BEHAVIOURAL RESPONSES OF BROILER CHICKENS FED DIETS CONTAINING DIFFERENT ENZYMES

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ABSTRACT
Animals used behaviour as one of the important tools of adapting to their physical and social environment. This study was conducted to examine the effect of enzyme on the behavioural response of broiler chickens fed with diets containing different enzymes.. The experiment was carried out for six weeks using one hundred and twenty-day old cobb 500 broiler chicks (n = 120). The birds were randomly distributed into four dietary treatment, such that there were 30 birds per each treatment and replicated thrice with ten birds each.. Treatment 1 (T1) (control diet without enzyme), T2 (diet with enzyme Nutrizymes NZ), T3 (diet with enzyme Nutrizymes NZ + Avizymes VZ) and T4 (diet with enzyme Nutrizymes NZ + Roxazyme PZ). Walking, lying, floor scratching, eating, wing flapping, feather pecking, dust bathing, preening and flying were not significantly ($p<0.05$) influenced by age while floor scratching, fighting and sleeping were significant ($p<0.05$). floor and fighting (3.20) behaviours were observed in broiler chickens atb5th week while birds appeared to sleep (0.81) more at 3rd week of age compared to the fifth week. Birds were seen lying down frequently (23.76) ($p<0.5$) in treatment 4 (NZ+PZ) while a higher occurrence of floor scratching (106.10) was observed on treatment 3 (NZ+VZ) diet which signifies better welfare. The interaction between enzyme and behaviour was significant ($p<0.05$) in birds on diet NZ+VZ with higher incidence of floor scratching (188.0) while fighting behaviour was seen on birds in T4 (NZ+PZ) diet. The study concluded that addition of enzymes in broiler diet enhances better behavioural response in NZ+PZ.
Keywords: Behaviour, Responses, Enzymes, Diet. Bro

INTRODUCTION

Broiler chickens originate from the jungle fowl of India subcontinent and are reared for meat production. Broiler feed is predominantly made up of cereal grains and protein with little amount of other nutrient and most of these feed ingredient contains non-digestive part (cellulose, xylose and galactonic acid) and some of these nutritive factors, effects feed utilization and birds performances, nutrition is part of welfare that motivated some specific behaviours that is typical of chicken such as pecking, scratching of floor, preening and dust bathing. Behaviour is the frequency used by experienced farmer to determined potential problem in birds, (Dawkins, 1999 and Duncan, 1998). The search for good welfare is important in poultry production (Moura *et al.,* 2006), because poultry behaviours is a reflex of their welfare status at a particular moment, and it is related to internal (physiological) and external (environmental) factors Ref.?. Several natural behaviours that favour welfare, as well as undesirable behaviours, may be stimulated by environmental enrichment (Coria, *et al*. 2022). The correct interpretation of poultry behaviours are expressed in frequency of what?, duration of what?, and sequence of what? which may be used to estimate the bird's behaviours (Li, *et al*. 2020). Welfare depends on how broiler chicken adapt to it environment, taking into account not only the physical aspects of the environment, but also the social aspects. In the last decades there has been a great improvement in poultry production based on the careful control of several aspects which include nutrition, management and ability to assess the bird's welfare which require a good understanding of the birds (Boissy, *et al*. 2007).

Good welfare can occur, provided the bird can adapt or cope with the constraints it is exposed to, hence welfare varies from very poor to very good and can be scientifically assessed. The word stress is used by some authors when there is failure to cope with it (Fraser and Broom, 1990; Humphrey, 2006), while others use it for any situation when the birds is forced to respond to environmental

challenge (Selye, 1980; Zulkifli and Siegel, 1995). The welfare of a farm animal can be considered in relation to the feed, housing and management conditions to which it is subjected (Madzingira, 2018; Webster and Margerison, 2022).

