PAT June, 2022; 18(1):105-113 ISSN: 0794-5213
Online copy available at www.patnsukjournal.net/currentissue
Publication of Nasarawa State University, Keffi

PERFORMANCE AND ECONOMICS OF PRODUCTION OF BROILER CHICKENS FED EXPONENTIAL LEVELS OF BITTER LEAF (*Vernonia amygdalina*) MEAL

Imouokhome, J.I. and Ilaboya, I.I.

Department of Animal Science, Benson Idahosa University, Benin City, Edo state, Nigeria
**Email: iilaboya@biu.edu.ng Tel +2348058768730*

Abstract
The study was carried out to determine the exponential feeding levels of dried bitter leaf (Vernonia amygdalia) (VA) leaf meal on the performance and economics of production of Arbor Acre broiler chickens as well as to compare the effects of colistin on the same parameters for six weeks. One hundred and ninety-eight chicks were used in the experiment, six dietary treatments, replicated three times with eleven chicks per replicate using a completely randomized design. Control treatment 1 (without colistin and VA), treatment 2 contains 0.77g colistin and 0% VA, treatment 3 to 6 contains 0.1%, 0.5%, 2.5% and 12.5% VA without colistin. Results showed a significant (P<0.05) difference between T1, T5, and T6 with regards to BWG with T1 having the highest (873.73g/bird). Treatments 5 and 6 had the least (P<0.05) BWG with 649.10 and 58.22g/bird respectively. The FCR indicated no significant differences (P>0.05) among birds in T1, T2, T3 and T4, with T1 having the best FCR (3.16). The results also revealed that total cost of feed intake per weight gain, T5 (₦1,668.12) and T6 (₦1,819.33) proved to be the best. Conclusively, VA at 2.5% is recommended as the best inclusion level having comparatively the lowest cost of production.
Keynote: Broiler chickens, Bitter leaf meal, Colistin, Exponential levels.

Introduction

Broilers play a significant role in the provision of animal protein required by man to meet his daily protein intake (Maidala and Istifanus, 2012). Feed constitutes the greatest and most costly input in any poultry farm. Thus, any significant reduction in the cost of feed will significantly reduce the overall cost of production and increase the profit margin of the farm (Owen and Amakiri, 2011). Due to the high cost of conventional drugs as well as protein ingredients like fish meal, groundnut cake, and soybean, research is now towards identifying non-conventional sources that are locally available with low human demands (Owen *et al.,* 2009). One of such conventional feed sources is bitter leaf (*Vernonia amygdalina*) meal (Mohammed and Zakariya 2012). *Vernonia amygdalina* (VA) is a shrub or small tree that grows throughout tropical Africa. It is popularly called bitter leaf because of its abundant bitter principles (Ekpo *et al.,* 2007). The leaves contain a considerable amount of anti-nutritive factors like high levels of tannic acid and saponin (Farombi and Owoeye, 2011). The findings by Akwaowo *et al.* (2000) reported that young leaves often preferred for human consumption, contain high cyanide (60.1mg 100-1 g DM) and tannin content (40.6mg 100-1 g DM) than older ones. Proximate composition of *Vernonia amygdalina* leaf meal (VALM) showed a chemical composition of 527.83 ME kcal/ kg, 86.40% DM, 21.50% CP, 13.10% CF, 6.80% EE, 11.05% Ash, and the result on mineral composition indicated that *V. amygdalina* has 3.85% Calcium, 0.40% Magnesium, 0.03% Phosphorus, 0.006% Iron, 0.33% Potassium and 0.05% Sodium (Owen, 2011).

With the emergence of drug-resistant strains of *Eimeria* in populations of commercial flocks and increasing public concern about drug residues in meat (Williams, 2006; Bafundo *et al*., 2008), alternative treatments are urgently needed (Dongjean *et al.,* 2011). Medicinal plants and herbs have been found useful for many years in the treatment of various diseases in animals and man.

Research has shown that *V. amygdalina* plant native to Nigeria has some beneficial effects on disease management of poultry, such as anti-coccidiosis, anti-bacterial and anti-parasitic (Nweze and Obiwulu, 2009; Gbolade, 2009; Tadesse *et al.,* 1993); as an anti-oxidant (Erasto *et al.,* 2007; Dakpogan, 2006) and as a growth promoter by enhancing the gastrointestinal enzymes thus, increasing feed conversion efficiency (Huffman *et al.,* 1996; Olobatoke and Oloniruha, 2009). Therefore, the objectives of this study were to determine the growth performance and economics of production of broiler chickens fed exponential levels of bitter leaf meal.

