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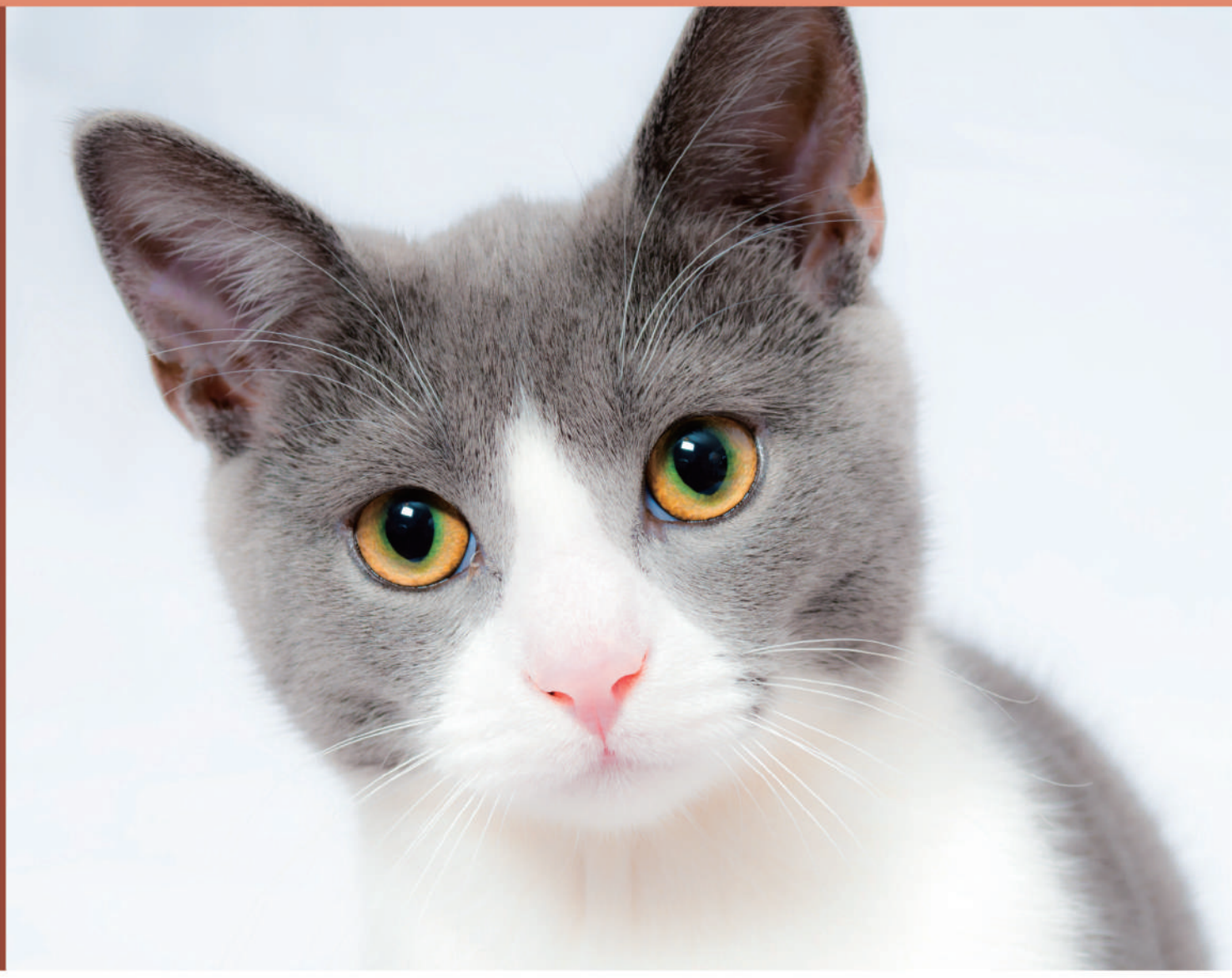

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ARTICLE

Response of Broiler Birds to Choline Chloride in Semi Arid Sokoto, Nigeria

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ABSTRACT

A study was carried out to evaluate growth performance and carcass characteristics of broiler birds fed with varying level of choline inclusion in their diets; T1 (control), T2 (10g/10kg) and T3 (20g/10kg). A total of 225 marshall broiler chicks were randomly divided into three (3) treatment group of 75 birds each. Each group was divided into five (5) replicates of fifteen (15) birds each laid in a completely randomized design. Feed and water was supplied ad-libitum and the experiment lasted for 49 days. The total feed intake (1316.75-14442.18) (24437.13-31999.76) for starter and finisher respectively, body weight gain (6227.30-8241.20) (10956.64-14182.96) for starter and finisher respectively, feed conversion ratio (1.73-2.26) (2.21-2.48) for starter and finisher respectively. Many (thigh, wings, back) of the carcass parameters measured were not significantly ($p>0.05$) affected by the treatments but significant difference ($p<0.05$) was observed in drum stick, breast and neck. However, significant difference ($p<0.05$) was also observed in gizzard, liver and bile, heart, lungs, legs and head. There was significant difference ($p<0.05$) in primal cuts per live weight, primal cuts per dressed weight, organs per live weight and organs per dressed weight. In view of the results obtained, it can be concluded that treatment two (10g/10kg) performed better in terms of total body weight (TBW) and feed conversion ratio (FCR). Also in the carcass characteristics, treatment two performed better in terms of breast yield, drumstick, percentage of primal cuts from live weight (P/LW) and percentage of primal cuts from dressed weight.

1. Introduction

Poultry industry has mainly two branches, which are; egg and meat production. Studies have indicated that broiler enterprise has great potential for increasing protein supply in Nigeria [7]. The hot-dry season is known to have the highest ambient temperature, long duration of sunshine and high relative humidity, making it thermally stressful to animals [13,21]. Heat stress in birds increase oxidative damage to cell evidenced by decrease in weight gain, feed intake and feed efficiency [11]. Broilers are strains of birds used for the purpose of producing a large quantity of chicken meat in a short pe-

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riod. Broiler chickens are raised from six to 10 weeks in poultry farms in Nigeria [1].

Nutrients are the nutritious components in foods that an organism utilizes to survive and grow. Macronutrients provide the bulk energy for an organism's metabolic system to function, while micronutrients provide the necessary cofactors for metabolism to be carried out. Both types of nutrients can be acquired from the environment (Whitney and Sharon, 2005). Only part of birds' nutrient requirements is provided by the natural feedstuffs in their diets. Nutrient supplements must therefore be included in feed formulations [14].

Choline, is a water soluble colourless compound with vitamin-like properties as not a metabolic catalyst but forms an essential structural component of body tissues [18]. Choline is ubiquitously distributed in all plant and animal cells, mostly in the form of the phospholipids, phosphatidylcholine (lecithin), lysophosphatidylcholine, choline plasmalogens and sphingomyelin - essential components of all membranes [28].Choline degrades in hot alkali creating trimethylamine. Choline has ability to form salts with many organic and inorganic acids. It is well soluble in water and ethanol, but not in ether. Choline is chemically a strong alkali and hygroscopic in nature. Choline is amino ethyl alcohol and have three methyl groups on the nitrogen atom, chemically termed as (2-Hydroxyethyl) trimethylammonium. Chemical formula of choline is $C_5H_{14}NO^+$ and of choline chloride is ($HOCH_2CH_2N$ (CH_3)3HCl) [6]. Cholinechloride have 139.63 g/mole molecular weight, 247^0c melting point, decompose on heating, 1.1 g/cm³ relative density at 20°C (70% choline chloride in water) and practically stable at 20-30^0C [6]. Choline is an essential nutrition for the poultry. One of its functions is to furnish methyl groups that can also be furnished by betaine and methionine [22,23].

It is important for researchers to reinvestigate the use of choline in poultry diets. In the past, several studies have looked at choline and betaine for their methionine sparing effects under ideal conditions (Rafeeq *et al.*, 2011a; Rafeeq *et al.*, 2011b), but it may also be important to evaluate choline for its primary function of supporting growth and not just as a methionine sparing molecule. Choline may also have added benefits during heat stress, since bird physiology is altered in this condition [16]. Therefore, the need for conducting this research.

In poultry industry, nutrition represents about 70% of total costs, thus constitutes a key factor in poultry production. Choline is classified as an essential vitamin for day-old chicks; it is usually added to diets for the purpose of furnishing the body with labile methyl group for formation of creatine and methionine. In addition, it also assists in the prevention of hemorrhagic kidney in different animal models and perosis in turkeys and broilers [25]. Choline's methyl group is available after the conversion to betaine in the liver. Choline has three essential metabolic roles, namely: As a constituent of phospholipids; secondly, it helps to prevent fatty liver; and thirdly, as a precursor for acetylcholine synthesis. Choline also has non-essential metabolic functions: As a labile methyl group, as well as prevention of perosis and fatty liver syndrome in broiler chicks (Workel *et al.*, 2002). Choline must be a part of the human and animal diets [8].

This research is aimed at assessing the response of broiler strain (marshal) to choline chloride at starter and finisher phase in semi-arid zone (Sokoto State), through thr following objectives:

(1) To assess the general performance of broiler birds fed with diet supplemented with choline chloride at starter and finisher phase.

(2) To assess carcass characteristics of broiler birds fed with diet supplemented with choline chloride at starter and finisher phase.

(3) To assess the level of inclusion of choline chloride that gives the best result in terms of performance and carcass quality.

2. Materials and Methods

2.1 Study Area

The study was carried out at the poultry production unit of the Department of Animal Science, Usmanu Danfodiyo University, Teaching and Research Farm situated at the veterinary clinic, Aliyu Jodi road in Sokoto metropolis, which lies between latitude 11°30N and 14°00N and longitude 4°00E and 6°40E. The state covers a total land area of 32,000km^2 (Tureta *et al.*, 2006), has a tropical continental climate and entirely falls within the semi-arid climatic environment. The annual rainfall is between 500 and 750mm with peak in August with mean monthly temperatures varying between 13°C in December/January and 42°C in April while the average annual temperature is 34°C (SERC, 2010). It has an estimated population of about 1,078,092 [19]. The demographic structure of the metropolis is cosmopolitan, albeit with the Hausas predominating, and Hausa is the common language. Occupation of the inhabitants includes trading; civil service while a reasonable proportion of the population works in organized private sectors [27].

2.2 Experimental Birds and their Management

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Two hundred and twenty five (225) Marshall Broiler chicks at day old were used for the experiment. The chicks were bought from a reputable dealer in Sokoto. The chicks were housed in a standard open-sided tropical house type. The housing unit was thoroughly clean and disinfected. Litter material e.g. wood shavings was used to cover the floor. Feed and water was served *ad libitum*. Vaccination was administered accordingly and disease outbreaks were adequately managed. Regular washing of the feeders and drinkers was carried out and sweeping of the pens were observed.

2.3 Experimental Feed Materials and Their Sourcing

The feeds to be used was self-formulated both starter and finisher feed. The birds were fed with commercial feed for the first three (3) days and afterwards introduced to the self-formulated feed without the test ingredient for the next four (4) days (adjustment week).Then from the second (2^{nd}) to seventh (7^{th}) week, they were fed with the self-formulated feed containing the test ingredient (choline chloride).

2.3.1 Sourcing of the Ingredients

The feed ingredients were bought from Sokoto central market e.g maize, wheat offal and salt. Other ingredients such as soya bean meal, groundnut cake, bone meal, lysine, methionine, limestone and premix was obtained from supply stores.

2.3.2 Gross and Calculated Chemical Composition of the Experimental Diets

Table 2.4.2 shows the gross composition and the calculated chemical composition of the feed ingredients for both starter and finisher diet.

2.4 Experimental Design

Two hundred and twenty five (225) chicks were randomly divided into three (3) treatment group of75 birds each. Each group was divided into five (5) replicates of fifteen (15) birds each. The three (3) treatment groups T_1 (control 0g/kg), T_2 (10g choline/10kg feed) and T_3 (20g choline/10kg of feed) was randomized in a completely randomized design (CRD) experiment. The birds were fed with starter diet for the first four (4) weeks and then finisher diet for the last four (4) weeks of age. The birds were housed in a deep litter system and good management was carried out. Feed and water was served *ad libitum*.

Table 1. Gross composition and calculated chemical composition of Feed Ingredients for both Starter and Finisher Diet

Ingredients	Starter (kg)	Finisher (kg)
Maize	50	49
Soya Bean Meal	18	22
Groundnut Cake	20	12
Wheat Offal	8	13
Limestone	1.5	0.5
Bone Meal	1.5	2.5
Premix	0.25	0.25
Lysine	0.25	0.25
Methionine	0.25	0.25
Salt	0.25	0.25
Total	100	100
Calculated Chemical Composition	**Starter**	**Finisher**
Energy (Kcal/kg)	3054	2941
Crude Protein (%)	23	21
Lysine (%)	0.9	1.1
Methionine (%)	0.6	0.6
Calcium (%)	1.0	0.9
Premix (%)	0.5	0.6
Fibre (%)	6.0	5.4

2.5 Data Collection

Data were collected on feed intake, body weight and feed conversion ratio and mortality. Data on carcass characteristics were primal cuts (thighs, drum sticks, wings, back and breast), the offal (heart, liver, gizzard, kidney, spleen, lungs, bile and intestine) and other organs such as the neck, head and legs. Data on weight and percentages (live weight, killed weight, plucked weight/carcass weight and their percentages) were also collected.

2.6 Data Analysis

Data collected were subjected to analysis of variance (ANOVA) and where difference among means was observed, means were separated using Least Significant Difference (LSD).

3. Results

Results on the performance and carcass characteristics of the experimental birds fed with feed supplemented with choline chloride is presented on tables 4.1, 4.2 and 4.3.

