.55^{b}	187.24^{a}	182.59^{a}	189.52^{a}	12.62	0.02
Ileum	128.28	130.73	132.35	131.75	133.04	133.91	4.70	0.12
Cell mitosis (number)								
Duodenum	711.00^{d}	728.31^{cd}	722.81^{cd}	738.00^{c}	756.88^{b}	804.44^{a}	9.42	0.01
Jejunum	593.94^{d}	618.96^{c}	638.69^{b}	650.13^{b}	671.81^{a}	681.56^{a}	6.32	0.01
Ileum	391.06^{b}	394.50^{b}	403.37^{b}	435.94^{a}	446.25^{a}	448.06^{a}	5.58	0.01

^{a,b,c,d}Data in the same row with different superscripts are significantly different (P<0.05).

and spoilage bacteria. Kukongviriyapan *et al.* (2013) showed that MP supplementation reduced blood pressure and improved hemodynamics in hypertensive induced rats. However, there is a dearth of data on broilers fed MP, with associated analyses of intestinal histology, specifically the intestinal villi. Duodenal and jejunal cell areas and cell mitosis in all small intestinal sections were significant result in broilers fed a 0.5% MP diet. Intestinal villus height, cell area and cell mitosis were reportedly related to intestinal function from ingested feed (Yamauchi, 2007). Moreover, cell mitosis in the intestine was proposed as an indicator of increased villus function (Langhout *et al.,* 1999).

From the present study, it is observed that an increased cell area and cell mitosis in the duodenum, jejunum and ileum was accompanied by increased FE. In this study, MP contained 29 g/kg fibre. Greater epithelial cell area and cell mitosis number observed may be attributed to the differences in physiochemical characteristics of MP and MP supplementation levels. Increased dietary fibre consumption may increase the epithelial cell area required for nutrient absorption (Cassidy *et al.,* 1982). Jin *et al.* (1994) also observed that high dietary fibre levels altered the intestinal morphology in growing pigs. Some reports also have indicated the favourable effects of moderate insoluble fibre doses in the diet (Adibmoradi *et al*., 2016). The inclusion of dietary fibre may improve HCL (Jiménez-Moreno *et al.,* 2010), bile acids, enzyme secretion (Hetland *et al.,* 2002), gizzard function (Hetland *et al.,* 2005), nutrient digestibility and performance traits in broilers (Adibmoradi *et al*., 2016). The presence of increased epithelial cell area and cell mitosis number might be stimulated by the combination of phytochemical and pharmacological potential of MP and physiochemical characteristics of fibre in MP. These observations suggest that MP can be supplemented to basal feed at 0.5% doses, to improve FE, dressing and breast percentages. Similarly, these improvements were more than likely due to the morphological changes observed in these broilers at the intestinal level.

CONCLUSION

In conclusion, supplementation of maoberry pomace (MP) in broiler diets resulted in enhanced feed efficiency and increased dressing and breast percentages. Increased cell area and mitosis were observed in the intestines of 0.5% MP-fed birds. These fundings suggest that MP can be used up to a level of 0.5% (5 g/kg) in broiler diets.

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