Materials and Methods

Management of birds and experiment design

The experiment was carried out in the Poultry unit of the Department of Animal Science Teaching and Research Farm of Benson Idahosa University, Ugbor, Benin City, Edo State.
A total of two hundred Arbor Acre strains of broiler chicks were used for the experiment. The chicks were obtained from a reputable hatchery in Ibadan. The chicks were distributed randomly into six dietary treatment groups of three replicates each with eleven birds in each replicate in a completely randomized design. The experimental design involved a control (without any level of antibiotic or bitter leaf) one of the treatments with antibiotics of choice which was colistin and four treatments with exponential levels of bitter leaf (0.1%, 0.5%, 2.5%, and 12.5%). Feed and water were routinely administered to the birds. The birds were raised in cages in an open-sided wire mesh constructed poultry house to allow for adequate ventilation. Colistin was only used as antibiotic in treatment two.

Sources and process of *Vernonia amygdalina*

Vernonia amygdalina leaves were collected from nearby bushes behind Benson Idahosa University Teaching and Research Farms and other surrounding areas within Benin City, Edo state. The bitter leaves were sun-dried for several days, crushed, and ground into meal using a commercial hammer mill. The meal was later added in different percentages to the different treatment groups. *Vernonia amygdalina* was later incorporated into the experimental diets at 0g/100g for treatments 1 and 2, 0.1g/100g, and 0.5g/100g, 2.5g/100g and 12.5g/100g for treatments 3, 4, 5, and 6 respectively. Table 1 shows the gross composition of experimental diets.

Data Collection

The body weight (BW) of the chicks was taken at the beginning of the experiment and subsequently on weekly basis using sensitive Camry electronic kitchen weighing scale (EK5350 model®). Feed intake (FI) was determined by the difference in the quantity of feed offered in each replicate and the left-over at the end of the week. Weight gain (WG) was calculated by subtracting the initial weight at the beginning of each week from the final weight at the end of each week. The feed conversion ratio (FCR) was computed as the total feed intake divided by the weight gain. Mortality per replicate was recorded throughout the experiment. Percentage mortality was calculated by dividing the number of dead birds by the total number of birds in the pen and multiplied by 100 as shown in equation 1

$$\%\ \text{Mortality} = \frac{Number\ of\ dead\ birds}{Total\ number\ of\ birds} \times 100 \quad \text{......................equation 1}$$

Table 1: Experimental Diets Composition

Ingredients	Without VA		With VA			
	T1	T2	T3	T4	T5	T6
	- Colistin	+ Colistin	0.10%	0.50%	2.50%	12.50%
Maize	45	45	45	45	44.4	38.6
Soybean meal	29.4	29.4	29.6	29.7	28.5	22.6
Wheat bran	9.9	9.9	9.7	9.3	8.3	7.4
Fish meal	6	6	5.9	5	6.5	9.1
Bitter leaf meal	0	0	0.1	0.5	2.5	12.5
Palm oil	5	5	5	5	5.1	6.1
Limestone	1.5	1.5	1.5	1.5	1.7	1
Dicalcium phosphate	2	2	2	2	2	2
sodium chloride	0.2	0.2	0.2	0.2	0.2	0.2
*Vitamins and premix	0.5	0.5	0.5	0.5	0.5	0.5
Methionine	0.3	0.3	0.3	0.3	0.3	0.3
Lysine	0.2	0.2	0.2	0.2	0.2	0.2
Antibiotics (colistin)	-ve	+ve	-ve	-ve	-ve	-ve
Total	100	100	100	100	100	100
Calculated Nutrient level						
ME kcal/kg	3,025.31	3,025.31	3,025.5	3,025.54	3,025.72	3,025.45
CP(g/kg)	220.15	220.15	220.29	220.21	220.65	220.43
Ca (g/kg)	10.64	10.64	10.62	10.64	10.28	10.94
Available Phosphorus	9.76	9.76	9.72	9.66	9.3	9.28

