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Effects of Dietary Supplementation with Peanut Skin and Bromelain on Egg Production, Blood Parameters and Liver Fat Accumulation in Middle-age and Aged Laying Hens

Jhy-Ming Li[1], Hsin-Tai Hong[2], Chean-Ping Wu[1]

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ABSTRACT

Background: This study examined the effects of a diet supplemented with peanut skin and bromelain on egg production, egg quality, blood parameters and fat accumulation in middle-aged and aged laying hens.

Methods: In Trial 1, 120, 62-week-old Hisex Brown laying hens were grouped into five treatments: A control and high and low concentrations of peanut skin and bromelain groups. In Trial 2, 96, 93-week-old Hisex Brown laying hens were grouped into four groups: control, high concentrations of peanut skin and bromelain and a peanut skin and bromelain combination. Egg production and quality were monitored over an 8-w period and included egg weight, shell thickness and yolk color. Blood samples were analyzed for lipid profiles and liver function. Fat accumulation was observed in the abdominal cavity.

Result: Egg production of hens receiving supplementation of peanut skin and bromelain improved substantially in 62-w-old hens (but not in 93-w-old hens) compared with the control group. Substantially reduced blood glucose levels and beneficial changes in yolk color were observed in the supplemented groups. The addition of peanut skin and bromelain to the diet positively affects the productivity and health of middle-aged laying hens.

Key words: Bromelain, Dietary supplementation, Laying hens, Peanut skin.

INTRODUCTION

Laying hens play a crucial role in agriculture and the global economy because their eggs serve as a vital source of essential nutrients for humans. Eggs are among the most affordable sources of animal protein worldwide (Bain *et al*., 2016). However, the production of older laying hens is associated with an increased incidence of broken eggs, resulting in considerable economic losses for the animal husbandry industry. Aging is a complex process molded by various environmental factors and molecular pathways; however, the regulatory mechanisms remain poorly understood. The primary reasons for chicken flock replacement are a decline in egg production and deterioration of eggshell quality, a trend often observed at approximately 72 w of age (Bain *et al*., 2016). In addition to a reduction in egg quantity and quality, various organs such as the ovaries age during egg production, (Liu *et al*., 2018), increasing the likelihood of the chickens developing osteoporosis (Beck and Hansen, 2004), diminished liver function and impaired feather conditioning (Su *et al*., 2006).

Fatty liver, also known as fatty liver hemorrhagic syndrome (FLHS), is a metabolic disorder that interferes with fatty acid metabolism in the liver and the synthesis and transport of low-density lipoproteins. FLHS is characterized by the accumulation of excessive intra-abdominal fat, a pale and fragile liver that may lack structural integrity and, in severe cases, liver rupture leading to massive intra-abdominal bleeding (Rozenboim *et al*., 2016). This syndrome commonly affects laying hens and causes abnormal accumulation of fat in the liver. These consequences often include reduced egg production, liver rupture and, in extreme cases, mortality. The factors contributing to FLHS are diverse and include genetics, nutrition, hormones and the environment (Squires and Leeson, 1988).

Peanuts (*Arachis hypogaea*) are technically classified as legumes and belong to the family Fabaceae. Although peanuts are legumes, they are commonly classified as an oilseed because of their high oil content. Peanuts are rich in protein, oil and fiber. Peanut skin (PS), in particular, is rich in antioxidants, such as anthocyanidins, catechin, epicatechin and resveratrol, which can decrease the rate of free radical-induced oxidation. PS is composed of cellulose (40.5%), hemicellulose (14.7%) and lignin (26.4%)

[1]Department of Animal Science, National Chiayi University, Chiayi City, Taiwan.
[2]Division of Animal Industry, Animal Technology Laboratories, Agricultural Technology Research Institute, Miaoli, Taiwan.

Corresponding Author: Jhy-Ming Li; Chean-Ping Wu, Department of Animal Science, National Chiayi University, Chiayi City, Taiwan. Email: jml@mail.ncyu.edu.tw; wcp@mail.ncyu.edu.tw
ORCID: 0009-0008-2786-6222

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(Bharthare *et al*., 2014). Toomer *et al*. (2019), showed that PS does not affect production performance or egg quality in 40-w-old Hy-Line W36 hens (Toomer *et al*., 2021).

Bromeliads (*Ananas comosus*) belong to the family Bromeliaceae and are known for their high bromelain content. Bromelain exhibits various beneficial properties including anticancer (Pillai *et al*., 2014), antithrombotic (Metzig *et al*., 1999) and anti-inflammatory effects (Sahbaz *et al*., 2015). Bromelain, derived mainly from pineapple stems, is a cysteine endopeptidase primarily associated with protein hydrolysis (Hale, 2004). Bromelain contains nine enzymes with proteolytic activities, including stem and fruit bromelain, bromelain phosphatase, glycosidase, peroxidase, cellulase, glycoprotease and ribonuclease, (Chobotova *et al*., 2010). Yenice *et al*. (2019) reported that bromelain does not affect laying performance or egg quality but could increase the blood concentration of antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase, in laying hens (Yenice *et al*., 2019). Similarly, Yan *et al*. (2020) used microorganisms to ferment pear peel waste, which was then added to the diet of laying hens. They found that bromelain had no discernible effect on the egg quality of laying hens (Yan *et al*., 2020).

