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Effects of Supplemental Japanese Pepper Seed on Thermoregulation, and Blood Monoamines in Heat Exposed Broiler Chicks

Maroof Khushdil[1, 2*], Oka Takao[1, 2], Himmat Jamaat Khan[2, 3] Fujihara Mika[2, 3], Bungo Takashi[1, 2]

[1]Department of Animal Science, Agriculture Faculty Nangarhar University, Nangarhar Afghanistan
[2]Laboratory of Animal Behavior and Physiology, Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima 739-8528, Japan
[3]Department of Basic Science, Medical Faculty Nangarhar University, Nangarhar Afghanistan
[4]Wakayama Prefectural Kihoku Livestock Hygiene Service Center, Wakayama 640-8483, Japan
Corresponding author: khmaroof@gmail.com

ABSTRACT

Background: This study was carried out to assess the effects of enriched feed with Japanese Pepper Seeds (1 and 2%) on plasma monoamine and thermoregulation in broilers.

Materials and Methods: A day old male broiler chicks were obtained from hatchery (Fukuda, Japan), and were kept in cages with floor of wire-mesh; 3 broilers in each cage. The surrounding heat was controlled at 30 ± 1 ^{0}C for three days in the beginning of experiment, and gently lowered to 26 ± 1 ^{0}C until broilers reached 11 days of age.

Findings: The study result indicated that after 6 days of feeding, their feed intake and body weight gain were not significantly different among groups of control and supplemented with Japanese pepper seeds. However, feed conversion ratio decreased significantly with feed of 1% Japanese pepper seeds against 2% in Japanese pepper seeds broilers ($P<0.05$). Subsequently, all groups were subjected to high heat at 38° C, for 3 hours with water but not feed. The tendency was in wing drop and panting during temperature exposure groups ($P>0.05$). With acute heat stress, the temperature of all groups was elevated. The effects of time and Japanese pepper seeds were significant ($P<0.05$) in temperature exposed broilers. The interaction between time and Japanese pepper seeds were measured to reflect a tendency of significance ($P<0.01$). The affinities were measured in rectal temperature of control group and 2.0% group of Japanese pepper seeds broilers to reduce after 2h, although they kept advancing in 1% group of Japanese pepper seeds. There were no significant differences in the level of plasma monoamines in 5- HT, Ad and NA among groups ($P>0.05$), although the level of DA in plasma in 2% Japanese pepper seeds in broilers was lower than control one ($P<0.05$).

Conclusion: Present investigation recommend that Japanese pepper seeds affect thermoregulation through the catecholaminergic system in broilers. Nevertheless, it may have adverse effects under long period high temperature in chicks.

Keywords: Broiler; Japanese pepper seed; Thermoregulation; Plasma Monoamines; High temperature

INTRODUCTION

Many of plant components gained the attention of nutrition specialist, which develop as anti-nutrition ingredients or elements, which should be eliminated or avoided, or their effects need to be improved. Nevertheless, even though, among the materials, that are concede to this class, it is mostly found that, in a proper status, plants components may have good effects. For example the ability of anti-carcinogenic properties of phyto-oestroneges (Migas & Krauze-Baranowska, 2015) and glucosinolates (Nugon-Baudon & Rabot, 1994), the anti-oxidative and anti-atherogenic effects of disuphide and allyl thiosulphinate and the potential coccidiostatic action of artmisin (Allen et al., 1999). While these possibly useful effects have reported mostly with health of human, it is possible, that some of components will be of worth in animal diet such as fit. Thermally stressed environment is a major concern in modern poultry farming. Various strategies reported to alleviate the negative effect of high temperature in broilers (Yahav et al., 1995; Sahin et al., 2003), but the badly behaved has not yet been explained. The nutritional manipulations are one of the countermeasures to cope with heat stress, and there have been in particular many investigations about supplementation of anti-oxidative compounds, such as vitamins (Takahashi et al., 1991; Lin et al., 2002). As shown in Table 1 in Chapter 1, JPS contains relatively much tocopherols (0.045 mg/g), and it is expected to negative effects of oxidation stress in animals. The present was investigated to explain the effects of Japanese pepper seeds on plasma monoamines and thermoregulation in high temperature exposed broilers.

