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RESEARCH ARTICLE

BLOOD MEAL SUPPLEMENT IMPROVES EXPLORATION BEHAVIOUR BUT INCREASES ESCAPE ATTEMPT

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ABSTRACT

Blood meal as an animal feed supplement promotes agricultural sustainability. Blood meal which is high in proteins, lacks certain nutrients hence is expected to give impact on the chicken behaviour and welfare. This study was carried out to determine the impact of blood meal supplement on chicken behaviour. The study involved 100 chickens which were bred in semi-opened poultry house for 6 weeks. At 6th week, chickens provided with fish meal (FM) only had a higher body weight compared to that of the group provided with FM and blood meal supplement (FBM). Normal behaviour such as walking, standing, feeding, drinking, dust bathing and lying down were not significantly affected by the changes in the meal ($p>0.05$). However, based on a single assessor assessment, FBM group displayed higher score in explore and escape characteristics, than the FM group. On the other hand, FM group displayed a slightly higher score for fear behaviour than the FBM. Findings in this study leads to the conclusion that blood meal supplement has influence on the welfare in chickens farming in terms of their exploration, fear, and escape behaviours. Therefore, amount of blood meal as animal feed supplement in poultry production should be determined carefully to avoid any potential detrimental effect on poultry welfare.

KEYWORDS

animal behaviour, *Gallus domesticus* L., poultry nutrition, protein meal, welfare

1. INTRODUCTION

Blood meal is favoured by animal nutritionists and environmentalists because of its high protein content and benefit as a sound disposal that promotes agricultural sustainability. Post-slaughtered blood of various poultry and fish are dried after coagulation and separation of the water content to prepare powdered blood meal (Beski et al., 2015). Benefits of blood meal as protein sources for poultry are attributed to the economic impact and productivity. Blood meal is rich in lysine and trace minerals (Ravindran et al., 2005; Seifdavati et al., 2008). Blood meal in balanced broiler diets resulted in better growth performance (Donkoh et al., 1999; Khawaja et al., 2007; Seifdavati et al., 2008; Makinde and Sonaiya, 2011; Adeyemi et al., 2012).

However, outcome of the blood meal varies, depending on the amount of blood meal added to the feed. The usage of less than 5% of blood meal in feed improved the performance of poultry (Anoh and Akpet, 2013). Chickens were found to have improved body weight (BW) gain with no adverse effect on their growth rate when 3% of blood meal was included in the feed (Khawaja et al., 2007). To the contrary, 3% or higher blood meal was shown to have no influence in the feed intake as well as the BW gain in chickens (Seifdavati et al., 2008). Despite the commercial benefit of blood meal in poultry production, the efficiency of meat production should not compromise health and wellbeing of the chickens.

In many instances, poultry nutrition is formulated without advocating the animal welfare (Oso et al., 2011; Kalmar et al., 2013; Goldberg, 2016; Wu et al., 2017). It can be noted that balanced diet formula promotes calm animals and consequently aids in the ease of handling, improved productivity and enhanced husbandry system. Blood meal supplements can cause imbalance of certain amino acids in poultry feed, which indirectly may affect chicken behaviour. Thus, adding blood meal into chicken's diet should be given careful consideration, given its unknown impact on chicken wellbeing.

2. MATERIALS AND METHODS

2.1 Animals, farm facilities and dietary treatments

This study was approved by the Institutional Animal Care and Ethics Committee (IACUC) at IIUM [Reference#/2017(3)]. The study was conducted for 6 weeks between end of February until early April 2017, at a farm in Kuantan, Malaysia. Cross-bred chickens were reared in a semi-opened, 32.4 m^2 poultry house, with netted walls for ventilation and wood shaven, saw dust and sand as beddings. For this study, a wall was placed to divide the area of the house into two compartments, for two study groups. Each compartment was provided with a feeder and a drinker to ensure *ad libitum* feed and water. Brown-coloured feed pellets (Cargill, Malaysia) containing fish meal (FM) as the source of protein feed were fed to all chicks. One hundred 3-day-old crossbred chicks, with initial BW of 70 ± 7 g, were distributed randomly into two groups (50 for each group): one was fed with FM only and the other group was fed with FM

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supplemented with 3% blood meal protein (FBM, Justlong Import & Export Co. Ltd., Dalian; source of blood, chickens). Both FM and FBM chicks were provided with the same base feed (FM) on the first week. On 2nd week onwards, FM chicks were given FM only feed and FBM chickens were fed with FM with blood meal supplement.

