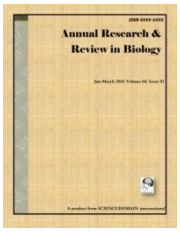

Annual Research & Review in Biology
4(4): 675-683, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Performance and Some Immunological Parameter Responses of Broiler Chickens to Licorice (*Glycyrrhiza glabra*) Extract Administration in the Drinking Water

Naser Moradi[1], Shahab Ghazi[1], Tahereh Amjadian[1], Hassan Khamisabadi[2] and Mahmood Habibian[1*]

[1]Department of Animal Science, Faculty of Agriculture, Razi University, Kermanshah, Iran.
[2]Department of Animal Science, Agriculture and Natural Resources Research Center, Kermanshah, Iran.

Authors' contributions

Major contribution for this study came from author SG and he carried out this study with the other assistants. Authors HK and NM took part in planning of the study. Author NM did the poultry examination, carried out this study and collected the data. Authors TA and MH took part in data collection and in preparation of the final manuscript.

Original Research Article

Received 12th June 2013
Accepted 4th October 2013
Published 13th November 2013

ABSTRACT

This study was conducted to evaluate the effect of *Glycyrrhiza glabra* root (licorice) extract (LE) administration through drinking water on the performance and some immunological parameters of broiler chickens. A total of 320 onedayold broiler chicks (Cobb 500) according to a completely randomized design were assigned into four treatment groups, namely 0LE, 0.1LE, 0.2LE and 0.3LE because the experimental treatments comprised a control (no inputs) and/or three levels of LE (0.1, 0.2 and 0.3 mg/L drinking water). There was not a significant difference in body weight; feed intake and feed conversion ratio among the birds given the control or the LE levels during the experiment ($P > .05$). Similarly, LE supplementation through drinking water had no significant ($P > .05$) effect on immunological parameters including antibody titers against Newcastle disease and Influenza viruses, heterophil and lymphocyte percentages and heterophil to lymphocyte (H/L) ratio as well as liver and lymphoid organ (bursa of Fabricius, thymus and spleen) weights.

**Corresponding author: Email: mahmood_habibiyan@yahoo.com;*

Keywords: Broiler; glycyrrhiza glabra; herb extract; growth; immunity.

1. INTRODUCTION

Glycyrrhiza (G.) glabra, family Leguminosae, is a plant which growth in Iran and other countries of the world. Its root possesses some nutritive value and medicinal properties. They are widely used as a cold beverage, in preparing some pharmaceutical preparations such as hematinic pills and to disguise the bitter taste of other remedies [1,2].

Phytochemical analysis of LE showed that it contains saponin triterpenes (glycyrrhizin, glycyrrhetinic acid and licorice acid), flavonoids (liquiritin, isoflavonoids and formononetin) and other constituents such as coumarins, sugars, amino acids, tannins, starch, choline, ascorbic acid, phytosterols and bitter principles [3-5].

Clinical animal studies on LE and the isolated active constituents revealed that both possess numerous pharmacological effects. Thus the extract has been used for the treatment of different diseases such as Addison's disease [6], bronchitis, cough, arthritis, rheumatism, hypoglycemia [3], inflammatory and allergic conditions [7], gastric ulcer [8, 9] and chronic hepatitis B and C [10].

In a previous study, Sedghi et al. [11] found that supplementation of LE (0.5, 1, or 2 g/kg) in broilers diets had no significant effect on body weights and feed efficiency of birds, whereas the addition of LE to broiler diets reduced abdominal fat content and serum concentrations of cholesterol and low density lipoprotein-cholesterol as compared to the control. Others reported that *G. glabra* plant was used with other medicinal plants as aqueous extract of plant mixture in broiler diets [12,13]. However, the effects of LE supplementation on the immunological responses of broiler chickens have not been well documented. Therefore, the present study was carried out to investigate the effect of LE administration through drinking water on the performance and some immunological parameters of broiler chickens.

