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RESEARCH ARTICLE

CORTISOL LEVEL, BEHAVIOR AND ECONOMICS OF RAISING PEKIN DUCK FED WITH FRESH AZOLLA RAISED UNDER INDUCED HEAT STRESS

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ABSTRACT

With Heat stress causes detrimental effects on duck production which generate negative economic impacts. Thus, raising Pekin duck as heat stress tolerant breeds and are well-adapted to Philippine climate and feeding azolla that is well-known for its high crude protein, lysine and glutamine that can combat heat stress due to immune-modulatory effect is indeed significant alternative strategy. The study aimed to determine the stress level, behavior and economics of pekin duck fed with different levels of fresh azolla raised under induced heat stress. Significant differences among dietary treatments were analyzed using Scheffe's Test. Findings showed that pekin ducks fed with azolla have significantly heavier final weights and lower blood and urine cortisol level. Moreover, feeding azolla did not trigger any aggressive behavior and molting and significantly reduced the feather pecking behavior. Pekin ducks fed with azolla raised under induced heat stress significantly improved the eating percentage. In general, pekin ducks provided with 50%-75% azolla have significantly higher incurred net income and ROI. Generally, provision of azolla up to 75% in dietary ration is recommended for farmer's adaptation to improved eating habit while lowering blood and urine cortisol and reducing aggression of ducks and increase profit even under induced heat stress.

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INTRODUCTION

Pekin duck (*Anas platyrhyncos domesticus*) are well known to be docile and could strive and well acclimatized to Philippine environment. Aside from one of the potential sources of high-quality protein, Pekin duck is one of the modern meat-type ducks with high capacity for fatty acid deposition in breast muscle and abdominal adipose tissue as compared with other duck breeds (Zhi-Guo *et al.,* 2015). However, pekin duck industry cannot reach its full potential due to unlimited exposure of ducks to various potential stressors (i.e., high temperature and relative humidity) during production. In addition, a long-term stress can have far-reaching behavioral changes and causes detrimental effects on poultry production (Virden and Kidd, 2009). As environmental temperature increases, food consumption, feed efficiency, growth rate, egg production, and survival ability decline (Kathirvelan *et al.,* 2015; Sohail *et al.,* 2012; Mashaly *et al.,* 2004). This causes an enormous economic loss in the duck industry. Strategy used by some duck raisers to reduce the negative economic impacts of heat stress is by raising heat stress tolerant breeds that are well-adapted to Philippine climate like Pekin duck and inclusion of azolla in the feeds that is well-known for its high CP content (25-35%) than most green forage crops and aquatic macrophytes, and contains essential amino acid – lysine, that is favorable for animal nutrition, and other nutrients like glutamine that can combat heat stress and had favorable immune-modulatory effect in poultry without any toxicity (Mishra *et al.,* 2016; Hasan and Chakrabati, 2009; Tran *et al.,* 2020).

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In addition, azolla produces a special kind of biomass and protein production yields of about 10 to 20 tons/acre/year largely exceeds that of soybean (Tran *et al.,* 2020; Hasan and Chakrabati, 2009). The severity of response of the animal to heat stress may vary depending on its breed, species, physiological and nutritional status, and genetic potential (Mishra *et al.,* 2016). Though poultry undergo thermoregulatory adaptations during periods of heat stress (Lara and Rostagno, 2013), studies showed that exposure to high ambient temperature could suppress immune system of birds and may lead to death and high mortality rates if coupled with high relative humidity (Lambio, 2010). Hence, this study was conducted to determine how feeding of azolla affects the performance of pekin duck breed under induced heat stress. This study aimed to determine the stress level and behavior of pekin duck fed with different levels of fresh azolla raised under induced heat stress. Specifically, it aims to determine the: (1) effect of fresh azolla on the blood and urine cortisol level as stress hormone of pekin duck raised under induced heat stress; (2) behavior of pekin duck fed with azolla raised under induced heat stress in terms of eating habit, feather pecking, aggressiveness, and molting; and (3) economics of raising pekin duck fed with fresh azolla raised under induced heat stress in terms of return on investment.