2.2 Growth

Weight gained was recorded on weekly basis using portable weighing scale (HB Series, New York). After adding blood meal powder to the feed pellets, the colour of the pellet changed slightly to red. To ensure that colours do not affect the amount of feed uptake, a preference test on colour was conducted (see S1) and weight gain were illustrated in Figure S2.

2.3 Experimental procedure

Birds were weekly monitored in the poultry house at the age of 2 weeks old and onwards, between 0900-1300, using a wide-angled video camera (Sony HD Handycam, San Diego) and a smartphone camera (OPPO, Dongguan). Every week, each episode of video recording involved 10 min of normal behaviour recording, followed by 1 min of each novel object test. Within the 10 min recording, normal behaviour was recorded for three repeats of 2 min cycles.

2.3.1 Normal behaviour assessment

Animals were subjected to visual observation using scan sampling (Newberry et al., 1987), in which the behaviour in each group was observed at 20 s intervals throughout 2 min recording. At the end of each 2 min cycle, the cumulative frequency of behaviour was recorded, and the cycle was repeated thrice. The mean for the frequency of normal behaviour were recorded and the proportion of chickens walking or standing (WS), feeding (FEED), drinking (DRNK) and dust bathing or lying down (DBL) over all behaviours were determined.

2.3.2 Novel object test

For testing, 2 types of stressors were introduced to the chickens: (i) a distraction by throwing an object; and (ii) an interference whereby a handler stood still or walked for 1 min in a consistent pattern inside the chicken compartment (Uzunova, 2007; Forkman et al., 2007). A rating system was designed to assess three characteristics of bird behaviour in novel situations: (i) fear; (ii) exploratory; and (iii) escape attempt. Induced behaviour was recorded by a single assessor according to the ethogram provided in Table 1.

Table 1: Ethogram used for novel object test.

Stressor	Behaviour	Definition
A thrown object (i.e. glove)	Explore	Approach and peck at the object, circle around it and look at it, with neck fully straightened forward towards the object
	Fear	Startle and quickly move away from the object.
	Escape	Attempt to jump over the partition/wall.
A handler (standing still or walking in the compartment)	Explore	Look at the (standing/non-moving) handler and walk towards him/her.
	Fear	Run away from the (walking) handler, with their wings flapping vigorously. Step on other chickens in their effort to avoid the (walking) handler.
	Escape	Attempt to jump over the partition/wall.

2.4 Statistical analysis

Data was analysed by GraphPad Prism (California). Mann-Whitney U test was performed to determine the colour preference for the feed. Independent *t*-test was used to analyse BW gained by weeks between experimental diets that are normally distributed. Descriptive statistics and two-way ANOVA was conducted to illustrate and analyse the normal behaviour of the chickens (which include normal activity such as WS, FEED, DRNK and DBL) in both treatment groups (FM and FBM). Homogeneity of variance was assessed, and appropriate corrections were made if necessary. All experiments were performed in a randomised fashion.

3. Results and Discussion

3.1 Effects of blood meal supplement on growth performance

This study examined the impact of blood meal supplement on the chickens' growth performance. Weekly BW gained for both FM and FBM groups were almost similar (ρ>0.05; Figure 1). The ranges of weekly weight gain were 67-319 g for FM group and 71-290 g for FBM group. Both groups saw the highest weight gain at week 5. Despite similar weight gain in both groups, at 6 weeks old, BW of chicks in FM group were greater than the FBM group (ρ=0.029). Colour of feed did not influence the feed uptake by the birds. It was noted earlier that the colour for FBM was reddish, and this may have some effects on the feeding, but our results found that there was no preference for colours of the feed among the birds (ρ=0.218; Supplementary Figure S1). Hence, we are satisfied that the significant low BW in FBM group was not due to the feed colour (nor low feed uptake). Earlier studies showed that 3% blood meal supplement improved faecal digestibility, as well as reducing relative cost per unit weight gain (Khawaja et al., 2007). Anoh and Akpet (2013) reported no significant differences when broilers were fed with FM only and FM with 5% bloodmeal. In contrast, weight gain was found to be compromised when broilers were fed with 5% bloodmeal (Caires et al., 2010). It could be suggested that these chickens lack the enzymes to break down the bloodmeal and the imbalance amino acids level hijacked the metabolism (Caires et al., 2010; Anoh and Akpet, 2013). The cumulative effect could be apparent at week 6, which would explain the low BW detected in FBM group.