3.1 Performance Characteristics at Starter Phase

Results on the performance of experimental birds fed diet

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supplemented with choline chloride at starter phase (2-4wks) is presented in table 2

Table 2. Performance Characteristics of Experimental Birds at Starter Phase

Parameters	T_1(0gC-C/10kg)	T_2(10gC-C/10kg)	T_3(20gC-C/10kg)	SEM
Total Feed Intake	14442.18	14252.52	13161.75	262.45
Average Feed Intake per Bird	1368.35	1295.68	1415.12	49.2
Average Feed Intake per Bird per Day	195.48	185.10	202.16	3.42
Total Body Weight	18155.72	19591.92	17745.36	385.80
Body Weight per Bird	1717.88^{b}	1781.08^{ab}	1907.59^{a}	33.29
Body Weight per Bird per Day	245.41^{b}	254.44^{ab}	272.51^{a}	4.76
Body Weight Gain	7620.75^{ab}	8241.20^{a}	6227.30^{b}	375.03
Body Weight Gain per Bird	719.30	749.20	648.67	20.26
Body Weight Gain per Bird per Day	102.76	107.03	92.67	2.89
Feed Conversion Ratio	1.90^{ab}	1.73^{b}	2.26^{a}	0.098

Note: ^{abc}means in the same row with different superscripts differ significantly; (P<0.05)= *

3.1.1 Total Feed Intake (TFI)

Results on total feed intake of the experimental birds shows that TFI (g/3wks) was lowest (13161.75) in T_3 and the highest was 14442.18 in T_1 (control). Furthermore, the intermediate was 14252.52 in T_2. However, there was no significant difference (p>0.05) among the treatments, thus, they are statistically similar. SEM recorded under TFI performance was 262.45.

A result of trend in ATFI (g/wk) of experimental birds under performance characteristics at starter phase is presented in appendix 1. Figures showed that ATFI (g/wk) increased in age of the birds from wk 2-4 (3133.84 - 6648.43) in T_1 (control), similar trend was observed in T_2 (3088.72 – 6290.54) and T_3 (2978.07 – 5523.52).

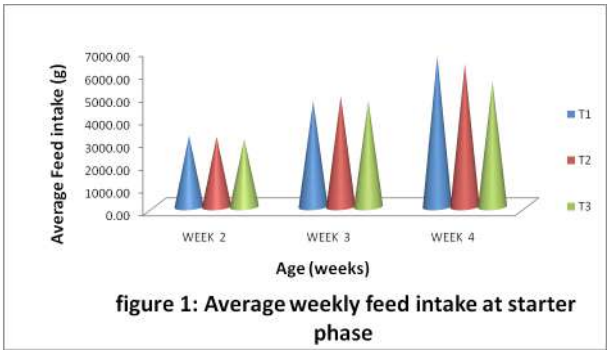

figure 1: Average weekly feed intake at starter phase

3.1.2 Average Feed Intake Per Bird (AFIB)

Results on average feed intake per bird per week indicated that AFIB (g/b) was lowest (1295.68) in T_2, highest (1415.12) in T_3 and intermediate (1368.35) in T_1. However, significant difference was not observed among the treatments (p>0.05) and the SEM recorded was 49.04.

3.1.3 Average Feed Intake Per Bird Per Day (AFIBD)

Lowest and highest value was obtained for results on AFIBD (g/b/d) 185.10 and 206.16 in T_2 and T_3 respectively. Intermediate value 195.48 was obtained in T_1. There was no significant difference (p>0.05) observed among the treatments and 3.42 was obtained as SEM.

3.1.4 Total Body Weight (TBW)

Results on TBW (g/3wks) indicated that T_2, T_1 and T_3 recorded highest (19591.92), intermediate (18155.72) and lowest (17745.36) respectively. Significant difference (p>0.05) was not observed among treatment means and SEM was 385.80.

Results of trend on AWkBW (g/b/wk) of the experimental birds under performance characteristics at starter phase from wk 2-4 is presented in appendix 3 and figure 3. It can be deduced from the figures that there was increase in body weight with increase in age of the birds from wk 2-4 across all treatments ; T_1(3347.10-9102.63), T_2 (3344.92-9779.00) and T_3 (3506.76-7765.10) respectively.

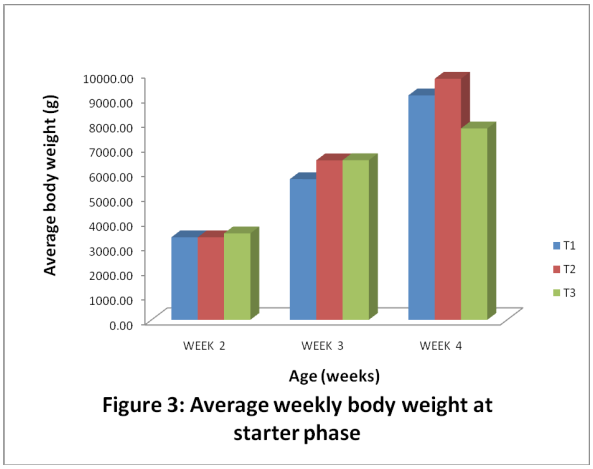

Figure 3: Average weekly body weight at starter phase

3.1.5 Body Weight Per Bird (BWB)

1717.88, 1781.08 and 1907.59 values was obtained for BWB (g/b) in T_1, $T_2$and T_3 respectively, with the highest value 1907.59 observed in T_3, intermediate 1781.08 in T_2 and lowest 1717.88 in T_1. Significant difference was observed among treatment means (p<0.05). SEM recorded was 33.29.

3.1.6 Body Weight Per Bird Per Day (BWBD)

Results on BWBD (g/b/d) recorded highest 272.51 in

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T_3 and lowest 245.41 in T_1 (control). Intermediate value (254.44) was recorded in T_2. There was significant difference ($p<0.05$) among the treatments and SEM value is 4.76.

3.1.7 Body Weight Gain (BWG)

Results on BWG (g/3wks) indicated that lowest (6227.30) and highest (8241.20) values were obtained in T_3 and T_2 respectively. Intermediate (7620.75) value obtained was in T_1. However, significant difference was observed between T2 and T3 ($p<0.05$) and SEM recorded was 375.03.

Results of trends on ABWG (g/b/wk) of the experimental birds under performance characteristics at starter phase are presented in appendix 5 and figure 5. Figures showed increase in ABWG with increase in age of the birds from wk 2-4 in T_1 (1865.22-3396.49) and T_2 (1807.12-3311.00) respectively. Similar trend was also observed from only wk 2-3 in T_3 (1968.96-2966.74) and sudden decrease from wk 3-4 (2966.74-1292.00).

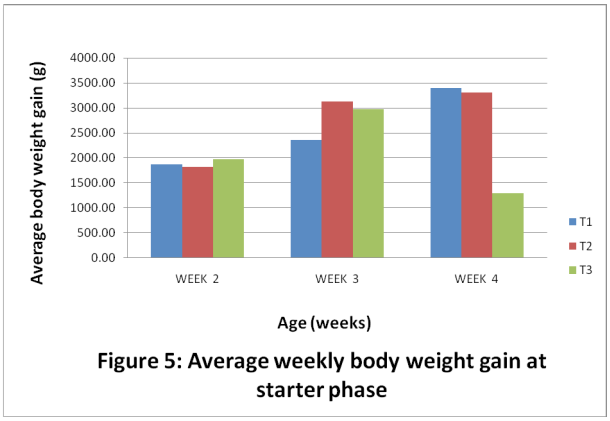

Figure 5: Average weekly body weight gain at starter phase

3.1.8 Body Weight Gain Per Bird (BWGB)

Result on BWGB (g/b) was lowest (648.67) in T_3, highest (749.20) in T_2 and intermediate (719.30) in T_1 respectively. There was no significant difference ($p>0.05$) among the treatments and SEM was 20.26.

3.1.9 Body Weight Gain Per Bird Per Day (BWGBD)

Result on BWG (g/d) was lowest (92.67), highest (107.03) and intermediate (102.76) in T_3, T_2 and T_1 respectively. No significant difference ($p>0.05$) was observed among the treatments and the SEM obtained was 2.89.

3.1.10 Feed Conversion Ratio (FCR)

Results on FCR (fi/bwg) shows that significant difference ($p<0.05$) existed among the treatments where T_3(2.26), T_1 (1.90) and T_2 (1.73) was highest, intermediate and lowest respectively. Significant difference existed between T3 and T1-T2. The SEM recorded was 0.098.

Weekly trend on AFCR (FI/BWG/Wk) of the experimental birds under performance characteristics at starter phase is presented in appendix 7 and figure 7. Figures shows increase in FCR with increase in age of the birds across all treatments; T_1 (1.68-2.04), T_2 (1.44-1.90) and T_3 (1.51-3.57). T2 has the best FCR across the weeks (1.44-1.57-1.90).

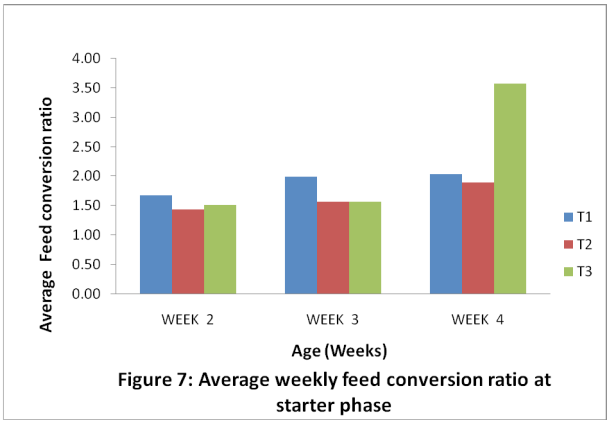

Figure 7: Average weekly feed conversion ratio at starter phase

3.2 Performance Characteristics at Finisher Phase

Results on performance of experimental birds fed with feed sup plemented with choline at finisher phase are presented in table 3.

Table 3. Performance Characteristics of Experimental Birds at Finisher Phase

Parameters	T_1(0gCC/kg)	T_2(10gC-C/10kg)	T_3(20gC-C/10kg)	SEM
Total Feed Intake	31214.32^{a}	31999.76^{a}	24437.13^{b}	1192.80
Average Feed Intake per Bird per Week	2952.69	3088.77	2697.07	85.90
Average Feed Intake per Bird per Day	421.81	441.25	385.30	12.27
Total Body Weight	58814.22^{ab}	61798.52^{a}	50887.42^{b}	1923.67
Body Weight per Bird per Week	5555.97	5945.22	5634.80	130.41
Body Weight per Bird per Day	793.71	849.32	804.97	18.63
Body Weight Gain	14182.96^{a}	13610.70^{ab}	10956.64^{b}	605.08
Body Weight Gain per Week	1344.96	1300.45	1217.18	41.90
Body Weight Gain per Day	192.14	185.78	173.88	5.99
Feed Conversion Ratio	2.21	2.48	2.22	0.12

Note: ^{abc}means in the same row with different superscripts differ significantly; ($P<0.05$)= *

3.2.1 Total Feed Intake

Results on TFI indicated significant difference ($p<0.05$) among the treatments where lowest (24437.13), highest (31999.76) and intermediate (31214.32) was recorded in T_3, T_2 andT_1 respectively. T_1 and T_2 were statistically the

same and significant difference existed between T_2 and T_3. SEM recorded was 1192.80.

Weekly trend of ATFI (g/wk) of the experimental birds under performance characteristics at finisher phase is presented in appendix 2 and figure 2. Figures shows increase in ATFI with increase in age of the birds from wk 5-6 (9297.91- 11366.22) in T_1 (control), similar trend was observed in T_2 (9679.78 – 12363.01) and T_3 (7587.51-9520.01). However, ATFI (g/wk) decreased in age of the birds from wk 6-7 in T_1 (11366.22 – 10568.17), T_2 (12363.01 -9956.97) and T_3 (9520.01-7329.60).

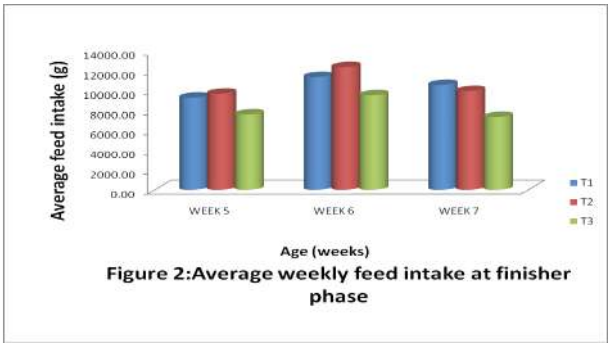

Figure 2:Average weekly feed intake at finisher phase

3.2.2 Average Feed Intake Per Bird (AFIB)

Results on AFIB (g/b) showed that lowest (2697.07), highest (3088.76) and intermediate (2952.69) were obtained in T_3, T_2 andT_1 respectively. However, significant difference ($p>0.05$) was not observed among the treatments and SEM recorded was 85.90.

3.2.3 Average Feed Intake Per Bird Per Day (AFIBD)

Results on AFIBD (g/b/d) indicated that lowest (385.30), highest (441.25) and intermediate (421.81) in T_3, T_2 and T_1 respectively. There was no significant difference ($p>0.05$) among the treatments and SEM recorded was 12.27.

3.2.4 Total Body Weight

Results on TBW indicated that significant difference ($p<0.05$) existed among the treatments. Significant difference existed between T_2 and T_3. Lowest (50887.42), highest (61798.52) and intermediate (58814.22) values was obtained in T_3, T_2 and T_1 respectively. SEM recorded was 1923.67.