This study aimed to examine the effects of diets supplemented with PS and bromelain on egg production, blood characteristics and fatty acid accumulation in middle-aged and aged laying hens.

MATERIALS AND METHODS

Animal treatment and experimental design

In Trial 1 and Trial 2, 120, 62-w-old and 96, 93-w-old Hisex Brown laying hens were purchased from a commercial breeding farm and housed in cages. In Trial 1, the hens were randomly assigned to five groups with different dietary treatments: normal control (NC) (Supplementary Table 1), high PS supplementation (HPS; 5 g/kg), low PS supplementation (LPS; 2.5 g/kg), high-bromelain (HBN; 50 g/kg) supplementation and low-bromelain (LBN; 25 g/kg) supplementation groups. In Trial 2, the hens were randomly assigned to four groups with different dietary treatments: basal diet (normal control, NC), high PS supplementation (HPS; 5 g/kg), high-bromelain (HBN; 50 g/kg) supplementation and a combination of PS powder (2.5 g/kg) and bromelain (25 g/kg) (PS and BN mixed; PSBNM) supplementation groups. Each treatment was performed in triplicate, with eight hens per replicate. The basal diet was purchased from a commercial feed mill and formulated according to the requirements of the laying hens. The hens had *ad libitum* access to food and water.

Egg production performance and quality

During the 8-w study period, feed consumption was recorded bi-weekly and egg production was documented daily. Egg production was expressed as the average daily production per hen, calculated by dividing the total number of eggs laid by the number of days and averaged per treatment. The egg production rate, feed-to-egg ratio and daily feed intake were calculated for each experimental phase and for the overall trial period. There were three replicates for each group and eight eggs per replicate were selected for egg quality analysis. The egg weight, Haugh unit (HU) and yolk color were determined using an egg multi-tester (Touhoku Rhythm Co. Ltd., Tokyo, Japan); eggshell breaking strength was assessed with an eggshell force gauge (Model II, Robotmation Co., Ltd., Tokyo, Japan); and eggshell thickness was measured using a dial pipe gauge (Ozaki MFG. Co., Ltd., Tokyo, Japan), considering the average thickness from the rounded, pointed and the middle ends and excluding the inner membrane.

Collection and examination of blood specimens

At the end of Trials 1 and 2, a 25G, 1.6 cm needle was used to collect blood samples from the wing veins of the 120 and 96 laying hens, respectively. The serum samples were separated by centrifugation at 3000 × g for 20 min at 4°C.

Supplementary Table 1: Ingredient and composition of the basal diet.

Ingredient, %	Basal diet
Corn meal	55.25
Full-fat Soybean meal, 45%	10.00
Dehulled soybean meal	16.00
DDGS[1]	4.00
Calcium carbonate (powders)	4.00
Calcium carbonate (particles)	4.50
Dicalcium Phosphate	0.50
Salt	0.20
sodium bicarbonate	0.10
Fish meal (Peru), 65%	1.50
Oyster shell	2.50
DL-Methionine 99%	0.30
Vitamin premix[2]	0.10
Mineral premix[3]	0.05
Palm oil	1.00
Calculated contents of feed	
CP,%	16.6
Metabolizable energy, MJ/kg 1	2800
Methionine, % 0.5	0.50
Methionine + Cystine, % 0.76	0.78
Lysine, % 0.83	0.88
Ca, % 4	4.70
Available P, % 0.4	0.48

[1]DDGS: Corn distiller's dried with solubles.

[2]Vitamin premix suppled per kilogram of diet: Vitamin A: 35,000,000 IU; Vitamin D_3: 13,500,000 IU; Vitamin E: 80,000 IU; Vitamin K: 8 g; Vitamin B1: 8 g; Vitamin B2: 20 g; Vitamin B6: 15 g; Vitamin B12: 80 mg; Niacin: 120 g; Pantothenate: 35 g; Folic acid: 4 g; Biotin: 400 mg.

[3]Mineral premix suppled per kilogram of diet: Fe ($FeSO_4 \cdot 7H_2O$, 20% Fe), 76 mg; Mn ($MnSO_4H_2O$, 32% Mn), 76 mg; Cu ($CuSO_4 \cdot 5H_2O$, 25% Cu), 12 mg; Zn (ZnO, 78%, Zn), 67 mg; Se ($NaSeO_3$, 2%Se), 0.3 mg. I (KI, 68% I) 1 mg; Co ($CoCO_3$) 0.25 mg.

Aliquots were stored at -80°C until analyzed for blood traits using colorimetric analysis on autoanalyzer (Automatic Biochemical Analyzer, RA-1000; Bayer Corp., Tarrytown, NY, USA).

Carcass traits and liver fat accumulation

At the end of Trials 1 and 2, six animals were randomly selected from each treatment group and their live weights were measured. Carcass data, including the weights of the carcass, wet liver, abdominal fat, ovaries, total reproductive tract, as well as the weight of other organs, were measured post-slaughter. Liver samples were scored using the fatty liver index (Supplementary Fig 1).

