01 2019 APRIL VOLUME 1 ISSUE 1

JOURNAL OF ZOOLOGICAL RESEARCH

Volume 1 | Issue 1 | 2019 April | ISSN 2630-5100 (Online)

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Volume 1 Issue 1 • April 2019 • ISSN 2630-5100 (Online)

Journal of Zoological Research

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Journal of Zoological Research
http://ojs.bilpublishing.com/index.php/jzr

ARTICLE

Effects of Supplementing Various Linoleic to α-linolenic Acid Ratios and Vitamin A on Production Performance and Egg Characteristics of Laying Hens during Summer Months

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ARTICLE INFO

Article history:
Received: 25 September 2018
Accepted: 19 November 2018
Published: 22 January 2019

Keywords:
Egg quality
Fatty acids
Hot climate
Layers performance
Vitamin A

ABSTRACT

In the present feeding trial, responses of laying hens that were kept at high ambient temperature and Fed with various dietary ratios of linoleic acid (LNA) to α-linolenic acid (ALA) and vitamin A levels on production performance and egg quality traits were evaluated. A total of 360 Leghorn laying hens at 40th week of age (average initial body weight; 1.79 ± 0.23 kg) were fed with various combinations of canola oil and linseed oil containing diets to achieve LNA to ALA dietary ratios of 20:1, 10:1, 4:1, 2:1, 1:1 and 1:2, each supplemented with 3000 or 10000 IU of vitamin A/kg of diet. The experiment was designed as a 6×2 factorial Completely Randomized Design that continued for 12 weeks. Feed intake, body weight gain, egg production and egg quality traits were recorded during the trial. Decreasing dietary LNA to ALA ratio or increasing poly unsaturated fatty acids (PUFA) in the diet decreased ($P<0.05$) body weight gain and yolk percentage in laying hens. Feed intake, hen-day and hen-housed egg production, feed conversion ratio (FCR) per dozen of eggs and shell quality remained unaffected ($P>0.05$) by dietary treatments. Feed conversion ratio per kg eggs, egg weight and egg-shell thickness showed a curvilinear ($P<0.05$) response to decreasing dietary LNA to ALA ratio. Although the dietary ratio of LNA to ALA of 4:1 or less could produce eggs by the hens with desirable quantities of n-6 and n-3 PUFA – that are characteristics of functional diets – the performance of laying hens in terms of body weight gain and egg-yolk percentage was slightly compromised. Therefore, a 4:1 or 2:1 LNA to ALA combination can make a borderline between the production traits and the feed economics.

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 DOI: https://doi.org/ 10.30564/jzr.v1i1.112

1.Introduction

There has been an increasing interest on the role of polyunsaturated fatty acids (PUFA) such as linoleic acid (LNA; n-6 PUFA) and α-linolenic acid (ALA; n-3 PUFA), since these hold the prime importance as being the precursors of long chain PUFA in the human diets[1]. The necessity of these two PUFAs as a part of functional foods[2], and their role in the propagation or inhibition of certain diseases has been well established[3-5]. The ratio between n-6 and n-3 PUFA in the diet appears to be more important for the fat and cholesterol metabolism in the body than their absolute concentrations[6]. The intake of n-6 and n-3 PUFA by humans in a ratio of 1 to 5:1 is recommended (EFSA, 2009). It is much easier to fix this ratio in poultry eggs[7-11] than other foods.

It is well established that in poultry diets a minimum quantity of LNA is required for optimum egg production and egg quality. This is why, LNA content has been considered an essential component while formulating commercial layer diets. The LNA content should be at least 1 % of the commercial egg layer diets[12], and by increasing ALA in proportion to LNA may cause a decrease in production performance due to the compromised LNA metabolism. The layer diets having high quantity of ALA but lower LNA may result into compromised production performance and egg characteristics[13]. Therefore, there is a need to evaluate production performance of laying hens while incorporating lower LNA to ALA ratios in the diets to produce omega-3-enriched eggs. An optimum ratio between LNA and ALA is needed to be figured out which is best to produce omega-3-eggs without compromising production performance and other egg quality traits in laying hens.

During summer months, feeding of high PUFA content to laying hens to produce PUFA-enriched eggs can accelerate the process of lipid peroxidation in the tissue linings of reproductive organs. It is well established that the increased lipid peroxidation, ultimately, results into lower production performance in laying hens. This situation demands some additional antioxidant in the layer diets containing high PUFA content to check the negative effects of lipid peroxidation on production performance. This may be possible through vitamin A supplementation, as an antioxidant, in the diet of hens higher than the normal level of 3000 IU/kg of diet[12]. Dietary vitamin A supplementation higher than recommendations[12]can increase the content of this vitamin in the eggs[14] and also restores the normal activity of reproductive organs in laying hens kept at high ambient temperature[15]. This increased vitamin A content in PUFA-enriched eggs can also reduce the egg quality deterioration due to lipid peroxidation of PUFA during storage. Considering the above-mentioned facts, the current study was planned and executed to evaluate the effects of various dietary LNA to ALA ratios and vitamin A levels on production performance and egg characteristics in laying hens kept at high ambient temperature during summer months.

2. Materials and Methods

2.1 Birds, Housing, and Experimental Diets

Three hundred and sixty White Leghorn laying hens, at 40th week of age (average initial body weight; 1.79 ± 0.23 kg), were randomly divided into 30 groups (each comprising 10 birds) and twelve treatments were randomly assigned to three groups representing three replicates per teatment. The experimental treatments i.e. layer rations included various combinations of canola oil and linseed oil to have LNA to ALA dietary ratios of 20:1, 10:1, 4:1, 2:1, 1:1 and 1:2, each combined with 3000 or 10000 IU vitamin A/kg of diet (6 × 2 factorial design, under Completely Randomized Design). The hens were kept in cages (2 birds/cage) providing 0.093 m^2 floor space area to each, and at a high ambient temperature in the range of 29.3 °C to 37.4 °C (diurnal temperature), humidity 17.7% and pressure 1.010 bar throughout the experimental period of 12 weeks. The light regime was 16 Light: 8 Dark for all treatment groups. The layer diets for all treatment groups were iso-nitrogenous and iso-caloric and formulated according to National Research Council [12] recommendations for dietary needs of laying hens (Table 1). The laying hens had ad-libitum access to feed and water throughout the experiment. The laying hens were vaccinated against Newcastle Disease Virus (NDV; live attenuated La Sota strain) through drinking water at 30th day of the study.

2.2 Data Collection and Statistical Analyses

Proximate components of feed ingredients were analyzed[16]prior to formulation of layer diets. The egg production was recorded daily, while, feed intake and egg weights were recorded weekly. The data, thus collected, were used to compute feed conversion ratio (FCR) per dozen of eggs and per kg egg mass. The egg-yolk weight, shell weight, shell thickness, and egg-shell breaking strength were measured on weekly basis for each replicate, separately. Egg-shell breaking strength was estimated by using egg-shell intensity meter's machine (Ogawa Seiki Co. Ltd., Japan). Haugh Unit Score and yolk indices were also calculated by using formula.. The data were analyzed by two-way analysis of variance using General Linear Model (Minitab 13.1, Minitab Inc., State College, PA) and means were compared by Tukey's honestly sig-

DOI: https://doi.org/ 10.30564/jzr.v1i1.112

Table 1. Ingredients and nutrient composition of layer diets (g/kg dry matter)

LNA:ALA[1]	20:1	10:1	4:1	2:1	1:1	1:2
Vitamin A[2]	1 & 2	1 & 2	1 & 2	1 & 2	1 & 2	1 & 2
Ingredients			g/kg			
Maize grain	687.0	597.6	520.0	520.0	480.0	310.0
Rice broken	0.0	61.0	121.0	115.0	133.5	240.0
Soybean meal	140.0	179.0	200.0	210.0	241.0	255.0
Fish meal 52%	56.0	0.0	0.0	0.0	0.0	0.0
Corn gluten 60%	35.0	60.0	47.5	40.0	20.0	10.0
Canola oil	0.0	10.0	16.0	10.0	3.0	0.0
Linseed oil	0.0	0.0	3.5	12.0	28.5	56.0
Wheat bran	0.0	0.0	0.0	0.0	0.0	34.0
Limestone	69.0	70.0	70.0	72.0	72.0	72.8
Di-calcium Phosphate	7.8	17.1	17.0	16.6	17.0	17.0
L-Lysine	0.6	1.6	1.2	0.9	0.4	0.0
DL-Methionine	0.3	0.6	0.7	0.7	0.9	1.0
Sodium chloride	1.0	0.1	0.2	0.7	0.9	0.9
Sodium bicarbonate	0.25	0.30	0.30	0.30	0.30	0.30
Vitamin/mineral premix[3]	2.5	2.5	2.5	2.5	2.5	2.5
Nutrient composition						
Crude protein	170.0	170.0	170.0	170.0	170.0	170.0
Ether extract	33.3	35.7	42.2	44.3	51.5	69.9
Crude fibre	25.9	32.7	38.1	38.1	40.8	52.0
ME (MJ/Kg)	12.14	12.14	12.14	12.14	12.14	12.14
Ca	32.5	32.7	32.6	32.9	32.8	32.4
P	4.0	4.1	4.0	4.1	4.2	4.0
Lysine	9.0	9.2	8.9	9.0	9.1	9.2
Methionine	3.8	3.9	3.7	4.0	3.8	3.7
Threonine	6.4	6.7	6.3	6.5	6.4	6.6
LNA	16.0	16.0	16.0	16.0	16.0	16.0
ALA	0.8	1.6	4.0	8.0	16.0	32.0
CP/EE[4]	5.11	4.76	4.03	3.84	3.30	2.43
ME/AA[5]	0.63	0.61	0.64	0.62	0.63	0.62

[1] LNA = Linoleic acid to ALA = α-Linolenic acid ratio

[2] rations containing either 3000 or 10000 IU/kg of diet vitamin A

[3] Vitamin/mineral premix, provided per kilogram of diet: Cholecalciferol, 1,250 IU; Vitamin E (dl-alpha-tocopheryl acetate), 12 IU; menadione, 2.5mg; riboflavin, 6 mg; calcium pantothenate, 8 mg; niacin, 15 mg; pyridoxine 2 mg; folic acid, 1 mg; cinocobalamine, 7µg; Mn, 50 mg; Zn, 55 mg; Fe 40 mg; Cu, 4 mg; I, 2 mg; Co, 0.3 mg; ethoxiquin, 150 mg.

[4] Dietary crude protein to ether extract ratio

[5] Dietary ME to total amino acid ratio

 DOI: https://doi.org/ 10.30564/jzr.v1i1.112

nificant difference test. Response differences were considered significant when P≤0.05.

3. Results

The decrease in dietary LNA to ALA ratio linearly decreased (P<0.05) body weight gain in hens during the study period, however, vitamin A levels had no effect (P>0.05) on weight gain of hens (Table 2). Varying dietary LNA to ALA ratios or vitamin A levels did not affect daily feed intake, hen-day and hen-housed egg production, and FCR per dozen of eggs in laying hens. The feed conversion ratio per kg egg mass differ significantly (P<0.05) at various dietary LNA to ALA ratios, however, vitamin A supplementation was not effective (P>0.05) to produce any difference in FCR/kg egg mass. The difference for FCR/kg egg mass between the hens at diets with dietary LNA to ALA ratio of 10:1 and 1:2 was significant; however, hens in all other dietary LNA to ALA ratio groups did not differ with each other. As a whole, the best FCR/Dozen and FCR/kg egg mass was observed in the hens at dietary LNA/ALA ratio of 1:2.