Phytase and protease, xylanase addition influences intestinal mucin production and also the composition of the mucin polymers (Fernandez *et al*., 2000). Addition of enzymes to poultry feed which are utilized currently in the poultry industry to improve nutrient utilization and reduce anti-nutritive factors, breakdown resistant starch, or phytate phosphorus, maintaining homeostatic balance between intestinal growth, cellular turnover, and bacterial colonization all of which relate to the health of the birds might likely induce stress or bring about other effects on the bird's behavior thus affecting their welfare (Choct, 2006; Erdaw, *et al*., 2016). The use of enzymes in the birds' diets had been successful in improving feed formulation (Choct, 2006), but little is known of the influence of enzymes on bird's behaviour (Negm, *et al*., 2023). In order to ascertain exogenous enzymes effect on bird's behaviour, this study was carried out to investigate more on the behavioural responses of broiler chickens to varying levels of dietary inclusion of enzymes.

MATERIALS AND METHODS

Experimental Site

The experiment was carried out in the poultry unit of the Directorate of University Farms of the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The study area is located on latitude 70 30' N and longitude 30 54' E. It lies within the humid lowland rainforest region with two distinctive seasons. The wet season extends from March to October while the dry season extends fromNovember to February. The vegetation has the characteristics of a tropical rain forest such as high forest and growth of massive trees and twinning shrubs. The forest is covered with a litter of fallen trees by both anthropogenic and natural activities (Ufoegbune and Fabiyi,2016)

Experimental Animals

A total number of one hundred and twenty (120) day old Cobb broiler chicks were purchased and brooded for two (2) weeks and were randomly distributed into four treatments of three replicate with 10 birds per replicate. Group one (1) was the control diet and the remaining three groups contained enzymes (NZ, NZ+VZ, NZ+PZ). Experimental diets and water were provided *ad libitum*. The birds' behaviours were observed at third and fifth weeks of the experiment. The behaviours of the broiler chickens were recorded using CCTV recording machine which was installed to each pen, recording the activities of the birds. Vaccination schedule and medication for birds was given when due. The experiment lasted for 42 days.

Experiment Diets

Commercial broiler starter feed was used for this experiment, supplemented with different enzymes based on the manufacturer's recommendation. Experimental diet one (T1) represent the control diet without enzyme, treatment two (T2) contained experimental diet with enzyme NZ while treatment three (T3) contained experimental diet with enzyme NZ and VZ and treatment four (T4) contained experimental diet with enzyme NZ and PZ.

Table 1: Nutrient composition of the experimental Diets

INGREDIENTS	T1	T2	T3	T4
ME (Kcal/Kg)	2700	2700	2700	2700
Crude protein	21%	21%	21%	21%
Fat	5.0%	5.0%	5.0%	5.0%
Ca	1.0%	1.0%	1.0%	1.0%
Available P	0.45%	0.45%	0.45%	0.45%
Fibre	5%	5%	5%	5%
Enzymes	No enzyme	NZ only	NZ + VZ	NZ + PZ
Inclusion rate	-	8.75kg/bag	12.5kg/bag	3.125kg/bag

Enzymes were added based on manufacturer recommendation NZ=0.35G/Kg, VZ= 0.5g/Kg, PZ=0.125g/Kg and RZ =0.1g/kg. T2- commercial broiler feed without enzyme, T3- commercial broiler feed with NZ, T3- commercial broilerfeed with NZ+NV T4 and commercial broiler feed with NZ+PZ.

Table 2: Nutrients composition of the experimental Diets

INGREDIENTS	T1	T2	T3	T4
ME (Kcal/Kg)	2700	2700	2700	2700
Crude protein	21%	21%	21%	21%
Fat	5.0%	5.0%	5.0%	5.0%
Ca	1.0%	1.0%	1.0%	1.0%
Available P	0.45%	0.45%	0.45%	0.45%
Fibre	5%	5%	5%	5%
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feed with NZ+NV T4 and commercial broiler feed with NZ+PZ.

Behavioral observations

The behaviors of the birds were observed and recorded at third and fifth weeks of age in the morning and afternoon according to the procedure used by Lewis *et al.* (1997). The behavioral observations included: walking, eating, drinking, preening, pecking, dust bathing and scratching the litter. The various behaviors were recorded on a purpose-designed table, and their respective frequencies were calculated as a percentage of the total observed behaviors.