Weekly trend of AWkBW (g/b/wk) of the experimental birds under performance characteristics at finisher phase is presented in appendix 4 and figure 4. Figures showed increase in ABW with increase in age of birds from wk 5-7 in T_1 (14170.47-23285.58) and T_2 (17052.20-23389.70). However, similar trend was also observed in T_3 (13483.28-18727.40) only from wk 5-6, while from wk 6-7 there was slight decrease (18727.40-18721.74).

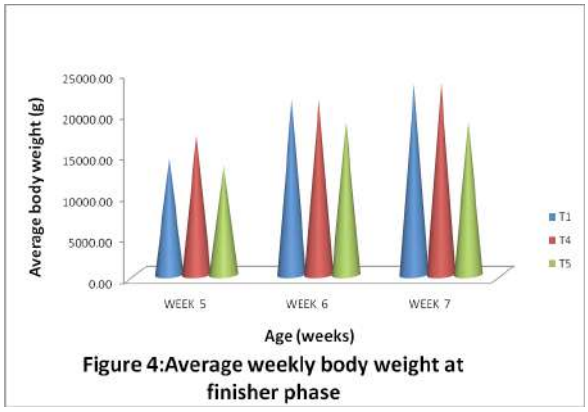

Figure 4:Average weekly body weight at finisher phase

3.2.5 Body Weight Per Bird (BWB)

Results on BWB indicated no significant difference ($p>0.05$) existed among the treatments. The lowest (5555.22), highest (5945.22) and intermediate (5634.80) were obtained in T_1, T_2 and T_3 respectively. SEM recorded was 130.41.

3.2.6 Body Weight Per Bird Per Day (BWBD)

Results on BWBD showed that lowest (793.7), highest (849.32) and intermediate (804.97) were obtained in T_1, T_3 and T_2 respectively. However, no significant difference ($p>0.05$) among the treatments and SEM recorded was 18.63.

3.2.7 Body Weight Gain (BWG)

Results on BWG showed that significant difference ($p<0.05$) was observed among the treatments between T_1 and T_3 with lowest (10956.64), highest (14182.96) and intermediate (13610.70) was recorded in T_3, T_1 and T_2 respectively. SEM recorded was 605.08.

Weekly trend of ABWG (g/b/wk) of the experimental birds under performance characteristics at finisher phase is presented in appendix 6 and figure 6. It can be deduced from the figures that BWG increased in age of the birds from wk 5-6 in T_1 (5067.84-7187.71) and immensely decreased from wk 6-7 (7187.71-1927.41). In contrast, BWG decreased in age of the birds from wk 5-7 in T_2 (7273.20-2033.08) and T_3 (5673.18-).

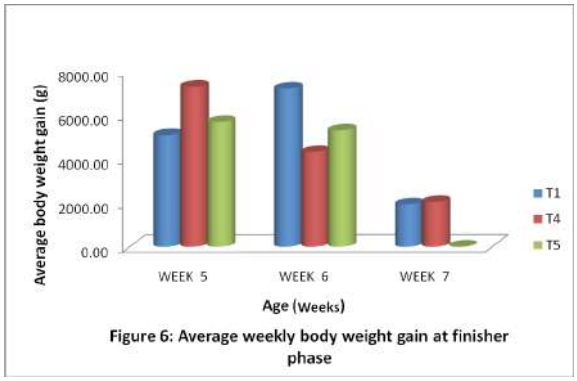

Figure 6: Average weekly body weight gain at finisher phase

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3.2.8 Body Weight Gain Per Bird (BWGB)

Results on BWG shows that lowest (1217.18), highest (1344.96) and intermediate (1300.45) values was obtained in T_3, T_1 and T_2 respectively. However, significant difference ($p>0.05$) was not observed among the treatments and SEM recorded was 41.90.

3.2.9 Body Weight Gain Per Bird Per Day (BWGBD)

Results on BWG indicated that no significant difference ($p>0.05$) existed among treatment means with lowest (173.88), highest (192.14) and intermediate (185.78) values in T_3, T_1 and T_2 respectively. SEM recorded was 5.99.

3.2.10 Feed Conversion Ratio (FCR)

Results on FCR shows that lowest (2.21), highest (2.48) and intermediate (2.22) values was obtained in T_1, T_2 and T_3 respectively. Furthermore, no significant difference ($p>0.05$) among the treatments thus, they are statistically the same. SEM recorded was 0.12.

Results of trend on AFCR (FI/BWG/Wk) of the experimental birds under performance characteristics at finisher phase are presented in appendix 8 and figure 8. Figures showed FCR decreased in age of the birds from wk 5-6 in T_1 (1.86-1.60) and immensely increased from wk 6-7 (1.60-5.89) for birds under control. In contrary, there was increased FCR in age of the birds from wk 5-7 in T_2 (1.33-5.39) and T_3 (1.31-9.18).

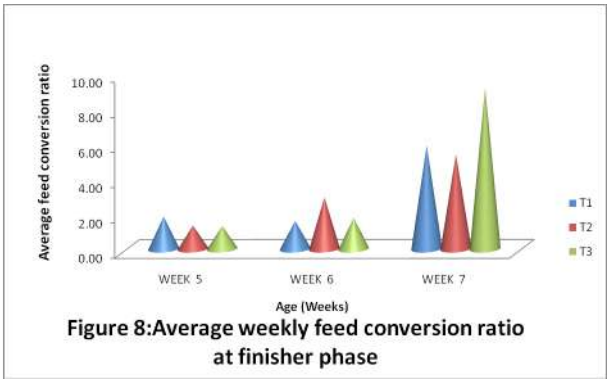

Figure 8:Average weekly feed conversion ratio at finisher phase

3.3 Carcass Characteristics at Finisher Phase

Results on carcass characteristics of experimental birds that consumed feed supplemented with choline at finisher phase is presented in table 4.3.

4. Primal Cuts

Drum Stick (g)

Results on DS indicated that significant difference ($p<0.05$) existed among treatments where T_2 and T_3 are statistically similar while significant difference existed between T2-T3 and T1. SEM recorded was 3.21. Highest (208.15), lowest (188.80) and intermediate (203.60) values was obtained in T_2, T_1 and T_3 for birds that consumed feed supplemented with 10g, 0g and 20g of choline respectively.

Thigh (g)

Results on thigh indicated that no significant difference ($p>0.05$) among the treatments. Thus, they are statistically similar with SEM 4.28. Lowest (251.80), highest (260.30) and intermediate (251.40) values was recorded in T_3, T_2 and T_1 for birds that consumed 20g, 10g and 0g of choline respectively.

Breast (g)

Results on breast indicated that significant difference ($p<0.05$) was observed among treatment means thus, they are statistically different and significant difference existed between T2 and T3, SEM recorded was 15.83. Highest (718.00), intermediate (660.90) and lowest (611.65) values were recorded in T_1, T_2 and T_3 for birds fed with feed supplemented with 0g, 10g and 20g of choline respectively.

Wings (g)

Results on wings shows that highest (168.45), intermediate (166.50) and lowest (161.50) was obtained in T_3, T_1 and T_2 for birds fed with feed supplemented with 20g, 0g and 10g of choline respectively. However, there was no significant difference ($p>0.05$) among the treatments and SEM was 2.23.

Back (g)

Results on back shows that no significant difference ($p>0.05$) observed among treatment means and SEM obtained was 2.29. Highest (167.70), intermediate (167.60) and lowest (166.20) values was recorded in T_1, T_3 and T_2 for birds fed with feed supplemented with 0g, 20g and 10g of choline respectively.

Neck (g)

Highest (80.15), intermediate (79.40) and lowest (70.30) values were obtained in T_3, T_2 and T_1 for birds fed with feed supplemented with 20g, 10g and 0g of choline respectively. However, significant difference ($p<0.05$) was observed between treatments 2-3 and treatment 1. SEM recorded was 1.80.

Total Primal (g)

Results on TP obtained was highest (1563.10), intermediate (1536.45) and lowest (1482.85) in T_1, T_2 and T_3 for birds fed with feed supplemented with 0g, 10g and 20g of choline respectively. No significant difference ($p>0.05$) was observed among treatment means and SEM recorded was 21.00.

Result on performance of primal cuts at the end of fin-

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isher phase is presented in figure 9 below.

Table 4. Carcass Characteristics of Experimental Birds at Finisher Phase

Parameters	T_1(0g) control	T_2(10g) CC	T_3(20g) CC	SEM
Drum Stick	188.80^{b}	208.15^{a}	203.60^{a}	3.21
Thigh	251.80	260.30	251.40	4.28
Breast	718.00^{a}	660.90^{ab}	611.65^{b}	15.83
Wings	166.50	161.50	168.45	2.23
Back	167.70	166.20	167.60	2.29
Neck	70.30^{b}	79.40^{a}	80.15^{a}	1.80
Total Primal	1563.10	1536.45	1482.85	21.00
Gizzard	49.10^{a}	39.40^{b}	42.70^{b}	1.49
Liver and Bile	45.50^{a}	41.70^{b}	48.55^{a}	1.28
Heart	8.00^{b}	7.05^{b}	10.00^{a}	0.40
Lungs	8.30^{b}	11.10^{a}	11.55^{a}	0.47
Crop	10.40	10.80	11.50	0.46
Spleen	1.70	1.45	6.55	1.56
Legs	64.10^{b}	65.60^{b}	81.15^{a}	2.26
Head	43.40^{b}	42.35^{b}	49.20^{a}	1.01
Intestines	124.20	108.50	131.70	6.24
Abdominal Fat	20.40	22.70	22.50	0.96
Total Organs	379.10^{ab}	350.65^{b}	410.40^{a}	9.72
Live Weight	2157.30	2091.20	2119.95	26.62
Killed Weight	2090.50	2027.45	2053.20	25.46
Dressed Weight	1661.70	1617.55	1574.10	20.99
Primal per Live Weight	72.43^{a}	73.47^{a}	69.97^{b}	0.46
Primal per Dressed Weight	94.05^{b}	94.99^{a}	94.18^{b}	0.16
Organs per Live Weight	17.61^{b}	16.76^{b}	19.36^{a}	0.41
Organs per Dressed Weight	22.87^{b}	21.67^{b}	26.09^{a}	0.66

Note: ^{abc}means in the same row with different superscripts differ significantly; (P<0.05)= *

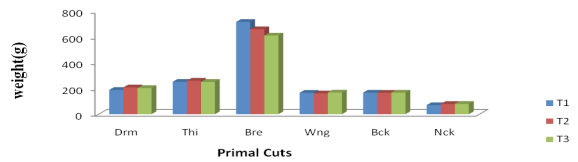

Figure 9: Performance of Primal Cuts at Finisher Phase

5. Organs

Gizzard (g)

Results on gizzard indicated that significant difference (p<0.05) was observed among treatment means thus, they are statistically different, T2 and T3 were statistically the same and significant difference existed between T1 and T2-T3 and SEM obtained was 1.49. The highest (49.10), intermediate (42.70) and lowest (39.40) was recorded in T_1, T_3 and T_2 for birds fed with feed supplemented 0g, 20g and 10g of choline respectively.

Liver and Bile (g)

Results on L&B shows that significant difference (p<0.05) was observed among the treatments where highest (49.50), intermediate (48.55) and lowest (41.70) values were recorded in T_1, T_3 and T_2 for birds fed with feed supplemented with 0g, 20g and 10g of choline respectively. SEM recorded under L&B was 1.28.

Heart (g)

Highest (10.00), intermediate (8.00) and lowest (7.05) values were recorded in T_3, T_1 and T_2 for birds fed with feed supplemented with 20g, 0g and 10g of choline respectively. However, significant difference (p<0.05) was observed among treatment means and SEM recorded was 0.40.

Lungs (g)

Results on lungs indicated that highest (11.55), intermediate (11.10) and lowest (8.30) values were recorded in T_3, T_2 and T_1 for birds fed with feed supplemented with 20g, 10g and 0g of choline respectively. Significant difference (p<0.05) existed among the treatments and SEM recorded was 0.47.

Crop (g)

Results on crop indicated that no significant difference (p>0.05) was observed among treatment means thus, they are similar statistically and SEM recorded was 0.46. Highest (11.50), intermediate (10.80) and lowest (10.40) was recorded in T_3, T_2 and T_1 for birds fed with feed supplemented with 20g, 10g and 0g of choline respectively.

Spleen (g)

Results on spleen indicated that highest (6.55), intermediate (1.70) and lowest (1.45) values were obtained in T_3, T_1 and T_2 for birds fed with feed supplemented with 20g, 0g and 10g of choline respectively. However, no significant difference (p>0.05) was observed among treatment means and SEM recorded was 1.56.

Legs (g)

The highest (81.15), intermediate (65.60) and lowest (64.10) values were recorded under T_3, T_2 and T_1 for birds fed with feed supplemented with 20g, 10g and 0g of choline respectively. However, significant difference (p<0.05) was observed among treatment means and 2.26 was recorded under SEM.

Head (g)

Results on head recorded highest (49.20), intermediate (43.40) and lowest (42.35) values under T_3, T_1 and T_2 for birds that consumed feed supplemented with 20g, 0g and 10g of choline respectively. In addition, significant difference (p<0.05) was observed among treatment means,

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were T_2 and T_1 are statistically similar. 1.01 was recorded under SEM.

Intestines (g)

Results on intestines indicated that no significant difference ($p>0.05$) existed among treatment means and 6.24 was recorded under SEM. Highest (131.70), intermediate (124.20) and lowest (108.50) was obtained in T_3, T_1 and T_2 for birds fed with feed supplemented with 20g, 0g and 10g of choline respectively.

Abdominal Fat (g)

There was no significant difference ($p>0.05$) observed among treatment means for abdominal fat thus, they are statistically the same and SEM recorded was 0.96. Highest (22.70), intermediate (22.50) and lowest (20.40) was recorded under T_2, T_3 and T_1 for birds fed with feed supplemented with 10g, 20g and 0g of choline respectively.