The egg weight varied significantly (P<0.05) with varying LNA to ALA ratios in the layers' diet being the heaviest for LNA to ALA ratio of 1:2, and the lowest for dietary LNA to ALA ratio of 10:1 (Table 3). However, the egg weight did not changed (P>0.05) with the increasing levels of vitamin A. The egg mass, egg-yolk weight, yolk index, and Haugh unit score were similar (P>0.05) for all dietary treatments (Table 3). However, Egg-yolk weight as a percentage of egg weight differed significantly (P<0.05) between different dietary LNA to ALA ratios. The highest egg-yolk weight as a percentage of egg weight was observed in the group fed diets with LNA to ALA ratio of 4:1 and the lowest for those with dietary LNA to ALA ratio of 1:2.

Neither the egg-shell weight nor egg-shell breaking strength was affected (P>0.05) by varying dietary treatments, however, different dietary LNA to ALA ratios significantly (P<0.05) affected the egg-shell thickness (Table 4). Higher shell thickness was noted for the hens fed on diets with LNA to ALA ratio of 2:1 and 1:1, compared to

Table 2. Effect of varying LNA to ALA ratio and vitamin A levels on body weight gain, feed intake, egg production and feed conversion ratio in laying hens.

Dietary treatment	Feed intake (g/bird)	Weight gain (g/bird)	HDP (%)[1]	HHP (%)[2]	FCR [3] (kg/dozen)	FCR (kg/kg eggs)
LNA: ALA[4]						
20:1	99.05	96.67[a]	80.61	79.73	1.47	2.03[ab]
10:1	99.38	91.33[a]	80.09	78.61	1.49	2.18[a]
4:1	98.81	84.33[a]	80.08	77.40	1.48	2.15[ab]
2:1	98.13	80.50[ab]	78.58	77.98	1.50	2.10[ab]
1:1	98.70	66.50[b]	78.16	77.51	1.52	2.09[ab]
1:2	98.58	46.50[c]	81.50	81.50	1.45	1.96[b]
SEM	0.320	3.740	1.307	1.573	0.024	0.050
Vitamin A (IU/kg diet)						
3000	98.85	78.78	79.73	78.28	1.49	2.10
10,000	98.70	76.50	79.94	79.29	1.48	2.07
SEM	0.185	2.159	0.755	0.908	0.014	0.029
Probability						
LNA:ALA	0.160	0.000	0.483	0.434	0.526	0.044
Vitamin A	0.573	0.463	0.840	0.441	0.713	0.522
LNA:ALA×Vitamin A	0.942	0.718	0.704	0.828	0.718	0.839

[a-c] means within a column with different superscripts differ significantly (P<0.05).

[1] Hen-day Egg Production

[2] Hen-housed Egg Production

[3] Feed Conversion Ratio

[4] Linoleic acid to α-Linolenic acid ratio

DOI: https://doi.org/ 10.30564/jzr.v1i1.112

Table 3. Effect of varying LNA to ALA ratio and vitamin A levels on egg quality parameters in laying hens.

Dietary treatment	Egg weight (g)	Egg mass[1] (g)	Yolk weight (g)	Yolk weight (% of egg wt)	Yolk index	Haugh Unit Score
LNA: ALA[2]						
20:1	60.66[ab]	48.91	17.02	28.08[ab]	0.415	91.69
10:1	57.00[b]	45.66	16.28	27.97[ab]	0.410	92.47
4:1	57.45[ab]	45.99	16.97	30.51[a]	0.403	93.42
2:1	59.60[ab]	46.83	16.56	28.00[ab]	0.406	89.70
1:1	60.51[ab]	47.32	16.38	26.50[b]	0.407	90.77
1:2	61.83[a]	50.36	16.72	26.30[b]	0.408	91.94
SEM	1.079	1.135	0.428	0.874	0.008	1.853
Vitamin A (IU/kg diet)						
3000	59.24	47.23	16.61	27.67	0.408	92.13
10,000	59.77	47.79	16.69	28.12	0.401	90.53
SEM	0.623	0.655	0.247	0.505	0.005	1.070
Probability						
LNA:ALA	0.026	0.057	0.772	0.031	0.949	0.363
Vitamin A	0.555	0.548	0.820	0.532	0.987	0.300
LNA: ALA×Vitamin A	0.913	0.791	0.889	0.927	0.880	0.401

[a-b] means within a column with different superscripts differ significantly (P<0.05).

[1] Average egg mass = Per cent HDP × Average egg weight in grams

[2] Linoleic acid to α-Linolenic acid ratio

Table 4. Effect of varying LNA to ALA ratio and vitamin A levels on egg-shell quality parameters in laying hens

Dietary treatment	Shell weight (g)	Shell weight (% of egg wt.)	Shell strength (kg/cm^2)	Shell thickness (mm)
LNA: ALA[1]				
20:1	7.28	12.00	1.93	0.368[ab]
10:1	7.16	12.56	2.77	0.373[ab]
4:1	6.43	11.19	1.45	0.348[b]
2:1	7.08	11.88	2.45	0.392[a]
1:1	7.35	12.15	2.60	0.396[a]
1:2	7.28	11.77	3.00	0.371[ab]
SEM	0.254	0.450	0.534	0.009
Vitamin A (IU/kg diet)				
3000	7.21	12.17	2.44	0.369
10,000	6.99	11.69	2.29	0.380
SEM	0.147	0.337	0.308	0.005
Probability				
LNA:ALA	0.153	0.155	0.359	0.007
Vitamin A	0.295	0.256	0.743	0.109
LNA: ALA×Vitamin A	0.401	0.501	0.327	0.511

[a-b] means within a column with different superscripts differ significantly (P<0.01).

[1] Linoleic acid to α-Linolenic acid ratio

 DOI: https://doi.org/ 10.30564/jzr.v1i1.112

other groups. Increasing vitamin A levels did not affect (P>0.05) egg-shell thickness. No significant (P>0.05) interaction between LNA to ALA ratios and vitamin A was observed for all egg characteristics.

4. Discussion

4.1 Production Performance

In the present study, the body weight gain of layers during the experimental period was negatively affected by lowering LNA to ALA ratio in the diets (Figure 1). The ration with LNA to ALA ratio of 1:2 had more than 5 % linseed oil in it and the lowest body weight gain was observed in the laying hens on this ration. Linseed oil is rich in ALA, and it was reported earlier that ALA had a negative effect on body weight gain in birds by enhancing Interleukin-1 and TNF release [17], as these factors reduce the growth of birds [18]. Increased amount of ALA in the ration might have caused the reduction in body weight gain in laying hens in the current study. The present results confirmed the previous findings depicting a decrease in body weight gain of laying hens fed on diets containing higher PUFA contents or lower LNA to ALA [19-20]. On the other hand, few studies showed no effect of feeding different LNA to ALA ratios on body weight gain in laying hens [21-22]. In the present study, higher level of vitamin A showed no influence on the body weight gain in layers which is similar to the previous findings [15,23].

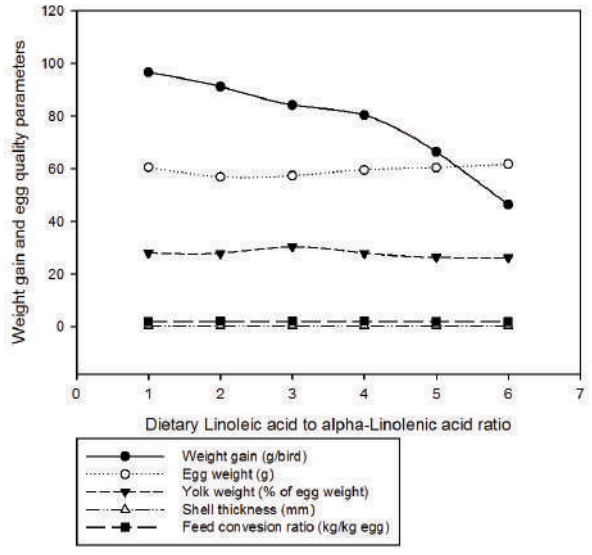

Figure 1. Graphical representation of weight gain, feed conversion ratio (kg per kg egg) and egg quality parameters. Number 1 to 6 on x-axis corresponds to dietary ratios of LNA to ALA of 20:1, 10:1, 4:1, 2:1, 1:1 and 1:2, respectively.

Since feed intake was similar for all dietary treatments, it may, therefore, be suggested that lower LNA to ALA ratios in the diet had no detrimental effects on feed intake in the laying hens. Feed intake data for hens on diets with various LNA to ALA ratios did not show any palatability problems, which is in agreement with the results of previous studies[24-25]. Similarly, no differences in the hen-day and hen-housed egg production were recorded for the laying hens at diets with decreasing LNA to ALA ratios or increasing vitamin A levels. Since, all the rations were iso-nitrogenous and iso-caloric, and the feed intake was similar in all treatment groups, this might explain the similar egg production for all treatment groups. Further, since the laying hens were reared into a similar environment but with comparatively cooler temperatures during early age than were recorded during the experimental period, they might have shown some biological adaptation to stress resulting from high temperatures. The present results proved that the combination of canola and linseed oil to attain desirable LNA to ALA ratios in the layer diets had no harmful effects on laying performance of hens. The results presented here confirmed the findings of previous studies showing no production problems when the hens were fed on different dietary sources of n-3 PUFA or various LNA to ALA ratios[24,26-29]. However, few researchers found an increase in egg production in hens with higher n-3 PUFA levels in the diet [19,30]. As the feed intake and the egg production were not affected by dietary treatments, the FCR per dozen of eggs was also unchanged, which supplements the previous research[21-22]. However, the FCR per kg egg mass differed significantly for groups on various LNA to ALA ratios in the diets, probably, due to the fact that the egg weight differed significantly among these groups.

Although all diets for this study were iso-nitrogen and iso-caloric, but the ether extract concentration increased, while crude protein to ether extract ratio decreased with decreasing dietary LNA to ALA ratio. Owing to the lower heat increment of lipid compared to either carbohydrate or protein, one might expect extra benefit of lipid inclusion in the diet to moderate heat stress in avian. However, when chickens were reared in a warm environment, the body weight response to increased dietary energy level occurred only when adequate amino acid levels were supplied[31]. Increasing dietary ME at particular amino acid to ME ratios significantly improves the growth and feed utilization of chickens kept at 18-26 and 25-35 ℃ ambient temperatures during the finishing period. The optimum amino acid to ME ratio varies with the dietary ME concentration in a hot, but not in a moderate environment. Relatively greater increases in feed intake and growth rate occur in a hot environment when dietary ME increases and the amino acid to ME ratio decreases. Increasing the

DOI: https://doi.org/ 10.30564/jzr.v1i1.112

dietary protein at particular ME concentrations had little or no effect on the feed intake and growth rate of birds kept at high temperatures [32]. The dietary amino acid to ME ratio were not altered for various dietary combinations in the present study, therefore, any effects of lipid supplementation to moderate heat stress can be excluded.

4.2 Egg Characteristics

The egg weight for different treatment groups showed a significant difference; it dropped when LNA to ALA ratio dropped from 20:1 to 10: 1 and then rose again with decreasing dietary LNA to ALA ratios (Figure 1). The group at LNA to ALA ratio of 1:2 produced heaviest eggs among all treatment groups; this increase in egg weight seemed to be an occasional observation. It is also important to describe that all LNA to ALA ratios were non-significant with each other from ratio of 20:1 to 1:1 in the diets. Apparently, egg weight tended to be almost similar for all dietary LNA to ALA ratios and for both vitamin A levels. Egg mass produced by the hens at various dietary LNA to ALA ratios and vitamin A levels was not different. From these results, it might be possible to suggest that the decrease in LNA to ALA ratio in the diet did not have any pronounced effect on egg mass in laying hens.

