

*Full Length Research Paper*

# Response of broiler chickens to diets of varying protein contents under *ad libitum* and skip-a-day feeding regimes

Olayode R. Folorunso<sup>1</sup>, Aanuoluwapo A. Adesua<sup>2</sup> and Gbenga E. Onibi<sup>2\*</sup>

<sup>1</sup>Department of Animal Production Technology, Federal College of Agriculture, Akure, Ondo State, Nigeria.

<sup>2</sup>Department of Animal Production and Health, The Federal University of Technology, Akure, Ondo State, Nigeria.

Accepted 18 October, 2019

A 3x2 factorial experiment was used to investigate the effect of varying dietary crude protein levels; optimum (control, 19.64% CP), low (17.72% CP) and high (21.66% CP) diets, fed *ad libitum* and on skip-a-day bases on the performance and carcass characteristics of broiler chickens. The diets were iso-energetic. A total of 180 3-week old broiler chicks were used at three replicates per treatment and 10 birds per replicate. The feeding trial lasted for 5 weeks. Weight gain was lowest for chickens on low protein diet compared with those fed the optimum ( $P>0.05$ ) and high protein diets ( $P<0.05$ ). Chickens fed *ad libitum* gained more weight ( $1.62\pm 0.13$  kg/bird) than those fed on skip-a-day basis ( $0.95\pm 0.07$  kg/bird). Chickens fed on skip-a-day basis had higher ( $P<0.05$ ) feed conversion ratio ( $2.86\pm 0.27$ ) than those fed *ad libitum* ( $2.59\pm 0.27$ ). Water intake of the birds significantly ( $P<0.05$ ) increased with increasing dietary protein. Abdominal fat deposition was promoted ( $P<0.01$ ) by the low protein diet but reduced ( $P>0.05$ ) by skip-a-day feeding method. Other carcass characteristics were not influenced ( $P>0.05$ ) by dietary protein levels but a higher energy to protein ratio promoted the development of organs. Higher values were recorded for carcass characteristics of birds on *ad libitum* feeding compared with skip-a-day feeding. This study reiterates the benefit of feeding a balanced (optimum) protein diet, *ad libitum* to broiler chicken finishers.

**Key words:** Broiler chickens, feed protein, feeding regime, performance.

## INTRODUCTION

Poultry raised intensively requires more nutrients than other stocks and their requirements are often specific as stated in poultry nutrition texts (Chiba, 2009). An ideal broiler diet is one that will maximize production at the least cost. Although a costly diet may produce phenomenal gains in livestock, the cost per unit of production may make the diet economically infeasible. Likewise, the cheapest diet will not always be the best since it may not allow for maximum production (Damron and Sloan, 1998). Many broiler producers in the developing countries raise their birds with low quality

feeds and this has persisted for a long time. Since feed alone accounts for about 60 to 80% of the total cost of production (Oluyemi and Roberts, 2000), the need to combine the available feed ingredients in the best possible way, becomes paramount. Oluyemi and Roberts (2000) also reported that the importance of feed to the livestock industry is to some extent a function of its quality, that is, the feed should be balanced. A balanced diet should fulfil all the requirements for maintenance, growth, work, production and reproduction. Of all the various feed constituents used in formulating poultry

**Table 1.** Gross composition of experimental broiler-chicken finisher diets.

Ingredients	Diets		
	Optimum protein (Control)	Low protein	High protein
Maize	50.00	52.00	50.00
Maize offal	13.25	15.25	7.00
Soybean meal	13.00	11.00	16.00
Groundnut cake	13.00	11.00	16.00
Brewer dried grain	5.30	6.00	5.47
Fishmeal (72%)	1.20	-	1.50
Bone meal	2.50	2.90	2.50
Oyster shell	0.50	0.45	0.40
Vit./min. premix	0.25	0.25	0.25
DL-Methionine	0.30	0.30	0.28
Lysine	0.20	0.35	0.10
Salt (NaCl)	0.50	0.50	0.50
Total	100.00	100.00	100.00
<b>Calculated composition</b>			
ME (kcal/kg)	2913.84	2910.22	2914.11
Crude protein (%)	19.64	17.72	21.66
Crude fibre (%)	4.58	4.63	4.64
Ether extract (%)	5.20	5.32	5.16
Ca (%)	1.25	1.27	1.24
P (%)	0.55	0.56	0.57
Methionine + cysteine (%)	0.60	0.58	0.60
Lysine (%)	1.04	1.05	1.07

diet, protein is the most important and most expensive. According to a report by the National Research Council (NRC, 1997), a reduction in protein content of feed or the use of less expensive protein supplements, could considerably reduce cost. In an effort to address this and associated problems of *ad libitum* feeding, feed restriction programmes are being advocated (Oyedeji and Atteh, 2003).