Statistical analysis

All groups were analyzed using a two-way analysis of variance, followed by Tukey's post-hoc test for pairwise comparison. The results in all experiments were considered significantly different at a *p*-value <0.05. Pearson's correlation coefficients were calculated to investigate the relationships between the physicochemical characteristics of peanut and bromelain and egg production, egg quality and blood biochemistry parameters of laying hens, with probabilities considered significant at the 5% level ($p<0.05$).

RESULTS AND DISCUSSION

Production performance

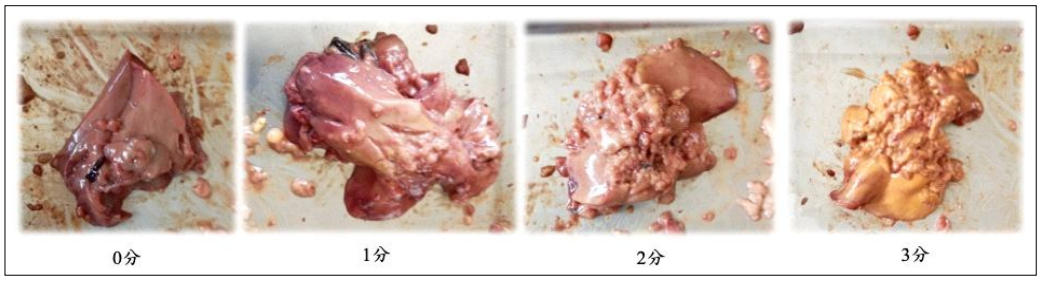
The effects of dietary supplementation with PS and/or bromelain on the egg production of 62- and 93-w-old laying hens are presented in Table 1 and 2. HPS and HBN supplementation resulted in an increase ($p<0.05$) in the daily feed intake of the 62-w-old laying hens (Table 1). Additionally, LPS and HBN increased ($p<0.05$) egg production and the number of eggs laid daily. There were no differences in average feed intake ($p>0.05$) among the 93-w-old laying hens (Table 2). However, hens in the HPS (but not in the HBN) group, had higher feed-to-egg ratios and lower egg production and daily laying rates compared to those in the NC group ($p<0.05$). Supplementing dietary intake with 5 g/kg PS led to decreased performance in hens in certain cases; however, this decrease was not observed in the bromelain group.

Egg quality

The effects of a dietary supplemented with PS and/or bromelain on egg quality in middle-aged and aged laying hens are presented in Supplymentary Table 2 and Table 3. Compared to the control group, there were no differences in albumen height, Haugh unit, eggshell strength, eggshell thickness, yolk height, yolk diameter, or yolk index among all groups ($p>0.05$) of the 62-and 93-w-old laying hens. However, dietary supplementation with PS and bromelain resulted in yellower yolk color compared to the NC group ($p<0.05$) in 62- and 93-w-old laying hens. The egg weight of the HBN group decreased ($p<0.05$) in the 93-w-old laying hens. PS and bromelain had no effect on most egg quality parameters in middle-aged and aged laying hens but enhanced yolk color.

Blood biochemistry parameters

The effects of a diet supplemented with PS and/or bromelain on blood plasma biochemical parameters of middle-aged and aged laying hens are presented in Table 4



Supplementary Fig 1: The standard for the fatty liver score used in the examination of fatty liver in this study was determined based on a color scale ranging from 1 to 4.

Table 1: Effects of peanut skin and/or bromelain on production performance in 62-week-old laying period.

Items[1]	NC	HPS	LPS	HBN	LBN	P value
Daily feed intake (g/hen/d)	104.44±7.13^{b}	118.96±0.55^{a}	111.95±2.40^{ab}	119.06±1.20^{a}	114.39±3.58^{ab}	0.0165
Egg production (%)	0.94±0.02^{b}	0.96±0.01^{ab}	0.99±0.004^{a}	0.98±0.01^{ab}	0.97±0.02^{ab}	0.0419
Daily laying egg	60.26±1.02^{b}	62.47±0.39^{ab}	64.35±0.30^{a}	64.33±0.64^{a}	61.95±1.30^{ab}	0.0026
Feed-to-egg-ratio(g of feed/g of egg)	1.73±0.09	1.90±0.003	1.74±0.04	1.85±0.03	1.85±0.10	0.0897

Abbreviations: NC- Normal control; HPS- High concentration peanut skin powder (5 g/100 kg); LPS- High concentration peanut skin powder (2.5 g/100 kg); HBN- High concentration bromelain (50 g/100 kg); LBN- Low concentration bromelain (25 g/100 kg).

^{A,b} Means within a row lacking a common superscript differ significantly ($p<0.05$).

[1]Each mean represents 8 layers/replicate, 3 replicates/treatment.

and Supplymentary Table 3. Compared with the control group, there were no differences in glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), total protein (TP), albumin, globulin, albumin/globulin ratio (A/G), creatinine and triglyceride (TG) among all groups (p>0.05) of the 62- and 93-w-old laying hens. However, the HBN and LBN group showed an increase in blood urea nitrogen (BUN) content and low density lipoprotein-cholesterol (LDL-C) levels in the 62-w-old laying hens (p<0.05).