MATERIALS AND METHODS

Materials: The study was conducted on March 27 to June 2, 2022 at Animal Production Project, Mindoro State University, Victoria, Oriental Mindoro while analysis for the blood cortisol and urine cortisol was conducted at Oriental Mindoro Provincial Hospital. Proximate analysis of the azolla utilized for the study was analyzed in the Animal Nutrition laboratory, Institute of Animal Science, College

of Agriculture and Food Science, University of the Philippine, Los Bańos. A total of 120, two months old pekin duck was utilized in the study, fresh azolla, commercial feed, 24-100 watts bulb, 24 cages, calculator, digital weighing scale, 24 pcs feeding trough, fumigant, ice box, record notebook and ballpen, shovel, syringe, thermometer, 24 vials and 24 pcs watering trough.

Methods

Experimental Design and Treatment: The ducks were equally and randomly distributed into four treatments, following Completely Randomized Design (CRD). Each treatment was replicated six (6) times with five (5) ducks per replication.

The treatments are as follows:

Treatment 1 = Pure commercial feeds
Treatment 2 = 75% commercial feeds + 25% fresh azolla
Treatment 3 = 50% commercial feeds + 50% fresh azolla
Treatment 4 = 25% commercial feeds + 75% fresh azolla

Treatment Implementation: After the arrival of the pekin ducks, it was randomly distributed into four treatments that were replicated six times with five ducks per replication. Restricted feeding was implemented. Supplementation of azolla began at the second day after the arrival of the ducks for acclimatization of the experimental birds. Aside from the treatments applied, using different levels of azolla, a 13-20^{o}C (55-68^{o}F) temperature requirement per duck (Sun *et al.,* 2019) was increased to 66 to 100% using lighting management. Each replication was given 100 watts of bulb that were serve as a stress inductor based on the trial-and-error sample of measuring temperature inside the cages (i.e., AM-29.5^{o}C, NN-30.23^{o}C, PM-26.9^{o}C) using thermometer and hygrometer.

Experimental Birds and Feeding Management: A total of one hundred twenty (120) heads of two-month-old pekin ducks was used for feeding trials for 60 days using different levels of azolla. The pekin ducks was provided with commercial feeds and different levels of fresh azolla according to corresponding treatment ratio.

Gathering of Raw Materials: Pekin duck was purchased at Dimaano Farm in Calapan City Oriental Mindoro. Commercial feeds were purchased in the nearest Agricultural Supplies at Victoria and/or in Calapan, Oriental Mindoro. Azolla were collected from the azolla ponds in Mindoro State University during the feeding trial. The collected fresh azolla was placed in a clean tray and were mixed to commercial feeds depending on their corresponding ratios based on the dry matter basis. Before the conduct of the study, 10 grams oven dried azolla (5 grams green and 5grams reddish brown) sample was analyzed for crude protein analysis and found out that it contains 25.97% CP. The provision of different levels of azolla in the concentrate based on treatment assignment started immediately after 7 days of acclimatization period and was provided for two months feeding trial. The weight of the 2 months old pekin duck after acclimatization served as the initial body weight and was randomly distributed to the assigned treatments. After weighing, analysis for the weight was done to assure that the experimental design before the conduct of the study was appropriate.

Ducks Management: *Preparation of House and Equipment*. A week before the arrival of the ducks, the experimental cages were prepared, cleaned, disinfected and fumigated to prevent growth of detrimental microorganism. The needed equipment that was used in this study were prepared a week before the arrival of the pekin ducks.

Feeding of the Experimental Birds: Finisher crumble was provided as feeds for the ducks upon the arrival until 60 days of feeding trial. Feeds and fresh azolla was weighed and mixed before feeding and was fed two times a day as restricted feeding from the start up to the end of the experimental feeding trial. Feeds were placed in plastic trough feeder type according to their corresponding ratio. Potable drinking water was provided at all times.

Animal Health and Sanitation: The experimental cages were thoroughly cleaned, disinfected and fumigated, one week before the arrival of the ducks. The feeders and waterers were also cleaned daily. Cleanliness of the area and even the surrounding of the experimental cages were maintained throughout the conduct of the study to avoid disease outbreak.