Table 3 the Ethogram of behavioural parameters observed.

Activity	Description
1. Walking	Moving from a direction by taking one or more steps at a time within the space allocated.
2. Lying	Head rested on the litters or breast touching the ground with neck stretched or lifted up.
3. Eating	The frequency in which the head is extended towards the feeder to feed.
4. Wing Flapping	Simultaneously lifting both wings from the body by flapping the wings.
5. Scratching floor	Scratching floor with feet usually associated with eating behaviour.
6. Sleeping	Bird's neck is fully stretched on the floor or sometimes tuck inside the web wing while the eyes are closed.
7. Dust bathing	Bathing the dust with the use of wings, head, neck and legs performing vertical wing-shaking, the behaviours is exhibited when there is environmental enrichment.
8. Drinking	The frequency the bird has contact with water by lowering the beak in the drinker.
9. Preening	The frequency at which the bird used its beak to scratch the plumage (feather).
10.Flying	The lifting of legs and wings from the ground level to another direction in the pen.
11. Wing/ leg stretching	Extending or stretching the right wing and leg at the same time.
12. Feather pecking	Birds raise their beak towards another bird by pecking the feather when standing close or lying down together.

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Results

The effect of age on the behaviours of broiler chickens at 3rd and 5th week

Table 4 shows the effect of age on the behaviours of broiler chickens. The effect of age on behaviours of broiler chickens were not significant ($p>0.05$) in the following parameters measured walking, eating, feather pecking, wing flapping, dust bathing, drinking, preening, Wing and leg stretching, flying except for floor scratching (38.20%) and fighting (3.20%) which were observed at 5th week ($p<0.05$) while sleeping behaviour was more evidence at 3rd week ($p<0.05$) than when the birds were at 5th week.

Table 4: The effect of behaviour of broiler chickens at 3rd and 5th week of age

Parameters	AGE		SEM	P value
	3 week	5 week		
Walking	26.75	30.60	8.80	0.68
Lying	10.08	15.30	3.85	0.21
Eating	7.45	9.10	3.06	0.62
Wing flapping	2.29	1.70	0.89	0.54
Floor scratching	9.77[b]	38.20[a]	5.24	0.00
Sleeping	0.81 [a]	0.00[b]	0.34	0.03
Feather pecking	0.34	0.00	0.23	0.18
Dust bathing	2.79	0.00	3.55	0.47
Drinking	4.50	5.10	2.33	0.81
Preening	26.80	24.40	9.36	0.81
Flying	2.88	3.70	1.28	0.56
Fighting	1.00[b]	3.20[a]	0.66	0.00

[ab] mean in the same row with different superscripts are significant (P<0.05). SEM=Standard error mean.

The effect of enzymes on behaviours of broiler chickens

The effect of enzymes on behaviours of broiler chickens (Table 5). The addition of exogenous enzymes did not significantly influence (p>0.05) some behaviour (walking, eating, feather pecking, wing flapping, dust bathing, drinking, preening, Wing and leg stretching, flying and fighting of broiler chickens. Lying down (23.76%) was more prominent in NZ+PZ enzyme inclusion diet while floor scratching (106.10%) were significantly higher in treatment NZ+PZ diet than other enzyme inclusion diet and control.

Table 5: The effect of enzyme on behaviours parameters of broiler chickens per hour

Parameters	Enzyme				SEM	P value
	CT	NZ	NZ+VZ	NZ+PZ		
Walking	28.28	27.97	22.51	37.51	10.51	0.49
Lying	12.58[b]	17.25[b]	2.26[b]	23.76[a]	4.60	0.00
Eating	15.93	5.54	2.86	8.67	3.66	0.22
Wing flapping	3.56	3.09	0.46	1.28	1.07	0.23
Floor scratching	2.18 [b]	1.33 [b]	106.10[a]	0.21 [b]	6.27	0.00
Sleeping	0.20	0.69	0.27	0.51	0.41	0.20
Feather pecking	0.12	0.30	0.18	0.19	0.28	0.59
Dusting bathing	0.28	1.17	0.16	0.21	4.35	0.17
Drinking	10.15	6.75	1.35	4.41	2.86	0.46
Preening	17.08	41.48	7.57	27.3	11.49	0.06
Flying	4.67	1.33	1.95	5.98	1.53	0.22
Fighting	2.49	0.65	0.07	6.41	0.79	0.15