Total Organs (g)

Results on TO indicated that there was significant difference ($p<0.05$) among the treatments, T_3 and T_1 are statistically similar as well as T_1 and T_2. Highest (410.40), intermediate (379.10) and lowest (350.65) values were obtained under T_3, T_2 and T_1 for birds fed with feed supplemented with 20g, 10g and 0g of choline respectively.

Result on the performance of organs at the end of finisher phase is presented in figure 10 below.

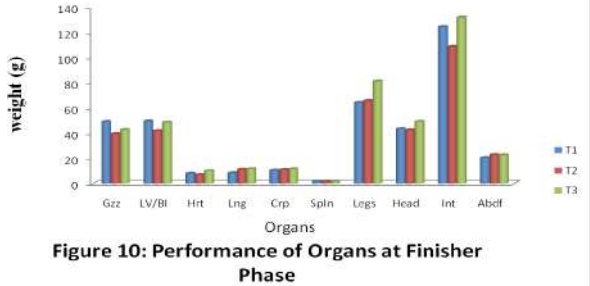

Figure 10: Performance of Organs at Finisher Phase

6. Weights and Percentages

Live Weight (g)

The results on LW as shown by the table indicated that no significant difference ($p>0.05$) was observed among the treatment means, thus, they are statistically the same with SEM 26.62. Highest (2157.30), intermediate (2119.95) and lowest (2019.20) values were recorded under T_1, $T_3$and T_2 for birds fed with feed supplemented with 0g, 20g and 10g of choline respectively.

Killed Weight (g)

Highest (2090.50), intermediate (2053.20) and lowest (2027.45) values were recorded under T_1, $T_3$and T_2 for birds fed with feed supplemented with 0g, 20g and 10g of choline respectively. However, there was no significant difference ($p>0.05$) among the treatments and SEM recorded was 25.46.

Plucked Weight (g)

Results on PW indicated that highest (2017.90), intermediate (1978.80) and lowest (1950.75) values were recorded under T_1, $T_3$and T_2 for birds fed with feed supplemented with 0g, 20g and 10g of choline respectively. In addition, there was no significant difference ($p>0.05$) among the treatment means and SEM recorded was 25.45.

Dressed Weight (g)

There was no significant difference ($p>0.05$) observed among the treatment means and SEM recorded was 20.99. Highest (1661.70), intermediate (1617.55) and lowest (1574.10) values were obtained under T_1, T_2 and T_3 for birds fed with feeed supplemented with 0g, 10g and 20g of choline respectively.

Primal Per Live Weight (%)

Results on P/LW shows that significant difference ($p<0.05$) existed among the treatment means with T_1 and T_2 statistically the same but statistically different from T3, while the SEM recorded under it was 0.46. Highest (73.47), intermediate (72.43) and lowest (69.97) values were obtained in T_2, T_1 and $T_3$for birds fed with feed supplemented with 10g, 0g and 20g of choline respectively.

Primal Per Dressed Weight (%)

Results on P/DW indicated that significant difference ($p<0.05$) was observed among the treatments where T_3 and T_1 are statistically the same but statistical difference existed between T2 and T1-T3 and the SEM recorded was 0.16. Highest (94.99), intermediate (94.05) and lowest (94.18) were obtained in T_2, $T_3$and T_2 for birds fed with feed supplemented with 10g, 20g and 0g of choline respectively.

Organs Per Live Weight (%)

Results on O/LW indicated that highest (19.36), intermediate (17.61) and lowest (16.76) values were recorded under T_3, T_2 and T_1 for birds fed with feed supplemented with 20g, 10g and 0g of choline respectively. However, significant difference ($p<0.05$) was observed among treatment means and 0.41 was recorded under SEM.

Organs Per Dressed Weight (%)

Results on O/DW indicated that significant difference ($p<0.05$) was observed among treatments where T_1 and T_2 were statistically the same and the SEM recorded was 0.66. Highest (26.09), intermediate (22.87) and lowest (21.67) values were obtained in T_3, T_1 and T_2 for birds fed with feed supplemented with 20g, 0g and 10g of choline respectively.

Result on weights of the experimental birds at the end of finisher phase is presented in figure 11 below.

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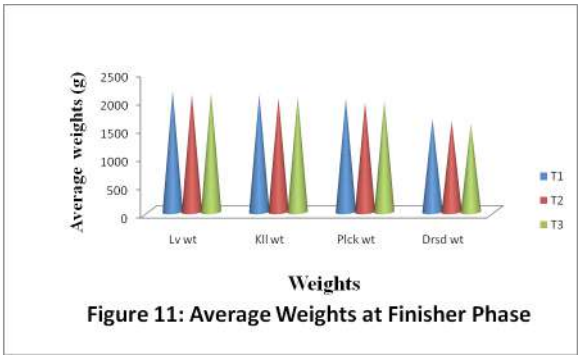

Figure 11: Average Weights at Finisher Phase

7. Discussion

7.1 Performance Characteristics at Starter Phase

7.1.1 Feed Intake

Although, there was no significant difference ($p>0.05$) among the treatments, there was reduction in feed intake across the different level of choline inclusion in diet; this could be as a result of choline inclusion in the diet. Thus, the more the choline, the lesser the feed intake. It agrees with Swain and Johri (2000) who recorded that choline supplementation alone did not elicit any response in the feed intake and feed utilization of broilers. Blair *et al.* [5] who found that choline had no effect on the feed consumption of broilers in the presence or absence of additional Methionine. The result contradict Shrivastav *et al.* [26] who stated the feed intake and feed efficiency ratio increased significantly with the increasing level of choline from 1500-2000mg/kg diet.

7.1.2 Body Weight

There was no significant difference ($p>0.05$) among treatments with response to different level of choline inclusion with respect to body weight. This was in accordance with Saarinen *et al.* (2000) who observed choline supplementation did not significantly affect growth performance of broiler chicks fed Methionine adequate or inadequate diets, their observation agrees with that of Swain and Johri (2000) who reported that dietary choline, at different inclusion level, had no significant effect on body weight and body weight change in broiler chicks. Which also contradict Hassan *et al.* [10] who observed that different levels of choline supplement had meaningful effect on average live body weight.

7.1.3 Feed Conversion Ratio

Significant difference ($p<0.05$) observed among treatments with response to different level of choline inclusion could be as a result of decreased in feed intake across the levels and significant body weight gain increment. This was in accordance with Hassan *et al.* [10] who found that supplementation of 0.3g/kg of choline increased feed conversion ratio by 3.3% compared to control diet.

7.2 Performance Characteristics at Finisher Phase

7.2.1 Feed Intake

Significant difference ($p<0.05$) that existed among treatment means could be as a result of increase in choline synthesis at finisher phase. This conforms with Fouladi *et al.* (2008) who found that using 500 or 1000mg/kg of choline chloride supplement of broiler diets significantly increased feed intake during 22-42 day-old age. This contradicts with Summer [29] who indicated no positive effects of dietary choline on broiler feed intake during the last three weeks of experimental study.

7.2.2 Body Weight

Significant difference ($p<0.05$) in body weight with response to different level of choline inclusion could be as a result of positive effect of feed intake and body weight gain. This conforms with Fouladi *et al.* (2008) who found that using 500-1000mg/kg of choline chloride supplement of broiler diets significantly increased live body weight during 22-42 day-old. Also Baranova [3] observed that inclusion of choline to the diet mixture improved growth rate with the inclusion rate of 500-700mg/kg of diet. Hassan *et al.* [10] also observed that different level of choline supplementation had meaningful effect on average live body weight. On the contrary, Rafeeq *et al.* (2011a) and Swain and Johri (2000) reported dietary choline at different level of inclusion had no significant effect on body weight and body weight change in broiler chicks.

Feed conversion ratio

No significant difference ($p>0.05$) was observed at different level of choline inclusion. This was in contrast with Waldroup *et al.* (2006) who noted that the supplementation of 1000mg/kg diet statistically improved feed conversion ratio over the chicks fed unsupplemented diets at 35, 42, 49 and 52 days of age. And Fouladi *et al.* (2008) who found that using 500-1000mg/kg of choline chloride supplement of broiler diets significantly increased feed conversion ratio during 22-42 day-old.

7.3 Carcass Characteristics at Finisher Phase

Significant difference ($p<0.05$) existed across different level of choline inclusion with respect to breast yield. This

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was in accordance with Waldroup and Fritis (2005) who reported an improvement in breast yield due to choline supplementation (1000g choline/ton diet) at 42 days of age and not at 49days of age in broiler chicks. Moreover, Waldroup *et al.* (2006) also observed that supplementation of 1000g choline/ton to the broiler diets resulted in significant improvements in breast yield at 42, 49 and 56 days of age.

7.4 Conclusion and Recommendation

7.4.1 Conclusion

It is concluded from the experiment that 10gCC/10kg performed better in terms of total body weight (TBW) and feed conversion ratio (FCR). Also in the carcass characteristics, treatment two performed better in terms of breast yield, drumstick, percentage of primal cuts from live weight (P/LW) and percentage of primal cuts from dressed weight.

7.4.2 Recommendation

Based on the result obtained from the research, it is therefore recommended from the experiment that 10g/10kg of choline chloride should be used. It is also recommended that more research should be carried out with lower levels of choline chloride.

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ARTICLE

Aquatic Beetles (Coleoptera: Dytiscidae, Haliplidae, Noteridae, Hydrophilidae) From Borujen and Lordegan (Chaharmahal and Bakhtiari Province, Iran)

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ABSTRACT

This research was aimed to study aquatic coleopteran faunas of Borujen and Lordegan (as two main towns of the Chaharmahal and Bakhtiari Province). Sampling was done at six stations between September 2017 to July 2018. The aquatic Coleoptera were identified to the species level with the help of keys and related references. Identification of samples was based on morphological characteristics such as taxonomic characters and external genitalia with appropriate entomological reference books and authors. A total of 12 species belonging to 11 genera and 4 families were identified. The greatest number of species identified were found in the family Dytiscidae Leach, 1815 and the least number in the family Hydrophilidae Latreille, 1802. Two species of *Agabus* Leach, 1817 namely, *Agabus conspersus* Marsham, 1802 and *Agabus bipustulatus* Linnaeus, 1767 were the most abundant insects.

1. Introduction

Aquatic Coleoptera known as water beetles, with more than 13,000 described species, is one of the most abundant aquatic insects [19]. They play an important role in freshwater ecosystems and are considered as a suitable bioindicator [3]. Since the maintenance of biological diversity (or biodiversity)- as a measure of the variety of all organisms- is one of the main goals of conservation for sustainable use of resources and animal survival, the identification and assessment of animal habitats are considered topics to be a priority for research. In this regard, aquatic Coleoptera as biodiversity indicators in freshwater ecosystems are of great importance [18]. The Dytiscidae with more than 4,000 described species is the most species family of water beetles which occur in virtually any freshwater habitat around the world [14]. The Hydrophilidae is the second most abundant family which are generally found in habitats of small shallow water bodies and they occupy in most kinds of stagnant waters, but also commonly inhabit streams, rivers, and seepage [4].

There is Little information about fauna of aquatic insects of Iran. For instance, [8-12] studied the aquatic beetle fauna of Fars, Guilan, Mazandaran and Khuzestan provinces. Ostovan and Niakan, [16] studied the diversity, abundance, and biology of aquatic insects, including the aquatic beetles in Fars province. The fauna of diving beetles was studied in Markazi province by Vafaei *et al.*, [20].

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The other most important publications on Iranian aquatic beetles are: Atamehr and Alaei [1], Mousavi *et al.* [15], Darilmaz *et al.* [2], Samin *et al.* [17], and Van Vondel *et al.*, [21]. Apart from a study conducted by Heydarnejad [7] on aquatic coleopteran of Choghakhor in 2010, no other study has not been done in Chaharmahal and Bakhtiari province so far. Thus, this study aimed to study aquatic coleopteran faunas of Borujen and Lordegan, Chaharmahl and Bakhtiari province, Iran.

2. Materials and Description of Sampling Station

Chaharmahl and Bakhtiari, one of the 31 Provinces of Iran, lies in the southwestern part of the country. Its capital is Shahrekord surrounded by the famous Zakros mountains. Due to the high mountainous nature, which is in the path of the wet winds of the Mediterranean systems, which makes these systems rise and discharge, the province has relatively good rainfall to the extent that this region, with only one percent of Iran's construction, is ten percent the country has water resources (Figure 1). Between September 2017 to July 2018 a field trip to Borujen and Lordegan (as two main towns of the province) was carried out by M.Taher. During this trip 6 sampling stations were examined that are listed below and presented on the map (Figure 2). The results are based on the study of 51 adults of Dytiscidae, 20 adults of Haliplidae, 17 adults of Noteridae, and 4 adults of Hydrophilidae. Identification of samples was based on morphological characteristics such as external taxonomic characters and external male genitalia with appropriate entomological reference books and authors [5,6,13,22]. All samples were deposited in the Zoological Museum, Shahrekord University (ZMSU).

Station 1: Zaghi River, substrate: muddy; 2136 m a.s.l.; near Dehno, 20 Km from the town of Borujen; 3202'54" N 5106'35" E; 23. IV. 2018, 16. V. 2018

Station 2: Kalbibak River, substrate: clay with dense aquatic vegetation; 2278 m a.s.l.; near Boldaji, 35 Km from the town of Borujen; 3153'40" N 5153'16" E; 23. IV. 2018,

Station 3: Gandoman Marsh, substrate: muddy with dense aquatic vegetation; 2219 m a.s.l.; near Gandoman, 15 Km from the town of Borujen; 3151'05"N 5105'34" E; 16. III. 2018, 16. V. 2018.