***Supplied per kg of diet-vitamin A-5000IU, vitamin D3 800iu, vitamin E-12mg, vitamin B6-.5mg, pantothenic acid 5mg, Biotin-0.02, vitamin B120.0mg, Folic acid 0.3mg, chlorine-chloride 50mg, manganese 60mg, iron 10mg, zinc15mg, copper 0.8mg, Iodine 0.4mg, cobalt 0.08mg, selenium 0.04mg and anti-oxidan40mg, colistin 0.77g**

Economic Analysis

At the end of the study period, the cost analysis and the gross margin analysis were carried out to determine the profitability of broiler birds fed exponential levels of *Vernonia amygdalina* using the following formulae:

(a) $\text{Cost/Kg Feed} = \Sigma \dfrac{\text{Proportion of each ingredient in the diet x cost per kg of the ingredient}}{100}$

(b) Feed cost/bird = Feed consumed x cost/kg feed

(c) Feed cost/kg live weight = cost/kg feed x feed: gain ratio

Statistical Analysis

Data collected were analyzed using the SPSS (2013) package version 16, and means were separated using the least significance difference (LSD) range test.

Statistical Model

Yijl = μ + Ti + ∑ij

Where: Yijl = Observed value of the dependent variable

μ = population mean Ti = effect of the ith treatment

∑ijl = Random residual error

Results

The results of growth performance characteristics of broiler chicken fed exponential levels of *Vernonia amygdalina* within the first three weeks of production presented in table 2, indicated that there was no significant difference (P>0.05) in feed intake across the dietary treatments with T4 having the highest value (916.00g/bird) and T6 having the lowest value (863.00g/bird). Body weight gain (BWG) and feed conversion ratio (FCR) showed no significant difference (P>0.05) among the treatments (T2, T3, and T4) but there was significant variation (P<0.05) between T1 (334.10g, 2.68) and the other treatments T5 (297.60g, 3.00), and T6 (235.80g, 3.66).

Table 2: Performance Characteristics of Broiler Chickens Fed *V. amygdalina* (0 – 3 Weeks)

Parameters	Without VA		With VA				
	T1	T2	T 3	T4	T5	T6	
	- colistin	+ colistin	0.10%	0.50%	2.50%	12.50%	SEM
Feed intake (g)	893.78	898.8	903	916	891.4	863	19.48
Weight gain (g)	334.10^{a}	357.60^{a}	342.10^{a}	341.60^{a}	297.60^{b}	235.80^{b}	14.79
FCR	2.68^{a}	2.51^{a}	2.64^{a}	2.68^{a}	3.00^{b}	3.66^{b}	0.34
Mortality (%)	1	0	0	1	0.5	0.5	

SEM = Standard error of mean, FCR = Feed conversion ratio, VA = *Vernonia amygdalina* Minus =No colistin + = with 0.77g colistin

The performance results characteristics of broiler chicken fed exponential levels of *Vernonia amygdalina* at 4 to 6 weeks of production presented in table 3, showed that there was no significance difference (P>0.05) in feed intake among the different diets groups with T5 having the highest value (1,953.00g/bird) and T4 having the lowest value (1,580.00g/bird). Body Weight Gain for T1 performed significantly (P<0.05) better (544.60g/bird) followed by T2 (466.37g/bird) compared to T3, T4, T4 and T6 which were not significantly (P>0.05) different. Feed conversion ratio for T1 and T2 were not significantly (P>0.05) different (3.43 and 3.39) but were significantly different (P<0.05) among T3 and T4 (4.42 and 4.02), the least was recorded in T5 and T6 (5.43 and 5.42).

Table 3: Performance Characteristics of Broiler Chickens Fed *V. amygdalina* (4-6 Weeks)

	Without VA		With VA				
	T1	T2	T 3	T4	T 5	T 6	
Parameters	- colistin	+ colistin	0.10%	0.50%	2.50%	12.50%	SEM
Feed intake (g)	1,868	1,581	1,724	1,580	1,953	1,926	195.6
Weight gain (g)	544.60[a]	466.37[ab]	390.05[b]	393.03[b]	359.68[b]	355.35[b]	23.87
FCR	3.43[a]	3.39[a]	4.42[b]	4.02[b]	5.43[c]	5.42[c]	3.81
Mortality (%)	0.5	2.5	1.5	0.5	0.5	0.5	