Table 2: Effects of peanut skin and/or bromelain on production performance in 93-week-old laying period.

Items[1]	NC	PS	BN	PSBNM	P value
Daily feed intake (g/hen/d)	100.47±12.74	96.32±15.01	98.55±11.91	98.75±19.07	0.2494
Egg production (%)	59±0.12^{ab}	52±0.1^{c}	58±0.12^{b}	62±0.12^{a}	<.0001
Daily laying egg	40.36±8.47^{a}	33.95±6.9^{c}	37.37±7.98^{b}	42.19±8.56^{a}	<.0001
Feed-to-egg-ratio (g of feed/g of egg)	2.49±0.77^{bc}	2.84±0.8^{a}	2.64±0.63^{ab}	2.34±0.78^{c}	0.0024

Abbreviations: NC- Normal control; PS- Peanut skin powder (5 g/100 kg); BN- Bromelain (50 g/100 kg); PSBNM- Peanut skin and bromelain mixed (2.5 g peanut skin powder+25 g bromelain).

^{A,b,c} Means within a row lacking a common superscript differ significantly (p<0.05, 0.001).

[1]Each mean represents 8 layers/replicate, 3 replicates/treatment.

Table 3: Effects of peanut skin and bromelain on egg quality in 93-week-old laying period.

Items[1]	NC	PS	BN	PSBNM	P value
Egg weight (g)	68.3±4.56^{a}	65.4±5.12^{ab}	64.7±4.73^{b}	68.0±5.95^{ab}	0.037
Albumen height (mm)	10.1±1.22	9.6±0.95	9.7±1.01	9.7±0.78	0.5424
Yolk color	7.6±0.57^{c}	8.4±0.68^{b}	8.7±0.59^{b}	9.5±0.44^{a}	<.0001
Haugh unit	97.8±5.6	96.4±4.44	96.6±5.16	96.2±3.5	0.7894
Eggshell strength (N)	2.93±1.08	3.37±0.94	2.66±0.96	3.40±1.26	0.0574
Eggshell thickness (um)	0.36±0.04	0.38±0.05	0.34±0.04	0.35±0.04	0.2724
Yolk height (mm)	21.0±1.27	19.9±1.4	20.0±0.73	20.5±1.11	0.4474
Yolk diameter (mm)	43.5±1.72	43.2±3.28	44.5±2.21	43.4±1.88	0.3441
Yolk index	0.483±0.03	0.465±0.07	0.451±0.03	0.472±0.03	0.2611

Abbreviations: NC- Normal control; PS- Peanut skin powder (5 g/100 kg); BN- Bromelain (50 g/100 kg); PSBNM- Peanut skin and bromelain mixed (2.5 g peanut skin powder+25 g bromelain).

^{A,b,c}Means within a row lacking a common superscript differ significantly (p<0.001).

[1]Each mean represents 8 layers/replicate, 3 replicates/treatment.

Table 4: Effects of peanut skin and bromelain on blood plasma biochemistry parameters in 62-week-old laying period.

Items[1]	NC	HPS	LPS	HBN	LBN	P value
Glu-Ac (mg/dl)	217.70±6.28	225.17±11.11	217.33±5.07	220.00±9.08	226.17±10.47	0.1477
GOT (U/L)	178.10±11.20	183.58±16.30	185.25±19.74	195.00±15.62	190.42±26.48	0.3160
GPT (U/L)	1.50±0.50	1.83±0.69	2.17±0.55	2.17±0.55	1.92±0.64	0.2528
T-P (g/dl)	5.91±0.57	5.64±0.53	5.67±0.32	5.83±0.31	5.93±0.39	0.5527
Alb (g/dl)	2.38±0.12^{b}	2.53±0.20^{a}	2.58±0.12^{a}	2.63±0.13^{a}	2.61±0.10^{a}	0.0002
Globulin (g/dl)	3.53±0.50	3.11±0.39	3.08±0.26	3.19±0.25	3.33±0.36	0.2202
A/G	0.67±0.09^{b}	0.82±0.09^{a}	0.86±0.08^{a}	0.83±0.07^{a}	0.79±0.10^{ab}	0.0407
BUN (mg/dl)	0.23±0.15^{b}	0.44±0.25^{ab}	0.37±0.23^{ab}	0.71±0.44^{a}	0.47±0.27^{ab}	0.0184
Creatinine (mg/dl)	0.20±0.03	0.21±0.01	0.19±0.02	0.22±0.02	0.19±0.02	0.0838
TG (mg/dl)	1831.10±248.52	1601.92±407.84	1837.33±284.43	1763.08±388.64	2023.58±510.72	0.1423
LDL-C (mg/dl)	11.30±2.61^{ab}	14.08±4.27^{ab}	10.50±3.07^{b}	13.25±3.42^{ab}	15.58±3.75^{a}	0.0097

Abbreviations: NC- Normal control; HPS- High concentration peanut skin powder (5 g/100 kg); LPS- High concentration peanut skin powder (2.5 g/100 kg); HBN- High concentration bromelain (50 g/100 kg); LBN- Low concentration bromelain (25 g/100 kg).