Station 4: Bizhgerd Spring, substrate: muddy; 2216 m a.s.l.; near Bizhgerd, 45 Km from the town of Borujen; 3146'55"N 5111'35" E; 16. III. 2018, 16. V. 2018.

Station 5: Barm malkhalife Spring, substrate: rubber cement; 1744 m a.s.l.; near malkhalife, 55 Km from the town of Lordegan; 3117'21"N 5115'58" E; 29. IV. 2018.

Station 6: Sendegan Spring, substrate: muddy; 1739 m a.s.l.; near Sendegan, 60 Km from the town of Lordegan; 3115'35"N 5117'00" E; 29. IV. 2018.

Figure 1. Location of Chaharmahl & Bakhtiari province within Iran

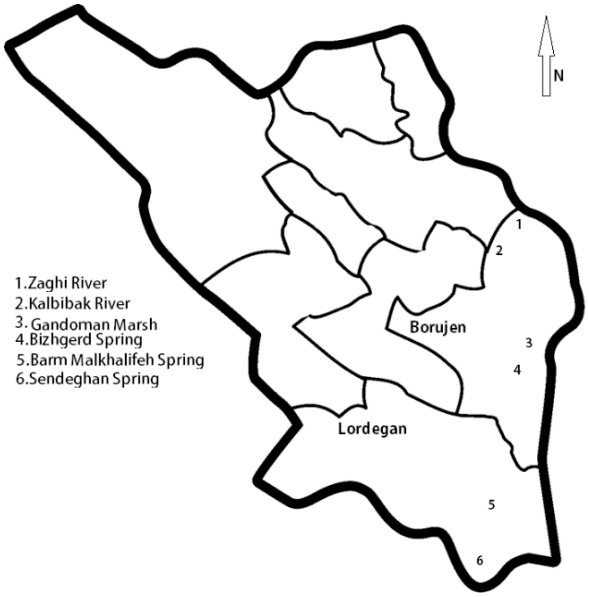

Figure 2. Location of 6 stations within Chaharmahl & Bakhtiari

3. Results

In this research totally 12 species of aquatic beetles from the families Dytiscidae, Haliplidae, Hydrophilidae, and Noteridae were collected and identified from some aquatic ecosystems in Borujen and Lordegan. The list of species is given as a checklist of the species and a list of the stations with the species collected.

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Table 1. List of a water beetles recorded from Borujen and Lordegan Species recorded from Chaharmahal and Bakhtiari province for the first time are indicated by an asterisk (*)

Species	Station
Dytiscidae	
Dytiscus persicus Wehncke, 1876	3
Laccophilus hyalinus (De Geer, 1774)	3,5
*Agabus conspersus** (Marsham, 1802)	1,4
Agabus bipustulatus (Linnaeus, 1767)	3,1,6
*Nebrioporus airumlus** (Kolenati, 1845)	4
*Hydroglyphus geminus** (Fabricius, 1792)	1,5
*Hydroporus inscitus** Sharp, 1882	4
Haliplidae	
Peltodytes caesus *(Duftschmid,1805)	3
*Haliplus obliquus** (Fabricius, 1787)	3
Hydrophilidae	
*Hydrobius fuscipes** (Linnaeus, 1758)	5
Noteridae	
*Noterus clavicornis** (De Geer,1774)	2

According to this checklist, 12 species of aquatic beetle are currently known from Borujen and Lordegan: Dytiscidae - eight species, Haliplidae - two species, Hydrophilidae - one species and Noteridae -one species.

List of stations with collected species

Station 1 :Dytiscidae: *Agabus conspersus, Agabus bipustulatus, Hydroglyphus geminus*

Station 2 :*Noteridae: Noterus clavicornis*

Station 3 :*Dytiscidae: Dytiscus persicus, Laccophilus hyalinus, Colymbetes fuscus, Agabus bipustulatus*; Haliplidae: *Peltodytes caesus, Haliplus obliquus*

Station 4 : Dytiscidae: *Agabus conspersus, Nebrioporus airumlus, Hydroporus inscitus*

Station 5 :Dytiscidae: *Laccophilus hyalinus, Hydroglyphus geminus*; Hydrophilidae: *Hydrobius fuscipes*

Station 6: Dytiscidae: *Agabus bipustulatus*

4. Discussion

This study investigated aquatic coleopteran faunas of Borujen and Lordegan, Chaharmahl and Bakhtiari province, Iran. From six stations surveyed, 11 genera and 4 families were found and identified. The most abundant families were Dytiscidae (51 samples), followed by Haliplidae (20 samples), Noteridae (17 samples), and Hydrophilidae (4 samples). In line with this research, the family Dytiscidae has been reported as the most abundant family in the study of aquatic Coleoptera by Hosseini [8-10], Ostovan and Niakan, [16] and Dong *et al.* [3]. Also, this study showed that *Agabus conspersus* and *Agabus bipustulatus* species were found in half of the stations where the samples were obtained. According to the results, Gandoman Marsh station is considered with the highest species richness. Among the total number of specimens collected respectively *Laccophilus hyalinus*, *Agabus conspersus*, *Agabus bipustulatus*, *Hydroporus inscitus*, *Peltodytes caesus* were dominant species. This indicates that the abundance of these species was more than 5% of the total number of specimens. *Colymbetes fuscus, Nebrioporus airumlus, Hydrobius fuscipes*, which contain 2 to 5% of all specimens, were semi-dominant species, but *Dytiscus persicus, Hydroglyphus geminus, Haliplus obliquus* which their abundance was less than 2% of the total number of specimens, were rare species [10].

5. Conclusion

This study, which was conducted to investigate aquatic coleopteran faunas of Borujen and Lordegan, Chaharmahl & Bakhtiari province, Iran, showed that Most species belong to the family Dytiscidae and the least to the family Hydrophilidae. Of the 12 species identified in this study, 11 species were reported for the first time in this province. Two species of Agabus with the names *Agabus conspersus* and *Agabus bipustulatus* were the most abundant specimens.

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ARTICLE

Combined Effects of Dietary *Bacillus subtilis* and Trans-cinnamic Acid on Growth Performance, Whole Body Compositions, Digestive Enzymes and Intestinal bacteria in Rainbow Trout (*Oncorhynchus mykiss*)

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ABSTRACT

In this study, the combined effects of dietary *Bacillus subtilis* (BS, 10^7 g/cfu) and different levels (0.025%, 0.050%, 0.075% and 0.150%) of trans-cinnamic acid (CA) on fish growth performance, whole body compositions, digestive enzymes, intestinal bacteria and internal organ index of rainbow trout (*Oncorhynchus mykiss*) were investigated. Six different experimental groups including control group (C), C+BS, 0.025%CA+BS, 0.050%CA+BS, 0.075CA+BS, 0.150%CA+BS) were established. According to the results obtained, growth performance, whole body compositions and digestive pH were not statistically significant among groups. Further, no significant differences were found between experimental groups in terms of the intestinal enzymes (trypsin, alkaline phosphatase and lipase) and gastric pepsin. Significantly higher levels of intestinal amylase were found in the control+BS, 0.025%CA+BS, 0.050% CA+BS, and 0.075%CA+BS compared to the control and 0.150%CA+BS groups. Moreover, coliform and *Enterobacteriaceae* counts were highest in the control+*B. subtilis* and lowest in the 0.150% CA + *B. subtilis* groups.

1. Introduction

In order to supply the increasing demand for fish consumption in the world, aquaculture facilities face the challenge of intensive production of fish species in recirculating aquaculture systems, which eases the outbreak of diseases posing significant treath in terms of limitation on production amounts, lowering economic development in many countries [1].

The use of antibiotics is one of the most common method to control disease treatment in aquaculture facilities. However, the uncontrolled and unconscious use of antibiotics increases waste and accumulation in the environment and consequently negative impacts on terrestrial animals

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as well as humans can be seen in near future. In addition, intensive use of antibiotics increases the resistance of fish to pathogens in aquaculture facilities. These negative conditions caused by antibiotics led researchers to search for alternative environmentally friendly feed additives [2-4]. Alternative feed additives such as prebiotics, probiotics, algae, fungi, microalgae, enzymes, organic acids, mycotoxin binders, photogenic or phytobiotic compounds and yeasts, may not only increase growth performance of fish, but also increase the immune response of the fish and improve health condition [5].

Microbial balance and optimal pH levels in the digestive system eliminate pathogenic microorganisms. This is necessary to keep fish health at the desired level and to achieve expected production levels [6]. Probiotics balance microbial flora in the digestive tract by enhancing host health with complementary microorganisms such as bacteria, fungi and yeast [7-13]. They also improve feed quality and enzymatic activity in digestion and improve animal health and nutrition by activating the immune response. Probiotics are important alternative feed additives with potentially positive impacts in aquaculture facilities [14-17].

Bacillus subtilis, used in this study, breaks down proteins and carbohydrates and metabolizes nutrients appropriately and produces B-group vitamins including B7 (biotin) and B12 (kobalamin) [18-19]. In addition, bacterial spores of B. subtilis are easy to add to fish diets since they can remain in feed for a long time [20-21].

Trans-cinnamic acid (CA) is a natural polyphenolic organic acid derived from plants and known to have anti-fungal [22], anti-microbial [23], anti-oxidant [24], anti-tumor [25] and anti-inflammatory [26] effects. In addition, CA has been reported to have antimicrobial effect against bacteria such as *Aeromonas hydrophila, Aeromonas salmonicida* and *Edwardsiella tarda* [27] and *Aeromonas sobria, Aeromonas salmonicida* ATCC 33658, *Listonella anguillarum* and *Yersinia ruckeri* [2,21].

Although CA is a suitable food additive due to its immune stimulating and antimicrobial effects [2,20,24,25], combined effects of dietary *Bacillus subtilis* and CA are limited with recent reports [43,48]. In this study, the combined effects of trans-cinnamic acid and *B. subtilis* on growth performance, nutrient composition, digestive enzymes and intestinal flora of rainbow trout (*Oncorhynchus mykiss*) were investigated.

2. Material and Methods

2.1 Fish and Experimental Design

Rainbow trout (*Oncorhynchus mykiss*) juveniles used in our study were obtained from a commercial trout farm (Keskin Alabalık Co., Bayramic-Canakkale). Before the start of the experiment, the fish were fed with commercial extruder diets (Anatolian Sea 50/4, Uğurlu Balık, Aydın-Turkey) for 2 weeks to adapt to the new conditions. *Basillus subtilis* (0486C, *Bacillus subtilis* subsp. *spizizenii* ATCC® 6633™* EZCFU) and trans-cinnamic acid (Aldrich W228826 trans-cinnamic acid natural, ≥99%, FCC, FG) used in the experiment were incorporated into the diets in fish oil at specified rates.

A total of 540 trout juveniles with a mean weight of 21.63±0.21 g were randomly allotted into 18-identical experimental tanks as 30 fish per tank (6 group × 3 replicate × 30 fish/tank). *B. subtilis* (BS) 10^7 cfu g^{-1} and cinnamic acid (CA) in ratios of 0.025%, 0.050%, 0.075% and 0.150% were added into the test diets. So the experimental groups were designed as 0% (control), 0% cinnamic acid + *B. subtilis* 10^7 cfu g^{-1}, cinnamic acid 0.025%+*B. subtilis* 10^7 cfu g^{-1}, cinnamic acid 0.050%+*B. subtilis* 10^7 cfu g^{-1}, cinnamic acid 0.075%+*B. subtilis* 10^7 cfu g^{-1} and cinnamic acid 0.150%+*B. subtilis* 10^7 cfu g^{-1}. At the end of the 60-day feeding experiment, fish growth performance, nutrient composition, total liver fat, internal organ indexes, intestinal and stomach enzymes, feed, stomach and intestinal pH amounts, and intestinal bacteria were analyzed.