[ab]mean in the same column with different superscripts are significant (P<0.05). CT- Commercial broiler diet with no enzyme, NZ – commercial broiler diet with enzyme NZ, NZ+VZ- commercial broiler diet with enzyme with NZ+PZ and commercial broiler diet with enzyme NZ+PZ. SEM=Standard error mean.

The effect of interaction between enzymes and behaviours of broiler chickens fed with exogenous enzymes

Table 6. shows the effect of interaction between behaviours and enzyme on broiler chickens. Interaction between behaviours and exogenous enzymes was significant (p<0.05) more in floor scratching (188.00%). The floor scratching was observed more in birds on treatment NZ+VZ diet which was higher (p<0.05) at 5th week while fighting behaviour was also seen on birds on enzyme

(NZ+PZ) at 5th week. Other parameter like walking, lying, feeding, drinking, preening, flying, sleeping, wing flapping and dust bathing were not significantly (p>0.05) influenced

Table 6: The effect of interaction between enzymes and behaviour of broiler chicken.

Parameters	WEEK 3							WEEK 5		
	CT	NZ	NZ+VZ	NZ+PZ	CT	NZ	NZ+VZ	NZ+PZ	SEM	P VALUE
Walking	23.56	29.94	18.03	35.01	33.00	26.00	27.00	40.00	7.43	0.98
Lying	9.66^{ab}	14.50^{ab}	2.52^{b}	18.52^{a}	15.50^{ab}	20.00^{ab}	2.00	29.00^{ab}	3.51	0.94
Eating	11.36	8.09	5.73	7.34	20.50	3.00	0.00	10.00	2.59	0.48
Wing flapping	2.63	4.19	0.93	2.55	4.50	2.00	0.00	0.00	0.76	0.54
Scratching floor	2.36^{c}	2.67^{c}	24.13^{b}	0.41	2.00^{bc}	0.00	188^{a}	0.00	4.43	0.00
Sleeping	0.41	1.38	0.54	1.02	0.00	0.00	0.00	0.00	0.29	0.92
Feather pecking	0.24	0.60	0.36	0.37	0.00	0.00	0.00	0.00	0.20	0.98
Dusting bathing	0.56	2.33	0.31	0.43	0.00	0.00	0.00	0.00	3.00	0.91
Drinking	8.79	4.51	2.70	4.82	11.50	9.00	0.00	4.00	2.13	0.90
Preening	14.17	33.97	14.14	38.78	20.00	49.00	1.00	16.00	7.92	0.76
Flying	3.84	2.65	0.90	3.95	5.50	0.00	3.00	8.00	1.08	0.56
Fighting	0.99^{b}	1.30^{b}	0.14^{b}	1.82^{b}	4.00^{ab}	0.00^{b}	0.00^{b}	11.00^{a}	0.55	0.00

abc mean in the same column with different superscripts are significant (P<0.05CT- Commercial broiler diet with no enzyme, NZ – commercial broiler diet with enzyme NZ, NZ+VZ- commercial broiler diet with enzyme with NZ+PZ and commercial broiler diet with enzyme NZ+PZ. SEM=Standard error mean.

Disscusion

Floor Scratching is a highly motivated behaviour of *Gallus gallus domesticus* which is important to bird's welfare according to Bracker and Hopster (2006); Hemsworth *et al.* (2020). This behaviour is very important to birds because the higher the intensity, duration and frequency, the healthier they are and the better the bird welfare. Birds preferred to scratch the litter when there was introduction of new substrate or after the substrate is been cleaned up or after feeding. Shields *et al.* (2005) revealed that scratching of floor is mostly evidenced after feeding and when the environment is enriched with bedding materials. Once there is no litter available for scratching it resulted into negative behaviour of feather pecking (de Haas *et al.* 2010; Cronin, *et al*. 2020) which was not evidence in these study. When analyzing the effect of enzymes on the behaviour, it related to the findings of Marquardt *et al.* (1996) where they reported that enzymes addition to poultry diets leads to improvement in their performance and welfare. The behavioural responses of floor scratching of behavioural responses was significant.Sutherland (2004) and Pariza and Cook, (2010), had reported that scratching behaviour is also associated with feeding in their research involving the use of enzymes in bird's diet to know its effect on behaviour. Scratching might have indicated that birds tends to feed more and is a natural behaviour exhibited in birds. This might tend to prove that the birds were under good management condition thus good welfare condition.