MATERIALS AND METHODS

Animals in Experiments

Male broilers (Chunky) at the age day old were obtained from hatchery (Okayama, Fukuda Hatchery, and Japan). Day old broilers were kept in cages (wooden) with floor of wire-mesh (18x25x20 cm) at density population of 3 broilers in each cage. Broilers were kept with 24 hours lighting in a room. The surrounding heat was controlled at 30±1 ^{o} C for three days in the beginning of experiment, and gently lowered to 26±1 ^{o} C until broilers reached 11 days of age. They had free access to a commercial starter feed (3100 kcal/kg of AME_n and 22% CP; Kobe, Japan, Nichiwa Sangyo Co. Ltd.) and water throughout the pre-experimental duration. The hold of broilers performed due to guidelines of the Animal Experiment Committee of Hiroshima University.

Body weight and feed intake

At day 4 of age, the broilers were placed in order into experimental sets based on body mass so the normal body weight was as similar as likely for each treatment. The broilers were kept separately in cages (13x25x25 cm) during experiment, had free access to water, and feed until the experiment ended. Commercial starter diet (basal diet) was fed to control group and the other groups were fed with ground Japanese pepper seeds added basal diet at 1.0 or 2.0%. The added level was determined at (5% Japanese pepper seeds feed may stimulate secretin of adrenaline, Japanese pepper seeds level was fixed to be less than half). Japanese pepper seeds was taken from Japanese pepper farm from prefecture of Wakayama, Japan. Feed intake and body weight gain measured in each day until the broilers were at age of 11 days.

Heat exposure treatment

Afterward of last determination of feed intake and body weight gain, all birds (11 days old) exposed to heat stress at 38 ^{o} C for 3 hours with water but not feed (experimental groups in the thermo-neutral zone were not set, because supplemental JPS had no effect on rectal temperature in previous experiments. Rectal temperature was measured hourly during the heat exposure test. During the heat test, the beginning times of excesses performances in wing drop and panting (Etches et al., 2008) in broilers were visually recorded and monitored. All broilers were bled at the end of the test by cardiac puncture and all samples were collected in heparinized tubes, and for 15 minutes centrifuged. Collected plasma samples were kept at -20 º C until analyzed.

Blood parameters

The levels of NEFA (non-esterified fatty acid), glucose, TG (triglyceride), total cholesterol, HDL (high density lipoprotein), LDL (low-density lipoprotein cholesterol), LA (lactic acid), calcium, ALT (alanine aminotransferase) and AST (aspartate aminotransferase) were determined by Beckman Coulter AU480 biochemistry analysis automatic system (CA, USA), which contain reagents prepared by producer.

Plasma monoamines were tested by the method of Terao et al. (2008), which used HPLC (high-performance liquid chromatography. Separation of monoamines were attained using a 5-µm reversed-phase Octadecyl-Silica column (Kyoto, Japan, CA-5 ODD; Eicom Co.). Column heat retained at 25 º C by a thermocontroller (Tokyo Japan, TSK CO-8000; Tosoh Co.). The solvent transfer system (Tokyo 105 Japan, TSK CCPD; Tosoh Co.) enclosed 20 µM Na_2EDTA and 2.5 mM 1-octanesulfonic acid sodium salt (SOS), and 12% methanol in a 0.1 M NaH_2PO_4"0.1 M Na_2HPO_4= 1000:85 (0.1M phosphate buffer solution). The pH of the buffer was adjusted to 3.5 with H_3PO_4. The buffer was filtered and degassed by degasser (Tokyo Japan, TSK SK-8022; Tosoh Co.) and then the flow rate adjusted to 150 µL/min. The electrochemical detector (Tokyo Japan, TSK EC-8020; Tosoh Co.) was set at 900 mV and peak height were measured using a computer integrator. All values were corrected for real recovery based on the extraction rate of the inner slandered isopropanol.

Statistical analysis

Collected data were examined via accessible package of StatView (SAS Institute, Version 5, Cary, USA, 1998). For numbers with normal distribution, ANOVA and Tukey-Kramer test were performed. If data were not normally distributed, they were subjected to Kruskal-Wallis variance analysis and Steel-Dwass post-hoc procedures. To compare rectal temperature at the different time-points, a linear to define the dose-response relations at separately time period. Changes were stated significant at $P < 0.05$, and a value of $P < 0.01$ was measured to reveal a tendency to significance.

RESULTS

Effects of Japanese pepper seeds on feed consumption, body weight and feed conversion ratio.

Table 1 showed the effects of supplement JPS on BW (body weight gain), feed consumption and feed FCR (conversion ratio) in broilers. Feed consumption and BW gain were none-significantly changed ($P > 0.01$) among Japanese pepper seeds and control one. Nevertheless, FCR decreased significantly in broilers fed with 1.0% Japanese pepper seeds feed compared to 2.0 % Japanese pepper seeds ($P < 0.05$) in broilers.