^{A,b}Means within a row lacking a common superscript differ significantly (p<0.05, 0.001).

[1]Each mean represents 8 layers/replicate, 3 replicates/treatment.

Carcass traits and fatty liver

The effects of dietary supplementation with PS and/or bromelain on the carcass traits of middle-aged and aged laying hens are presented in Supplymentary Table 4 and Table 5. There were no differences in liver, abdominal fat, carcass, ovarian, reproductive tract weights, or other organ weights among all groups (p>0.05) when compared to the control group. The highest wet liver weight was observed in

Table 5: Effects of peanut skin and/or bromelain on carcass traits in 93-week-old laying period.

Items	NC	PS	BN	PSBNM	P value
Live weight (g)	1982.26±254.37	1953.72±263.86	2031.57±201.65	1969.58±191.2	0.7115
Wet weight of Liver (g)	39.61±7.09^{b}	41.89±7.75^{b}	44.64±10.05^{a}	41.63±6.77^{b}	<0.001
Abdominal fat weight (g)	41.13±25.54	46.04±17.56	54.71±23.53	47.16±24.3	0.2801
Other organs weight (g)	402.26±81.12	421.9±72.53	435.09±85.72	417.92±57.54	0.5399
Carcass weight (g)	1354.34±123.18	1303.77±187.86	1423.85±125.14	1367.83±133.49	0.2081
Ovarian weight (g)	33.78±10.84	38±14.29	36.14±16.43	36.45±12.27	0.7712
Reproductive tract weight (g)	167.69±37.24	110.36±28.12	107.95±29.79	113.41±20.99	0.2717

Abbreviations: NC- Normal control; PS- Peanut skin powder (5 g/100kg); BN- Bromelain (50 g/100 kg); PSBNM, peanut skin and bromelain mixed (2.5 g peanut skin powder+25 g bromelain).

^{A,b} Means within a row lacking a common superscript differ significantly (p<0.001).

[1]Each mean represents 8 layers/replicate, 3 replicates/treatment.

Supplymentary Table 2: Effects of peanut skin and bromelain on egg quality in 62-week-old laying period.

Items[1]	NC	HPS	LPS	HBN	LBN	P value
Egg weight (g)	63.8±5.38	64.85±5.07	65.26±5.10	65.74±5.07	63.73±5.56	0.2580
Albumen height (mm)	8.15±1.51	7.59±1.94	7.58±1.70	7.34±1.46	7.33±1.34	0.1318
Yolk color	6.25±0.50^{b}	6.75±0.45^{a}	6.56±0.46^{a}	6.66±0.36^{a}	6.70±0.38^{a}	<.0001
Haugh unit	88.59±8.97	84.19±13.17	84.45±11.33	82.99±10.55	83.91±8.31	0.1449
Eggshell strength (N)	3.97±1.12	4.36±0.95	4.22±0.80	4.11±1.12	4.05±1.23	0.4808
Eggshell thickness (um)	0.40±0.04	0.40±0.03	0.40±0.03	0.41±0.03	0.41±0.03	0.0955
Yolk height (mm)	17.11±1.29^{ab}	17.48±0.83^{a}	17.44±1.00^{a}	17.23±1.16^{ab}	16.79±1.21^{b}	0.0246
Yolk diameter (mm)	41.85±3.11	43.43±10.80	43.08±3.00	41.81±2.13	42.34±2.02	0.0605
Yolk index	0.41±0.05	0.41±0.05	0.41±0.03	0.41±0.03	0.40±0.03	0.2044

Abbreviations: NC- Normal control; HPS- High concentration peanut skin powder (5 g/100 kg); LPS- High concentration peanut skin powder (2.5 g/100 kg); HBN- High concentration bromelain (50 g/100 kg); LBN- Low concentration bromelain (25 g/100 kg).

^{A,b} Means within a row lacking a common superscript differ significantly (p<0.05; 0.001).

[1]Each mean represents 8 layers/replicate, 3 replicates/treatment.

Supplymentary Table 3: Effects of peanut skin and bromelain on blood plasma biochemistry parameters in 93-week-old laying period.

Items[1]	NC	PS	BN	PSBNM	P value
Glu-Ac (mg/dl)	167±32.09^{a}	140±24.94^{b}	138±30.59^{b}	134±31.27^{b}	<0.001
GOT (U/L)	202±25.42	220±27.8	227±44.52	212±21.37	0.1003
GPT (U/L)	2±0.6	2±0.92	2±0.77	2±0.74	0.8177
T-P (g/dl)	6.8±1	7.0±1.43	6.8±1.03	6.4±0.69	0.4128
Alb (g/dl)	2.5±0.15	2.6±0.14	2.6±0.14	2.5±0.17	0.4391
Globulin (g/dl)	4.3±0.99	4.4±1.41	4.2±1.06	3.9±0.61	0.4691
A/G	0.6±0.13	0.6±0.12	0.7±0.15	0.7±0.09	0.6401
BUN (mg/dl)	0.4±0.23^{b}	0.4±0.13^{b}	0.5±0.21^{a}	0.5±0.24^{a}	0.0184
Creatinine (mg/dl)	0.17±0.04	0.16±0.04	0.16±0.03	0.17±0.03	0.8005
TG (mg/dl)	1702±661.89	1942±274.65	2053±796.81	1811±472.42	0.3175
LDL-C (mg/dl)	19±5.59	21±8.38	23±20.73	17±4.47	0.4294

Abbreviations: NC- Normal control; PS- Peanut skin powder (5 g/100 kg); BN- Bromelain (50 g/100 kg); PSBNM- Peanut skin and bromelain mixed (2.5 g peanut skin powder+25 g bromelain).