2.2 Growth Performance and Feed Utilization

The following analyzes were used to calculate feed utilization [28]:

Relative growth rate, RGR (%) = final weight, g - initial weight, g) / initial weight × 100

Specific growth rate, SGR (%Day^{-1}) = [Ln (final avarage weight, g) – Ln (initial avarage weight, g)] / trial days × 100

Feed conversion rate, FCR = feed consumption (g) / weight gain (g) × 100

2.3 Chemical Nutrient Analysis

2.3.1 Dry Matter Analysis

First, the internal organs of the fish were removed and fish were weighed. Fish were then dried in an oven at 70 °C until the constant weight was reached [29]. The samples were homogenized by grinding for protein, fat and ash analysis. Dry matter was calculated according to the following formula:

Dry matter (%) = 100 - [(sample weight + weight of foil pot) - (pot weight after drying)] / [(sample weight + weight of foil pot - weight of foil pot)] × 100

2.3.2 Protein Analysis

Kjeldahl method was used to determine the amount of

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protein [29]. Approximately 0.5 g of samples was taken into glass cylinder tubes and 1 catalyst tablet and 15 ml of sulfuric acid (H_2SO_4) were added. Protein digestion process was performed in BUCHI mark K-436 model infrared burning system. After cooling, the samples were taken to BUCHI mark K-350 model distillation system. Then, it was titrated with 0.1 moles of Hydrochloric acid (HCl). The percentage of protein was calculated according to the following formula:

Crude protein % = (discharge at titration - blind sample) × 0.1 (Normality of HCI solution) × 14.007 (Milliequivalent weight of nitrogen) × 6.25(Factor) / sample weight × 100

2.3.3 Fat Analysis

In fat analysis, 0.5 g of fish and feed samples and 0.25 g of liver samples were weighed in test tubes with lids and methanol/chloroform mixture was added. The samples were kept in the dark for 1 night. Then the samples were filtered and taken to the first weighed test tubes. methanol/chloroform was removed in a 40 °C water bath with a nitrogen evaporator. Afterwards, the tubes were taken into the desiccator and weighed [30]. The amount of crude fat was calculated according to the following formula:

Crude fat amount % = weight change of glass bubble (g) / sample weight (g) × 100

2.3.4 Ash Analysis

For the analyses of ash content, 0.5 g of samples were taken and put into pre-tared porcelain crucibles. Then, the crucibles were fired in the incinerator at 525 °C for 12 hours [29]. The ash content was calculated according to the following formula:

Crude ash content % = weight change of porcelain crucible (g) / sample weight (g) × 100

2.4 Biometric Indices

Biometric indices were calculated using the following equations:

Visceral fat index (VFI) = {wet weight of visceral fat (g) / [wet body weight (g) − wet weight of visceral fat (g)]} × 100

Hepatosomatic index (HSI) = {wet weight of liver (g) / [wet body weight (g) − wet weight of liver (g)]} × 100

Viscerosomatic index (VSI)

= {wet weight of viscera and associated fat (g) / [wet body weight (g) − wet weight of viscera andassociated fat (g)]} × 100

Bile–somatic index (BSI) = {wet weight of bile (g) / [wet body weight (g) − wet weight of bile (g)]} × 100

Spleen–somatic index (SSI) = {wet weight of spleen (g) / [wet body weight (g) − wet weight of spleen (g)]} × 100

2.5 Intestinal Bacteria and pH Analysis

Total bacteria, total yeast, mold, coliform and lactic acid bacteria were counted in order to determine the effects of cinnamic acid and *Bacillus subtilis* on intestinal bacteria. For 1 g intestine sample, 3 fish were taken from each tank and the anterior intestine was combined. Sterile PBS was added to the intestinal samples at a rate of 9 times and homogenized with glass homogenizers. Then, dilution was applied at a rate of 9 times. The counts were applied by smear and pouring plate methods [31] and methods previously reported in trout were used [32-34].

Microbiological analyses were performed as (a) determination of total heterotrophic and mesophilic aerobic counts on Tryptic Soy Agar (TSA; Merck) at 22 °C for 36 h, and 37°C for 24 h respectively; (b) determination of total yeast and mold counts on Potato Dextrose Agar (PTA; Sigma-Aldrich) at 22 °C for 120 h; (c) determination of total coliform counts on MacConkey Agar (MA; Merck) at 37°C for 24 h; (d) determination of total *Enterobacteriaceae* counts on Violet Red Bile Glucose Agar (VRBG; Merck) at 37°C for 24 h; and (e) determination of total *Lactobacillus* counts on de Man, Rogosa, and Sharpe Agar (MRSA) at 37°C for 120 h. Bacterial counts were expressed as log CFU g^{-1} of wet matter.

The pH values were measured with tabletop pH meter in fish feed, intestine and stomach (HI 2221).

2.6 Intestinal and Stomach Enzymes

Prior to analysis, stomach and intestinal samples were homogenized in cold pure water and was centrifuged at 30000 rcf for 30 minutes at 4 °C. The supernatants were stored at -80 °C until use in the assays. Protein ratio for each sample was determined by Bradford [35] method analyzes. The concentration of trypsin [36], amylase [37], lipase [38], alkaline phosphatase and pepsin [39] was estimated according to the methods previously conducted and described in our laboratory [62].

3. Results

3.1 Growth Performance

At the end of the experiment, average initial weight (IW), average final weight (FW), relative growth rate (RGR), feed conversion rate (FCR) and specific growth rate (SGR) results are given in Table 1. According to the results of the study, no statistical significance in growth performance was observed in rainbow trout (*O. mykiss*) juvenils fed ex-

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perimental diets compared to the control group (p>0.05).

3.2 Whole Body Composition and Liver Fat

At the end of the experiment, nutritional composition of *B. subtilis* and cinnamic acid+*B. subtilis* groups were evaluated and presented in Table 2. According to the data obtained, it was found that there were no statistically effects on dry matter, protein, fat and ash and liver fat (p>0.05).

3.3 Intestinal Bacteria

At the end of the experiment, the dietary incorporation of *B. subtilis* and cinnamic acid + *B. subtilis*, did not show any significant influence on the intestinal bacteria (p>0.05). However, it was observed that *B. subtilis* was isolated back in the intestines in all groups with the addition of *B. subtilis* (Table 3). When mesophilic bacteria, coliform and *Enterobacteriaceae* data were evaluated, the highest values were found in the control + *B. subtilis* group, while the lowest values were found in the group containing 0.150% CA + *B. subtilis*.

3.4 Feed, Stomach and Intestinal pH

The effects of dietary incorporation of *B. subtilis* and cinnamic acid + *B. subtilis* on pH values of the feed, intestine and stomach are shown in Table 4. Stomach and intestinal pH values were similar in all groups at day 60 (p>0.05).

Table 1. Growth performance and feed evaluation of rainbow trout juvenil at the end of the trial

	Experiment Groups					
	Control	**Control+ *B.subtilis***	**%0.025 CA+*B. subtilis***	**%0.050 CA+*B. subtilis***	**%0.075 CA+*B.subtilis***	**%0.150 CA+*B.subtilis***
IW (g)	21.10±2.96	21.53±3.41	21.81±3.38	21.81±3.38	21.54±3.34	22.00±3.30
FW (g)	55.52±4.30	53.55±4.28	55.16±3.80	54.41±3.66	55.74±3.75	56.17±3.07
RGR (%)	168.83±20.25	156.54±24.94	161.29±27.30	157.88±27.35	167.46±28.19	164.51±30.81
FCR	1.16±0.10	1.21±0.05	1.17±0.03	1.20±0.03	1.14±0.03	1.16±0.02
SGR (% day^{-1})	1.64±0.12	1.56±0.16	1.58±0.17	1.56±0.17	1.62±0.17	1.60±0.19

Table 2. Biochemical composition of fish meat and liver fat (excluding internal organs)

	Experiment Groups					
	Control	**Control+ *B.subtilis***	**%0.025 CA+*B.subtilis***	**%0.050 CA+*B.subtilis***	**%0.075 CA+*B.subtilis***	**%0.150 CA+*B.subtilis***
Dry Matter* (%)	29.23±0.24	29.10±0.20	30.00±0.52	29.10±0.20	30.00±0.52	29.10±0.20
Protein (%)	16.53±0.26	16.23±0.21	16.53±0.26	16.23±0.21	16.53±0.26	16.23±0.21
Fat (%)	9.76±0.30	9.79±0.27	9.76±0.30	9.79±0.27	9.76±0.30	9.79±0.27
Ash (%)	2.73±0.07	2.71±0.04	2.73±0.07	2.71±0.04	2.73±0.07	2.71±0.04
Liver Fat (%)	6.43±0.19	5.88±0.45	5.57±0.31	5.16±0.25	5.94±0.42	5.86±0.43

Note: *The percentages of protein, fat and ash results are expressed as % in dry matter.

Table 3. Total counts of bacterial groups and yeasts and Moulds (log CFU g^{-1}) in the intestines

	Experiment Groups					
	Control	**Control+ *B.subtilis***	**%0.025 CA+*B. subtilis***	**%0.050 CA+*B. subtilis***	**%0.075 CA+*B. subtilis***	**%0.150 CA+*B. subtilis***
***Total Heterotrophic Aerobic Bacteria**	5.49±0.57	6.73±0.15	5.96±0.67	4.90±0.42	5.86±0.72	5.49±0.29
***Mesophilic Bacteria**	3.61±0.34	4.93±0.39	3.31±0.31	3.47±0.62	3.47±0.77	2.74±0.02
B.subtilis	-	5.49±0.26	5.29±0.06	5.49±0.12	5.80±0.49	5.53±0.17
Yeast and Mould	3.78±0.77	3.57±0.23	4.12±0.10	4.06±0.39	4.47±0.68	4.09±0.36
Coliform	2.49±1.15	3.73±0.37	1.56±0.98	1.53±1.09	2.67±1.19	0.54±0.16
Enterobacteriaceae	2.83±1.20	4.39±0.16	2.29±0.95	2.03±1.49	3.33±1.52	1.20±0.75
Lactic Acid Bacteria	0.36±0.06	0.74±0.13	0.56±0.04	0.52±0.14	0.57±0.13	0.65±0.17

Note: * While the number of mesophile bacteria and total heterotrophic aerobic bacteria were calculated in the groups containing B. subtilis, the amount of B. subtilis was not added to the count since it was given separately.

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3.5 Intestinal and Stomach Enzyme

At the end of the experiment, trypsin, amylase, lipase, alkaline phosphatase and pepsin values of intestinal and gastric enzymes of *B. subtilis* and cinnamic acid + *B. subtilis* groups were analyzed and findings are shown in Table 5. There was no statistically significant difference between the groups of intestinal enzymes trypsin, alkaline phosphatase, lipase and gastric pepsin ($p>0.05$). However, it was found that the amount of intestinal amylase was higher in the groups control+B.subtilis, 0.025%CA+B.subtilis, 0.050%CA+B.subtilis and 0.075%CA+B.subtilis compared to the control and 0.150%CA+B.subtilis groups ($p<0.05$).

3.6 Internal Organ Index

Visceromatic index (VSI), hepatosomatic index (HSI), visceral fat somatic index (VFSI), bile somatic index (BSI), spleen somatic index (SSI) and heart somatic index (HSI) values of the internal organ indexes of groups fed diets incorporated with *B. subtilis* and CA+B.subtilis are given in Table 6. At the end of the study, it was found that VSI, VFSI and GSI values of the experimental groups were similar to the control group ($p>0.05$). HSI ratio was found to be lower in group 0.075%CA+*B. subtilis* than the control -and the 0.050%CA+B.subtilis groups ($p<0.05$). SSI ratio was found to be statistically higher in group 0.150%CA+B.subtilis than all other groups ($p<0.05$). HSI values were found to be statistically lower in group 0.075%CA+B.subtilis than the control group ($p<0.05$).

4. Discussion

The combination of probiotic and organic acids at dietary incorporation levels tested in this study, did not influence growth performance of rainbow trout. This was in agree-

Table 4. Changes in pH values of feed, stomach and intestines

	Experiment Groups					
	Control	**Control+ *B.subtilis***	**%0.025 CA+*B.subtilis***	**%0.050 CA+*B. subtilis***	**%0.075 CA+*B.subtilis***	**%0.150 CA+*B.subtilis***
Feed pH	5.90	5.89	5.86	5.83	5.75	5.74
Stomach pH	6.81±0.04	6.88±0.03	6.92±0.02	6.87±0.04	6.91±0.02	6.93±0.03
Intestine pH	7.07±0.01	7.07±0.01	7.06±0.02	7.05±0.02	7.04±0.01	7.08±0.01

Table 5. Changes of trypsin, amylase, lipase and alkaline phosphatase in the intestine and pepsin enzymes in the stomach

	Experiment Groups					
	Control	**Control+ *B.subtilis***	**%0.025 CA+*B. subtilis***	**%0.050 CA+*B. subtilis***	**%0.075 CA+*B. subtilis***	**%0.150 CA+*B. subtilis***
Trypsin (U/mg protein/ min)	1.66±0.26	1.57±0.16	1.66±0.15	1.43±0.14	2.02±0.19	1.53±0.12
Amylase (mU/mg protein)	57.48±6.71^{b}	249.79±33.60^{a}	207.65±21.67^{a}	250.62±26.65^{a}	190.86±20.28^{a}	62.83±6.65^{b}
Lipase (uMol/mg protein/min)	0.28±0.02	0.22±0.02	0.22±0.01	0.20±0.02	0.26±0.05	0.20±0.02
Alkaline phosphatase (U/mg protein/min)	0.23±0.03	0.21±0.03	0.26±0.03	0.17±0.02	0.20±0.03	0.24±0.03
Pepsin (U/mg protein/ min)	34.59±6.34	30.12±4.82	31.20±3.08	39.46±4.31	32.29±3.65	26.74±2.24

Table 6. Changes in internal organ indexes

	Experiment Groups					
Parameters	**Control**	**Control+ *B.subtilis***	**%0.025 CA+*B. subtilis***	**%0.050 CA+*B. subtilis***	**%0.075 CA+*B. subtilis***	**%0.150 CA+*B.subtilis***
VSI	15.31±0.63^{ab}	16.21±0.59^{ab}	14.35±0.50^{b}	17.07±0.23^{a}	14.62±0.54^{ab}	15.68±0.86^{ab}
HSI	1.56±0.08^{a}	1.42±0.05^{ab}	1.34±0.06^{ab}	1.59±0.08^{a}	1.25±0.05^{b}	1.46±0.06^{ab}
VFSI	4.00±0.30^{a}	4.31±0.20^{a}	3.86±0.23^{a}	4.88±0.10^{a}	4.50±0.31^{a}	4.12±0.29^{a}
BSI	0.21±0.02^{a}	0.18±0.03^{a}	0.13±0.01^{a}	0.20±0.03^{a}	0.14±0.01^{a}	0.12±0.01^{a}
SSI	0.34±0.02^{b}	0.34±0.01^{b}	0.33±0.03^{b}	0.36±0.04^{b}	0.33±0.01^{b}	0.47±0.02^{a}
HSI	0.23±0.01^{a}	0.21±0.01^{ab}	0.19±0.02^{ab}	0.22±0.02^{ab}	0.17±0.02^{b}	0.22±0.01^{ab}

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ment with a recent study on rainbow trout (*Oncorhynchus mykiss*), where dietary inclusion of *Bacillus subtilis* did not show any impact on fish growth [18]. No significant differences in terms of fish growth were found in flounder fed diets incorporated with a mixture of organic acids [40]. In another study, giant grouper *Epinephelus lanceolatus* demonstrated the lowest growth performance when fish was fed a diet with 1% lactase addition compared to the other test groups [41]. In contrast to these reports, Hassaann et al. [6], reported significantly higher values for weight gain (WG), specific growth rate (SGR) and Feed conversation ratio (FCR) on nile tilapia (*Oreochromis niloticus*), fed a combination of *Bacillus subtilis* and malic acid compared to the control group. Further, Nesara et al. [42] also found higher growth rate in *Labeo rohita*, fed diets incorporated with *Lactobacillus plantarum* and citric acid combinations over the control group without dietary treaments.The probiotic combination of *B. subtilis* and *B. licheniformis* increased growth rate of rainbow trout (*O. mykiss*) [1], and experimental diets with *Bacillus subtilis* addition also increased growth rate in red sea bream (*Pagrus major*) [14].