Internal egg quality parameters like yolk weight, yolk index and Haugh unit score were not affected by dietary manipulation. However, egg-yolk weight as percentage of egg weight was decreased significantly for dietary LNA to ALA ratios of 1:1 and 1:2. Yolk weight % showed a decreasing trend in the hens with the decrease in LNA to ALA ratio in the feed except for the hens at dietary LNA to ALA ratio 4:1 which showed highest yolk weight percentage. The decrease in yolk weight in response to n-3 PUFA diets might be related to the influence of n-3 PUFA on hepatic lipid metabolism and particularly to the decrease in the level of circulating lipids of birds which are the source of yolk lipids[33]. Van Elswyk[34] unrevealed that feeding linseed to laying hens decreased yolk weight and proposed that yolk weight reduction is related to changes in circulating estradiol brought about by either n-3 PUFA. Vitamin A did not influence the yolk weight % of hens in present study. The Present results are in agreement with findings of[35-37] but, in disagreement with few previous reports showing no effect of dietary LNA to ALA ratios or higher PUFA contents on internal egg quality[38-39].

The response of laying hens to various dietary treatments for egg shell quality parameters like egg-shell weight and egg-shell breaking strength was non-significant. But, the egg-shell thickness differed significantly between various LNA to ALA ratios with no effect of vitamin A on it. Although the egg-shell thickness differed significantly at different LNA to ALA ratios, no clear trend for increase or decrease was found. From these results it could be hypothesized that higher levels of PUFA or lower LNA to ALA ratios, and vitamin A in the diets had no relation with egg-shell quality, similar were the findings of previous studies [38,40].

Richter et al.[41]in five experiments with a large number of laying hens from 21st to 72nd week of age found lower feed intake, egg production, egg weight and feed efficiency per 100 g egg weight without vitamin A supplementation. Their studies included evaluation of vitamin A supplementation ranging from 0–10000 IU per kg mixed feed and concluded that 2500 IU vitamin A per kg feed are required for optimal egg production, that 4000 IU vitamin A are required for optimal egg production including a body reserve, and the supplementation of 6000 IU per kg mixed feed are recommended considering a safety disk.

5. Conclusion

From the results of the present study, it is evident that feeding high PUFA with lower LNA/ALA ratio in the diet of laying hens can result into PUFA-enriched eggs, with negligible negative effects on few of the production performance and egg quality traits – mainly body weight gain and egg-yolk percentage. Since no clear trends were observed for various production and egg quality traits with varying LNA/ALA ratios, a 4:1 or 2:1 combination can make a borderline between the production traits and the feed economics. It is further suggested that higher amount of vitamin A in the diet has off-set lipid peroxidation, if any, induced by heat stress and higher dietary PUFA contents.

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  DOI: https://doi.org/ 10.30564/jzr.v1i1.112

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 DOI: https://doi.org/ 10.30564/jzr.v1i1.112

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Journal of Zoological Research
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ARTICLE

Dry matter degradation kinetics of selected tropical forage in Nili-Ravi buffalo and Cholistani cows at heifer and lactating stages using NorFor in situ standards

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ARTICLE INFO

Article history:
Received: 17 October 2018
Accpetaed: 21 November 2018
Published: 11 March 2019

Keywords:
Animal species
Cattle feed
Dry matter degradability
Developmental stage
Tropical forage

ABSTRACT

Current methods of ruminant ration formulation in Pakistan use foreign-based nutrient availability values. These values may not be optimal for all geographic areas, as variation in environment, agronomic factors, animal species, and diet characteristics may not be considered. The aim of present study was to establish a database of the chemical composition and dry matter degradation parameters of tropical forage commonly fed to ruminants in Pakistan and South Asian countries using Nili-Ravi buffalo and Cholistani cattle at heifer and lactating stages. Six cereal grain and four legume species were grown in 3 locations under standard agronomic conditions and sampled at booting and at 50% flowering stage for cereal and legumes, respectively. Dried and milled feeds were analyzed for chemical composition and in situ dry matter degradation parameters using 1 g samples in bags placed in the rumen of 2 Nili-Ravi buffalo heifers, 2 lactating Nili-Ravi buffaloes, 2 Cholistani heifers, and 2 lactating Cholistani cows. The forage family (cereal vs. legumes), species, and geographic location of growth significantly influenced ($P < 0.001$) chemical composition and in situ degradation fractions. Animal species and developmental stage showed no effect on degradation fractions ($P > 0.05$). Legume-by-heifer interactions significantly increased ($P < 0.05$), and legume-by-lactating cow interaction tended ($P = 0.065$), to increase the rate of degradation (Kd). The selected forages were degraded to a similar extent independent of animal species or developmental stage, and legumes are degraded at higher rates and to a greater extent than are cereals. A moderately significant relationship between Kd and effective dry matter degradability (DMD) suggests that Kd could be the single most important predictor of forage degradability in the rumen.

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DOI: https://doi.org/10.30564/jzr.v1i1.151

1. Introduction

The rate and extent of dry matter (DM) degradation in the rumen is a major determinant of energy and nutrient supply to ruminants from fiber-rich forage. This information forms the basis for ration formulation and for the prediction of metabolizable nutrient and energy intake in feed evaluation systems such as that of the National Research Council[1], the Cornell Net Carbohydrate and Protein System[2], and the Nordic Feed Evaluation System[3]. The pattern of rumen degradation has been reported to affect rumen function and fiber digestion, microbial protein and milk fat synthesis, and overall animal performance and health[4-5].

Current ration formulation methods in Pakistan[6] are based on nutrient availability values reported in feed evaluation systems developed for feeds grown in temperate conditions and fed to animals common to those areas. These values may not be optimal in other geographic locations, as variations in environment, agronomy, animal species, and diet characteristics are not considered. As a consequence, animals may be under- or over-fed, resulting in lower feed efficiency and economic losses. Accurate estimates of the coefficients of nutrient degradation of locally produced feeds in the rumen are required. The in situ technique is widely used to study the fractional rate of ruminal degradation of feed DM and nutrients[7]. Despite some limitations, it utilizes the ruminal environment[8] and is considered to produce a more reliable measure of rumen degradation than do in vitro techniques.

In situ degradation of forage is primarily influenced by plant genotype, agronomic conditions, climate, and post-harvest processing[9]. Various animal-related factors that affect the in situ degradation of forage have been reported including intake level, forage to concentrate ratio, nutrient composition and degradation rate of the concentrate feeds, feeding frequency[10], and animal species[11] and developmental stage[12]. Internationally, substantial research has been conducted to quantitatively evaluate the effects of these factors[13-15], but information is lacking for tropical areas such as Pakistan.

The objectives of the present study were to: 1) evaluate chemical composition of commonly used forage plants; 2) assess the effects on DM degradability of forage family, geographic location of growth, and animal species and developmental stage; and 3) determine the relationship between rate and extent of degradation in situ.

2. Materials and Methods

The animals were kept at the Livestock Farm of Islamia University of Bahawalpur (IUB) and maintained according to the criteria of Animal Care and Management Committee (The IUB Bioethics and Animal Use Committee, 2015).

2.1 Forage Sampling

Ten forage species, comprising 6 cereals and 4 legumes, were evaluated (Table 2). Summer (*maize Zea maize, millet Pennisetum glaucum, sorghum sorghum bicolor, lucerne Medicago sativa and jantar sesbania bispinosa*) and winter (*barley Hordeum vulgare, oats Avena sativa, wheat Triticum aestivum, berseem Trifolium alexandrinum, and mustard brassica napus*) crops were used for the analyses. Summer crops were sown on the same date in mid March 2015 and winter in late November and grown under uniform recommended agronomic conditions. Each species was sown in 3 plots separated by ~100 m apart with the trial replicated in Rawalpindi (33.598° N, 73.04° E), Lahore (31.55° N, 74.35° E), and Bahawalpur (29.39° N, 71.68° E) representing northern, central, and south-ern regions of Punjab Province of Pakistan, respectively. Herbage samples (~10 kg each) from locations within an area were harvested on the same date, when the cereals were at booting and legumes were at 50% flowering stage, chopped with a chaff cutter (Toka 510, Patiala Agri-Industries, Faisalabad, Pakistan) to a nominal length of 20 mm, and spread under shade to reduce moisture content within the recommended range for drying of 3 to 7 days. The dried samples were transported to the Livestock Production and Management section at the IUB, forced through a 2-mm screen using a hammer mill (POLYMIX PX-MFC, Kinematica AG, Germany) and stored in small plastic jars at room temperature for in situ experiments. The samples for chemical analyses were ground through a 1 mm screen.

2.2 Maintenance of Cannulated Animals

Eight rumen-cannulated (Bar Diamond, Parma, ID, USA) animals including 2 lactating Nili-Ravi buffaloes, mean live weight (LW) = 509 ± 43.4 kg, milk yield = 5.63 ± 0.207 kg/day, age = 2225 ± 49.5 days; 2 Nili-Ravi heifers LW = 531 ± 48.8 kg, age = 1913 ± 123.7 days; 2 lactating Cholistani cows, LW = 289 ± 29.4 kg, milk yield = 3.34 ± 0.271 kg/day, age = 1115 ± 21.9 days; and 2 Cholistani heifers LW = 312 ± 35.4 kg, age = 867 ± 64.3 days were used for the in situ incubations. The animals were offered a standard diet at maintenance level as per NorFor standards for cannulated-animals throughout the experiment [3]. Ingredients and mean chemical composition of the diets are presented in Table 1. The animals were confined to individual stalls, individually fed and given access to fresh clean water as per requirements.

 DOI: https://doi.org/10.30564/jzr.v1i1.151

Tables and figures

Table 1. Ingredients and mean chemical composition of the diets offered to rumen-cannulated animals (g/kg DM unless otherwise stated).

Item	Lactating cows	Heifers
No. of samples = 5, no. of statistical replicates = 2, Total no. of observations per feed = 10		
Ingredients (as fed basis)		
Sorghum	844	771
Lucerne hay	88	195
Cotton seed cake	30	0
Concentrate mixture	37	34
Forage to concentrate ratio (DM)	80:20	90:10
Chemical composition (DM)		
DM	302	360
CP	58	60
EE	18	17
NDF	583	567
NFC	227	241
Ash	113	113

DM = dry matter; CP = crude protein; EE = ether extract; NDF = amylase-treated neutral detergent fiber; NFC = non-fiber carbohydrates

Table 2. Effect of forage family, species, and geographic location of growth on chemical composition of Cereal and legumes fodder sown in 3 locations in Punjab Province. The values are presented as least square means (g/kg DM) with Standard error of mean (SEM) unless otherwise stated .