^{A,b} Means within a row lacking a common superscript differ significantly (p<0.05, 0.001).

[1]Each mean represents 8 layers/replicate, 3 replicates/treatment.

the NBN group (in 93-w-old laying hens). Bromelain and PS have protective effects against non-alcoholic fatty liver disease. The 93-w-old laying hens treated with bromelain had a lower lesion score (1.48±1.12) compared to the NC (1.61±0.98) and PS (1.86±1.08) and PSBNM groups (1.87±1.03) (Table 6).

Effect of peanut skin and bromelain on egg production

In this study, the middle-aged laying hens had higher egg production in the LPS group than the NC group, whereas in the HPS group, middle-aged hens demonstrated better performance than aging hens. Because fat accumulation causes irreversible liver damage in aging laying hens, PS supplementation cannot improve the decline in egg production caused by fatty liver in these hens. A previous study investigated the enrichment of consumable food products by incorporating waste byproducts rich in polyphenolic compounds into poultry feed rations (Toomer *et al*., 2021). Enhancing the value of agricultural waste byproducts, such as incorporating PS into poultry feed, can foster sustainable agriculture and offer innovative solutions for utilizing these byproducts, which often has considerable residual nutritional value.

Effect of peanut skin and bromelain on egg quality

The decline in egg production is attributed to health after prolonged egg production in the late-laying stage (Vlèková *et al*., 2019). The LPS and HBN group of 62-w-old laying hens had higher egg production than the control, HPS and LBN groups. Although overall egg quality parameters showed no substantial improvements over the control group, the PS and bromelain groups of 62- and 93-w-old laying hens showed enhancements in yolk color. These findings suggest that PS and bromelain supplementation might offer specific benefits to egg quality. Contrary to a previous report by (Toomer *et al*., 2021), egg yolk color in the current study was deeper in hens fed a diet containing PS and bromelain. β-carotene is a naturally occurring compound that serves as a precursor to vitamin A and functions as an antioxidant (Mezzomo and Ferreira, 2016). β-carotene has been found in bromelain (Ataide *et al*., 2017) and peanut oil (Pattee and Purcell, 1967). A previous report indicated that the supplementation of tomato powder to the diet of laying hens could sufficiently transfer carotenoids to the yolk, thereby improving both yolk color and oxidative stability (Akdemir *et al*., 2012). Although we did not evaluate the carotenoid content in the yolk in the current study, the diet supplemented with PS and bromelain substantially enhanced yolk color. This finding implies that PS and bromelain may transfer and retain β-carotene in the yolk, leading to improved yolk pigmentation.

Effect of peanut skin and bromelain on blood biochemistry parameters

Xiang *et al*. (2020) reported that PS extract could decrease inflammation and maintain gut microbiota homeostasis, thereby improving the symptoms of type 2 diabetes mellitus in mice (Xiang *et al*., 2020). Bromelain substantially decreases fasting blood glucose levels in mice with streptozotocin- induced type 1 diabetes (Abo El-Magd *et al*., 2021). PS is rich in flavonoids, which mitigate glucose intolerance and enhance insulin sensitivity to regulate blood glucose. These findings suggest that although PS and bromelain supplementation did not affect most blood biochemical parameters, they were effective in lowering

Supplymentary Table 4: Effects of peanut skin and/or bromelain on carcass traits in 62-week-old laying period.

Items[1]	NC	HPS	LPS	HBN	LBN	P value
Live weight (g)	1772.58±119.96	1741.75±139.72	1889.58±111.00	1956.83±214.73	1849.67±139.93	0.4371
Wet weight of Liver (g)	39.50±5.19	39.17±4.84	39.17±4.30	45.50±5.53	38.33±4.53	0.1651
Abdominal fat weight (g)	60.17±18.58	61.17±19.73	90.17±27.65	100.17±30.71	72.67±17.99	0.0636
Other organs weight (g)	294.17±27.61	301.17±45.25	336.33±45.11	336.83±60.69	308.00±39.11	0.4272
Carcass weight (g)	1320.08±75.89	1265.17±93.71	1368.08±69.98	1439.58±146.64	1361.82±112.43	0.1359
Ovarian weight (g)	48.50±9.25	46.17±3.24	48.00±9.27	54.33±10.56	45.17±12.17	0.5871
Reproductive tract weight (g)	129.67±12.28	118.83±13.36	133.17±15.07	142.17±26.42	129.33±29.68	0.5227

Abbreviations: NC- Normal control; HPS- High concentration peanut skin powder (5 g/100 kg); LPS- High concentration peanut skin powder (2.5 g/100 kg); HBN- High concentration bromelain (50 g/100 kg); LBN- Low concentration bromelain (25 g/100 kg).
[1]Each mean represents 8 layers/replicate, 3 replicates/treatment.