2. MATERIALS AND METHODS

2.1 Birds and Experimental Treatments

All experimental protocols adhered to the guidelines of, and were approved by, the Animal Ethics Committee of Razi University (Kermanshah, Iran). From a commercial broiler chick hatchery, located in Kermanshah Province, Iran, a total of 320 one-day-old broiler chicks (Cobb 500) were purchased and were used for this study from February through March 2011. The broilers were housed in identical-sized floor pens (16 pens; 10 males and 10 females per pen; 0.21 m^2 per bird) containing straw as a litter base. Environmental temperature was set at 33 ± 2°C for the first week and 30 ± 2°C for the second week, which was further decreased to 23 ± 2°C until the end of the experiment. Relative humidity was not measured and uncontrolled throughout the study. During the first week, the light regimen was continuous, which was reduced to 23 hours of light afterward.

The birds were randomly divided into 4 groups and kept to 42 days of age. Each group had 4 replicates. Birds were fed *ad libitum* and diets contained no antimicrobial growth promoters. The basal diet with ingredient composition is shown in Table 1. Water was supplemented with 0.1, 0.2 and 0.3 mg of LE/L of drinking water for those chickens in the treatment groups. Control birds received tap water throughout the experiment. Water not treated with chlorine

was premixed with LE in the water tank and was delivered through a water line to a nozzle. The LE was obtained from Zagros Company (Kermanshah, Iran: http://www.zagros-licorice.com). Water was provided ad libitum throughout the study.

2.2 Growth Performance

Data on feed intake and body weight were recorded on days 1, 21 and 42, whereas mortality was recorded daily throughout the experimental period. Feed intake was corrected for mortality.

2.3 Humoral Immune Response and Blood Cell Counts

All chicks were intramuscularly immunized with killed vaccine of Newcastle and Avian Influenza (H9 N2) viruses at age of 8 days. Live Newcastle disease vaccine (Nobilis ND Lasota) was administered orally (drinking water) at day 22. On days 21 and 42, eight chicks were selected randomly from each treatment (two chicks per replicate) and blood samples were collected from the wing vein into a 5-ml syringe. Part of the blood was placed in tubes with heparin as anticoagulant in order to heterophil and lymphocyte enumeration based on the procedures of Gross and Siegel [14]. Briefly, two drops of blood were placed on a slide, spin prepared, and stained with May-Grünwald-Giemsa stain. All slides were coded, and heterophils and lymphocytes were counted to a total of 100 cells per slide by the same individual, and the heterophil to lymphocyte (H/L) ratio was calculated. The other part of the blood was allowed to coagulate at room temperature for 1–2 hours. The serum was separated by centrifugation at 1,500 g for 5 minutes, and stored at −20°C until used for analysis. The serum antibody titers against Newcastle and Influenza viruses were determined by hemagglutination inhibition (HI) test [15]. All titers were expressed as the $\log_2$ of the reciprocal of the highest dilution giving visible hemagglutination.

2.4 Organ Collection

Eight chicks per treatment (4 male and 4 female) were weighed and killed at the end of experiment. Thymus, spleen, bursa of Fabricius, and liver were collected and individually weighed. Relative lymphoid organ and liver weights were calculated as a proportion of live body weight.

2.5 Statistical Analysis

The data were analyzed using the general linear model procedure of SAS software [16] as a complete randomized design. Differences among treatment means were determined using the Duncan's multiple-range test. Before analysis, antibody titers were transformed to $\log_2$ and mortality percentage to arc sine square roots. The experimental unit differed according to the parameter measured. For performance characteristics, the experimental unit was pen, whereas individual chick data were used for immunological parameters.

Table 1. Ingredients and nutrient level of basal diets

Ingredients (%)	Starter (1-21)	Grower and Finisher (21-42)
Corn Seed	58.91	69.94
Soybean meal, CP 48 %	35.82	24.19
Soybean oil	1.59	0.50
Oyster shell	1.32	
Dicalcium phosphate	1.47	1.57
Salt	0.29	0.40
Mineral-vitamin premix[1]	0.50	0.50
DL-methionine	0.10	0.15
Nutrients composition		
Metabolizable energy (kcal/kg)	2,919	3,000
Crude protein	21.00	18.75
Calcium	0.91	0.84
Available phosphorus	0.41	0.33
Methionine + cysteine	0.82	0.67
Lysine	1.28	1.09

[1]Mineral-vitamin premix provided the following per kilogram of diet: vitamin A, 9,000 IU; vitamin D_3, 2,100 IU; vitamin E, 30 mg; nicotinic acid, 30 mg; vitamin B_{12}, 0.12 mg; calcium pantothenate, 10 mg; vitamin K_3, 5 mg; thiamin, 1.1 mg; riboflavin, 4.5 mg; vitamin B_6, 2.0 mg; folic acid, 0.5 mg; biotin, 0.5 mg; Fe, 50 mg; Cu, 10 mg; Mn, 70 mg; Zn, 50 mg; Co, 0.2 mg; I, 1.0 mg; Se, 0.3 mg; butylated hydroxytoluene (BHT),150 mg.