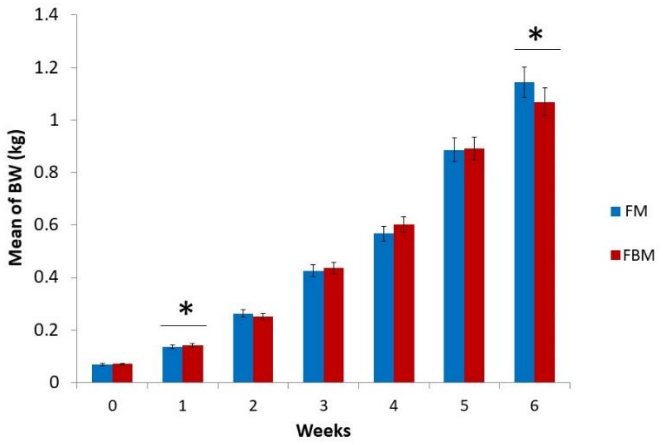

Figure 1: Distribution of the mean of chicken's BW over 6 weeks growth in both chicken groups. BW, body weight; FBM, fish-blood meal; FM, fish meal. *ρ-value < 0.05

3.2 Impacts of blood meal supplement on chicken behaviour

3.2.1 Normal behaviour

In this study, the impact of FM and FBM was studied in normal and induced behaviours. No significance difference was observed in FM and FBM groups, in terms of feeding (p=0.25), drinking (p=0.98), and dust bathing or lying down activities (p=0.81; Table 2). Only walking or standing activity was different significantly between the treatment groups (p=0.02; Figure 2). Increased walking and standing activity maybe associated with increased heat stress or by high body temperatures (Costa et al., 2012). However, there was no increase in drinking activity for the FBM group to support heat stress as the cause of the increased WS activity in FBM group (Table 2). Young chicks in the FBM group are found to walk and stand up more than the ones in FM group although the reason for this is unclear. In fact, walking activity was varied between week 1 and 3 for chickens in FBM group, and between weeks 1, 2 and 4 in the FM group. However, when comparing the proportions of normal behaviour over total behaviour, all activities were statistically similar in both groups, suggesting that the bloodmeal supplementation does not affect the normal behaviour of the chickens, including the WS activity. Our study also agreed with previous study that all activities including FEED, DRNK and WS had declined significantly with increasing age of chickens (Newberry et al., 1987).

Table 2: Comparison between feed type and behaviour (T-test, n=30)

Behaviour category	Proportion of behaviour¥		
	Fish meal (%)	Fish-blood meal (%)	Significance
WS	36.8±11.9	44.2±18.9	NS
FEED	10.1±9.0	11.6±5.7	NS
DRNK	2.9±2.0	2.7±2.5	NS
DBL	48.9±16.0	46.6±14.8	NS

¥ Percentage of number of chickens over total of chickens observed in the group.

NS, not significant. FEED, feeding; DBL, dust bathing or lying; DRNK, drinking; WS, walking & standing.