Marketing: The ducks were harvested after 60 days of feeding trial, and sold to different farmers of Alcate, Victoria Orienal Mindoro to get the net income of pekin duck and return on investment.

Data Gathered: *Behavior.* Behavior of pekin ducks was evaluated twice a day (6 am, and 5 pm) from first day to 60 days of feeding trial. The behavior was observed and recorded based on 3-hedonic scale as follows.

Aggressiveness
3-Very aggressive (90% are aggressive)
2-Aggressive (70-80% are aggressive)
1- Inactive (30-50% are aggressive)
Eating Habit
3- Very often (90% are eating)
2- Often (70-80% are eating)
1- Sometimes (30-50% are eating)
Feather Pecking
3- Very often (90% are feather pecking)
2- Often (70-80% feather pecking)
1- Sometimes (30-50% feather pecking)
Molting
3-Very often (90% are molting)
2-Often (70-80% are molting)
1- Sometimes (30-50% are molting)

Blood Collection: After 60th days of feeding trial, blood was collected at the wing vein and jugular vein. Blood sample was collected for one pekin duck per replication. It was analyzed for cortisol level and brought to laboratory for analysis.

Urine Collection*:* After 60th days of feeding trial, urine and feces was collected using improvised collecting container. Urine and feces sample was collected per replication and were purified using centrifuge to separate the feces. Urine was brought to laboratory for analysis of cortisol level.

Cost and Return*:* The cost of producing pekin ducks fed with different levels of azolla was recorded and compared with the control. The net income of duck and return on investment was computed using the following formula:

Net Income = Total sales – Total cost

$$ROI = \frac{\text{Net Income}}{\text{Total Expenses}} \times 100$$

Statistical Analysis: The data collected was analyzed using Analysis of Variance (ANOVA) for Completely Randomized Design (CRD). Significant differences between treatments were further determined using Scheffe's Test at 1% and 5% levels of significance.

RESULT AND DISCUSSION

Body Weight: Findings revealed that initial body weight among pekin ducks used in the experiment are comparable (p>0.05). However, analysis on the final body weight showed that weight of pekin ducks fed with azolla regardless of proportion in the dietary ration have significantly heavier (p<0.01) weights than the ducks not provided with azolla (Table 1). This can be attributed to the higher protein content (25-35%) of azolla than most green forage crops and aquatic macrophytes, and contains essential amino acid – lysine, vitamins (vitamin A, vitamin B12 and Beta Carotene) growth promoter,

vitamin A showed improved performance of broiler under heat-stress when supplemented in drinking water (Kamalasanan *et al.,* 2002; Vierden and Kidd, 2009), that is favorable for animal nutrition, thus a valuable protein supplement for many animal species, including ruminants, poultry, pigs and fish (Cabaral and Rieta, 2020; Tran *et al.,* 2020; Hasan and Chakrabarti, 2009; Kamalasanan *et al.,* 2002). In addition, results was in line to the findings reported by Cabaral and Rieta (2020), Tran *et al.,* (2020), Rieta and Cabaral (2018), Norwan (2015), Balaji *et al.,* (2010), Namra *et al.,* (2010), Balaji *et al.,* (2009), and Lumpkin *et al.,* (2003). Moreover, broilers (Rieta and Cabaral, 2018; Taaroa, 2013; Minh, 2005) and pekin ducks (Cabaral and Rieta, 2020) fed with 15-30% and higher proportion either fresh and/or dried azolla has high growth performance with higher body weights, ADG and FCR compared to birds without supplementation and thus has high potential as alternative feedstuffs for expensive protein source like soybean.

Table 1. Initial and final weights of pekin duck fed with azolla raised under induced heat stress

LEVEL OF AZOLLA	INITIAL BODY WEIGHT	FINAL BODY WEIGHT
Pure commercial feeds	2.533[a]	2.733[b]
75% commercial feeds+25% fresh azolla	2.533[a]	3.067[a]
50% commercial feeds+50% fresh azolla	2.567[a]	3.167[a]
25% commercial feeds+75% fresh azolla	2.500[a]	3.150[a]

Means within column having different superscripts are significantly different ($p<0.01$).