3. RESULTS AND DISCUSSION

3.1 Growth Performance

As shown in Table 2, the addition of LE did not significantly influence broilers body weight and weight gain during both the starter (1-21 days) and grower (21-42) periods of the study ($P > .05$). This is not unexpected as LE supplementation has been shown numerous times to not influence body weight of chickens [11, 17]. The LE addition through drinking water also had no effect on feed intake ($P > .05$). Similarly, the broilers receiving the different levels of supplemental LE were identical in feed conversion ratio ($P > .05$). These results are comparable with those reported recently by other researchers [11, 17], who studied the effect of dietary supplementation of LE on broiler chickens (0.5, 1.0 and 2.0 g/kg) and Japanese quails (0.2 g/kg). However, previous studies with other animal species have shown that licorice flavonoids (0.5-2.0% of diet for 8-12 weeks) suppress body weight by reducing body fat content [18-20]. They suggested the enhancement of fatty acid oxidation and reduction in biosynthesis of fatty acids as the possible mechanisms for the reduction of abdominal fat and lower body weight gains. Recently published kinetic data [21] have confirmed this proposition and have indicated that LFO is more effective in obese animals and overweight subjects rather than normal healthy subjects.

Table 2. Effect of licorice extract supplementation via drinking water on body weight (g), weight gain (g/chick/day), feed intake (g/chick/day), feed conversion ratio (g/g) and mortality rate (%) of broiler chicks[1]

		Licorice extract (mg/L)			
Item	**Control**	**0.1**	**0.2**	**0.3**	***P* values**
Body weight					
Day 1	42.6 ± 0.26	41.6 ± 1.52	43.1 ± 0.19	41.1 ± 0.69	.39
Day 21	850.5 ± 9.83	857.7 ± 16.04	862.9 ± 8.22	845.3 ± 17.26	.80
Day 42	2667.8 ± 47.31	2604.5 ± 77.11	2629.6 ± 45.83	2611.6 ± 52.82	.86
Weight gain					
Days 1-21	38.5 ± 0.47	38.9 ± 0.70	39.0 ± 0.39	38.3 ± 0.85	.83
Days 21-42	86.5 ± 1.78	83.2 ± 3.07	84.1 ± 1.87	84.1 ± 2.07	.75
Days 1-42	62.5 ± 1.13	61.0 ± 1.80	61.6 ± 1.09	61.2 ± 1.26	.87
Feed intake					
Days 1-21	70.9 ± 0.38	72.0 ± 0.60	71.7 ± 0.47	70.3 ± 0.66	.16
Days 21-42	160.3 ± 1.01	160.6 ± 0.58	160.4 ± 1.78	163.2 ± 0.62	.24
Days 1-42	115.6 ± 0.69	116.3 ± 0.26	116.1 ± 0.67	116.8 ± 0.27	.47
Feed conversion					
Days 1-21	1.84 ± 0.026	1.86 ± 0.045	1.84 ± 0.028	1.84 ± 0.025	.97
Days 21-42	1.86 ± 0.039	1.94 ± 0.066	1.91 ± 0.021	1.95 ± 0.045	.50
Days 1-42	1.85 ± 0.035	1.91 ± 0.054	1.89 ± 0.023	1.91 ± 0.037	.70