Similar to our findings in this study, Yilmaz et al., [43] did not find significant change in dry matter, protein, fat and ash contents of rainbow trout fed diets with cinnamic acid incorporation.

Microbial diversity affects the digestive system and probiotic application plays an important role in regulating and functioning of this system [43]. Earlier studies have also reported that *B. subtilis* may support growth performance and survival of animals and humans [44-45-46]. In this study, however, experimental treatment groups with *B. subtilis* did not show significant changes on the intestinal bacteria, which is in agreement with rhe findings of Wu et al., [44], who investigated the effects of *B. subtilis* on the intestinal bacteria and found similar counts for the total aerobic and facultative anaerobic bacteria in all experimental groups including the control.

Organic acids, mineral absorption, nutrient digestion and accumulation of H^+ ions reduce the level of pH in the digestive tract and can positively affect growth performance [43-47]. In our study, it was observed that pH levels in the digestive system did not cause any changes between experimental groups. However, Yılmaz et al., [43] reported that cinnamic acid supplements decreased the pH levels of the stomach and intestines 4 hours after feeding. Culture media containing cinnamic acid did not show antimicrobial effect on *B. subtilis* [48]. This shows that cinnamic acid provides acidic condition to improve *B. subtilis* in the digestive tract. Therefore, low doses of *B. subtilis* and cinnamic acid mixtures are encouraged to be investigated.

Digestive enzyme activities are important data for digestive capacity and growth performance [44,49,50]. In our study, no statistically significant differences were found between trypsin, alkaline phosphatase, lipase and pepsin groups from intestinal and stomach enzymes. Similarly, in a study on rainbow trout, amylase, lipase and trypsin values did not differ between experimental groups [43]. However, it was observed that cinnamic acid supplementation increased stomach pepsin activity [43]. In the study with *Ctenopharyngodon idella*, it was observed that the addition of *B. subtilis* Ch9 increased amylase and lipase activity in the intestine [44]. Another study investigating the effects of malic acid in fish diets, reported increased levels for gastric pepsin activity and growth performance in tilapia (*Oreochromis niloticus*) [51].

Enterobacteriaceae is usually found in the gastrointestinal tract of fish and its presence in fish farming can cause serious problems for human health [52]. Degree of contamination of coliform bacteria gives information about fish quality [53]. So, according to the studies, contamination of enteric bacteria in the intestinal micflora [54] of human or animal may cause food spoilage [55]. Faecal coliforms in fish also show the level of pollution in the environment, because coliforms are not found in a normal flora of fish [56]. In our study, the coliform and *Enterobacteriaceae* counts were highest in the "control+*B. subtilis*" and lowest in the "0.150% CA + *B. subtilis*" groups. In combination with *B. subtilis* and high doses of cinnamic acid, the bacterial count is reduced. So, cinnamic acid can suppress harmful bacteria when used within appropriate doses.

Internal organ indexes may increase or decrease in case of unhealthy conditions [57]. Spleen, an important organ in fish, is the place where erythrocytes and neutrophils are produced and matured [58-59]. Spleen has an important role in the immune response in fish [58-60]. In our study, SSI value increased in the group containing 0.150CA + *B. subtilis*. In an earlier study conducted on the effect of herbal supplement in sea bass diets, SSI values in experimental fish were increased compared to the control group [58]. In another study, the spleen size was found to be positively influenced by disease resistance [61]. SSI value can provide information about fish health and immunity. HSI values were lowest in the group containing 0.075% CA+*B. subtilis* compared to the control+*B. subtilis*. Similarly, HSI values were reduced in the study conducted on sea bass [58], this could be linked to some improvements in the organs.

In our study, the combination of *B. subtilis* and cinnamic acid did not show statistically significant effects on growth performance, whole body composition values, and gastrointestinal system pH values. However, according to internal organ index and intestinal bacteria results, we have observed that it reduces coliform and *Enterobacte-*

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riaceae counts when used at appropriate doses. Also, organic acid and probiotics may be used as additives for the healing of internal organs.

Experimental conditions such as feeding periods, type and size of fish, different organic acid or probiotics used and dosage ranges could be explained as reasons for the differences between different studies. Therefore, further investigations on different conditions are encouraged to find out best results.

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REVIEW

Summary of Animal and Plant Quarantine Objects in the Imported Parcels and X-ray Pattern Identification

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ABSTRACT

The quantity of the imported parcels in China has increased rapidly,becoming an important means for criminals to hide the prohibited animal and plant quarantine objects.The x-ray applied to the port of post inspection can speed up inspection and better protect the ecological safety of China by mastering its method of judging animal and plant quarantine objects.

1. Introduction

With the development of e-commerce and overseas purchasing,international exchanges and personnel exchanges are increasing type growth [1]. The rapid increase and convenience of the imported parcels, while also making it more diversified and subtle. The presence of these characteristics has made it possible for criminals to hide contraband items, raw beef from BSE areas,venomous poisonous snakes and plants containing quarantine pests in infected areas can be entered by mail. This risk of carrying harmful organisms and spreading epidemic diseases is increasing, and the ecological safety of our country, production of agriculture,forestry and the health of the people are seriously threatened. Quarantine of the imported parcels is to effectively prevent infectious, parasitic, dangerous,pests and weeds and other pests from being carries by mail into the country [2].

At present,the X-ray equipment is used in all post inspection points to identity the imported parcels on-line, and combined with artificial opening sensory judgment.

2. Animal and Plant Quarantine Objects in the Imported Parcels

2.1 Species of Animal and Plant Quarantine Objects

At present,the main law enforcement basis for quarantine of the imported parcels in China is the Entry-exit Animal and plant Quarantine Law and its implementing regulations, The list of prohibited articles under quarantine shall be prescribed by the Catalogue of animals and plants and products whose carriage is prohibited by the people's Republic of China and sent by mail to China (Announcement NO. 1712). Divided into animals, plants and other three categories.Animals include live animals, raw and mature meat and its products, animal milk and milk products,

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eggs and products, bird's nest, hoof horn and products, etc. Plants include fresh fruits, vegetables, tobacco leaves, seed seedlings, organic culture media, soil and so on. A review of the interception data from the national postal inspection shows that the share of animal and plant quarantine products in all dangerous source articles entering China with the imported parcels is the largest,Apart from common meaty plants,all kinds of raw and cooked meats and their products,sea cucubers in bird's nest,It also includes a lot of seed seedings and live animals, including radiation turtles,ivory,earth incense and other internationally endangered protected animals and plant products.

2.2 Harm from Illegal Mailling of Animals, Plants and Products

Animals and plants and their products that enter illegally with the imported parcels will cause animal and plant diseases.Based on the data intercepted in the past,the most virulent poison was detected from HongKong mail, eggs containing highly pathogenic avian influenza virus found in Vietnamese mailings, the content of lavender teddy bear from Australian mail detected the detection of epidemic pests: Avena sterilis L. subsp. Sterilis and Avena ludoviciana Durieu, detection of quarantine pest Hypothenemus coffee from India, Brazil,Vietnam and other postal products, have appeared in the imported parcels. The invasion of alien pests will lead to new ecological system damage, ecological imbalance,resulting in economic losses and environmental damage.

3. Method for X-ray Mapping of Animal and Plant Quarantine Objects

Inspection of the imported parcels by X-ray at the inspection site at the post office, The information of the inspected object can be quickly and rich,at the same time the inspection staff can avoid the risk of the unknown in the postal object.

3.1 X-ray Imaging Principles

X-ray is a highly penetrating eletromagnetic wave, when it strikes a subject, partially passes through it, while the other part is emitted. The internal structure of the object is reflected on a fluorescent screen by an image of the X-ray through various components and density materials by signal conversion.

3.2 Imaging Characteristics of Animal and Plant Quarantine Objects and Mapping Methods can be Identified by Colour, Angle, Density and Shape

Depending on the density of the object and the x-ray absorption characteristics of the material, the orange,green and blue colors represent organic matter, mixture and no machine respectively, the classification and coloring of images make different substances easier to distinguish. Most of the animal and plant quarantine products take in X-rays and they appearorange, organic. A few special images, such as ivory, dried sea cucumber, animal hoof angle, appear green.

The same animal and plant quarantine objects present different images at different passing angles depending on the angle in which they are placed in the parcels.The sausage if a horizontal image shows a high-density array of dots, this is the top view: if the tile is vertical in a parcel it shows the outline of the sausage.

Since the electronic density and atomic number of most of the itens are determined [3]. The thickness and density of the clolor in X-ray images correspond to the thickness and density of the object being inspected. The size of the density is used to determine the number of animal and plant quarantine objects, and the density of the object can also be determined by the to identify the presence of animal and plant quarantine objects somewhere. A seed in a book hidden away in mail, The book will appear different depth of x-ray images, you can identify the inner sandwich, and the corresponding density of the clip, can identify whether the amount contained is more or less.

Compared with other articles in the imported parcels, animal and plant quarantine products have their specific shapes. The x-ray imaging has strong appearance identification and characteristic.

4. Summary

The physical mechanism of X-ray interaction with matter provides an important and comprehensive guarantee for public safety.With the rapid development of electronic commerce,the safety of inbound postal materials is particularly important.A thorough understanding of the X-ray method for the identification of animal and plant quarantine objects in the imported parcles can help the inspectors at mail ports to identify them quickly and further improve the efficiency of inspection.

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REVIEW

Advances in Terrestrial Mammal Movement Ecology: An Overview

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ABSTRACT

As a research field which is blooming quickly in recent years, movement ecology has been a worldwide concern and interest. However, movement ecology is so comprehensive and complicated that many articles only focus on few aspects or species. As tracking technologies and methods of movement data analysis develop, the abundance of movement data becomes available for demonstrating more scientific facts about animal movement. This article is aimed to summarize the advances of terrestrial mammal movement ecology in the past years to show its critical and potential research fields, as well as trying to ascertain direction of these advances.

1. Introduction

Movement ecology is defined as a kind of ecology which focuses on the relationship between organism movement and environment (biotic and abiotic). Research on terrestrial mammal movement ecology can cover many themes and aspects [1]. For example, based on an individual's movement data, the researcher can know about when and where animals moved, activity patterns and conclude motion capacities. Beyond this, given the movement path and home range of animals, accompanied with environment factors and food patch information, researchers can demonstrate habitat selection of animals [2]. With new technology of animal sensors, individual physiological states and behaviours can be recorded [2], enabling the researcher to understand further what drives animal movement. If the tracking of an animal lasts its whole life, the researcher can draw the life-movement and behaviour map of individuals, which can represent the life history of this individual[3]. This special sequence is simillar to a DNA strand [3], it may become an ID of this species, or even this individual. If the individual samples are enough and data is fitted, even social relationships of a group can be explored[4]. Population ecology can also be supported by movement data, reflecting population distribution and competition. Movement of prey and predator is also connected in population movement ecology. For some species, long-term and large spatial scale tracking may reveal the relationships between animal migration and global climate change.

Nowadays, movement ecology is becoming more and more generalized and concerning at many objects wildly,

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from dispersal of seed to migration of blue whine. As the development of tracking technology, it is no doubt that the number of research papers about movement ecology is increasing in recent years. Holyoak et al. (2008) figured out that at least 2,6000 papers referd to movement ecology in 1997-2006. He selected 1,000 articles randomly and found that 12% of them aiming at mammal movement ecology, which was just lower than plants and birds (both of them are 19%) [5]. It suggest that mammal, whose majority is terrestrial mammal, is an important taxa in movement ecology research.

We searched papers as terms such as "migration", "dispersal", "movement pattern", "home range", "activity" and filter them with the limit of "terrestrial mammals" to summarize the papers about terrestrial mammal movement ecology. We found that probably 1,800 papers about terrestrial mammal movement ecology were published during 2017-2020. To descript the history of movement terrestrial mammal ecology, we also reviewed previous papers.

In China, rare species such as Giant Panda (*Ailuropoda melanoleuca*) and Golden Monkey (*Rhinopithecus* spp.) have had their movement patterns and habitat use explored [6,7]. Ungulates are also a hot taxonomic class in China and North America [8]. Some smaller wild animals are little focused because their sampling is very challenging [9]. Some larger animals like Black bear (*Ursus thibetanus*) and African elephants (*Loxodonta*) have been tracked with Global Positioning System (GPS) collars to achieve their movement trace [10,11]. Many feline animals like Amur tiger (*Panthera tigris altaica*), Cheetah (*Acinonyx jubatus*), lynx (*Lynx lynx*), Jaguar (*Panthera onca*) and African Lion (*Panthera leo*) also has been tracked[12-16]. Rodents like Mice (*Muridae*) and Squirrel (*Sciuridae*) are also important target species[17]. Many research themes has been explored compared to other species but internal state and navigation capacity of individuals during their movement are seldomly mentioned.