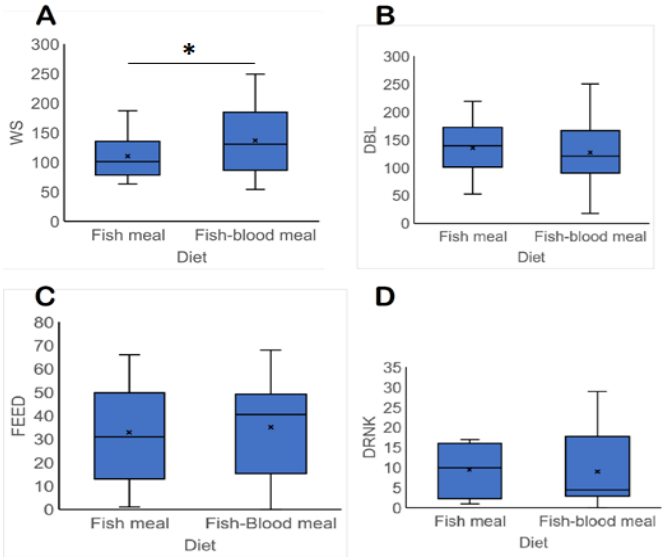

Figure 2: Box plots showing the range of data and standard deviations for the frequency of chicken's behaviour in each group: (A) WS, walking and standing; (B) DBL, dust bathing or lying; (C) FEED, feeding; and (D) DRINK, drinking. Both groups were similar in terms of the frequency of normal behaviours, except for in (A) where group provided with blood meal supplement showed higher WS activity than the fish meal only group.

Injury due to feather pecking was observed in two birds from the FBM group, and 1 bird from the FM group between week 3 and 4. It is likely that the injury involves not only pecking, but also a possible removal of the feathers of one bird by another (Costa et al., 2012), and this behaviour is considered by Bracke and Hopster (2006) as a symptom of negative welfare status. In fact, authors noted that these injuries were severe, due to the excessive number of feathers being pulled out, and the presence of blood from the injuries. Various parts that were affected by feather pecking include the neck, wing, and at the back. Severe feather pecking is considered detrimental to bird welfare because it causes pain (Gentle and Hunter, 1991), and the blood from the injuries may lead to cannibalism (Duncan and Hughes, 1972). Authors also noted that injured chickens were less sociable and demonstrated a tendency to isolate themselves from the group. However, since birds from both groups were injured, no conclusive findings were available to link the feather pecking behaviour with the blood meal supplement.

3.2.2 Induced behaviour

Induced behaviour was assessed by novel object tests. Behavioural scores for FBM group are higher for explore and escape characteristics (Table 3). FM chickens scored higher for fear characteristic than the FBM chickens. Exploration behaviour is said to be an essential for animal survival, thus a quality trait in animal welfare. This alone may support the use of blood meal in feed. However, escape attempt is associated with animal agitation in response to stress, as well as a sign of fear (EFSA et al., 2019). Hence the two characteristics might be contradicting observation. Fear, on the other hand, can be seen as the animals' reaction to a perceived danger which can be harmful to health and affect productivity in husbandry systems (Zulkifli and Siti Nor Azah, 2004; Agnvall et al., 2014; Meuser et al., 2021). In this study, the novel object and handler were perceived as a threat and the chickens responded by avoiding and withdrawing from the novel object. This behaviour could also be attributed to active/pro-active fight and flight response (Armstrong et al., 2020).

On the contrary, the opposite to active response is the reactive/passive response with individuals displaying a freezing-type fear response (Armstrong et al., 2020). A delayed reaction, where chickens were seen slowly reacting to the novel objects is also an indication of a fear response. The fear characteristic appears to be diminished as the chickens became familiar with the stressors. The behavioural score for week 5 was lower than week 3 for both groups (Table 3). A decreased reaction of fear towards the stressor can be seen as an indicator for a good adaptation (Agnvall et al., 2014; Meuser et al., 2021). However, FBM group displayed increased escape behaviour in week 5 whereby more than 6 chickens successfully flew over the partition wall, suggesting an increased agitation in that group. However, escape propensity was described as a more proactive behavioral type instead of fearfulness in red junglefowl (Zidar et al., 2017; Rubene and Løvlie, 2021).