[1] *Values are treatment means (±SE). Data from 80 birds per treatment.*

3.2 Antibody Responses against Newcastle Disease and Influenza Viruses

Hemagglutination inhibition antibody titers against Newcastle disease and Influenza viruses are shown in Table 3. The level of LE in the drinking water did not influence antibody titers ($P > .05$). These results did not consistent with the previous report by Khaligh et al. [13], which found higher antibody titers against Newcastle disease virus in broilers fed combined dietary supplementation of alfalfa leaves meal, cinnamon, burdock root and licorice root (10 g/kg of diet at equal ratios). The same opinion was expressed by Yu-ying et al. [12] when chicks fed diet containing different supplementary levels of four Chinese herbal extract. Our results (Table 3) did also not match the findings of an increased survival rate after intraperitoneal administration of Glycyrrhizin (0.2 ml of a saline solution/mouse 1 day before infection and 1 and 4 days postinfection) in mice infected with 20 and 10 LD_{50}s of influenza virus (H2 N2) [22]. At present, the exact mechanism of these differences is still not clear. Nevertheless, factors such as the route and the level of administration, differences in background of the targeted populations, age and sex of the animal, overall farm hygiene, stressor severity and level of stress response may influence the efficacy of herbal extracts application.

Table 3. Effect of licorice extract supplementation via drinking water on antibody titer against Newcastle (log_2 HI titer) and Influenza virus (log_2 HI titer) at different ages[1]

		Licorice extract (mg/L)			
Item	**Control**	**0.1**	**0.2**	**0.3**	***P* values**
Newcastle					
Day 21	4.00 ± 0.408	4.00 ± 0.707	2.75 ± 0.629	3.25 ± 0.629	.41
Day 42	6.25 ± 0.629	5.00 ± 0.913	6.75 ± 0.479	6.50 ± 1.041	.44
Influenza virus					
Day 21	1.00 ± 0.408	1.75 ± 0.479	0.75 ± 0.250	1.75 ± 0.250	.16
Day 42	2.75 ± 0.854	2.25 ± 0.250	3.75 ± 1.250	3.50 ± 0.646	.57
Mortality	1.00	0.90	1.02	0.97	.53

[1] *Values are treatment means (±SE). Data from 8 birds per treatment.*

3.3 Heterophils, Lymphocytes, and H/L Ratio

The H/L ratios obtained in the present study are shown in Table 4. Percentages of heterophils and lymphocytes and the H/L ratio at days 21 and 42 were unaffected by LE treatments ($P > .05$). These results are in accordance with the findings of Sedghi et al. [11] who found no significant effects on heterophil, monocyte and lymphocyte percentages as well as H/L ratio or red blood cell proliferation as a result of dietary LE (0.5, 1.0 and 2.0 g/kg) supplementation. However, Al-Daraji [23] reported a significant decrease in H/L ratio due to drinking water supplementation of LE (0.15, 0.30 or 0.45 mg/L) in heat-stressed broiler chickens. An increased H/L ratio is a reliable indicator of stress in the chicken [14], and it has been demonstrated that heat stress is one of the most important stressors resulting in increased percentage of heterophils and H/L ratio [24-26]. This may explain different results that we have obtained in the present study.

3.4 Lymphoid Organ Weights

Bown [27] reported that licorice root is a very sweet herb that detoxifies and protects the liver, lymphoid organs and other visceral tissues, and is also powerfully antiinflammatory agent. However, in the present study none of these organs (thymus, bursa, spleen, or liver)

were affected significantly (P > .05) by the level of LE in drinking water (Table 5). These results were consistent with those of the study by Sedghi et al. [11], which showed no significant differences in liver, spleen, bursa or heart weights of the broilers fed the control or diets containing the LE. Similar results were reported in Japanese quails [17] and type 2 diabetic mice [28].

Table 4. Effect of licorice extract supplementation via drinking water on heterophil and lymphocyte numbers and heterophil to lymphocyte (H/L) ratio at different ages[1]

Item	Control	Licorice extract (mg/L)			*P* values
		0.1	0.2	0.3	
Heterophils					
Day 21	32.00 ± 2.483	37.00 ± 4.123	34.00 ± 3.227	29.75 ± 2.462	.43
Day 42	53.75 ± 2.898	53.00 ± 3.342	49.25 ± 5.677	44.00 ± 3.764	.34
Lymphocytes					
Day 21	68.00 ± 2.483	63.00 ± 4.123	65.50 ± 3.227	70.25 ± 2.462	.43
Day 42	46.25 ± 2.898	47.00 ± 3.342	50.75 ± 5.677	56.00 ± 3.764	.34
H/L ratio					
Day 21	0.47 ± 0.053	0.61 ± 0.107	0.53 ± 0.076	0.43 ± 0.050	.40
Day 42	1.18 ± 0.133	1.16 ± 0.166	1.04 ± 0.229	0.81 ± 0.124	.41