Table 6: Effects of peanut skin and bromelain on fatty liver in 93-week-old laying period.

Items	NC	PS	BN	PSBNM
Average fatty liver score	1.61±0.98	1.86±1.08	1.48±1.12	1.87±1.03
Fatty liver score g”2 (NB)	12	14	10	14
Number of slaughtered chickens	22	21	24	23
Fatty liver rate (%)[1]	52%	64%	48%	58%

[1]Fatty liver rate (%) = Number of chickens with fatty liver score ≥2/Total number of slaughtered chicken.

blood glucose content in aged laying hens, indicating their potential benefits for metabolic health.

Effect of peanut skin and bromelain on carcass traits and fatty liver

In this study, the results indicated a high prevalence of FLHS lesions among aged laying hens exposed to PS. Nevertheless, bromelain tended to promote the repair of fatty liver in aged laying hens. As a substantial portion of the lipids in egg yolk is synthesized by the liver, aging laying hens are more prone to developing fatty liver syndrome (FLS) (Squires and Leeson, 1988; Shini *et al*., 2019). Fatty liver disease in laying hens is induced by free fatty acids through the inhibition of AMPK signaling (Huang *et al*., 2022). Our results showed that PS increased, while bromelain decreased, the percentage of hepatic lipid accumulation in aging laying hens, respectively. Therefore, bromelain may be correlated with fatty acid formation in the liver *via* the AMPK signaling pathway.

Pineapple (*Ananas comosus*) is a short-stemmed, terrestrial bromeliad and an edible member of *the Bromeliaceae* family. Bromelain, a protease extracted from pineapple, is used as a medicinal herb in several indigenous cultures (Mondal *et al*., 2011). A mixture of PS extracts substantially improved hepatic lipid accumulation in mice (Yi *et al*., 2023). Our findings indicate that PS, but not bromelain, led to lower production (lower egg production, daily laying rate and feed-to-egg-ratio) and increased liver lipid accumulation in laying hens, which is inconsistent with previous reports (Toomer *et al*., 2021; Yi *et al*., 2023). Although the daily feed intake in the current study was similar in both groups, the high tannin and low calorie content of PS may have contributed to the downregulation of egg production in aged laying hens. However, the LPS group with low tannin content improved the production of middle-aged laying hens. In the past, tannins were considered antinutritional compounds in diets for monogastric animals. However, low concentrations of tannins can improve feed intake, nutrition and growth performance in monogastric animals (Schiavone *et al*., 2008). A combination of LPS and HBN might improve the performance of young and middle-aged hens.

CONCLUSION

In conclusion, this study demonstrated that PS and bromelain could improve egg production performance and enhance yolk color through β-carotene in middle-aged laying hens. This can be beneficial in increasing vitamin A supplementation in egg consumers. In future research, we will investigate the supplementation of new compounds in combination with PS or bromelain in the diet of laying hens.

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Informed consent

All experimental procedures involving animals adhered to the guidelines of the Institutional Animal Care and Use Committee of National Chiayi University.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Abo El-Magd, N.F., Ramadan, N.M. and Eraky, S.M. (2021). The ameliorative effect of bromelain on STZ-induced type 1 diabetes in rats through Oxi-LDL/LPA/LPAR1 pathway. *Life Science*. **285:** 119982. doi: 10.1016/j.lfs.2021.119982.

Akdemir, F., Orhan, C., Sahin, N., Sahin, K. and Hayirli, A. (2012). Tomato powder in laying hen diets: Effects on concentrations of yolk carotenoids and lipid peroxidation. *British Poultry Science*. **53:** 675-680.

Ataide, J.A., de Carvalho, N.M., Rebelo, M.A., Chaud, M.V., Grotto, D., Gerenutti, M., Rai, M., Mazzola, P.G. and Jozala, A.F. (2017). Bacterial nanocellulose loaded with bromelain: Assessment of antimicrobial, antioxidant and physical-chemical properties. *Scientific Reports*. **7:** 18031. doi: 10.1038/s41598-017-18271-4.

Bain, M.M., Nys, Y. and Dunn, I.C. (2016). Increasing persistency in lay and stabilising egg quality in longer laying cycles. What are the challenges? *British Poultry Science*. **57:** 330-338.

Beck, M.M. and Hansen, K.K. (2004). Role of estrogen in avian osteoporosis. *Poultry Science*. **83:** 200-206.

Bharthare, P., Shrivastava, P., Singh, P. and Ttiwari, A. (2014). Peanut shell as renewable energy source and their utility in production of ethanol. *International Journal of Advanced Research*. **2:** 1-12.

Chobotova, K., Vernallis, A.B. and Majid, F.A. (2010). Bromelain's activity and potential as an anti-cancer agent: Current evidence and perspectives. *Cancer Letter*. **290:** 148-156.

Hale, L.P. (2004). Proteolytic activity and immunogenicity of oral bromelain within the gastrointestinal tract of mice. *International Immunopharmacology*. **4:** 255-264.