Items[1]		DM	CP	EE	NDF	Ash	NFC
No. of samples = 3, no. of statistical replicates = 2, Total no. of observations per feed = 6							
Cereal fodders							
Barley		954.1 ^{a}	53.5 ^{c}	16.3 ^{b}	614.3 ^{a}	127.2 ^{b}	189.3 ^{cd}
Oat		946.0 ^{b}	62.3 ^{b}	21.4 ^{a}	549.4 ^{c}	110.2 ^{bc}	257.2 ^{a}
Wheat		954.2 ^{a}	74.5 ^{a}	15.4 ^{b}	532.4 ^{d}	149.3 ^{a}	228.3 ^{b}
Maize		945.1 ^{b}	65.7 ^{b}	10.3 ^{c}	605.2 ^{a}	94.0 ^{d}	225.3 ^{b}
Millet		939.3 ^{c}	66.6 ^{b}	15.3 ^{b}	606.2 ^{a}	102.5 ^{c}	209.4 ^{c}
Sorghum		935.1 ^{d}	50.6 ^{c}	17.0 ^{ab}	584.3 ^{b}	88.3 ^{d}	260.4 ^{a}
Legume fodders							
Barseem		948.2 ^{b}	133.0 ^{a}	18.8 ^{ab}	405.4 ^{c}	183.6 ^{a}	260.2 ^{b}
Lucerne		940.3 ^{c}	135.5 ^{a}	18.2 ^{ab}	410.3 ^{c}	120.3 ^{b}	316.3 ^{ab}
Mustard		956.0 ^{a}	110.3 ^{c}	23.2 ^{a}	498.2 ^{a}	123.9 ^{b}	245.1 ^{b}
Jantar		943.1 ^{c}	119.6 ^{b}	20.8 ^{ab}	448.2 ^{b}	74.3 ^{c}	337.3 ^{a}
SEM		4.93	5.37	2.02	17.20	8.03	19.90
Family	Cereals	945.1	62.2	15.9	581.8	111.9	228.2
	Legumes	946.2	113.9	18.3	481.9	120.5	265.3
Location	Bahawalpur	934.6	109.6	19.1	482.0	116.2	273.1
	Lahore	951.8	78.0	15.8	583.8	116.3	206.1
	Rawalpindi	951.1	76.6	16.4	529.8	116.1	261.0
Significance							
	Forage specie	0.044	<0.001	0.016	<0.001	<0.001	<0.001
	Family	0.514	<0.001	0.047	<0.001	0.007	0.023
	Location	<0.001	<0.001	0.168	<0.001	0.287	<0.001
Interactions[2]	T × L	0.467	<0.001	0.904	0.032	0.016	0.594

[1] For abbreviations see Table 1

[2] Effect of main factor interactions (Family × Location).

Different lower-case superscripts in a column indicate significant difference ($P < 0.05$).

DOI: https://doi.org/ 10.30564/jzr.v1i1.151

2.3 In Situ Incubations and Degradation Profiles

The incubations continued from June to October, 2016. The assessment of DM degradation of fodder samples was conducted according to NorFor standards[3,16]. In brief, air dried and milled (2 mm screen; POLYMIX PX-MFC, Kinematica AG, Germany) fodder samples (1 sample per incubation period and animal) were incubated in the rumen of each rumen-cannulated animal in a sewn and glued polyester (Dacron) bag measuring 11 × 8.5 cm (10 × 7.5 effective size), pore size 33 µm (PES material 140/37) with 25% open bag area (Sefar AG, Hinterbissaustrasse 12, 9410 Heiden, Switzerland). Samples of approximately 1 g, allowing 15 mg of sample per cm^2 of bag surface area, were incubated for 0, 4, 8, 16, 24, or 48 h. All bags were placed in the rumen at the same time and removed according to specified duration of incubation (all-in system). At the conclusion of each incubation period, the bags were removed, washed with tap water, and stored at -18 °C. After all bags from all incubation periods had been retrieved, they were thawed and washed in a washing machine twice for 12 min each with tap water at 25 °C. The residues in the bags were dried at 100 °C for 24 h to determine DM loss.

2.4 Chemical Analyses

Fresh forage and dry feed including commercial concentrate, lucerne hay and cotton seed cake fed to animals were sampled fortnightly throughout the experiment. The DM content of fresh chopped forage was determined at 60 °C for 48 h and that of dry feeds at 105 °C for 16 h [Association of Official Analytical Chemists (AOAC),[17]; method 7.003]. Ash was analyzed by incinerating the samples at 525 °C for 6 h ([17]; method 923.03), crude protein (CP) (6.25 × N) by Kjeldahl method ([17]; method 7.015), and ether extract (EE) by 6-h extraction with petroleum ether ([17]; method 7.062). The amylase-treated neutral detergent fiber (NDF) was determined using the method of Van Soest et al.[18] as modified by Mertens et al.[19] with the addition of sodium sulfite and heat-stable alpha-amylase (CAS No. 9000-90-2, Junsei Chemicals, Japan). The non-fiber carbohydrates were calculated as [NFC (g/kg DM) = 1,000 – (CP + EE + NDF + ash)].

2.5 Data Analysis and Curve Fitting

The in situ degradation data was categorized as particle loss or washable fraction (*a*, 0 h values for washed samples) and non-washable fraction. The non-washable fraction was sub-divided into the potentially degradable (*b*) and the indigestible fraction, represented as the degradation and residue at the final incubation interval, respectively as described by Ørskov and McDonald [7]. The in situ degradation data were fitted to a first-order kinetic model (Equation 1) assuming the steady state degradation and passage conditions

$$Y_t = a + b(1\text{-}exp(\text{-}K_d t)) \quad \text{Equation 1.}$$

The model was fitted using Table Curve 2D (ver. 5.0, SPSS Inc. NY). Y_t denotes the degraded fraction at a given time t, and K_d denotes the fractional degradation rate of fraction b. Effective ruminal DM degradability (DMD) was calculated according to Ørskov and McDonald[7] as

$$DMD = a + b \times K_d/(K_d + K_p) \quad \text{Equation 2}$$

assuming the fractional rate of passage (K_p) to be 0.05/h for forage (a 20-h rumen retention time) as used in several protein evaluation systems[20]. A second-order (DMD1) was calculated from the in situ data according to a 2-compartment model (Equation 3) as suggested by Allen and Mertens [21]

$$\text{DMD1} = = a + [(b \times K_d)/(K_d + K_p) \times (1 + K_p)/(K_d + K_r)] \quad \text{Equation 3}$$

where K_p = [1/(0.6 × 20) = 0.083/h], and K_r is the fractional rate of release from the non-escapable fraction to the escapable fraction [1/(0.4 × 20) = 0.125/h was used]. This implied a total rumen residence time of 20 h for forage distributed between the 2 compartments at a ratio of 40:60.

3. Statistical Analyses

The statistical analyses were performed using the GLM procedure of Minitab 16.1.1.0. The data on chemical composition of rations were analyzed using the model (Equation 4)

$$Y_{ijkl} = \mu + F(T)_i + T_j + L_k + E_{ijk}. \quad \text{Equation 4}$$

The data on in situ parameters were analyzed considering each buffalo and cow/heifer an experimental replicate using the model (Equation 5)

$Y_{ijklmn} = \mu + F(T)_i + T_j + L_k + A_l + P_m + E_{ijklm}$,(Equation 5) in which Y_{ijklmn} is the dependent variable, μ is the overall mean, $F(T)_i$ shows the effect of ith forage species nested under forage family, T_j shows the effect of the jth family of forage (cereal vs. legume), L_k shows the effect of *kth* location, A_l shows the effect of *lth* animal species, P_m shows the effect of *mth* developmental stage of the animal, and E_{ijklm} is the residual error. Results were considered significant when $P \leq 0.05$ and are presented as least square means with standard error of the means. The pairwise comparisons were made using Tukey's test.

4. Results

4.1 Chemical Composition

Table 2 shows the chemical composition of forage by family, species, and growing location of collected samples. The CP ranged from 50.6 (sorghum) to 74.5 g/kg

 DOI: https://doi.org/ 10.30564/jzr.v1i1.151

DM (wheat) for cereals; and from 110.3 (mustard) to 135.5 g/kg DM (lucerne) for legumes, and was significantly influenced ($P < 0.001$) by family, species, location, and family by location interaction. The NDF ranged from 532.0 (wheat) to 614 g/kg DM (barley) for cereals, and from 405.0 (berseem) to 498.0 g/kg DM (mustard) for legumes and was significantly influenced ($P < 0.001$) by family, species, and location. The ash and NFC averaged 112.0 and 228.0 g/kg DM, respectively, for cereals and 126.0 and 289.0 g/kg DM, respectively, for legumes, and varied significantly ($P < 0.05$) by family and species.

4.2 Degradation Parameter Estimates and Effective DMD as Influenced by Forage Family,Species, and Growing Location

Table 3 shows the DMD parameters of the forages. The forage family, species, and location significantly influenced ($P < 0.001$) all degradation fractions. The a-fraction ranged from 0.26 (maize) to 0.34 (wheat) in cereals and from 0.28 (jantar) to 0.46 (lucerne) in legumes. The b-fraction ranged from 0.50 (wheat and millet) to 0.59 (oats) in cereals and from 0.31 (mustard) to 0.44 (jantar) in legumes (mean 0.36). The Kd ranged from 0.05 to 0.06/h for cereals and from 0.09 to 0.12/h for legumes. The DMD varied from 0.53 (millet) to 0.61 (oats) for cereals and from 0.56 (mustard) to 0.68 (lucerne) for legumes. The DMD1 varied from 0.79 (millet) to 0.93 (oats) for cereals and from 0.70 (mustard) to 0.82 (lucerne) for legumes. The forage species ranked in order of decreasing DMD and DMD1 are oats > wheat > barley > maize > sorghum > millet (cereals) and lucerne > berseem > jantar > mustard (legumes).

4.3 Degradation Parameter Estimates and Effective DMD as Influenced by Animal Species and Developmental Stage

The Kd was significantly influenced ($P < 0.05$) by animal species, but other fractions were not ($P > 0.05$). The DMD and DMD1 did not differ ($P > 0.05$) between heifers and lactating animals although fraction b and Kd differed significantly ($P < 0.05$). Legume-by-heifer interactions significantly increased ($P < 0.05$) the value of Kd, whereas legume-by-lactating cow interaction tended ($P = 0.065$) to increase the value of Kd however, the interaction effects for other analyzed parameters remained non-significant ($P > 0.05$).

4.4 Relationship of DMD to Degradation Parameters

Figures 1 and 2 show relationships of K_d with DMD and DMD1 respectively. We found a moderate ($R^2 = 0.43$) but significant ($P < 0.001$) positive relationship in cereals, and a low ($R^2 = 0.02$) but significant ($P < 0.001$) positive relationship in legumes, between DMD and K_d (Fig. 1). Figure 2 shows a borderline ($R^2 = 0.05$) significant ($P < 0.01$) relationship between DMD1 and K_d in cereals but no correlation in legumes. The relationship of DMD to *a* and *b* fractions was also investigated and showed a low and no correlation, respectively (data not shown).

5. Discussion

5.1 Chemical Composition

The CP values for cereals agree with Sarwar et al.[22], whereas those for legumes were lower. Sarwar et al.[22] may have harvested leguminous crops at more advanced stages of growth. For all forages, the content of EE was less than 30 g/kg DM, which is typical of forage plant material. With the exception of CP, the nutrient composition of the analyzed forage plants fell within the range of typical ruminant diets[1] (Table 2).

5.2 Degradation Parameter Estimates and Effective DMD as Influenced by Forage Family, Species, and Growing Location

Our observation that the legumes were degraded more rapidly and to a greater extent than were cereals agree with results of Sarwar et al. [22] in cannulated Nili-Ravi buffalo calves. The values for K_d and DMD reported in our study were comparable to those of Sarwar et al.[22] for sub-tropical cereal and legume forage plants, although differences from Sarwar et al.[22] in CP values were found. The K_d and DMD values for legumes were also in agreement with findings of Aufrère et al.[23] for temperate lucerne.

5.3 Degradation Parameter Estimates and Effective DMD as Influenced by Animal Species and Developmental Stage

A review of literature data reporting rumen degrad ability and K_d in situ in species such as Bubalus bubalis and Bos taurus showed varying results that were not consistent with respect to either feedstuffs or animal species. Sarwar et al.[11] compared digestibility characteristics of cattle and buffalo for various forage plants and agro-industrial by-products using the in situ nylon bag technique. They reported greater digestibility and K_d for DM and NDF of grasses in rumen-cannulated buffaloes than in cattle during 48 h incubation. For leguminous forage and agro-industrial by-products, no differences in extent and rate of DM and NDF degradation with respect to animal species were reported. Similarly, Bhatia at el.[24] reported higher DM and NDF in situ digestion rates of berseem hay in buffaloes compared to those in cattle; however, overall effective degradability values did not differ with

DOI: https://doi.org/ 10.30564/jzr.v1i1.151

Table 3. Effect of plant and animal factors on in situ dry matter degradation kinetics and effective degradability of cereal and legume fodder sown at 3 locations in Punjab Province. The values are presented as least square means (g/kg DM) with standard error of mean (SEM) unless otherwise stated.