Compared with *ad libitum* feeding, several approaches (restricted feed supply, short-term feed removal, nutrient dilution, etc) have been employed to restrict nutrient or caloric intakes in broilers in order to reduce the cost of feeding, improve feed efficiency and reduce fat depositions. Since birds are able to regulate their dietary energy intake, the adaptability of broilers to extreme long-term and presumably stressful SAD feeding regime, under varying dietary protein contents has not been investigated. Of what benefits would it be to broiler chicken producers in term of labour savings, and would dietary protein contents play any role in productivity and fat deposition of the broiler chickens under this feeding regime? These uncertainties informed this study on the performance and carcass characteristics of broiler chicken finishers offered diets with varying dietary crude protein contents, and fed *ad libitum* and on skip-a-day regimes for a five-week period.

## MATERIALS AND METHODS

### Experimental birds and layout

Day-old Hubbard broiler chicks were obtained from CHI-Ajanla Farms (Nig.) Ltd, Ibadan. The feedstuffs used in feed formulation were obtained from a reputable feedmill in Akure, Nigeria. The study involved the use of 3 experimental diets (optimum, low and high protein) which were formulated at the Teaching and Research Farm of the Federal College of Agriculture, Akure, Nigeria. Dietary composition and proximate chemical composition of diets are shown in Table 1. The optimum protein diet which served as the control was a conventional broiler finisher diet consisting of 19.64% CP and 2913.84 kcal/kg ME which was regarded as the optimum protein-optimum energy diet. The low protein diet had lower crude protein content (17.72% CP) than the control diet while the high protein diet had the highest protein content (21.66% CP). All the diets were iso-caloric.

The chicks were brooded for 3 weeks during which they were fed commercial broiler starter diets (22% CP) *ad libitum*. At the end of the 3-week brooding period, 180 chicks were selected and assigned to one of the three experimental diets under two feeding regimes of *ad libitum* (ADL) and skip-a-day (SAD) representing a 3 × 2 factorial experiment in completely randomized design. There were 3 replicates per treatment and 10 chicks per replicate. The chicks were selected such that there were 5 males and 5 females per replicate and the mean weight per treatment did not vary by more than 20 g. The feeding troughs had hoods that prevented spillage. Standard broiler management procedures were followed and all necessary vaccinations and other medications were administered at the appropriate periods. Feed intake, weight gain

**Table 2.** Performance characteristics of the broiler chickens placed on diets of varying dietary protein and feeding regimes.