This article is aimed to focus on the terrestrial mammals as object species of movement ecology, find the access to get and analyse movement data and summarize the critical and potential research fields of terrestrial mammal movement ecology to try to find the trend and missing parts in terrestrial mammal movement ecology.

2. Method and Themes of Research

2.1 Tracking Technology

Tracking technology is the supporting of the development of terrestrial mammal movement ecology. The original method of animal tracking is observing animals in the field or checking the animal traces such as footprints on the snow ground. This method is very precise but will take much time and bring many difficulties to researchers. This kind of movement data is generally short-term data [18].

Nowadays, researchers often use Very-High-Frequency (VHF) telemetry or GPS to track terrestrial mammals. VHF is also known as radio tracking, whereby researchers can deploy VHF transmitters onto animals and carry the corresponding VHF receiver to the field to locate animals periodically and draw the location map depending on the dirction of observation [19]. However, GPS technology is the most commonly used tracking technology at the present time. The devices can be built as collars, ear tags or some other gadgets. After deploying the devices, they send location data to satellites in a particular frequency and researchers can retrieve data from specific websites or software [20]. Differ from bird or fish tracking , tracking of terrestrial animals usually needs shorter fix interval and higher precision because there are much canopy on the ground. This requir bigger battery and more advanced positioning device than other species.

Additionally, there are other state of the art technologies which record animal internal state and behaviour, and even the environmental factors can be recorded along the animal's pathway at the same time [21]. Such a technological revolution must bring more opportunities and possibilities to terrestrial mammal movement research.

2.2 Research Themes

Individual movement is fundamental in terrestrial mammal movement ecology. Nathan et al. (2008) proposed the paradigm of individual movement ecology, which contains four frameworks (internal state, motion capacity, navigation capacity and external factors) which can affect an animal's movement path [3]. He also listed five links between them as the movement ecology research direction. The following research themes mentioned in this paper will be fitted to these frameworks to facilitate future research.

2.2.1 Movement Pattern and Locomotion

Movement pattern and locomotion is the most common theme in individual movement ecology, and has been broadly explored in previous times. This theme focuses on the motion capacity. Movement pattern can be demonstrated by activity quantity and active time, which can reflect which time period this individual prefers to move. Motion capacity can be measured by day range [22] and home range [23] and is affected by the physiological condition and life period of individuals or environment factors.

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Home range is a common metric which is necessary for various studies [24] and seasonal home range is becoming a new focus [25,26].

Behaviour during the movement is a critical component of individual movement because they reflect the goal of animal movement. This theme mainly focuses on internal factors and external factors. When individuals stay, are they foraging or bedding? When individuals move, are they walking toward a food patch or seeking mating? Although animal behaviours can be partly deduced from their movement data, there will be error due to the environmental change and the observation [27]. Besides getting actual behaviour data to support movement analyses, State-Space Model (SSM) and Hidden Markov Model (HMM) are excellent tools to integrate individual hypothetical behaviours and movement [28].

2.2.2 Habitat Selection and Landscape use

Using movement data to explore habitat selection of animals is very popular in individual movement ecology studies. Internal state and external factors of individuals are more concerned in this field. Resource Select Function (RSF) is the usual method to study habitat selection. However, it ignore the nature of continuously movement data and has difficility in making "avaliable point" to match movement data. The appearance of Step Select Function (SSF) solved this problem and it is becoming the most common tool in habitat selection study with movement data. The integration of more metrics of SSF is making it more powerful [29].

Concerning external factors and animal movement paths, the landscape of the animal movement is a environmental factor that cannot be ignored. Landscape structure is proven to affect animal movement, which can be found in movement behaviour, home range change, dispersal progress and so on. The distribution, shape and area of different kinds of patches and the existence of corridors is all relevant and increases the variance [30,31]. Shepard et al. (2013) transform the landscape to energy cost distribution, indicating that energy landscapes shape animal movement [32]. Landscape connectivity is under focus in recent times [33]. For some rare species such as Amur tigers whose habitat fragmentation is obvious, corridor research is also important. Additionally, artificial landscapes of human activities are expanding and becoming part of animal habitat. Animal movement in artificial landscapes is also a focus of movement ecologists.

Spatiotemporal scales represent the minimum space and time unit within movement research. For example, the time interval of GPS data could be the temporal scale, and step, movement phase or movement path could be the spatial scale. The most suitable spatiotemporal scales in research differ across species, even differ within same species research. For example, multiple movement modes were observed in large herbivores by different spatiotemporal scales [34]. Accuracy of habitat selection of Giant panda is also different among different scales [35]. Sometimes different scales can bring new views of research and lead to new findings.

2.2.3 Migration, Dispersal and Homing

Navigation capacity of individual movement is often focused on the migration and dispersal phenomenon research. Certainly, external factors like heterogeneity of landscape also affects the result of migration and dispersal. In terrestrial mammals, elephants (*Elephas maximus*) [36] and some ungulates in Africa and North America are known for their long-distance migration [37,38]. Why do animals migrate and how can they navigate during their trip are the critical problems in this field. When a new group arrives at a new habitat, their distribution process is defined as dispersal. There is a hypothesis which assumes that animal movement is "random walk" in a homogeneous environment [39]; analyses of animal movement in heterogeneous environments are based on this hypothesis [40].

Memory and homing behaviour are themes related to internal states and navigation of individuals. Animal memory is always a problem researchers are interested in [41]. How can animals remember the location of the feeding points, water resources and their nests? It is sure that navigation and memory capacity contributes to it, but the environmental factors must drive their movement. Homing behaviour is a kind of memory which has been widely studied [42]. Revisitation of specific locations is a another kind of memory and has been used to prove animal habitat preference [43].

2.2.4 Population Movement and Intra-species Relationship

Sometimes, individuals' movement can reflect spatial organization of populations, but must be considered at suitable scales and with enough samples. In some conditions, individual behaviours can be extrapolated to population spacing patterns [44]. Even population dynamics can be derived from reproduction behaviour and mating related movement. Movement ecology is expected to connect with population ecology more widely.

Besides fundamental population ecology like population distribution and population size dynamics, gene flow is another theme of population movement ecology. Based

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on the spatial distribution and movement information of populations, combined with genetic samples of individuals, the change of population distribution and gene-flow can be revealed [10]. Certainly, progress of dispersal and mating is always accompanied with gene flow, which should be concerned in this field.

Tracking individuals of a group or family, researchers can find the social relationship between individuals by their interactive behaviours. These research directions often choose domestic animals as samples because their society information is known previously [45]. For terrestrial mammals, it gives great access to explore parenting behavior, battle behaviour for mating priority and obeying behaviour to higher-level individuals.

Intraspecific and interspecific relationships are also a focus of population movement ecology. Movement data provides a new access to explore competition between individuals in the same group and the pressures between prey and predator. Many metrics can measure competition between individuals, such as overlap of home range of individuals [46]. The predation pressure between prey and predator can be reflected by avoidance by prey and chasing behaviour of predator. Eriksen et al. (2011) compared moose and wolves (*Canis lupus*) activity patterns but found no correlation between them [47]. This indicates that co-analysing of prey and predator movement data may be not easy. Tracking critical movement behaviour of the target animals and keeping prey and predator in the same small research area may make such ecological pressures clearer to us [48].

2.2.5 Applications of Movement Ecology

There are some applications of movement ecology which can offer great ecological benefits. For example, the spread of infectious disease among animals is a concerned problem nowadays. Besides the information of the pathogen and ways of transmission, animal population distribution and movement is also necessary to build the transmission model[49]. But sometimes movement data cannot surrogate epidemiological indicators. Podgórski et al. (2018) found that no movement metric of wild boar contributed to swine fever outbreak in Europe [50].

Monitoring movement paths for rare species and candidate reintroduction animals is very supportive to their conservation. Movement monitoring can inform animal physiological state, and home range area can visually depict and demarcate high-quality habitat to guide and make convenient the implementation of in-situ protection. Movement monitoring of rare species also benefits education and publicity in animal conservation activities.

Making use of animal movement monitoring data to protect crops and control pest animals is a great application in agriculture. For example, a case study of American research used camera trap and GPS tracking of wild boar mark the breakpoints of farmland, and thus, were able to protect crops from invading wild boar. Jarolímek et al. (2014) tried to develop a similar platform whereby farmers and hunters can know about the movement of wild boar for hunting and protection against crop damage [51].

Climate change is a concerning topic nowadays that can be revealed by movement ecology. For some terrestrial mammals like Elk, their long-distance migration change may reflect the changing climate [37]. For some environmentally sensitive animals like Moose (*Alces alces*) who have the adaptive fitness to cold climates, their behaviour or habitat selection change may also be the sign of climate change[38]. Thus, we suggest that studying the effects of climate change is a potential direction of movement ecology.

2.3 Analysis of Movement Data

To solve specific movement ecology problem, researchers developed many metrics and modles to fit movement data. Day range and home range are normal metrics in terrestrail movement ecology. Day range can be measured by culcalate daily travel distance and can be modeled. There are many methods to calculate home range, including Minimum Convex Polygon (MCP), Kernel Density Estimation (KDE) and Brownie Bridge Movement Model (BBMM). MCP is a kind of geometry method. Due to the lack of distribution density estimation, MCP is rarely used nowadays. KDE is the most commonly used method to calculate home range now and has much reformation and expansion to fit most situations [52]. BBMM is suitable for drawing corridors between patches and has been more considered to estimate home range area [53].

To study habitat selection with movement data, the most popular and suitable method is Step Select Function (SSF). SSF are powerful models to study habitat selection during animal movement. A defined random steps from two distributions established from observation of step lengths and turning angles of monitored individuals. Compared to Resource Selection Functions (RSF), used steps are contrasted with a limited domain of random steps that characterize what is 'available' to the animal during its movement through the environment[54]. Researchers often use Conditional logistic regression as modeling approach to these "actual steps" and "random steps" [55]. At different spatial scales, Path Select Function (PathSF) is also suitable. Zeller et al. (2015) use PathSF to calculate landscape resistance surfaces of of pumas (*Puma concolor*) and found that PathSF is more suitable than SSF for this spe-

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cies [56].

State-Space Model (SSM) and Hidden Markov Model (HMM) are excellent tools to integrate individual hypothetical behaviours and movement. In movement ecology, SSM is a kind of model which can predict animal state in the future from the previous location and behaviour [57]. Forester et al. (2007) utilize SSM to quantify how elk (*Elaphurus davidianus*) respond both to local conditions and to their internal state in heterogeneous landscapes at different spatial scales [58]. HMM is a diversity process which can link the chain of movement and behaviour to environmental factors [59,60] and has been broadly used in movement ecology, like SSM. Movement and behaviour will be more steadily combined in future research.

3. Research Prospects

This article summarized the papers about terrestrial mammal movement ecology published rencently to find the main themes and object species of this research field. Generally, research themes of terrestrial mammal movement ecology are as follows: Movement pattern and locomotion, habitat selection and landscape use, migration, dispersal and homing and polpulation movement ecology. Despite of some small-size mammals, many terrestrial mammals are concerned in this field. As the development of tracking technology, there are more access to detailed information about animal movement. Terrestrial mammal movement ecology will keep on going basing on these technologies.

Compared to other species, terrestrial mammal share a big part in movement ecology. Maybe it's because the convenience of tracking of most terrestrial mammals or the amount of classes of terrestrial mammals, many research themes has been explored. However, we can't ignore that complexity and difficulty of terrestrial mammal movement research. It's a challenge to determine the internal state and behaviour of terrestrial mammal during their movement on heterogeneous landscape. In the same time, there are too many factors to impact animal movement path. This is the critical and difficult part of terrestrial mammal movement research. Secondly, because there are many shelters on the ground, movement data can be not accurate and have missing point, which bring troubles in movement data analysis. Researchers can choose more precise devices to track animals.

There are some trends and gaps in current terrestrial mammal movement research. According to the paradigm of movement ecology, most research only focuses on motion capacity and external factors; navigation capacity and the internal state are rarely studied. Maybe this is due to the associated difficulty of monitoring physiological state and navigation capacity. As animal behaviour and physiological state monitoring technologies develop, internal state will likely be more considered to demonstrate what drives animals to move [61]. Navigation mechanisms are studied to explain how animals locate and move with some directions. Accompanied with the finding of environment markers such as magnetic fields and the help of nerve and brain science, the researcher can get a deeper understanding of animal navigation [62].

On the other hand, some species are missed in current research. Some large-size terrestrial mammals are easier to track and are of more concern, but small-sized terrestrial mammals such as Mustelidae (*Mustelidae*), including mink (*Martes* spp.), badger (*Meles* spp.) and rodents (*Glires*) are harder to track and more ignored. Actually, small-sized terrestrial mammals also play important roles in ecological systems and their movement deserves more attention.

There are many potential research themes in terrestrial mammal movement ecology research. For animal individuals, movement and landscape are considered grand themes. With the expansion of human utilised land area, lots of artificial landscapes such as roads, farmland and residential areas will likely be highly influential on terrestrial mammal movement; habitat fragmentation caused by this cutting up of the natural landscape is also a problem to animal dispersal and distribution. Movement data is direct data to reflect animal movement affected by these landscape factors. The internal state factors during animal movement is also potential dirction of terrestrial mammal, even the personality can be inclued to factors to affect space use of terrestrial mammals [63].

For animal populations, combining movement data with gene samples to predict animal distribution changing and migration is very powerful. If an animal's reproduction and mortality information is collected, population numbers can also be predicted. Additionally, disease transmission and climate change are also potential fields to which movement data may be applied. Once supported by animal movement and contact information, disease transmission models will predictably be more accurate. Climate change must be felt by polar animals firstly; their adapted movement behaviours or changes of migration can inspire researchers to understand climate change.

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