In situ[1] items		*a*	*b*	K_d	DMD[2]	DMD1[3]
No. of samples = 3, no. of statistical replicates = 8, Total no. of observations per feed = 24						
Cereal fodders						
Barley		0.30 ^{ab}	0.54 ^{b}	0.05	0.57 ^{ab}	0.86 ^{b}
Oat		0.31 ^{ab}	0.59 ^{a}	0.06	0.61 ^{a}	0.93 ^{a}
Wheat		0.34 ^{a}	0.50 ^{c}	0.06	0.61 ^{a}	0.87 ^{b}
Maize		0.26 ^{b}	0.54 ^{b}	0.06	0.55 ^{b}	0.84 ^{b}
Millet		0.27 ^{b}	0.50 ^{c}	0.05	0.53 ^{b}	0.79 ^{c}
Sorghum		0.26 ^{b}	0.52 ^{bc}	0.06	0.54 ^{b}	0.81 ^{c}
Legume fodders						
Barseem		0.42 ^{b}	0.36 ^{b}	0.12	0.67 ^{a}	0.81 ^{a}
Lucerne		0.46 ^{a}	0.34 ^{b}	0.12	0.68 ^{a}	0.82 ^{a}
Mustard		0.36 ^{c}	0.31 ^{c}	0.09	0.56 ^{b}	0.70 ^{b}
Jantar		0.28 ^{d}	0.44 ^{a}	0.10	0.56 ^{b}	0.75 ^{ab}
SEM		0.008	0.014	0.007	0.008	0.011
Family	Cereals	0.29	0.53	0.06	0.57	0.85
	Legumes	0.38	0.37	0.11	0.62	0.77
Location	Bahawalpur	0.36	0.48	0.09	0.66	0.87
	Lahore	0.29	0.47	0.07	0.55	0.78
	Rawalpindi	0.37	0.40	0.08	0.58	0.79
Animal species	Bubalus bubalis	0.34	0.45	0.08	0.59	0.82
	Bos taurus	0.34	0.44	0.09	0.60	0.81
Developmental stage	Heifers	0.34	0.44	0.09	0.59	0.80
	Lactating animals	0.33	0.46	0.08	0.60	0.82
Significance	Forage species	<0.001	<0.001	0.042	<0.001	<0.001
	Family	<0.001	<0.001	<0.001	<0.001	<0.001
	Location	<0.001	<0.001	0.001	<0.001	<0.001
Interactions[4]	Animal Species	0.562	0.153	0.021	0.337	0.199
	Developmental stage	0.231	0.020	0.040	0.378	0.131
	T × L	0.001	<0.001	0.008	<0.001	<0.001
	T × A	0.902	0.699	0.065	0.973	0.568
	T × P	0.989	0.786	0.011	0.986	0.754
	A × P	0.315	0.134	0.037	0.222	0.296

a = washable fraction representing the portion of dry matter (DM) that had disappeared at time 0; b = potentially degradable DM fraction. The estimate of Kd from the in situ method represents the fractional rate of degradation of fraction b; DMD = dry matter degradability.

[1] Degradation parameters described according to the model by Ørskov and McDonald [7]

[2] Effective DMD calculated from data assuming the fractional rate of passage (K_p) to be 0.05/h for forage as used by protein evaluation system of Hvelplund and Weisbjerg [20]

[3] Effective DMD1 calculated according to a 2-compartment model as suggested by Allen and Mertens (1988).

[4] Effect of main factor interactions (Family × Location), (Family × Animal species), (Family × Developmental stage) and (Animal species × Developmental stage).

Different lower-case superscripts in a column indicate significant difference ($P < 0.05$).

 DOI: https://doi.org/ 10.30564/jzr.v1i1.151

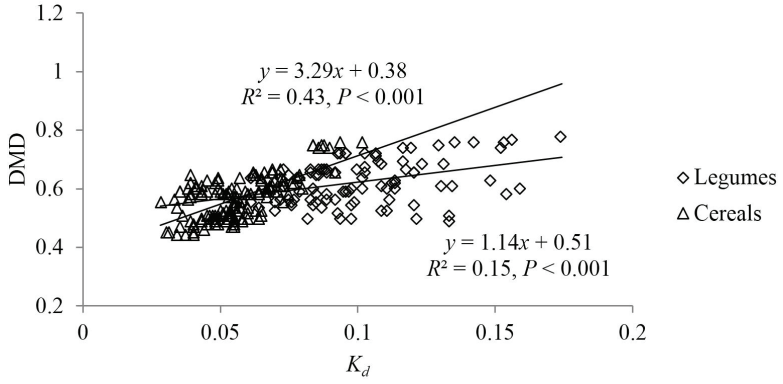

Fig. 1. Relationship between dry matter degradability (DMD) and rate of degradation (Kd/h) for cereal and legume fodders.

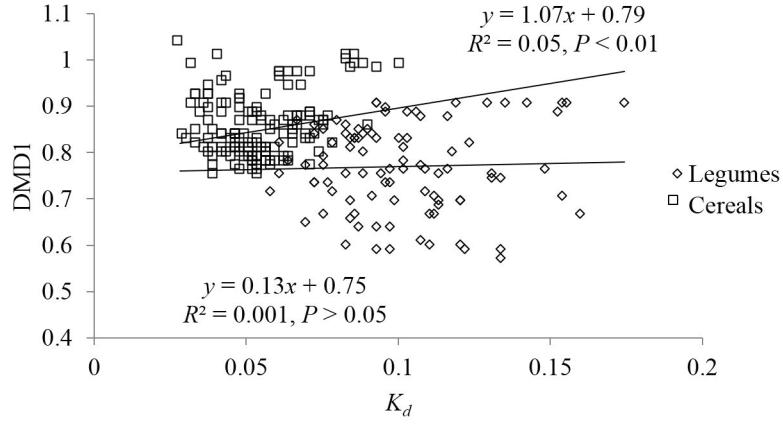

Fig. 2. Relationship between dry matter degradability (DMD1) and rate of degradation (Kd/h) for cereal and legume fodders.

animal species. Franzolin and Dehority [25] reported no differences with respect to rumen DM or NDF degradation in cannulated riverine buffaloes vs. cows feeding on tropical forage grasses, however, K_d values were higher in buffaloes than in cows.

Nandra et al.[26] reported that parameters of DM degradation for forage and concentrate feeds showed no differences between sheep and cattle with no significant species effect and no interaction of species with either feed or experimental period. They further suggested that a single curve for each test feed in both sheep and cattle may be used to represent DM degradation in the rumen. The results of the present study are also in accordance with the findings of Huntington and Givens[27] who observed no differences between host species on in situ DM degradation of hay, soybean- and fish-meal. Uden and Van Soest [28] also found that mature ruminant species degrade the fiber fraction of feeds similarly.

Lactating animal energy and protein requirements are different from those of heifers[1]. We hypothesized that the lactating animals would better utilize the available feed resources, based on their requirements for milk and their developed capacity to extract nutrients from within the rumen digesta[29]. The present study did not support the hypothesis, but suggest that the feedstuffs studied are equally nutritionally important for different ruminant species at different developmental stages. The energy and protein requirements of the studied species and developmental stages might be a reflection of body mass. Further data of feed consumption and production parameters can be combined with the in situ degradation data to assess feed efficiency.

5.4 Relationship Between DMD and In situ Parameters

Our data and that of other in situ studies[9] show that K_d is the single most important parameter describing ruminal degradability of tropical forages. Many feed evaluation systems using K_d values in predicting the feed intake and nutrient utilization in dairy cows are inaccurate when legumes form a substantial portion of the forage fraction[1-2]. This reflects atypical degradation in the rumen. Although they are characterized by larger quantities of soluble and rapidly degradable protein[2] and greater K_d[3], legumes

DOI: https://doi.org/ 10.30564/jzr.v1i1.151

present a lower extent of degradation compared to grasses due to the greater K_p[30].

5.5 Comparison of In situ DMD and In vivo Data

All methods of nutritive evaluation in ruminants attempt to mimic in vivo methods, because they are reliable and preferred for a range of ingredients and nutrients. Despite limitations described by several researchers[31-32], in situ is considered a more accurate and reliable method for quantifying rumen degradation parameters than other techniques[33-34]. It is not only a powerful tool for ranking the relative degradation of feedstuffs, but may also be used to increase understanding of the processes of rumen fermentation, although the in situ method has rarely been validated in vivo. Madsen and Hvelplund[35] observed a close relationship between in vivo and in situ measurements of protein degradation in concentrate feedstuffs. However, Offner and Sauvant [36] presented a high slope bias for predicting starch digestion in the rumen from in situ effective degradability data of Offner et al.[37]. The in situ method tends to overestimate starch degradation rates, and, consequently, effective degradation values for rapidly-degraded feedstuffs, and to underestimate the rate of starch degradation in feedstuffs that are degraded slowly[36,38].

Vanzant et al. [39], Gosselink et al. [33] and Di Marco et al. [40] did not observe significant difference between in vivo and in situ degradability of forage, although their in vivo data contained large standard errors and mean prediction errors. Adesogan et al.[41] and Di Marco et al.[42] found a poor relationship and large prediction error for DM degradation between in vivo and in situ methods for whole plant wheat and maize silage, and sweet sorghum[40] but their in vivo values determined in sheep are similar to our DMD findings. Comparing in vivo and alternative techniques, Damiran et al.[43] found greater values of forage DMD and lower of NDF degradation with the in situ technique in wether sheep and steers compared to in vivo data.

6. Conclusions

Availability of nutrient components such as CP and NFC in tropical forage were highly influenced by forage family and species along with geographic location of growth. No differences were found between buffaloes and cattle or their developmental stages, suggesting that these feedstuffs can be equally efficiently utilized by various species of large ruminants at different life stages. A moderately strong relationship between K_d and DMD suggests that the K_d may be the single most important predictor of rumen degradation of forage plants. The in situ technique, with all its limitations, more closely mirrors in vivo measurements than do other common techniques.

Acknowledgements: The authors thank the Higher Education Commission (HEC) of Pakistan for their financial support for this research.

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 DOI: https://doi.org/ 10.30564/jzr.v1i1.151

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REVIEW

Progress in Single Cell Sequencing Technology

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ARTICLE INFO

Article history
Received: 1 April 2019
Accepted: 1 April 2019
Published: 30 April 2019

Keywords:
Single cell isolation
Single cell sequencing
Whole genome
Transcriptome

ABSTRACT

Cells are the basic unit of life structure and life activities. Because of the complex micro-environment of cells, the content of components that play a key role is relatively small, so single-cell analysis is extremely challenging. In recent years, single-cell sequencing technology has been developed and matured. Single-cell sequencing can reveal the composition and physiological diversity of cells, and the existing single-cell separation technology, single-cell whole genome amplification technology, single The principles and applications of cell whole transcriptome amplification technology and single cell transcriptome sequencing are summarized and summarized.

1. Introduction

Cells are the basic unit of life activity, with complete vitality. Most living organisms are multicellular. However, single-cell living organisms can carry out life activities separately because cells have a complete set of self-regulating devices[1,2]. In multicellular organisms, although the cells as a whole coordinate and control the function of each cell, each cell has a relatively independent set of metabolic systems, and each cell body performs specific functions in a cell-based unit. of. Only when the cells have a suitable growth environment, each isolated single cell can grow and multiply in vitro. The cell is the basic functional unit of life, and indirectly explains the laws of metabolism and regulation of the body. Therefore, research on single cells has been receiving public attention.