SEM = Standard error of mean, FCR = Feed conversion ratio, VA = *Vernonia amygdalina* ; - = No colistin, + = with 0.77g colistin

Table 4 showed the cumulative results of the six weeks experiment recording no significant difference (P>0.05) in feed intake among the various treatments (T1 – T6), however, there were significant differences (P<0.05) between T1, T5, and T6 with regards to BWG. Body weight gain was highest with T1 (873.73g/bird) followed by T3, T2, and T4 (having 766.80g, 744.40g, and 721.30g/bird), respectively. Treatment 5 and T6 had the least significance (P<0.05) body weight gain with 649.10g and 58.22g/bird respectively. Feed Conversion Ratio indicated no significant (P>0.05) difference among birds in the first four treatments, however, diet treatments 5 and 6 showed a significant difference (P<0.05) with lesser performance (4.3 and 4.7) respectively with treatments T1 having the best FCR 3.16).

Table 4: Performance Characteristics of Broiler Chicken Fed *V. amygdalina* (0-6weeks)

	Without VA		With VA				
	T1:	T2:	T 3:	T 4:	T5:	T 6:	
Parameters	- colistin,	+ colistin	0.10%	0.50%	2.50%	12.50%	SEM
Feed intake (g)	2,761	2,479	2,627	2,496	2,844	2,789	210
Weight gain (g)	873.73[d]	744.40[b]	766.80[b]	721.39[b]	649.10[c]	538.22[c]	111.8
FCR	3.16[a]	3.33[b]	3.43[b]	3.46[b]	4.38[c]	4.37[c]	0.48
Mortality (%)	1.5	2	1.5	1.5	1	1	

SEM = Standard error of mean, FCR = Feed conversion ratio, VA = *Vernonia amygdalina*, - =No colistin + = with 0.77g colistin

The analysis of broiler chickens reared for six weeks and fed exponential levels of VA is presented in table 5. The results revealed that feed cost ranges from ₦352.13/kg to ₦382.06/kg, total feed cost range from ₦947.51/kg to ₦1,084.24/kg, while the feed cost per gain weight range between ₦1,668.12/kg to ₦1,324.35kg gain.

The results shows that the total feed intake was highest in chickens fed T5 and T6 (2.84kg and 2.79kg) respectively, while the lowest was recorded in T2 (2.28kg). The lowest feed cost was found in T6 (₦352.13). The highest total weight gain was observed in chickens fed T1 (0.87kg) while T6 (0.54kg) recorded the lowest. The highest value of feed cost per kg gain was observed in T4 (₦1,324.35/kg) while the lowest was in chicken fed T5 (₦1,668.12/kg).

Table 5: Economic Analysis of Broiler Chickens Fed Exponential Levels of *Vernonia armygdalina*

	Diets Without VA		With VA			
	T1	T2	T3	T4	T5	T6
Parameters	- colistin	+ colistin	0.10%	0.50%	2.50%	12.50%
Initial weight (g/bird)	38.85	38.75	39	38.8	39.1	39
Final weight (g/bird)	912.58	783.15	805.8	760.19	688.2	567.22
Total feed intake (kg/bird)	2.76	2.48	2.63	2.5	2.84	2.79
Cost of feed (₦/kg)	382.06	382.06	381.97	381.41	381.79	352.13
Cost of total feed intake (₦)	1,054.49	947.51	1004.58	953.53	1084.28	982.44
Total weight gain (kg)	0.87	0.74	0.77	0.72	0.65	0.54
feed cost/kg gain (₦)	1,212.06	1,280.42	1,304.65	1,324.35	1,668.12	1,819.33

- =No colistin, + = with 0.77g colistin

Discussion

The result pertaining to FCR in this study is in line with the findings of Olobotoke and Oloniruha (2009) who reported that the inclusion of VA powder in cockerels feed, significantly improved FCR. This could be associated with its effect on enhancing the gastrointestinal enzyme thereby improving digestion and assimilation of nutrients (Adaramoye, *et al.,* 2008). The findings by Windisch (2007) also reported improved growth performance of animals fed VA. Furthermore, the report of Mohammed and Zakariya (2012) supported the observations made by Abubakar *et al*., (2010) that phytogenic feed additives are often associated with the improvement of flavor and palatability of feed, thus bitter leaf extract enhances the production performance of birds. However, the observations made by Mohammed and Zakariya (2012) pertaining to improvement of weight gain and FCR in broilers are contrary to the present result. This may be attributed to the levels of inclusion of VA in diet as well as the interaction of genotype and environmental effect of the animals in the different locations.