Protein level	FMT	Initial LW (kg/chick)	Final LW (kg/bird)	Feed intake (kg/bird)	Weight gain (kg/bird)	Feed conversion ratio	Water intake (litre/bird)
Optimum protein	ADL	0.53 ± 0.01	2.20± 0.09	4.61 ± 0.17	1.66 ± 0.10	2.79 ± 0.25	7.96 ± 0.31
	SAD	0.53 ± 0.01	1.57 ± 0.11	2.84 ± 0.03	0.99 ± 0.04	2.86 ± 0.02	6.99 ± 0.25
Low protein	ADL	0.51 ± 0.01	2.01 ± 0.05	3.74 ± 0.24	1.48 ± 0.07	2.52 ± 0.06	7.56 ± 0.28
	SAD	0.52 ± 0.01	1.42 ± 0.04	2.68± 0.25	0.88±0.05	3.06±0.29	6.48±0.13
High protein	ADL	0.52 ± 0.02	2.29 ± 0.17	4.23 ± 0.45	1.73 ± 0.09	2.45 ± 0.37	7.93 ± 0.23
	SAD	0.52±0.01	1.49±0.04	2.55±0.17	0.97±0.05	2.65±0.27	7.09±0.29
<b>Statistical significance</b>							
Protein level		NS	*	*	**	NS	*
Feeding method		NS	***	***	***	*	***
Protein level × feeding method		NS	NS	NS	NS	NS	NS
<b>Mean separation</b>							
<b>Protein level effect</b>							
Optimum protein		0.53 ± 0.01	1.88 ± 0.36 <sup>a</sup>	3.73 ± 0.58 <sup>a</sup>	1.33 ± 0.37 <sup>a</sup>	2.82 ± 0.18	7.47 ± 0.39 <sup>ab</sup>
Low protein		0.51 ± 0.01	1.72 ± 0.33 <sup>d</sup>	3.21 ± 0.42 <sup>d</sup>	1.18 ± 0.34 <sup>d</sup>	2.79 ± 0.35	7.02 ± 0.42 <sup>d</sup>
High protein		0.51 ± 0.01	1.89 ± 0.45 <sup>a</sup>	3.39 ± 0.67 <sup>b</sup>	1.35 ± 0.42 <sup>a</sup>	2.55 ± 0.31	7.51 ± 0.58 <sup>a</sup>
<b>Feeding method effect</b>							
Ad libitum		0.52 ± 0.02	2.17 ± 0.16 <sup>a</sup>	4.19 ± 0.47 <sup>a</sup>	1.62 ± 0.13 <sup>a</sup>	2.59 ± 0.27 <sup>a</sup>	7.82 ± 0.32 <sup>a</sup>
Skip-a-day		0.52 ± 0.01	1.49 ± 0.18 <sup>d</sup>	2.69 ± 0.19 <sup>d</sup>	0.95 ± 0.07 <sup>d</sup>	2.86 ± 0.27 <sup>d</sup>	6.85 ± 0.31 <sup>d</sup>

Values are mean ±SD; NS = not significant (P>0.05), \* =P<0.05; \*\*=P<0.01; \*\*\*=P<0.001; FMT = feeding method, ADL = *Ad libitum*, SAD = Skip-a-day; means with different subscripts within the same column and for the same parameters are significant (P<0.05).

and feed conversion ratios were determined at weekly intervals for a period of five weeks.

#### Carcass measurements, chemical and statistical analyses

At the end of the 5-week feeding trial, three birds of about the average group weight were selected from each replicate, humanely slaughtered, dressed, eviscerated and cut into parts for carcass characteristics and organ measurements. Proximate composition of diets was determined by the methods of AOAC (1990). The data obtained from the study were subjected to 3 × 2 factorial

analysis of variance (ANOVA) using the Minitab (ver. 10.2, Minitab Inc., USA) Statistical Package. Significant differences between treatment means were separated using least significance difference (LSD) using the same statistical software.

#### RESULTS

The performance characteristics of broiler chickens are shown in Table 2. Initial live weights of the chicks were not significantly different (P>0.05). The final live weight, feed intake, feed

conversion rate, weight gain and water intake were significantly (P<0.05) influenced by experimental treatments. There was no significant (P>0.05) feeding-method by diet-type interaction so the overall effect of diet-type and feeding-method were tested for significance. Chickens on high protein diet had the highest(1.89±0.45 kg/bird) final live weight, though this was not significantly (P>0.05) different from those on optimum protein diet. The lowest final live weight (1.72±0.33 kg/bird) was found in chicken on low protein diet (1.88±0.36 kg/bird). Chickens fed *ad*

*libitum* had higher ( $P<0.05$ ) final live weight ( $2.17\pm 0.16$  kg/bird) compared with those fed on skip-a-day basis ( $1.49\pm 0.18$  kg/bird). Chickens fed the optimum protein diet had the highest ( $3.73\pm 0.98$  kg/bird) feed intake while the lowest ( $3.21\pm 0.62$  kg/bird) ( $P<0.05$ ) was found in chickens on low protein diet. Chickens fed *ad libitum* ate more feed ( $4.19\pm 0.47$  kg/bird) than their counterparts on skip-a-day regime ( $2.69\pm 0.19$  kg/bird) ( $P<0.001$ ). Weight gain was significantly lower for chickens fed on the low protein diet compared with those fed the optimum or high protein diets. Chickens fed *ad libitum* gained more weight ( $1.62\pm 0.13$  kg/bird) than those fed on skip-a-day basis ( $0.95\pm 0.07$  kg/bird). The feeding method significantly ( $P<0.05$ ) influenced the feed conversion ratios with chickens fed on skip-a-day converting feed less efficiently ( $2.86\pm 0.27$ ) compared with those fed *ad libitum* ( $2.59\pm 0.27$ ). Dietary protein levels influenced ( $P<0.05$ ) the water consumption pattern of the chickens. Birds on high protein diet drank the highest quantity of water ( $7.51\pm 0.58$  litre/bird), though this was not significantly ( $P>0.05$ ) different from those on optimum protein diet ( $7.47\pm 0.39$  litre/bird). The least water intake ( $7.02\pm 0.42$  litre/bird) was found in birds on low protein diet. Broiler chickens on *ad libitum* feeding drank more water ( $7.82\pm 0.32$  litre/bird) which was significantly ( $P<0.001$ ) higher than their counterparts fed on skip-a-day basis ( $6.69\pm 0.31$  litre/bird).