Single-cell sequencing technology is the analysis of single-cell DNA or RNA, revealing the genetic structure and expression of individual cells, changes in cell growth and development, genetic material status, and heterogeneity between cells and cells. Development is slower than other disciplines [3, 4]. It plays an irreplaceable role in tumor [5], developmental biology [6], stem cell biology [7], microbiology [8], neuroscience [9] and so on. Single cell sequencing separates, sequences, etc. cells in a series of steps. However, there are certain difficulties in isolating single cells, single cell genomes, and amplification of transcriptomes. This paper mainly summarizes and analyzes the research progress of single cell genomic amplification technology, transcriptome amplification technology and single cell separation technology and their advantages and disadvantages in single cell sequencing technology.

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DOI: https://doi.org/10.30564/jzr.v1i1.715

2. Single Cell Separation and Technology

Single cell sequencing analysis is the sequencing of the genes of the cells of interest extracted from the tissue. In recent years, many researchers have continued to explore and improve the separation method of single cells. Currently, the techniques commonly used for single cell separation are: gradient dilution, micromanipulation, fluorescence activated cell sorting, Microfluidic technology, laser capture microdissection and so on.

2.1. Gradient Dilution Method

Serial dilution is used to clone stem cells or premature cells from different tissues in vitro. The cell suspension is diluted by a series of different gradients until a single cell is obtained. By gradient dilution calculation, single cells cannot be sorted intuitively. This method requires less equipment and operation, but the success rate of separating single cells is not high, and it is easy to make mistakes [10-13].

2.2. Micromanipulation

Micromanipulation is mainly applied under the condition that the target cells are sparse. The single cells are visually selected by micromanipulator or combined microscope and mouth pipette, and further research is carried out. This method has little effect on the activity and state of cells, and has low cost, but it has time-consuming shortcomings, which is easy to cause RNA degradation, low flux, and more manpower, which is not conducive to large-scale application [14].

2.3. Fluorescence Activated Cell Sorting Technology

In recent years, the development of immunological techniques and flow cytometry has rapidly achieved the goal of sorting cells by means of flow cytometry, which is debugged by a computer. The principle is that the cells to be tested are placed in a dye-stained sample tube, and the cells are in a single-row cell column under the action of air pressure, and then the fluorescence signal excited by the laser is captured by the optical system, and then detected according to the optical system. The fluorescence signal intensity sorts the cells. This technology is a technique for rapid quantitative analysis and is the most commonly used single cell separation technique. The advantages of fluorescence activated cell sorting (FACS) are high accuracy and throughput, uniform standards, mature experimental methods, and the ability to analyze individual cells for rapid detection of cellular physiology. Chemical parameters; the disadvantages of the process are complex, the demand for raw materials is large, and the spatial resolution is insufficient [15-17].

2.4. Micro Control Flow Technology

Microfluidics are the separation and capture of individual cells within a micron-scale channel. Due to the closedness of the operation space, this technology can greatly reduce the pollution. Due to the particularity of the operating device, the concentration of the sample is high, and the consumption of the detection reagent is small, and the operation error can be reduced. Low, this completes the data with higher reliability and better reaction efficiency, and has a good application prospect [18-22].

2.5. Laser Capture Microdissection Technology

Laser capture microdissection (LCM) is the precise separation of single cells from a smear of frozen or paraffin-embedded tissue-slurry thermoplastic films using a UV (320-400 nm) laser under a microscope. The biggest advantage is that the target cells to be preserved can be captured without destroying the tissue structure, and the degree of automation is high, and the application prospect is wide. However, the disadvantage of this technique is that the instrument is expensive and expensive, and for some fixed and uncovered tissue sections, the visual resolution is limited, resulting in inaccurate cutting, and may also lose nuclear genetic material due to the operation time [23- 25].

3. Single Cell Whole Genome Sequencing

Single-cell gene sequencing refers to the process of separating single cells from tissues by tissue separation technology, then extracting the single-cell DNA and sequencing them to obtain a single-cell genome-wide map, but sometimes the single-cell genome DNA. As low as 6pg, and the copy number of each gene is only two [26], and often occurs due to lack of band, multi-band, etc. Therefore, it is not feasible to use a common method to amplify a single-cell genome, in order to meet the second-generation sequencing. The minimum DNA content is required for efficient amplification of genomic DNA.

3.1 Single-cell Whole Genome Amplification Technology

Whole genome amplification (WGA) is a technique for efficient amplification and sequencing of single-cell genomes. The principle is to efficiently amplify the micro-genomic DNA of isolated single cells to obtain high-coverage single-cell genome technology. Used to reveal cell population differences and cell evolutionary

 DOI: https://doi.org/10.30564/jzr.v1i1.715

relationships. So far, PCR-based whole genome amplification methods include: degenerate oligonucleotide primed PCR, DOP-PCR, ligation mediated PRC, LM-PCR, primer extension pre-amplification, PEP. There are multiple displacement amplification based on isothermal reaction, but not based on PCR, MDA, multiple annealing and looping-based amplification cycles, MALBAC [26-28].

3.1.1 PCR-based Approach

Among them, in the PCR-based method, due to the inevitable error or uncontrollable external factors in PCR, the fragment size or secondary structure and GC content of DNA are affected, and the coverage of the genome is insufficient. Complete, and the sequence of amplification results may be false positive, false negative and non-specific amplification products, resulting in impure product, amplification bias [29-31].

3.1.2 MDA and MALBAC Technology

The most commonly used techniques today are multiple displacement amplification techniques, amplification techniques for multiple annealing loops. MDA technology is a constant-temperature amplification method that relies on the principle of strand displacement amplification, highly amplified DNA, and utilizes the strong template binding ability of phi29 DNA polymerase and template for strand displacement amplification [32]. The advantages are simple operation, low error rate, high amplification coverage, and the amplified DNA fragments are 10~100 kb in length; the disadvantage is that non-specific amplification occurs during amplification, and a high initial template amount is required. At low times, the amplification bias is large and there is sequence deviation [33]. MALBAC technology uses the advantages of PCR amplification technology and MDA amplification technology. Through special primers, the end of the amplicon is complemented to form a loop, which avoids the exponential amplification of genomic DNA, and the amplification bias is low. The product increased coverage of more than 93% of the whole genome [33]. And the detection rate of MALPC in SNPs alleles can reach 70%, compared with MDA, the improvement effect is obvious [34-35].

3.2 Single-cell Whole Genome Sequencing Technology

After efficient amplification of single-cell whole-genome DNA, a high-coverage genome is obtained, and single-cell genomic DNA is sequenced. So far, under the premise of ensuring the accuracy of sequencing, high-throughput sequencing technology is constantly optimizing the operating procedures, which is thousands of times higher than the traditional methods, which can greatly reduce the cost of gene sequencing. Single-cell genome sequencing has been used to analyze the recombination patterns and aneuploidy of human single germ cells, blastocysts and polar bodies pre-implantation screening, and heterogeneity of genomes in tumor cells and circulating tumor cells. . The current high-throughput sequencing next-generation technologies include: Roche 454 pyrosequencing, Illumina Solexa sequencing, and Ion torrent ion semiconductor sequencing.

3.2.1 Roche 454 Pyrosequencing

Roche454 pyrosequencing technology uses a magnetic bead emulsification polymerase chain reaction technique based on pyrosequencing, which produces pyrophosphate when a base is paired with a template, and fluorescein and fluorescein under the action of ATP sulfated enzyme. The enzyme oxidizes to fluorescein, and the release of the optical signal is determined by the high-sensitivity charge-coupled device CCD to determine the base sequence of the template [36]. The technology has an accuracy of 99.96%, a coverage rate of 96%, and a long read length of up to 400bp. The disadvantage of this technique is that the continuous incorporation of bases cannot terminate the penetration of the same base due to the lack of termination elements. The length of the same base can only be inferred from the strength of the signal. In this process, errors of insertion or deletion may occur, and the cost is high, and the flux is low [37, 38].

3.2.2 Illumina Solexa Sequencing

Illumina Solexa sequencing technology utilizes "DNA clusters" and "reversible end terminations" [39]. By recovering 100 bp-200 bp DNA fragments randomly interrupted by physical methods, a linker is added at both ends to dilute at a certain concentration, and then the single-stranded DNA is complementary to the single-stranded primer on the surface of the chip, and after 30 rounds of amplification, a single sheet is formed. Clone "cluster". After linearization of the amplicon, four fluorescently labeled dNTPs (these dNTP "reversible terminators") were used for sequencing while sequencing, and the 3'-OH with a chemical cleavage site allowed only one base to be added. Then, after fluorescence is obtained by pyrosequencing, the 3'-OH terminal group is chemically cleaved, and the 3' viscosity is lowered, and the next round of reaction sequencing is performed [40]. The advantage of Illumina Solexa sequencing technology is that it can obtain higher throughput, requires less sample volume, high precision, avoids insertion or missing errors,

DOI: https://doi.org/10.30564/jzr.v1i1.715

less manual operation, disadvantages of long running time, and sequence reading due to optical signals and other reasons. The length is short and the error rate accumulates as the chain lengthens [37, 41].

3.2.3 Ion Torrent Ion Semiconductor Sequencing

Ion torrent ion semiconductor sequencing technology is based on synthetic sequencing, using chemical and digital information technology, combined with nucleic acid chemistry and semiconductor technology, to directly complete non-optical application model DNA sequencing in integrated circuits [42-43]. The advantages of Ion torrent ion semiconductor sequencing technology are that DNA synthesis and detection can be performed under natural conditions, and the cost is low, the time is short, the flexibility is fast, and the flux measurement is fast, and the disadvantage is that misreading of polybases is easy.

4. Single Cell Whole Transcriptome Analysis

The transcriptome was first proposed by Veclalesuc and Kinzler et al[44], in 1997. It is a collection of genes expressed in the genome, which refers to the sum of all RNAs processed and transcribed by a living unit or tissue under certain conditions (including messenger RNA and non-coding RNA). In a single cell, mRNA has thousands of copies [45], and sequencing analysis requires that mRNA is reverse transcribed into cDNA and then amplified by PCR. Therefore, efficient and unbiased amplification is reverse transcription. Sequencing is a key factor [46].

4.1 Single Cell Whole Transcriptome Amplification Technology

At present, the methods for amplifying single cell cDNA mainly include: (1) PCR index amplification; (2) linear amplification in vitro; (3) Phi29 polymerase amplification.

4.1.1 PCR Index Amplification

The first is PCR amplification using cDNA with anchor sequences (left and right primers). The enzymes used in the synthesis of the second cDNA strand can be classified into mRNA-Seq [47], Smart-Seq [48], Smart-Seq2 [49], STRT-Seq [50], and SMA [51]. The anchor sequence at the 3' end of the transcript can be introduced by oligo (dT) primers during reverse transcription. Methods such as Smart-Seq, Smart-Seq2, and STRT-Seq utilize reverse-transferase terminal transferase and template-switching activities to synthesize complementary cDNA strands by template mapping. This method can avoid the interference of ribosomal RNA (rRNA) and transport RNA (tRNA), but can not detect various RNAs without poly(A) tail, the transcriptome with low level expression may be lost and the instability of Smart technology Wait.

The SMA semirandom primed PCR-based mRNA transcriptomeamplification technique uses oligoribonucleotides with a hairpin structure as primers [52]. The full-length sequence of mRNA can be obtained, but the starting amount, sensitivity, and stability are different, and the range of use is also different.

4.1.2 In Vitro Transcription Linear Amplification

The second is to sequence the entire transcript, and the in vitro transcription (IVT) method [53] for linear amplification. IVT amplification avoids PCR bias but requires multiple rounds of reverse transcription to achieve the initial amount. The advantage of this technique is that the exponential amplification of cDNA is faster, but slower than the PCR process, the result is more accurate, and it is easy to cause Primer dimer and byproduct accumulation [54].