The feed cost per kg was lowest (₦352.13) in T6 compared with the other chicken diets used in the experiment. This could be attributed to the high inclusion level (12.5%) of VA as well as the low cost of sourcing and processing it. The cost of total feed intake was highest (₦1,054.49) in T1 (control diet without colistin) while the lowest value (₦947.51) in T2 (control diet with colisten, without VA). This report is in agreement with the report of Mubarak and Ahmed (2019) when an appraisal of the performance of broiler birds fed with different commercial feed was carried out in Kano state of Nigeria. It is also in tandem with the report of Isikwenu *et al*. (2008), when broiler chicks fed groundnut cake with urea fermented brewer's dried grain. T4(0.5% VA inclusion) had the best total cost of feed intake (₦953.53) than others, but in terms of the total cost of feed intake per weight gain, T5 (₦1,668.12) and T6 (₦1,819.33) revealed the best when compared with others. This result is in disagreement with the report presented by Raji *et al.,* (2021) showing that the control diet had the best cost of feed intake than others but in terms of cost of feed intake per weight gain 10% and 20% replacement levels of sun-dried cassava starch pulp revealed the best result.

Conclusion

In conclusion, the six weeks experiment shows that apart from T5 and T6 (with 2.5% and 12.5%) inclusion which showed the least performance in terms of body weight gain, there was no marked significant differences between T2 (control with colistin as an antibiotic) and the ones with 0.1% and 0.5% VA inclusion levels (T3 and T4) but T1 (control without colistin and VA) had the best performance in terms of weight gain (873.73g/bird) and FCR (3.16). However, the economic analysis revealed that T4 (0.5% VA inclusion) had the best total cost of feed intake (₦953.53) than others, but in terms of the total cost of feed intake per weight gain, T5 (₦1,668.12) and T6 (₦1,819.33) had the best when compared with others. The experiment however showed that VA is a good feed additive that could be used as an alternative source of colistin as an antibiotic, with little or no advert effects on boiler birds. Therefore, *Vernonia amygdalina* at 2.5% will be recommended as the best inclusion level having comparatively the lowest cost of production.

References

Abubakar, A.L., Malik, O.O., Bashar, Y.A., and Muffau, M. (2010). Growth Performance of Broiler finishers fed Garlic (*Allium salivium*) supplemented diets. *Proc. 35th conf. Nig. Soc. for Anim. Prod.* UNIBADAN, Oyo state; 372-374

Akwaowo, E. U., Ndon, B. A., and Etuk. (2000). Minerals and antinutrients in fluted pumpkin (*Telfaria accidentalis* Hook F.). *Food Chem*; 70: 235-240.

Bonsi, M. L .K. Osuji, P. O., Tuah, A. K. and Umunna, N. N. (1995). *Vernonia amygdalina* as a supplement to teff straw (Eragrostistef) fed to Menz sheep. *Agro For. Syst*; 31:229-241.

Bafundo, K.W, Cervantes, H.M, Mathis, G.H. (2008). Sensitivity of *Eimeria* field isolates in the United States: responses of nicarbazin-containing anticoccidials. *Poultry Sci.* 87: 1760-1767.

Farombi, E.O. and Owoeye, O. (2011). Antioxidative and Chemopreventive Properties of *Vernonia amygdalina* and Garciniabi flavonoid. *International journal of Environ. Res. Publ. Health* 8: 2533 – 2555.

Dongjean Y, Sang S. K., Dong, W. K,, Sang H. K., Hyun, S. L., and Wongi, M. (2011). Protective effects of Aloe vera-based diets in Eimeria maxima-infected broiler chickens. Experimental Parasitology. 127(1): 322-325

Dakpogan, H.B. (2006). Free range chick survivability in improved conditions and the effect of 3 medicinal plants on *Eimeria tenella*. M.Sc. Thesis, Department of Veterinary Pathobiology, The Royal Veterinary and Agricultural University, Denmark.

Ekpo, A., Eseyin, O.A., Ikpeme, A.O., and Edoho, E.J. (2007). Studies on Some Biochemical Effects of *Vernonia amygdalina* In Rats. *Asia Journal of Biochemistry*; 2(3): 193- 197.