Selected carcass and organ characteristics are presented in Table 3. Once again, there was no significant ( $P>0.05$ ) feeding-method by diet-type interaction (except for relative weight of the abdominal fat) so the overall affect of diet type and feeding method were tested for significance. The results revealed that the relative weight of the abdominal fat and heart were significantly ( $P<0.01$ ) influenced by varying dietary protein levels. Chickens on the low protein diet had significantly ( $P<0.05$ ) higher abdominal fat than those on optimum and high protein diets. The feeding method had no influence ( $P>0.05$ ) on abdominal fat weight. Similarly, chickens on low protein diet had the highest heart weight, though not significantly ( $P>0.05$ ) different from those on optimum protein diet. Birds fed the high protein diet had significantly ( $P<0.05$ ) smaller hearts compared with the other two groups. The relative weights of the breast ( $P<0.001$ ) and gizzard ( $P<0.05$ ) of the birds were significantly influenced by the feeding methods. Chickens on *ad libitum* feeding regime had higher ( $P<0.001$ ) breast weights but lower ( $P<0.05$ ) gizzard weights compared with those on skip-a-day feeding. Generally, higher values were recorded for carcass muscle weights and lower values were recorded for organ weights for *ad libitum* feeding compared with skip-a-day feeding.

## DISCUSSION

The significantly ( $P<0.05$ ) higher final live weight and

weight gain found in birds on high protein diet compared with those recorded for birds on low protein diet is attributable to the reduced quantity of dietary protein supplied by the latter. Adequate protein in term of quantity and quality is essential for muscle growth. Birds on the optimum protein diet had similar final live weight and weight gain as those on the high protein diet revealing the adequacy of nutrients in the optimum protein diet. These measures of growth performance re-emphasised the importance of feed to the livestock industry as earlier reported by Ogunwolere and Onwuka (1997) and Oluyemi and Roberts (2000). For feeding method, birds fed *ad libitum* had higher final live weight gain, feed intake and weight gain compared to their counterparts on skip-a-day feeding regime. This could be attributed to the fact that birds fed *ad libitum* had more access to feed and thus the adequacy of nutrients to support better performance. Birds on control diet (optimum protein) had the highest feed intake compared with those on either low or high protein diets. The observed low feed intake recorded for birds on low protein diet could be due to an imbalance in energy to protein needed for optimum growth hence inhibiting greater intake. Generally, poultry fed low protein diets could reduce their feed intake due to an excess of energy intake. The reduced intake of birds on high protein diets could also be attributed to an imbalance in energy to protein. However, the adequacy or excess of the dietary protein supported high live weight gain. The reduction in feed intake by chickens fed on alternate days was most likely a direct response to birds' limited access to feed and inability to eat more compared with their counterparts fed *ad libitum*, corroborating the findings of Akpa et al. (1999). The feed utilization was not significantly ( $P>0.05$ ) influenced by varying dietary protein levels. However, it was observed that the high protein diet supported the best growth performance with lowest quantity of feed. *Ad libitum* feeding significantly ( $P<0.05$ ) promoted better feed utilisation (lower feed conversion ratio) compared with restricted feeding (skip-a-day) suggesting that a consistent supply of feed to broiler finishers was better in promoting growth performance. Water intake of the birds significantly ( $P<0.05$ ) increased with increasing dietary protein level. This observation agrees with the statement made by Francesch and Brufau (2004) that increased protein consumption increased water consumption. Birds on *ad libitum* feeding significantly higher ( $P<0.05$ ) water intake compared with those on skip-a-day feeding, attributable to higher feed intake under *ad libitum* feeding and the need for more water necessary for digestion of nutrients and excretion of wastes. This corroborates the findings of Leeson and Summer (1997) and Damron and Sloan (1998) that water intake behaviour of a bird is closely associated with feed intake, hence, any factor affecting feed intake will affect the water intake.