[1] *Values are treatment means (±SE). Data from 8 birds per treatment.*

Table 5. Effect of licorice extract supplementation via drinking water on the relative weight of lymphoid organs and liver of 42-day-old broiler chicks[1]

Item	Control	Licorice extract (mg/L)			*P* values
		0.1	0.2	0.3	
Bursa of Fabricius	0.12 ± 0.017	0.15 ± 0.031	0.16 ± 0.027	0.13 ± 0.016	.60
Spleen	0.12 ± 0.020	0.18 ± 0.017	0.15 ± 0.021	0.14 ± 0.025	.27
Thymus	0.29 ± 0.045	0.33 ± 0.044	0.30 ± 0.036	0.31 ± 0.053	.94
Liver	1.79 ± 0.023	1.77 ± 0.025	1.83 ± 0.329	1.85 ± 0.052	.98

[1] *Values are treatment means (±SE). Data from 8 birds per treatment.*

4. CONCLUSION

The results of the present study showed that the LE supplementation through drinking of broilers had no considerable effect on their growth performance throughout the study. Similarly, LE supplementation through drinking water had no considerable effect on their immunological parameters. Based on these observations we cannot offer supplementation of LE extract for broiler chickens and further studies must be done, especially with large numbers of animals, such as those found in actual production.

ACKNOWLEDGEMENTS

This work was supported by Razi University (Kermanshah, Iran) and Agriculture and Natural Resources Research Center of Kermanshah Province (Kermanshah, Iran) that kindly appreciated.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Fenwick GR, Lutomski J, Nieman C. Liquorice, glycyrrhiza glabra L.—composition, uses and analysis. Food chem. 1990;38:119–143. Doi:2561/10.1016/0308-8146(90)90159-2
2. Shalaby MA, Ibrahim HS, Mahmoud EM, Mahmoud AF. Some effects of glycyrrhiza glabra (liquorice) roots extract on male rats. Egypt J Natural Toxins. 2004;1:83–94.
3. Snow J. Glycyrrhiza glabra. Monograph. Protocol J Botanical Med. 1996;1:9–14.
4. Fukai T, Cai BS, Maruno K, Miyakawa Y, Konishi M, Nomura T. An isoprenylated flavanone from Glycyrrhiza glabra and rec-assay of licorice phenols. Phytochem. 1998;49:2005–2013. Doi:10.1016/S0031-9422(98)00389-6
5. Arystanova, TP, Irismetov MP, Sopbekova AO. Chromatographic determination of glycyrrhizinic acid in Glycyrrhiza glabra preparation. Chem Natural Comp. 2001;37:89–90. Doi: 10.1023/A:1017675115337
6. Doeker BM, Andler W. Liquorice, growth retardation and Addison's disease. Hormone Res Paediatr. 2000;52:253–255. Doi: 10.1159/000023470
7. Račková L, Jančinová V, Petríková M, Drábiková K, Nosáľ R, Štefek M, Košťálová D, Prónayová N, Kováčová M. Mechanism of anti-inflammatory action of liquorice extract and glycyrrhizin. Natural Prod Res. 2007;21:1234–1241. Doi: 10.1080/1478641070137 1280
8. Alkofahi A, Atta AH. Pharmacological screening of the anti-ulcerogenic effects of some Jordanian medicinal plants in rats. J Ethnopharmacol. 1999;67:341–345. Doi: 10.1016/S 0378-8741(98)00126-3
9. Khayyal MT, El-Ghazaly MA, Kenawy SA, Seif-El-Nasr M, Mahran LG, Kafafi YAH, Okpanyi SN. Antiulcerogenic effect of some gastrointestinally acting plant extracts and their combination. Arzneimittelforschung. 2001;51:545–553. Doi: 10.1055/s-0031-1300 078
10. Al-Qarawi AA, Abdel-Rahman HA, El-Mougy SA. Hepatoprotective activity of licorice in rat liver injury models. J Herbs Spices Med Plants. 2001;8:7–14. Doi: 10.1300/J044v08n 01_02
11. Sedghi M, Golian A, Kermanshahi H, Ahmadi H MA. Effect of dietary supplementation of licorice extract and a prebiotic on performance and blood metabolites of broilers. South Afric J Anim Sci. 2010;40:371–380.
12. Yu-ying L, Shin-shan Q, Li-mei Q. Effects of Chinese medical herbs on the growth and immunity of Yinxiang chickens. Guangxi Sci. 2010;4:038.
13. Khaligh F, Sadeghi G, Karimi A, Vaziry A. Evaluation of different medicinal plants blends in diets for broiler chickens. J Med Plants Res. 2011;5:971–1977.
14. Gross WB, Siegel HS. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Dis. 1983;27:972–978. Doi:10.2307/1590198
15. Thayer SG, Beard CW. Serological procedure. In: Thayer SG, Beard CW, editors. A Laboratory Manual for the Isolation and Identification of Avian Pathogens. 4th ed. American Association of Avian Pathologists, Kennett square, PA; 1998.
16. SAS Institute. SAS user's guide. Version 9.1 reviews. SAS Institute, Cary, NC: USA; 2003.
17. Myandoab MP, Mansoub NH. Comparative effect of liquorice root extract medicinal plants and probiotic in diets on performance, carcass traits and serum composition of Japanese quails. Glob Vet. 2012;8:39–42.
18. Armanini D, Palo CB, Mattarello MJ, Spinella P, Zaccaria M, Ermolao A, Palermo M, Fiore C, Sartorato P, Francini-Pesenti F, Karbowiak I. Effect of licorice on the reduction of body fat mass in healthy subjects. J Endocrinol Invest. 2003;26:646–650. PMID: 14594116