Table 1. Effect of Japanese Paper seed on body weight and feed intake in broiler checks

	Control (5)	1.0 % JP (5)	2.0 % JP (5)	P value
Initial BW (4 d)	67.5±0.98	66.6±0.75	66.7±0.43	0.665
Final BW (11 d)	207.4±9.08	215.7±8.16	198.7±8.01	0.393
BW gain (g)	140.0±8.75	149.1±8.27	132.0±8.28	0.388
Feed intake (g)	175.0±10.31	176.6±8.86	165.7±8.68	0.679
FCR	1.25±0.030 [a]	1.19±0.007 [b]	1.26±0.018 [a]	0.048

JP: Japanese paper seeds, BW: body weight, FCR: Feed conversion ratio. Values are Mean±SE of the number of checks in parentheses. Means with different letters are significantly different at $p < 0.05$.

Effects of JPS on body temperature and behavioral response during heat challenge

Fig. 1. Indicated the result of JPS supplemented feed on tendency in wing-drop or panting throughout high temperature test. Bot heat dissipation behaviors were not significantly different between the Japanese pepper seeds (P > 0.01) and control one.

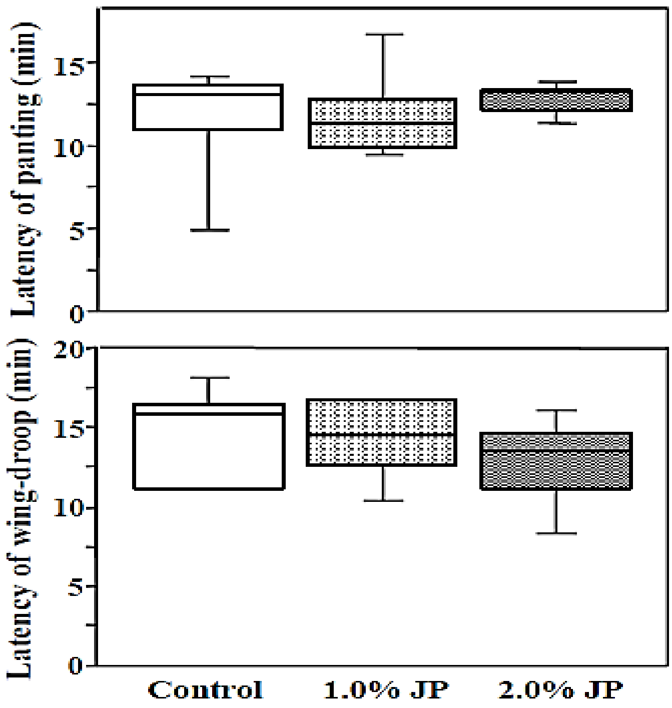

Fig. 1. Effects of Japanese pepper seed on thermoregulation behavior (panting and wing-droop) in heat exposed checks. Box plots show the median (center line) and the interquartile range from the 25th to the 75th percentile. Whiskers above and below the box indicate the 10th and 9th percentiles and circles indicate outliers.

The result of JPS supplemented feed on rectal temperature in heat-exposed chicks publicized in **Fig. 2** number 2. Before the high temperature exposure, rectal heat in control, 1.0 or 2.0% JPS chicks was 40.7±0.1, 40.8±0.1, 40.9±0.1 º C, respectively. The temperature each group were increased by high temperature. The effect of feed and time were significant in high temperature exposed broilers (P<0.05, P<0.0001). An association between feed and time was measured to return a tendency to significance (P<0.01). Rectal temperature at 2 and 3 h heat exposure indicated quadratic changes (2 h: y= 41.78 (SE 0.135, P<0.0001) +1.370 (SE 0.345, P=0.0018) X -0.630 (SE 0.166, P=0.0025) X^2, R2=0.497, P=0.0065; 3 h: y=41.60 (SE 0.241, P<0.0001) + 1.570 (SE 0.614, P=0.0251) X-0.750 (SE 0.295, P=0.0292)X^2, R2=0.245, P=0.0735). There were trends for rectal temperature of control ones and 2.0% Japanese Pepper Seeds broilers group the reduction after 2 h though that of 1.0% Japanese pepper seeds reserved advancing.