Results of the selected carcass characteristics (except for abdominal fat) showed that there was no significant

**Table 3.** Selected carcass and organ characteristics of broiler chickens placed on diets of varying dietary protein and feeding regimes.

Protein level	FMT	Percent dressed weight	Percent eviscerated weight	Relative weights (g/kg live weight)							
				Breast	Thigh	Drumstick	Back	Abdominal fat	Heart	Gizzard	Liver
Optimum protein	ADL	87.73±8.65	78.66±7.85	185.7±32.62	47.88±5.48	45.65±5.12	56.92±8.25	14.15±2.07	5.03±1.02	23.09±1.41	17.58±1.15
	SAD	83.53±10.1	74.83±9.49	134.2±25.03	46.28±4.01	43.01±6.83	55.36±9.41	13.78±3.43	4.29±0.79	25.34±1.89	17.41±3.95
Low protein	ADL	84.34±5.61	77.27±5.50	171.0±26.11	46.04±4.27	45.31±3.67	53.41±8.19	17.14±2.54	4.57±0.84	22.94±4.45	16.90±3.18
	SAD	87.90±6.15	78.12±4.54	148.3±16.31	45.70±3.53	43.03±6.96	54.15±10.6	13.81±4.01	5.59±1.19	27.50±4.22	19.6±1.89
High protein	ADL	83.75±11.4	75.08±11.4	167.4±46.04	45.41±5.14	43.04±6.61	52.15±10.9	12.94±2.28	3.68±0.76	22.85±1.33	16.53±3.40
	SAD	84.72±10.1	76.79±8.29	138.4±23.31	46.96±4.17	45.92±7.04	51.58±9.72	11.97±1.96	3.96±0.76	23.42±3.33	17.52±2.22
<b>Statistical significance</b>											
Protein level		NS	NS	NS	NS	NS	NS	**	**	NS	NS
Feeding method		NS	NS	**	NS	NS	NS	NS	NS	*	NS
Protein level x feeding method		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
<b>Mean separation</b>											
<b>Protein level effect</b>											
Optimum protein		85.13±10.7	76.24±11.4	159.9±38.61	47.08±5.13	44.33±6.31	56.14±9.62	13.96±2.14 <sup>a</sup>	4.66±0.95 <sup>a</sup>	24.22±1.98	17.49±2.77
Low protein		86.62±6.10	77.7±4.83	159.7±23.93	45.87±4.20	44.18±5.44	53.75±9.05	15.48±3.22 <sup>b</sup>	5.08±1.12 <sup>a</sup>	25.22±4.77	18.27±2.87
High protein		84.72±10.31	75.93±9.59	153.0±38.01	46.19±4.93	44.36±6.82	51.87±10.8	12.45±2.01 <sup>a</sup>	3.82±0.66 <sup>b</sup>	23.14±2.44	17.03±2.79
<b>Feeding method effect</b>											
<i>Ad-libitum</i>		85.93±9.59	77.14±9.46	174.81±34.7 <sup>a</sup>	46.44±4.66	44.66±6.16	54.18±9.71	14.74±3.58	4.43±0.98	22.96±2.63 <sup>b</sup>	17.00±2.64
Skip-a-day		85.38±10.3	76.63±8.97	140.29±21.4 <sup>b</sup>	46.31±4.05	43.99±6.75	54.00±16.8	13.18±3.11	4.61±1.14	25.42±3.53 <sup>a</sup>	18.19±2.86

Values are mean ±SD; NS = not significant (P>0.05), \* =P<0.05; \*\*=P<0.01; FMT = feeding method, ADL = *Ad libitum*, SAD = skip-a-day; means with different subscripts within the same column and for the same parameters are significant (P<0.05).