Blood and Urine Cortisol: Analysis showed significant ($p<0.01$) differences on the blood and urine cortisol of pekin ducks fed with azolla raised under induced heat stress. Pekin ducks fed with 50% azolla in the dietary ration has significantly ($p<0.01$) lower blood and urine cortisol level compared to the cortisol level of ducks fed with 25% and 75% azolla and ducks fed with pure commercial feeds (Table 2). Results can be attributed to the high crude protein content of azolla that are highly digestible (Cagauan *et al.,* 2000 and Kamalasanan *et al.,* 2002) and other nutrients like iron, probiotics and lysine (essential amino acid) that enhance nutrient absorption, its glutamine content and favorable immune-modulatory effect on poultry that can combat heat stress without any toxicity (Mishra *et al.,* 2016); it essential amino acid Met, Thr, Arg, Val, Trp, Cys, Gly, Ser, Tyr, Phe, Ala, His, Pro, and Asp, content are glucogenic that can be converted to glucose (Huggins, 2007; Leterme *et al.,* 2009; Murthy *et al.,* 2013; Parashuramulu *et al.,* 2013; Vierden and Kidd, 2009), thus potential still exists for electrolytes to play a role in physiological stress reduction. Because glucocorticoid and catecholamine function causes increased in urinary electrolyte excretion, perhaps electrolytes deficiencies occur during physiological stress. Additionally, azolla contain vitamins (vitamin A, vitamin B12 and Beta Carotene) growth promoter intermediaries. Heat-stressed broilers supplemented with vitamin A in the drinking water showed improved performance (Kamalasanan *et al.,* 2002; Vierden and Kidd, 2009), that subsequently lower the level of blood cortisol. Likewise, results can be associated to the findings of Cabaral and Rieta (2020) and Bhattacharyya *et al.,* (2016) who reported that azolla feeding in poultry diet has significantly ($p<0.05$) lower bad cholesterol while increasing good cholesterol which result in reduced triglycerides, hence a potential feedstuff to relieved stress that consequently enhanced feed intake and improved productivity.

Table 2. Blood and urine of pekin duck fed with azolla raised under induced heat stress

LEVEL OF AZOLLA	BLOOD CORTISOL	URINE CORTISOL
Pure commercial feeds	21.000[a]	11.660[a]
75% commercial feeds+25% fresh azolla	20.000[a]	9.310[b]
50% commercial feeds+50% fresh azolla	3.000[c]	5.780[c]
25% commercial feeds+75% fresh azolla	5.000[b]	8.700[b]

Means within column having different superscripts are significantly different ($p<0.01$).

Behavior: Analysis revealed comparable ($p<0.05$) aggressiveness and molting behavior among pekin ducks fed with azolla regardless of proportion in the dietary ration raised under induced heat stress. Results showed that including azolla in the dietary ration did not trigger any aggressive behavior and molting in pekin ducks. Moreover, results revealed highly significant ($p<0.01$) differences on the feather pecking of pekin ducks raised under induced heat stress and fed with azolla. Pekin ducks fed with pure commercial feeds showed almost 70-80% ducks that are pecking feathers ($p<0.05$) compared to ducks that fed with azolla regardless of proportion in the dietary ration raised under induced heat stress (Table 3). On the other hand, findings on the eating habit of pekin ducks raised under induced heat stress and fed with azolla regardless of level has significantly ($p<0.05$) higher percentage (70-80% are eating) of ducks eating than the ducks provided with pure commercial feeds (30-50% are eating). Results can be associated to the significantly ($p<0.01$) lower cortisol level of ducks fed with azolla even under heat stress both in blood and urine that is known as the stress hormone; glutamine content of azolla that has favorable immune-modulatory effects to combat heat stress without any toxicity (Mishra *et al.,* 2016); it essential amino acid Met, Thr, Arg, Val, Trp, Cys, Gly, Ser, Tyr, Phe, Ala, His, Pro, and Asp, content are glucogenic that can be converted to glucose (Huggins, 2007; Leterme *et al.,* 2009; Murthy *et al.,* 2013; Parashuramulu *et al.,* 2013; Vierden and Kidd, 2009). Thus potential still exists for electrolytes to play a role in physiological stress reduction. Because glucocorticoid and catecholamine fuction cause increases in urinary electrolyte excretion, perhaps electrolytes deficiencies occur during physiological stress. Additionally, azolla contain vitamins (vitamin A, vitamin B12 and Beta Carotene) growth promoter intermediaries. Heat-stressed broilers supplemented with vitamin A in the drinking water showed improved performance (Kamalasanan *et al.,* 2002; Vierden and Kidd, 2009). Providing comfort and reduced stress; essential amino acid and vitamin content of azolla that might help the ducks cope with stress by reducing electrolytes excretion while improving electrolytes absorption during heat-stress condition, thus relaxes the animals which consequently reduces the incidence of stress and contributes to the efficiency to convert feeds into kilogram of meat and body weight gain, hence a potential feedstuff for stress alleviation that consequently enhanced feed intake and improved productivity.