Erasto, P., Grierson, D.S., and Afolayan, A.J. (2007). Evaluation of Antioxidant activity and the fatty acid profile of the leaves of *Vernonia amygdalina* growing in South Africa. *Food Chem;* 104:636-642.

Gbolade, A. A. (2009). Inventory of antidiabetic plants in selected districts of Lagos State, Nigeria. *J. Ethnopharmacol;* 121:135-139.

Huffman, M. A., Koshimizu, K., and Ohigashi, H. (1996). Ethnobotany and Zoopharmacognosy of *Vernonia amygdalina*, A Medicinal Plant Used by Humans and Chimpanzees in the Wild. In: Compositae: Biology and Utilization, Caligari, P.D.S. and D.J.N. Hind (Eds.). The Royal Botanical Garden, Kew; 351-360.

Isikwenu, J.O., Omeje, S.I., Okagbare, G. and Akpodiete, O.J. (2008). Effect of replacing groundnut cake with urea fermented brewer's dried grains in broiler chick diets. Animal Research International, 5 (1): 795 – 800.

Maidala, A. and Istifanus, J.A. (2012).The role of micro livestock in alleviating protein deficiency and poverty reduction in Nigeria. *Proc. 2nd National conference of school of vocational and technical education,* College of Education, Azare. Bauchi state.

Mohammed, I., and Zakariya, R. (2012). Bitter leave (*Vernonia amygdalina*) as a feed additive in broiler diets. *Research Journal of Animal Sciences*, 6(3):38 – 41

Mubarak, H. and Ahmed, A. (2019). An Appraisal of performance of broiler birds fed with different commercial feed in Kano state of Nigeria. *Journal of Agricultural Research and Development.*18:57-62.

Nweze, N.E., and Obiwulu, I.S. (2009). Anticoccidial effects of *Ageratum conyzoides*. *Journal of Ethnopharmacol.* 122: 6-9.

Olobatoke, R. Y., and Oloniruha, J. A. (2009). Haematological assessment of bitter leaf (*Vernonia amygdalina*) efficiency in reducing infections in cockerels. *Proc. World Congress on Medicinal and Aromatic Plants,* Cape Town, South Africa; 472-473.

Owen, O. J., Amakiri, A. O., David, E. U., Nyeche, V. N., and Ndor, L. (2009). Proximate composition, energy content, and mineral profile of *Vernonia amygdalina* (Bitter Leaf) meal. *Proc.14th Ann. Conf. Animal Science Association of Nigeria* LAUTECH, Ogbomoso, Oyo State; 173-176.

Owen, O. J., and Amakiri, A. O. (2011). Serological and haematological profile of broiler finishers fed graded levels of bitter leaf (*Vernonia amygdalina*) meal. *Adv. Agric. Biotechnology*; 1: 77-81.

Owen, O. J. (2011). Proximate Composition, Energy Content and Mineral Profile of *Vernonia amygdalina* (Bitter Leaf) Meal Advance In Food and Energy Security; 1:36-39.

Raji, M.O., Adams, T.O., Oloko, B.A., Fasari, M.O., Adebayo, H.D., Usman, A.B., Oso, A.O., and Bamgbose, A.M. (2021). Evaluation of performance characteristics and cost implication of broiler chicks fed varying levels of sundried cassava starch extract pulp. *Journal of Animal Production Research* 33 (1):97-103.

SPSS (2013). Statistical Package for Social Sciences. SPSS Version 22 IBM Inc. 444 Michigan Avenue, Chicago, IL60611, USA.

Tadesse, A. A., Gebie-Hiwot, K., Asres, M., and Djote, F. D. (1993). The *in vitro* activity of *Vernonia amygdalina* on Leishmaniaa ethiopica. *Ethiop. Med. J*; 31: 183-189.

Williams, R.B. (2006). Tracing the emergence of drug-resistance in coccidia (*Eimeria sp.*) of commercial broiler flocks medicated with decoquinate for the first time in the United Kingdom. Vet. Parasitol. 135: 1-14.

Windisch, W., Schedle , K., Plitzer, C., and Kroismayr, A. (2007). Use of phytogenic products as feed additives for swine and poultry. *J. Anim. Sci;* 86:140-148