(P>0.05) difference due to quantity of dietary crude protein. This suggested that the experimental diets influence were similar in terms of relative carcass traits of broilers. The higher abdominal fat due to feeding low protein diet is attributable to higher energy to protein ratio in the low protein diet compared with the other diets. Expectedly, the abdominal fat was reduced (P>0.05) by skip-a-day feeding method similar to

earlier report (Oyedemi et al., 2003), for the organs, the relative weight of the heart significantly (P<0.05) decreased with increasing dietary protein. A similar trend was observed with the other organs. A higher energy to protein ratio could have promoted the development of these organs. The generally higher values recorded for carcass characteristics of birds on *ad libitum* feeding compared with skip-a-day feeding could

be attributed to better supply of nutrients for muscle accretion when birds are allowed unrestricted access to feed. It has been reported that adequate nutrition influences muscular growth and carcass traits while malnutrition causes an increase in protein degradation in chicken (Schreurs, 2000). Conversely, relative organ weights were promoted by skip-a-day feeding compared with *ad libitum* feeding. This

may be attributed to the higher musculature of these organs as they would tend to work more to enhance feed digestion and utilization every other day, when feed was provided under the skip-a-day feeding regime. This observation reiterates the fact that the volume of digesta in the gastro-intestinal tract could cause an increase in size and length of the gut parts and muscles of the gizzard.

## Conclusion

The better performance of birds on optimum (19.64% CP) and high (21.66% CP) protein diets compared with low (17.72% CP) protein diet re-emphasised the importance of adequate dietary protein supply in term of quantity and quality to actively growing birds like broiler chickens. However, feeding the 19.64% CP diet was adequate, above which no significance improvement in performance was achieved. *Ad libitum* feeding of broilers produced better growth performance characteristics than skip-a-day feeding. Abdominal fat deposition was promoted by the low protein diet. Other carcass characteristics were not influenced by dietary protein levels but a higher energy to protein ratio promoted the developments of organs. Generally higher values were recorded for carcass characteristics of birds on *ad libitum* feeding compared with skip-a-day feeding. This study reiterates the benefit of feeding a balanced (optimum) protein diet, *ad libitum* to broiler finishers.

## REFERENCES

- AOAC (1990). Association of Official Analytical Chemists. Official methods of analysis (15<sup>th</sup> edition), Washington DC.
- Akpa MO, Ozoude DC, Odo BI (1999). Effects of alternate days feeding programme on the performance of two broiler strains. Book of Proceedings, 26<sup>th</sup> Annual Nigerian Society of Animal Production Conference, held at the University of Ilorin between 21<sup>st</sup> - 25<sup>th</sup> March, 1999.
- Chiba LI (2009). Poultry Nutrition and Feeding. Section 12: Poultry Nutrition and Feeding. Animal Nutrition Handbook, pp. 316-330.
- Damron BL, Sloan DR (1998). Small Poultry Flock Nutrition. Fact Sheet PS-29. Pp 4. [http://mysrf.org/pdf/pdf\\_poultry/p5.pdf](http://mysrf.org/pdf/pdf_poultry/p5.pdf) accessed 29th May, 2012.
- Francesch M, Brufau J (2004). Nutritional factors affecting excreta/litter moisture and quality. *World's Poult. Sci. J.* 60:64-75.
- Leeson S, Summers JD (1997). Water consumption of poultry. *Commercial Poultry Nutrition*. 2nd ed. P. 102.
- NRC (1997). National Research Council. Nutrient Requirement of Poultry. 1st ed. Washington D.C. National Academy of Sciences. National Research Council.
- Ogunwolere YO, Onwuka CFI (1997). Assessment of some qualities of commercial livestock feeds. *Nig. J. Anim. Prod.* 24(2):137-142.
- Oluyemi JA, Roberts FA (2000). *Poultry Production in Warm Wet Climates* (2<sup>nd</sup> Ed). Spectrum Books Limited, Ibadan, Oyo State, Nigeria. P. 244.
- Oyedeji JO, Atteh JO (2003). Response of broiler to 3 weeks feed resistance initiated at different time periods. *Nig. J. Anim. Prod.* 30:157-162.
- Oyedeji JO, Atteh JO, Adedeji SA (2003). Response of broilers to skip-a-day (SAD) feeding. *Nig. J. Anim. Prod.* 30:163-172.
- Schreurs FJG (2000). Post-mortem changes in chicken muscle. *World Poult. Sci. J.* 50:319-346.