Huang, C., Gao, X., Shi, Y., Guo, L., Zhou, C., Li, N., Chen, W., Yang, F., Li, G., Zhuang, Y., Liu, P., Hu, G. and Guo, X. (2022). Inhibition of hepatic AMPK pathway contributes to free fatty acids-induced fatty liver disease in laying hens. *Metabolites*. **12:** 825. doi: 10.3390/metabo12090825.

Liu, X.T., Lin, X., Mi, Y.L., Zeng, W.D. and Zhang, C.Q. (2018). Age-related changes of yolk precursor formation in the liver of laying hens. *Journal of Zhejiang University-Science*. **19(5):** 390-399.

Metzig, C., Grabowska, E., Eckert, K., Rehse, K. and Maurer, H.R. (1999). Bromelain proteases reduce human platelet aggregation *in vitro*, adhesion to bovine endothelial cells and thrombus formation in rat vessels *in vivo*. *In Vivo.* **13(1):** 7-12.

Mezzomo, N. and Ferreira, S.R. (2016). Carotenoids functionality, sources and processing by supercritical technology: A review. *Journal of Chemistry*. **7:** 1-16.

Mondal, S., Bhattacharya, S., Pandey, J. and Biswas, M. (2011). Evaluation of acute anti-inflammatory effect of *Ananas comosus* leaf extract in rats. *Pharmocologyonline*. **3:** 1312-1315.

Pattee, H.E. and Purcell, A.E. (1967). Carotenoid pigments of peanut oil. *Journal of American Oil Chemists Society*. **44:** 328-330.

Pillai, K., Ehteda, A., Akhter, J., Chua, T.C. and Morris, D.L. (2014). Anticancer effect of bromelain with and without cisplatin or 5-fu on malignant peritoneal mesothelioma cells. *Anti-Cancer Drug*. **25:** 150-160.

Rozenboim, I., Mahato, J., Cohen, N.A. and Tirosh, O. (2016). Low protein and high-energy diet: A possible natural cause of fatty liver hemorrhagic syndrome in caged white leghorn laying hens. *Poultry Science*. **95:** 612-621.

Sahbaz, A., Aynioglu, O., Isik, H., Ozmen, U., Cengil, O., Gun, B.D. and Gungorduk, K. (2015). Bromelain: A natural proteolytic for intra-abdominal adhesion prevention. *International Journal of Surgery*. **14:** 7-11.

Schiavone, A., Guo, K., Tassone, S., Gasco, L., Hernandez, E., Denti, R. and Zoccarato, I. (2008). Effects of a natural extract of chestnut wood on digestibility, performance traits and nitrogen balance of broiler chicks. *Poultry Science*. **87:** 521-527.

Shini, A., Shini, S. and Bryden, W.L. (2019). Fatty liver haemorrhagic syndrome occurrence in laying hens: Impact of production system. *Avian Pathology*. **48:** 25-34.

Squires, E.J. and Leeson, S. (1988a). Aetiology of fatty liver syndrome in laying hens. *British Veterinary Journal*. **144:** 602-609.

Squires, E.J. and Leeson, S. (1988b). Aetiology of fatty liver syndrome in laying hens. *British Veterinary Journal*. **144:** 602-609.

Su, G., Kjaer, J.B. and Sorensen, P. (2006). Divergent selection on feather pecking behavior in laying hens has caused differences between lines in egg production, egg quality and feed efficiency. *Poultry Science*. **85:** 191-197.

Toomer, O., Vu, T., Wysocky, R., Moraes, V., Malheiros, R. and Anderson, K. (2021). The effect of feeding hens a peanut skin-containing diet on hen performance and shell egg quality and lipid chemistry. *Agriculture*. **11:** 894. https://doi.org/10.3390/agriculture11090894.

Vlèková, J., Tùmová, E., Míková, K., Englmaierová, M., Okrouhlá, M. and Chodová, D. (2019). Changes in the quality of eggs during storage depending on the housing system and the age of hens. *Poultry Science*. **98:** 6187-6193.

Xiang, L., Wu, Q., Osada, H., Yoshida, M., Pan, W. and Qi, J. (2020). Peanut skin extract ameliorates the symptoms of type 2 diabetes mellitus in mice by alleviating inflammation and maintaining gut microbiota homeostasis. *Aging (Albany NY)*. **12:** 13991-4018.

Yan, H., Adrizal, A., Nova, P.Y. and Mahata, M.E. (2020). The response of laying hens fed fermented pineapple peel waste by indigenous microorganisms from bamboo sprout. *Revista Brasileira de Saúde e Produção Animal*. **21:** 1-8.

Yenice, G., Iskender, H., Dokumacioglu, E., Kaynar, O., Kaya, A., Hayirli, A. and Sezmis, G. (2019). Dietary bromelain supplementation for improving laying performance, egg quality and antioxidant status. *European Poultry Science*. **83:** 1-16.

Yi, M., Fasina, O.B., Li, Y., Xiang, L. and Qi, J. (2023). Mixture of peanut skin extract, geniposide and isoquercitrin improves the hepatic lipid accumulation of mice *via* modification of gut microbiota homeostasis and the TLR4 and AMPK signaling pathways. *International Journal of Molecular Science*. **24:** 16684. doi: 10.3390/ijms242316684.