19. Nakagawa K, Kishida H, Arai N, Nishiyama T, Mae T. Licorice flavonoids suppress abdominal fat accumulation and increase in blood glucose level in obese diabetic KK-A(y) mice. Biol Pharm Bull. 2004;27:1775–1778. Doi: 10.1248/bpb.27.1775
20. Tominaga Y, Tatsumasa M, Mitsuaki K, Yoshiro S, Hideyuki I, Nakagawa N. Licorice flavonoid oil effect body weight loss by reduction of body fat mass in overweight subject. J Health Sci. 2006;52:672–683. Doi: 10.1248/jhs.52.672
21. Aoki F, Honda S, Kishida H, Kitano M, Arai N, Tanaka H, Yokato S, Nakagawa K. Suppression by licorice flavonoids of abdominal fat accumulation and body weight gain in high-fat diet-induced obese C57BL/6J mice. Biosci Biotechnol Biochem. 2007;71:206–214. Doi: 10.1271/bbb.60463
22. Utsunomiya T, Kobayashi Mo, Pollard RB, Suzuki F. Glycyrrhizin, an active component of licorice roots, reduces morbidity and mortality of mice infected with lethal doses of influenza virus. Antimicrob Agents Chemother. 1997;41:551–556. PMID: 905599
23. Al–Daraji HJ. Influence of drinking water supplementation with licorice extract on certain blood traits of broiler chickens during heat stress. Rep Opin. 2012;4:56–60.
24. McFarlane JM, Curtis SE. Multiple concurrent stressors in chicks. 3. Effects on plasma corticosterone and the heterophil: lymphocyte ratio. Poult Sci. 1989;68:522–527. Doi: 10.3382/ps.0680522
25. Yalçın S, Özkan S, Çabuk MC, Siegel PB. Criteria for evaluating husbandry practices to alleviate heat stress in broilers. J Appl Poult Res. 2003;12:382–388.
26. Habibian M, Ghazi S, Moeini MM, Abdolmohammadi A. Effects of dietary selenium and vitamin E on immune response and biological blood parameters of broilers reared under thermoneutral or heat stress conditions. Int J Biometeorol. 2013;(In press). Doi: 10.1007/s00484-013-0654-y
27. Bown D. Encyclopedia of Herbs and their Uses. Dorling Kindersley: London, ISBN 0–7513– 020–31; 1995.
28. Ma Z, Xu Y. Zhang Y, Dong L, Li Q, Duan Q, Xu J, Ni J. Anti-metabolic syndrome effect of Licorice flavonoid dispersible tablets in a high fat diet-induced obesity/type 2 diabetes mouse model. Asian J Tradit Med. 2010;55:89–101.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=316&id=32&aid=2500