Table 3: Rating scores for induced behaviour (Score: 0, no such behaviour; 1, least likely towards the behaviour; 2, likely towards the behaviour; 3, very likely towards the behaviour)

Behaviour	**Stressor**	**Behavioural score$^{\Delta}$**	
		FM	**FBM**
Explore	Object	2	3
	Standing/non-moving handler	1	3
Score		3	**6***
Fear	Object	3	3
	Walking handler (Week3) (Week 5)	 3 1	 2 1
Score		**7***	6
Escape	Object	0	0
	Handler (Week 3) (Week 5)	 2 1	 2 3
Score		3	**5***

$^{\Delta}$ For each behaviour, higher scores between the two groups are indicated in asterisk (*) and bold.

FM, fish meal group; FBM, fish meal with blood meal supplement group.

Feed composition and unavailability of macro- or micro-nutrients could contribute to different animal behaviours towards certain. Metallic taste and odour from the iron of haemoglobin from the blood meal supplement are repugnant in chicken diet. Aggressive behaviour could also be due to specific restrictions in animal proteins. For example, experiments have shown that protein-deficient diets can result in growing pigs mutilating the bodies of other individuals by biting off their tails (Fraser, 1987; Fraser et al., 1991). Jensen et al. (1993) found that protein-deprived growing pigs will spend more time directing foraging behaviour at straw than individuals who are able to meet their protein requirements. Malnutrition can lead to aggressive behaviour and boldness as seen in many studies on insects. Aggressive behaviour is particularly predicted to vary with level of protein intake (Wilder and Rypstra, 2008). In cannibalistic species such as Mormon crickets, protein deficiency is also likely to increase the expression of aggressive behaviour because the deficit of proteins leads to increased frequency of cannibalism (Simpson et al., 2006). Blood meal supplement in poultry feed results in the imbalance of certain amino acids in chickens.

The importance of the current study is not limited to chicken welfare. Certain cultures and religious adherents have strict diet, which include forbidden in the consumption of blood. The *halal* (permissibility) in Islam is often mentioned with *tayyibat* which means good, pleasant, delightful, delicious, sweet, pure and clean. According to the Islamic jurisprudence, any form of blood that flows out of the body, such as during slaughtering, is deemed as impure or filth (Nurdeng, 2009). It is perhaps because of this reason, the use of blood meal in animal feed could be opposed in *halal* diet (Ofori and Hsieh, 2011; Shahdan et al., 2016). Certain religions advocate eating food which is not only good for the body, but also the mind and spirit. Hence, the use of blood meal in chicken feed may need to be appraised if the industry is considering to promote this as an alternative for, say, *halal* meat production which tenet includes prohibition to consume meat and meat products that could be injurious to one's physical health and detrimental to the character and spiritual faculties of man (Nurdeng, 2009).

4. Conclusion

This study provides an observation on the impact of blood meal supplement in chicken's diet on fear, exploration and escape attempts. These three traits of behavior are important from husbandry point of view in ensuring efficient production and animal welfare. Blood meal supplement did not influence the normal behavior of the chickens. Although chickens from FBM group had higher frequency of walking and standing than the FM group, when looking at individual activity over the entire normal behavior, both FM and FBM groups had similar normal behaviour. We adapted scoring system as a tool for novel object tests to assess free range chickens. The tests demonstrated that FM group had a slightly higher fear behaviour than the FBM. However, FBM group displayed more escape attempts than the FM.

This could suggest that both groups experienced fear in novel situations, but FBM group had an overwhelming flight response to cope with the fear. In addition, FBM group demonstrated higher exploration behaviour than the FM, indicating a positive behavior. In husbandry setting, it is important to reduce fear and escape behaviours to improve the animal welfare. Exploration behaviour indicates positive adaptation of the chickens to novel situation and this would enhance productivity. Formulating animal diet must take into account the aspect of animal welfare, and behavioural tests is one of the tools for such assessments. We concluded that induced behaviour is a better approach to examine the impact of diet, and future studies may consider other behaviours such as wing flapping and tonic immobility as the test parameters. Indeed, blood meal is a good source of protein and environmentally a sustainable option for poultry producers. However, poultry producers may consider other factors including welfare of the chickens, as well as the *halal* market, when making a choice for an affordable and quality protein feed.

Declaration

We declared that this manuscript is an original work, and at present, has not been sent, in part or in whole, for publication to another scientific journal.

Conflict of Interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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