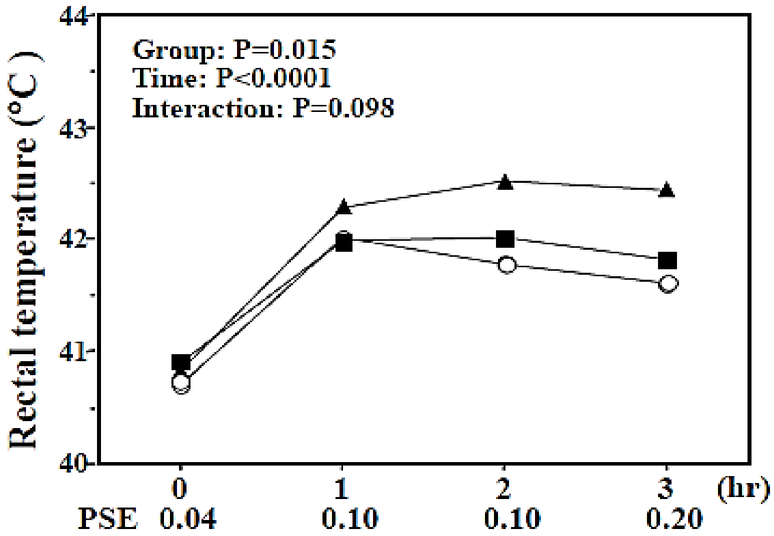

Fig. 2. Effects of Japanese pepper seed on rectal temperature of heat exposed checks during 3h. PSE, pooled SE, open circles; control, solid triangles; 1.0% JP, Solid Square; 2.0% JP. The number of checks in each group was as follows; control 5; 1.0% JP, 5; 2.0% JP, 5.

Effect of JPS on blood parameters after warm air challenge

The effect of supplemental JPS on blood parameters after high temperature stress revealed in **Table. 2**. Non-significant differences were find in all blood parameters (AST, ALT, and glucose, NEFA, T-Cho, TG, HDL, LDL, LA, Ca and IgA) among the groups (P > 0.01).

Table 2. Effects of Japanese pepper seed on plasma parameters in heat exposed chicks

	Control (5)	1.0 % JP (5)	2.0 % JP (5)	P-value
AST (U/L)	167.1±7.6	157.5±6.9	1667.6±4.6	0.484
ALT (U/L)	3.64±0.40	3.88±0.39	3.90±0.87	0.944
Glucose (mg/dL)	235.9±10.2	240.1±11.2	226.0±5.2	0.559
NEFA (mEq/L)	681.3±98.2	903.7±57.6	815.8±132.9	0.327
T-Cho (mg/dL)	130.6±8.6	123.8±8.4	130.9±3.4	0.738
TG (mg/dL)	16.5±1.9	13.9±0.8	17.1±0.6	0.209
HDL (mg/dL)	90.4±5.8	83.7±6.0	88.6±2.4	0.635
LDL (mg/dL)	13.0±1.4	12.9±1.3	15.3±0.6	0.296
LA (mg/dL)	25.1±5.6	25.4±2.5	28.0±3.7	0.863
Ca (mg/dL)	8.46±0.43	8.62±0.25	8.45±0.35	0.931
IgA (mg/dL)	2.30±0.13	2.14±0.09	2.12±0.10	0.458

JP: Japanese pepper seeds, AST: Aspartate aminotransferase, ALT: alanine aminotransferase, NEFA: Non-esterified Fatty acid, T-Cho: Total cholesterol, TG: Triglycerides, HDL: High-density lipoprotein cholesterol, LDL: Low-density lipoprotein cholesterol, LA: Lactic acid, Ca: Calcium, IGA: Immunoglobulin A. Values are means± SEM of the numbers of chicks in parentheses.

Table 3 explained the result of Japanese pepper seeds in plasma monoamines in heat-exposed broilers. Although, significantly difference were not found in Ad, 5-HT and N A between groups (P > 0.01), the amount of DA in plasma in 2% Japanese pepper seeds broilers was not high then control one (P < 0.05).

Table 3. Effects of Japanese pepper seed on plasma monoamines in heat exposed chicks

	Control (5)	1.0 % JP (4)	2.0 % JP (5)	P-value
NA (ng/mL)	8.33±2.41	9.08±3.35	5.78±1.62	0.617
Ad (ng/mL)	6.27±1.24	20.46±8.29	10.98±5.60	0.229
DA (ng/mL)	1.36±0.61 ^{a}	0.44±0.36 ^{ab}	0.22±0.06 ^{b}	0.046
5-HT (ng/mL)	14.92±10.20	3.08±1.60	6.18±3.30	0.463

JP: Japanese pepper seeds, NA: noradrenalin, AD: adrenalin, DA: dopamine, 5-HT: serotonin. Values are means ± SEM of the number of chicks in parentheses. Means with different letters are significantly different at P < 0.05.