Table 3. Behavior of pekin duck fed with azolla under induced heat stress

Level of Azolla	Aggressiveness	Feather pecking	Eating habit	Molting
Pure commercial feeds	1.207[a]	1.300[a]	1.214[b]	1.469[a]
75% commercial feeds+25% fresh azolla	1.282[a]	1.000[b]	1.681[a]	1.080[a]
50% commercial feeds+50% fresh azolla	1.213[a]	1.000[b]	1.574[ab]	1.058[a]
25% commercial feeds+75% fresh azolla	1.213[a]	1.000[b]	1.791[a]	1.081[a]

Means within column having different superscripts are significantly different ($p<0.05$).

Economics

In general, analysis revealed that pekin duck provided with 50% and 75% azolla in the dietary ration has the highest incurred sales, net income and return on investment that was highly significant ($p<0.01$) from the income of raising pekin ducks provided with 25% azolla and pure commercial feeds (Table 4). Results can be attributed to the highly significant ($p<0.01$) final body weight of pekin ducks provided with 50% and 75% azolla in the diet even under induced heat stress. In addition, higher ROI incurred in ducks fed with 50% and 75% azolla as an alternative protein source is due to its low-cost of production input compared to pure commercial feeds which is considerably expensive, hence decreases the total costs that consequently improves the net income and ROI.

Table 4. Economics of raising pekin duck fed with azolla under induced heat stress

LEVEL OF AZOLLA	TOTAL SALES	TOTAL COST	NET INCOME	RETURN ON INVESTMENT
Pure commercial feeds	1093.333	780.354	312.979	40.225[c]
75% commercial feeds +25% fresh azolla	1226.667	825.012	401.655	48.644[b]
50% commercial feeds +50% fresh azolla	1266.667	837.540	429.127	51.485[a]
25% commercial feeds +75% fresh azolla	1260.000	837.076	422.924	50.622[a]

Means within column having different superscripts are significantly different (p<0.01).

CONCLUSION

Generally, pekin ducks fed with 25% to 75% azolla in the dietary ration have significantly heavier average final weights. Feeding of 50% azolla in the dietary ration of pekin ducks raised under induced heat stress has significantly lower blood and urine cortisol level. Including 25% to 75% azolla in the dietary ration did not trigger any aggressive behavior and molting in pekin ducks. Inclusion of azolla in the dietary ration of pekin ducks raised under induced heat stress reduces the feather pecking behavior of ducks compared to ducks fed with pure commercial feeds. On the other hand, findings showed that eating habit of pekin ducks raised under induced heat stress and fed with azolla regardless of level can increased the eating percentage of ducks. In general, pekin ducks provided with 50% to 75% azolla in the dietary ration significantly increases the net income and return on investment.

Recommendation

Based on the result of the study, the researcher recommended the use of 50% to 75% azolla in dietary ration since it enhances average final weight, eating habit while lowering the stress hormone cortisol in terms of blood and urine cortisol level of the ducks. Moreover, provision of azolla up to 75% in dietary ration can be adapted by the farmers to improved eating habit while lowering blood and urine cortisol level and reducing aggression of various avian species even under induced heat stress with higher economic returns.

Conflict of Interest: We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

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