The frequency of behaviour tends to increase with age, because at week five birds spent more time in sleeping either in standing, sitting or lying position, and this lying behaviour enhanced body weight of birds which agree with the report of Blokhuis, (1984). Rest and sleeping were strongly associated with energy conservation, tissues restoration and growth. Sleeping or resting is related to age of broiler chicken because with increase in age, birds spend more time to lie-down (Malleau, *et al*. 2006; Forslind, *et al*. 2021). This was evidenced in this finding where birds slept more with increase in age adhering to the fact that the birds were conserving their energy for better tissue restoration?.

Fighting behaviour observed in the birds in this study was not in terms of aggressive behaviour but to establish hierarchy among the flock in the replicate which cannot be avoided especially during feeding, floor scratching and sleeping space and it can be influenced by some factors like age, colour, sex and size of comb (Mench, 2009). In this study, it was influenced by the age of the birds.

Zhang *et al.* (2005) and Mench (2001), reported that enzyme inclusion into broiler diet tends to improve their behavioural activities. Several studies have tried to encourage expression of more natural behaviour in broiler chickens through environmental enrichment. With the result of this study, birds fed enzymatic diets increased in their activities as their age increases leading to pronounce behavioural responses as seen with the floor scratching increase in fighting which was influenced significantly.

Despite the fact that some behaviour were not influenced by age or enzyme, the effect of age on the behaviours of broiler chickens at 5th week of age reflected increase in walking, lying and flying behaviours and these activities were seen more in NZ+PZ inclusion diet that support the finding of Bessei, (1992); Baxter *et al*. (2021), who reported that, lower growing strain of broiler walking

ability increases because of their less body weight while lying down which is related to the age of the bird, while birds on in NZ diet at 3rd week had increased in feather pecking, dust bathing and preening. Regarding body care and comfort behaviour of broilers, many studies have shown that comfort behaviour is important in body maintenance and care of the feather such as wing flapping, wing and leg stretching and preening to keep broiler feathers and it skin in healthy condition (Shields and Duncan, 2004). Estevez and Leone, (2008); Dawson *et al*. (2021) investigated the impact of enzymes on the broiler behaviours and reported that, these behaviours are very important to the birds because the higher the intensity, duration and incidence the better the bird welfare. Observed wing flapping, feeding and drinking of the birds on control treatment were more, the birds were eating and drinking more, there was no positive effect of enzyme diets on the body weight of broiler chicken.
As regard this present study, enzymes inclusion into broiler diets might have provided a kind of enrichment for the birds leading to good welfarism observed through the birds' behaviour. Furthermore, Baxter et al. (2019); Lourenço da Silva et al. (2021), reported that by enriching broiler chickens' environment, it will result into increased behavioural responses in birds as well as increase in general activity level. Enzyme inclusion in diet shows that there is higher probability of lying when birds spend time in feeding and drinking behaviour. This finding agreed with report of Razek and Tony, (2013).

CONCLUSION

From this study it could be concluded that enzymatic diets influence on behavioural responses were higher in floor scratching and sleeping. Interaction between behaviours and enzymes were significant in scratching floor and fighting. The floor scratching was observed more in birds on treatment NZ+VZ diet which was higher ($p<0.05$) at 5th week while fighting behaviour was also seen on birds on enzyme (NZ+PZ) at 5th week. Other parameter like walking, lying, feeding, drinking, preening, flying, sleeping, wing flapping and dust bathing were not significantly ($p>0.05$) influenced

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