DISCUSSION

A sequence of metabolic and physiological changes in broilers occurred such as painting, increasing of body heat, alkalosis in respiration, through which metabolic status bring out and reduce level of plasma triiodothyronine (Deyhim & Teeter, 1991). Growth promoters are expecting to increase growth rate and improve gut health ensuing in better-feed consumption and reducing FCR (feed conversion ratio) (Visek, 1978). Birds outside of normal temperature may suffer physiological changes, such as decrease feed intake and egg production. Fowl is delicate to heat as related ecological challenges such as heat stress. Heat stress elevate steroidogenesis, which effects tissues and metabolism. Understand and control of environment is threat to success poultry production and welfare (Lucas & Marcos, 2013). However, the current study indicates that there is no effect of JPS on feed consumption and body weight gain supplemented as feed additives, but FCR was significantly lower in 1% JPS compare to other groups (**Table 1**). Deduction in FCR in 1% JPS might be because of positive phenolic outcome of JPS as antioxidant which improved FCR, improved intestinal mucosal lining and improved digestion. Our study is supported that enhancement in FCR might be due to the active ingredient of medicinal plant which increase fat metabolism and energy utilization (Smeets & Lejeune, 2005). This may effect of herbal plant and enhance the digestive system function in assimilation of feed, and role of the polyphenol that possessed antioxidant activities (Papoutsi et al., 2005). This may effect of herbal plant and enhance the function of digestive system in adjustment of feed, and character of the polyphenol that keep antioxidant activities (Papoutsi et al., 2005). Plants and phytogenic property may control and enhance the growth of various pathogenic and nonpathogenic species of bacteria in chick's gut, which may affect to a better digestion and consumption of feed, results in enhanced FCR (Bedford, 2000). Plants derived feed additives may have helpful effect on digestion and increase absorption volume of gastric mucosa (Gonzalez et al., 1998). Supplementation of JPS as feed extracts might have helpful effect on digestion of feed and further active on nutrition, and due to deviations in intestinal ecosystem and absorption of digestive materials, thus FCR decreased in 1% JPS.

In the present study plasma Dopamine significantly lowered in 2% JPS compare to control and 1% JPS (**Table 3**). High doses of JPS decreased Dopamine level in broiler chicks. It could be support of JPS and removal of adverse influence of high temperature on adrenal lipid peroxidation at cell membrane (Edens & Siegel, 1975).

The reduction in plasma Dopamine could be due to effect of JPS tocopherol on heat stress, α-tocopherol of plants decrease the adverse effect of high temperature in broilers (Young et al., 2003), the inclusion of medicinal plant in the ration lessened the synthesis of corticosterone hormone and prevented their negative effect (Kutlu & Forbes, 1993). Phenylalanine and tyrosine are precursor of dopamine, epinephrine and norepinephrine. Phenylalanine first converted to tyrosine and serves as precursor of epinephrine, norepinephrine and dopamine. Epinephrine and norepinephrine stimulated glucose uptake of muscle, and norepinephrine activity stimulated by stress (Choochote et al., 2009).

Under heat stress, addition of JPS as feed additives in broilers diets prevent negative effect of corticosteroid hormone by decreasing their synthesis, which improved performance of broiler chicks under heat stress. Dopamine is highly correlated with faster and stronger adaptation of chicken to heat stressors (Hester et al., 1996a, 1996b). The effect of group (feed) and time were significant in high temperature exposed broilers ($P<0.05$) is shown in **Fig 2**. Supplementation of the diet with JPS improved group (feed) and time of heat stressed may be due to decreasing the synthesis of corticosteroid hormones and prevented their adverse effects. The significant in feed and time interaction indicated that JPS had more strong positive effect in heat stress group then non-heat stressed broilers. Supplementation of feed with acidifiers has the ability to increase chicken presentation under high teperature situation (Daskiran et al., 2004).

CONCLUSION

In conclusion, the present investigations recommend that Japanese pepper seeds distress temperature regulation through catecholaminergic system in broilers, although, it make possible thermoregulation through the catecholaminergic system in chicks but it may convert the opposing effect under the long time high temperature in chicks.

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Conflict of Interest

No, conflict of interest among all authors

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