4.1.3 Phi29 Polymerase Amplification

The third is Phi29-mRNA amplification (PMA) [55] using the high fidelity and high amplification rate of Phi29 DNA polymerase used in whole genome amplification technology, with excellent strand displacement and continuous The characteristics of the synthesis, the cyclized cDNA is subjected to multiple generations of continuous amplification, and the enzyme also has 3,-5, exonuclease reading activity. This reaction can be amplified at room temperature, which not only strongly avoids the influence of DNA degradation on the quality of the amplified product at high temperature, but also reduces the amplification advantage caused by the difference in GC content. However, the amplification uniformity of this method is not high.

4.2 Single Cell Transcriptome Sequencing Technology

Single-cell RNA-sequencing method, scRNA-seq can obtain a single-cell genome-wide expression profile, which can effectively avoid the cellular heterogeneity of conventional transcriptome analysis technology in biological systems and tissues, and can directly distinguish different organisms. The difference in gene expression and transcriptional characteristics between cell types can reflect the expression and regulation of all genes from the overall level of cells, revealing the molecular composition in cells and tissues, and can be clear about the randomness of gene expression [56].

General transcriptome sequencing is the choice to utilize high-throughput sequencing or microarray analysis [57-59]. The advantage of microarray analysis is that it can

 DOI: https://doi.org/10.30564/jzr.v1i1.715

provide transcriptome information and regulate network information, and high-throughput sequencing can reveal dynamic information more accurately from multiple angles [60]. In both methods, the ultimate goal is to first obtain the original RNA molecule, and then effectively perform consistent and stable amplification, and finally analyze the RNA sequence according to the experimental purpose. In addition, a new generation of sequencing methods is also constantly exploring and developing, single molecule fluorescence in situ hybridization, fluorescence in situ hybridization RNA sequencing and intracellular transcript analysis [26].

5. Outlook

So far, single-cell separation technology is still immature, whole-genome amplification bias, non-specific amplification, pollution during database construction, high technical noise, operational errors, low reproducibility, poor non-coding RNA detection The lack of barriers to splicing software still exists. Perfecting and improving sequencing analysis methods understand cell heterogeneity, randomness and synergy at the cellular level and play an important role in grasping the body's growth and development process and biological key events. With the continuous advancement of sequencing technology, single-cell sequencing technology is widely used in the field of tumors, which can analyze the heterogeneity between solid tumors and circulating tumor cells from a large scale, and use this technology to predict and monitor diseases. Detection of drug resistance, etc[61]. In the process of growth and development of tissues and organs, search for new cell types and marker genes; in the nerve and immune system, it can clearly understand the advantages of cell heterogeneity; it also has important applications in the fields of artificial insemination and stem cell transplantation. In order to achieve true proteomics and metabolomics analysis, with the continuous optimization of technical means, these problems are solved just around the corner. Single-cell sequencing technology will become an important technical means for humans to explore the growth and development of the body and various clinical fields.

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DOI: https://doi.org/10.30564/jzr.v1i1.715

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ARTICLE

Patterns of Species Richness and Abundance in Badingilo National Park in South Sudan

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ARTICLE INFO

Article history
Received: 8 October 2019
Accepted: 14 October 2019
Published Online: 30 October 2019

Keywords:
Habitat types
Jack1 estimator
Rarefaction curves
Rank abundance curves
South Sudan

ABSTRACT

The study was carried out to determine patterns of birds' species richness, alpha and beta diversities; and abundance in Badingilo national park using a 10 m fixed-radius point count method. A total of 2670 individuals were recorded from 182 points in the park. The highest expected number of species (Jack1 estimator) was observed in the Riverine habitat and least was in the Agriculture and Human settlement habitat type. The total number of species observed in the park was 63; however Jack1 estimator indicated that there were 68 species in the park. The majority of the birds observed during the study were resident species, few migratory and Palaearctic bird species. Few birds observed in the park were abundant. The most abundant species was the village weaver (381 individuals), and the rarest species were black-bellied bustard, barn owl, black scimitar bill and tree pipit (one individual each).

1. Introduction

Avian community is an important component of all forest ecosystems. Birds play a major role as pollinators, consumers, and dispersers of plant seeds and predators of many invertebrates and small vertebrates [24]. Research on birds' communities to design and strategize for biodiversity friendly development is of paramount importance [10,22]. Community ecology is the study of grouping of species, their distribution and interactions between them and physical as well as biological components of the environment [23,28]. According to Cody [11] birds' community is directly associated with habitat; and as such can serve indicators of environmental changes. Likewise, Mills et al. 1989 found a strong relationship between breeding bird community structure and vegetation in Arizona. Similarly, Ikin et al. [21] reported a multi-scale association between vegetation cover and woodland bird communities in South-west Wales in Australia. Although compared to mammals and amphibians, birds are regarded as excellent conservation indicators, yet some species are classified as Data Deficient in the IUCN Red list [8,19,32].

As yet birds are seriously threatened. These threats affect their distribution and diversity both locally and regionally. These threats arise from both natural factors and anthropogenic activities [2,4,9,27]. Illegal activities such as fishing, logging, and agricultural practices within the park can be detrimental to birds' species diversity in the long term (Birds Life International 2010). Habitat destruction as a result of anthropogenic activities is the major problem affecting diversity, abundance and species richness

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 DOI: https://doi.org/10.30564/jzr.v1i1.1284

of most birds [1,2,9,10,16]. Fahring [16] in a simulation study showed that habitat loss has a profound effect on extinction. Also, Zitske et al. [34] found that survival of migrant warblers in a forest mosaic was inversely proportional to habitat loss in New Brunswick in Canada. The objectives of this study were; first, to determine species richness of birds' in the park. Second, to determine abundance of birds in Badingilo National park and how this abundance is distributed across habitats.

2. Materials and Methods

2.1 Study Area

Badingilo National park is situated in South Sudan's equatorial region, within central equatorial state. The bordered by Bor in the north and Lafon to the east of White Nile. The Park was gazetted in 1986 and covers approximately an area of 8,400Km^2. The park is situated on a swamp 40 km east of Mongalla, and provides a dry season refuge for mammal populations [28]. It is surrounded by a large area of mostly waterless plains (Figure 1).

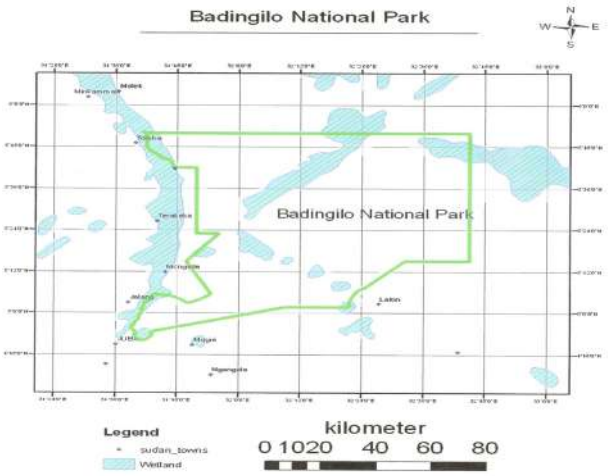

Figure 1. Map of Badingilo National Park (Green rectangle)

The climate of the area is characterised by two seasons wet and dry as it lies near the equator, temperature are hot throughout the year with the hottest maximum temperatures reaching 38^{0}C. Total annual rainfall ranges between 1000 to 1,500mm [33]. The fauna of the park includes white-eared kob (*Kobus kob leucotis*), the tiang (*Damaliscus lunatus tiang*), the Mongalla gazelle (*Gazella rufifrons albonotata*), reticulated giraffe (*Giraffa camelopardalis*), zebra (*Equus burchelli*), Grant's gazelle (*Gazella granti*), lesser kudu (*Tragelaphus imberbis*), Beisa oryx (*Oryx beisa*), warthog (*Phacochoerus africanus*), Bohor reedbuck (*Redunca redunca*), lion (*Panthera leo*), spotted hyena (*Crocuta crocuta*), wild dog (*Lycaon pictus*), leopard (*Panthera pardus*) and black-blacked jackal (*Canis mesomelas*) and several bird species [26,31]. The dominant tree species in the park includes white thorn (*Acacia seyal*), pod mahogany (*Afzelia quanzensis*), desert dates (*Balanites aegyptiaca*), stink wood (*Celtis sp.*), Sodom apple (*Calotropis procera*), Bush-willow (*Combretum sp.*), African fan palm (*Borassus aethiopium*), Bell-flowered mimosa (*Dichrostachys cinerea*), African ebony (*Diospyros mespiliformis*), Kaffir boom (*Erythrina sp.*), Fig (*Ficus sp.*), Sausage tree (*Kigelia africana*), Black plum (*Vitex doniana*), Christ thorn (*Ziziphus spina-christi*), Tamarind (*Tamarindus indica*), and Neem (*Azadirachta indica*) [5]. The main types of grasses found in the area consist of swamp meadow, with dense low growing stoloniferous grasses, antelope grass (*Echinochloa pyramidalis*), and thatching grass (*Hyparrhenia rufa*).

2.2 Sampling Design and Data Collection

Survey area was divided into five (5) different habitats based on the physiognomy and land use type as follows: first,_mixed Woodland which is characterised by wooded landscapes with dominant woody layers about 50-90% canopy cover [4]. Mixed woodland habitat type is mostly dominated by white thorn (*A. seyal*), pod mahogany (*A. quanzensis*), desert dates (*B. aegyptiaca*), and bush-willow (*Combretum sp.*) among others [5]. Second, is Wetland/seasonal flooded grassland habitat type which is seasonally inundated by water from rivers and rain shed/ land where saturation of water is the dominant factor determining the nature of soil development and the types of plants and animals living in the soil and on its surface [4]. Wetland habitats are mostly dominated by the following grass species wild rice (*Oryza longistaminata*), Guinea grass (*Panicum sp.*), thatching grass (*H. rufa*) and antelope grass (*E. pyramidalis*). Third, is desert dates (*B. aegyptiaca*) and Christ thorn (*Z. spina-christi*) woodland habitats which is characterised by dense woody species mostly desert dates and Christ thorn as well as some grasses and shrubs adjacent to river courses [4]. Fourth, Riverine habitats is found along the riverside and its edge, these habitats are mostly dominated by nut-grass or water-grass (*Cyperus papyrus*), reed (*Phragmites sp.*) and reedmache (*Typha domingensis*) swamp among others [4]. Fifth, Agricultural and human settlement habitat is characterised by agricultural activities and human settlement. The area is dominated by open fields of most groundnuts, few maize and some beans. Within each habitat type 15-40 10 m radius circular plots were placed. Birds seen within the plots were identified to species and counted. Identification was done with the help of binoculars. Bird identification was done according

DOI: https://doi.org/10.30564/jzr.v1i1.1284

Nikolaus [28] and Sinclair & Ryan [31].

2.3 Data Analysis

To determine, species richness for the whole park and each habitat type, rarefaction curves were generated using 50 randomizations and sampling without replacement implemented in Estimate S v9 [13]. This was done using Jack-knife1 estimator chosen as the most applicable to our data. Rarefaction curve is a standardization procedure that calculates expected species accumulation curve and allows comparison of species richness among samples of different sites or habitats [13]. Avian species abundance in Badingilo National park and within each habitat type was assessed using species rank abundance curves. Here species are ordered from the most to lest abundant. This enables the pattern of abundance to be discerned.

3. Results

3.1 Species Richness and Diversity

In total, 2857 birds were recorded from 182 point samples of 10 m radius across five different habitats in the park over the course of two weeks birding periods. The highest expected number of species (Jack 1 estimator) was observed in the Riverine habitat (51). The lowest was in the Agriculture and Human settlement habitat type (35) (Figure 2).

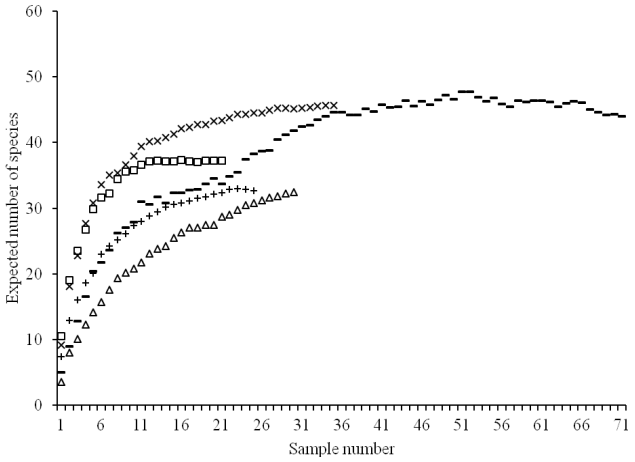

Figure 2. Rarefaction curves of birds in different habitat types (x RVRNE □ WTL + B & ZWL - MWL Δ AGRIC)

A total of 63 species was observed in the park. However, Jack 1 estimator puts the number of bird species in the park at 68 which is slightly higher than the observed number of bird species in the park (Figure 3).

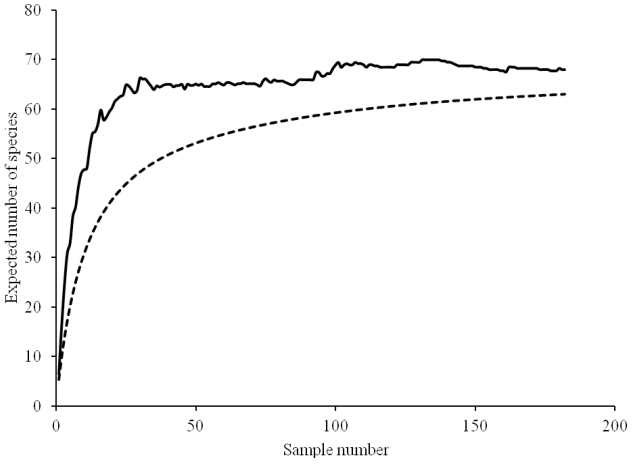

Figure 3. Species richness of birds in the park (----- observed number of species S(est) estimated number of species (Jack1)

3.2 Bird Species Abundance

Most species of birds observed in each habitat type were rare (Figure 4).

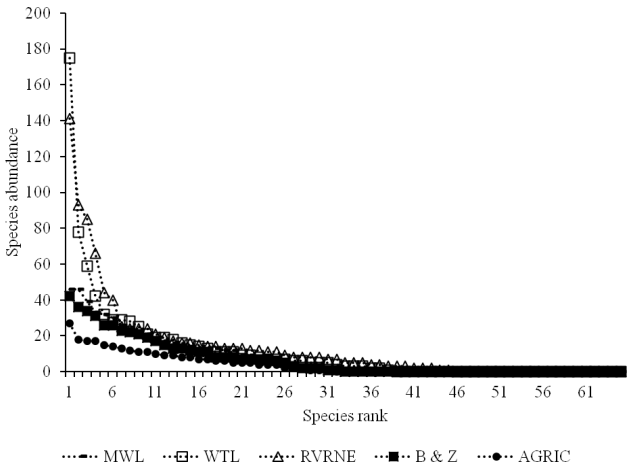

Figure 4. Species rank-abundance of birds in different habitat types in Badingilo National park (•••-••• MWL •••□••• WTL •••Δ••• RVRNE •••■••• B & Z WL •••●••• AGRIC)

Likewise the global species rank-abundance curve of birds indicates that the number of rare species outnumbered that of abundant birds in Badingilo National Park. The village weaver was the most abundant bird species and each of black-bellied bustard, barn owl, black scimitar and tree pipit were rare (Figure 5).

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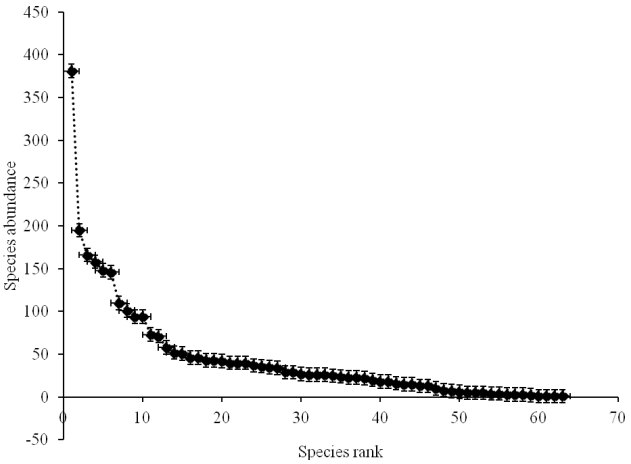

Figure 5. Species rank-abundance curve of birds in Badingilo National park

4. Discussion

Both the observed and estimated number of bird species in the park may represent a tiny fraction of birds found in Badingilo National Park in a relatively short sampling period. Nevertheless, it provides the first checklist of bird species recorded during the duration of this study in the National park. In addition, it indicates that Badingilo National Park may be harbouring a relatively rich avian community as has been reported by Evans and Fishpool [15].

Although, 63 bird species were observed in the park, Jack 1 estimator puts the figure at 68 indicating that the park may be harbouring more species. The underestimation may be due to the low sampling effort and thick vegetation present in some habitat types. Besides, the timing of sampling August may have contributed to low species richness. Evans and Fishpool put the number of birds in Badingilo National Park at 85. Although this species richness of birds may be higher than the 63 (74%) observed in this study, it suggests that the number of individuals observed in that study was higher than those in the current study. In spite of biases that may be associated with the sampling procedure, the Jack1 estimate is probably the correct estimate of the avian community in Badingilo National Park as it is considered a less bias estimator [14]. Moreover the Jack1 estimator has started to level off indicating that avian sampling over the two weeks has been exhausted. The occurrence of high number of species in the riverine habitat type [7,29] may be explained by the presence of several microhabitats thereby supporting many bird species [12,20,23,28]. For example, the riverine habitat type encompasses pockets of woodland, grasslands and plenty of water making it attractive to many bird species. The observed lowest species diversity of birds in the agriculture and human settlement habitat may be due to high anthropogenic activity. For example, the bird survey period coincided with the harvest time for the groundnuts, thus there was a lot of activity in the agriculture and human settlement habitat type. In addition, this habitat type was mainly open with few scattered trees making it less attractive for many bird species as they may become exposed to predators [3, 18].

The riverine habitat type had the highest number of individuals of birds dominated by the village weaver suggest that it is the main habitat type. The high number of rare species observed in this study is similar to that reported in other studies where many of the species found were rare and few were abundant [6,17,25,30]. This suggests many birds in Badingilo National Park occur in small numbers. In conclusion, the patterns of avian diversity observed in this study should be interpreted with caution because of the limited sampling effort. In addition, further research with sampling period extended over the dry and wet seasons should be undertaken to ascertain the species richness and abundance of birds in Badingilo National Park.

Acknowledgements

Thanks to Dr. Hassan A. Babikir for providing upkeep, field assistance, birds' field guide, and binoculars. Thanks also to the Ministry of Wildlife Conservation and Tourism, Republic of South Sudan for the permit to work in the Park, College of Natural Resources and Environmental Studies, Department of Wildlife Science, and the Wildlife Conservation Society, South Sudan Office for logistical support.

Appendix

Table 1. List of birds observed in Badingilo National Park

Serial number	Local name	Scientific name	Status
1	Mourning dove	*Streptopelia semitorquata*	MB
2	Cape turtle dove	*Streptopelia semitorquata*	R
3	Village weaver	*Ploceus cucullatus*	RB
4	Rüppell's starling	*Lamprotornis purpuropterus*	RB
5	Sedge warbler	*Acrocephalus schoenobaenus*	PW
6	Red-cheeked cordon-bleu	*Uraeginthus bengalus*	RB
7	African pigmy kingfisher	*Ispidina picta*	MB
8	Malachite kingfisher	*Alcedo cristata*	R
9	Cat-throat finch	*Amadina fasciata*	RB
10	Tree pipit	*Anthus trivialis*	PW

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11	Superb starling	*Lamprotornis superbus*	R
12	Common bulbul	*Pycnonotus barbatus*	RB
13	Lesser grey shrike	*Lanius minor*	P
14	Northern red bishop	*Euplectes franciscanus*	RB
15	Speckled mousebird	*Colius striatus*	RB
16	Senegal coucal	*Centropus senegalensis*	RB
17	Beautiful sunbird	*Cinnyris pulchellus*	RB
18	Pied kingfisher	*Ceryle rudis*	RB
19	Greyhooded kingfisher	*Halcyon leucocephala*	MB
20	Little bee-eater	*Merops pulsillus*	RB
21	Northern redbilled hornbill	*Tockus erythrorhynchus*	RB
22	Yellow-fronted canary	*Serinus mozambicus*	RB
23	Helmeted guineafowl	*Numida meleagris*	RB
24	Black-headed gonolek	*Laniarius erythrogaster*	RB
25	Purple glossy starling	*Lamprotornis purpureus*	RB
26	Black-bellied bustard	*Eupodotis melanogaster*	R
27	Red-billed quelea	*Quelea quelea*	MB
28	Tawny-flanked Prinia	*Prinia subflava*	RB
29	Cardinal quelea	*Quelea cardinalis*	R
30	Barn owl	*Tyto alba*	RB
31	Marsh owl	*Asio capensis*	M
32	Black kite	*Milvus migrans*	MB/PW
33	Pin-tailed whydah	*Vidua macroura*	RB
34	Eastern paradise whydah	*Vidua paradisaea*	R
35	Fork-tailed drongo	*Dicrurus adsimilis*	RB
36	Hadeda ibis	*Bostrychia hagedash*	R
37	White-faced whistling duck	*Dendrocygna viduata*	MB
38	Spotted flycatcher	*Muscicapa striata*	P
39	Bronze mannikin	*Spermestes cucullata*	RB
40	Great reed warbler	*Acrocephalus arundinaceus*	P
41	Eurasian reed warbler	*Acrocephalus scirpaceus*	PW/MB
42	Northern carmine bee-eater	*Merops nubicus*	MB
43	African paradise flycatcher	*Terpsiphone viridis*	MB
44	Black scimitarbill	*Rhinopomastus aterrimus*	RB
45	African golden oriole	*Oriolus auratus*	M
46	African fish eagle	*Haliaeetus vocifer*	RB
47	Lizzard buzzard	*Kaupifalco monogrammicus*	RB
48	Cattle egret	*Bubulcus ibis*	MB/P
49	Hammerkop	*Scopus umbretta*	RB
50	Goliath heron	*Ardea goliath*	RB
51	African yellow white eye	*Zosterops senegalensis*	RB
52	Copper sunbird	*Cinnyris cupreus*	RB
53	Jacobin cuckoo	*Clamator jacobinus*	M?
54	Rufous-rumped lark	*Pinarocorys erythropygia*	M
55	Lesser swamp warbler	*Acrocephalus gracilirostris*	RB
56	Swallowtailed bee-eater	*Merops hirundineus*	R
57	Namaqua dove	*Oena capensis*	MB
58	Dark chanting goshawk	*Melerax metabates*	RB
59	Village indigobird	*Vidua chalybeata*	RB
60	Flappet lark	*Mirafra rufocinnamomea*	RB
61	African thrush	*Turdus pelios*	RB
62	Red-backed shrike	*Lanius collurio*	P
63	Orange river francolin	*Scleroptila levaillantoides*	R

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